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1- Engineering Education, 2 - Civil and Environmental Engineering, 3 – Biological Sciences 4 - Crop and Soil Environmental Sciences, 5 - Geosciences

Host Lab: www.lewas.centers.vt.edu

LEWAS LAB
LabVIEW Enabled Watershed Assessment System

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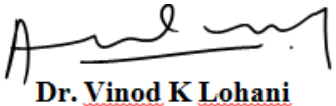
We would like to express our sincere thanks to REU/NSF Site Research Mentors for their kind cooperation and excellent mentoring during summer 2013. Our thanks are also to our graduate student mentors, REU Fellows for their dedication and excellent performance, laboratory staff, seminar speakers, professionals who assisted in field trips and all other individuals who directly/indirectly contributed to the success of our 10-week research program at VT. The program is supported by the National Science Foundation (NSF-REU Grant No. 1062860).

Editorial Staff



Dr. Vinod K Lohani

Summary

This Research Proceedings includes papers of undergraduate research that was conducted at Virginia Tech during summer 2013 as part of an NSF/REU Site on Interdisciplinary Water Sciences and Engineering. This was the 3rd and final year of our 2011-14 NSF/REU Site. This Site follows a very successful NSF/REU Site that was implemented at VT during 2007-09. Research Proceedings of 2007, 2008, 2009, 2011, and 2012 Sites are available at: www.lewas.centers.vt.edu. At the end of summer 2013, 56 REU Fellows (33 women and 23 men) have graduated from our 2007-09 and 2011-14 Sites. This Site has been renewed for another 3 years (2014-16).

Exposing qualified undergraduates to interdisciplinary research issues in water sciences and engineering is the key goal of this REU Site. Faculty members from five departments (Engineering Education, Civil and Environmental Engineering, Biological Sciences, Geo-sciences, and Crop and Soil Environmental Sciences) at Virginia Tech mentored 11 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 – 2013 Research Work

Ms. Holly Clark (an REU Fellow from University of Idaho) and her co-authors investigated the response of an urban sub-watershed (i.e., Webb branch; ~3 km^2) of the Stroubles Creek Watershed in south-west Virginia to acute toxicity events using high frequency water quantity and quality measurements recorded at an outdoor lab called the LabVIEW Enabled Watershed Assessment System (LEWAS). A variety of sensors including a rain gauge, a water quality Sonde, and an Acoustic Doppler Current Profiler are used for data collection. In addition, the location and attributes of local storm-water catchments are surveyed and recorded using GIS software to determine the area that's contributing runoff at the LEWAS site. A case study examining the effects of road deicing salts on this urban watershed is also presented. In a

study conducted by Ms. Brittany Flittner (an REU fellow from University of Rochester) and her co-authors, the possibility of combating *Legionella pneumophila* (LP), an opportunistic pathogen found within premise plumbing, is explored by selection of plumbing materials. LP can cause Legionnaire's Disease (severe pneumonia) in immunocompromised individuals, hospitalizing 8,000 to 18,000 people each year. The overall goal was to show the relationship between free copper ion levels and LP inactivation, with an expectation that higher levels of free copper will reduce LP. Mr. Quinn Hull (an REU fellow from Oberlin College) and his co-authors conducted a study to characterize Mn behavioral dynamics in the downstream of Smith Mountain and Leesville Lakes, a dual reservoir hydroelectric system created by the impoundment of the Roanoke River in Southwest Virginia. Results show that hydrologic condition (i.e., stage) controlled Mn behavior from a major tributary (Goose Creek) whereas a complex set of parameters ('reservoir dynamics') controlled Mn behavior from Leesville Lake. Mr. David Koser (an REU fellow from University of Iowa) and his co-authors examined the potential of an emulsified soybean oil to treat soil contaminated with tetrachloroethene (PCE) and trichloroethene (TCE) compounds. An experiment was set up to estimate the amount of time for carbon levels to decrease by 90% in aquifer sediment after injection of the oil. In a study conducted by Ms. Arianna Nasser (an REU fellow from N C State Univ.) and her co-authors examined filter performance in removing manganese and lead from drinking water containing varying mineral levels. Three water types, one with low hardness and TDS, one with high TDS, and one with high hardness, were augmented with 1 mg/L Mn(II) and 0.15 mg/L Pb(II) and two types of filters were used to treat each. Results indicate that filter efficiency decreases as each filter reaches capacity and filer performance varied significantly. Mr. Olumayokun Odukale (an REU fellow from Morgan State University) and his co-authors examined the impacts of nonylphenol ethoxylates (NPEs) that are generally discharged in large quantities into aquatic environments either directly from untreated effluents or indirectly from sewage treatment plants. The major transformation product of NPEs is 4-nonylphenol (4-NP), which is an endocrine disruptor in many organisms. The authors tested water and sediment samples taken upstream and downstream of the Tazewell Wastewater Treatment Plant and the Richlands Regional Wastewater Treatment Facility (both located in Tazewell County, Virginia) along the Clinch River and 4-NP was detected in the water and sediment samples at a maximum of 0.2μg/L and 160μg/kg (dry weight basis), respectively. The effects of 4-NP on one freshwater mussel species inhabiting the Clinch River, Medionidus Conradicus (Cumberland moccasinshell) were also investigated. In a study conducted by Mr. Arun Rai (an REU fellow from Virginia Tech) and his co-authors, the possibilities to automate the analysis of weather data recorded at the LabVIEW Enabled Watershed Assessment System (LEWAS) were examined. Three key components of the study were to: (i) create a database specific to the parameters measured by the LEWAS Lab, (ii) create a connection between the real-time LabVIEW system and the database, and (iii) designing a data-retrieval web-interface. A number of software related challenges were encountered in completing the study. Mr. Kenneth Sears (an REU fellow from Rowan University) and his co-authors studied surface water-groundwater (SW-GW) exchange in a stream's floodplain during periods of inundation. They examined the effects of seasons and soil moisture on SW-GW exchange during controlled floodplain inundation in Stroubles Creek, Blacksburg, VA. Results showed that no significant difference of SW-GW exchange was observed between spring and summer flood events when antecedent moisture conditions were similar. An REU fellow from University of Wisconsin-Platteville (Ms. Nancy Streu) and her co-authors used the inundation distance as an indicator of storm intensity in a coastal storm. They estimated this distance using rectified photographs instead of using survey equipment. The method was tested at Duck, NC, where coastal features were measured using the images with an accuracy of about 75%, indicating its usefulness during site visits. Ms. Sydney Sumner (an REU fellow from James Madison University) and her co-author evaluated the density and distribution of benthic macroinvertebrates in Stroubles Creek, used this data to assess the effectiveness of a stream restoration project implemented by an engineering

department, and compared benthic macroinvertebrate diversity in Stroubles Creek to that of Toms Creek. The macroinvertebrate data indicate continued impairment of Stroubles Creek. However, the extent of impairment found in Stroubles Creek was not uniform. An REU fellows from Humboldt State University in California (Ms. Christina Urbanczyk) and her co-authors studies algal blooms in four local reservoirs including Falling Creek Reservoir (FCR) in south-west Virginia. The primary goals were to determine the limiting nutrient for phytoplankton growth and the effect of copper sulfate on phytoplankton. Results indicate that the primary limiting nutrient for phytoplankton in FCR is phosphorus, and the minimum concentration of copper sulfate needed to reduce phytoplankton concentrations 24h after application is between 0.125mg/L and 0.250mg/L, though its effectiveness diminishes by 72h after application.

REU Fellows created three YouTube videos to document their experiences of 10-week work at VT:

https://www.youtube.com/watch?v=O3TGJLqDnl4
https://www.youtube.com/watch?v=AqugvXwHAJU
https://www.youtube.com/watch?v=zLXgAAriFb0

Disclaimer

The opinions, findings, and conclusions or recommendations expressed in this proceedings are those of the authors and do not necessarily reflect the views of the National Science Foundation or Virginia Tech.

Research Papers

Investigating the Response of the Stroubles Creek Watershed to Acute Toxicity Events via Real-Time Data Analysis

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ABSTRACT

This study investigates the response that the Stroubles Creek Watershed has to acute toxicity events. Meteorological and water quantity and quality measurements are taken at a high-temporal resolution (.5 – 3 minutes) via a quartet of in-situ instruments, including a weather station, rain gauge, water quality Sonde, and an Acoustic Doppler Current Profiler. These sensors, located in Stroubles Creek and maintained by the LabView Enabled Watershed Assessment System Lab (LEWAS) on the Virginia Tech campus, make real-time monitoring possible. Continuous measurements of specified water and weather parameters are taken and relayed through a network to LabView software and on to an end-user interface. The location and attributes of local storm-water catchments are surveyed and recorded using GIS software to determine the overall area of the Stroubles Creek Watershed. This fieldwork results in the construction of an accurate storm-water network map, which in turn allows sources of sedimentation and pollution to be pinpointed. The efficiency of the LEWAS Lab's protocols are examined and compared to the suggested operations of the USGS. This will improve the quality of research that the lab performs. A case study examining the effects of road deicing salts on this urban watershed using data collected this past spring is presented. This analysis characterizes the creek's metabolic response to this event by illustrating the consequential "spike" in the water's specific conductivity measurements and the implications of saline runoff for native freshwater organisms.

Keywords: Watersheds, acute toxicity, real-time data, data analysis, monitoring, flow measurement, data collection, hydrologic data

I. Introduction

Background: Stroubles Creek Watershed and the LEWAS Lab

Stroubles Creek, which flows approximately 9 miles from Blacksburg, Virginia to the New River, has been classified as a second order stream with a watershed that is part of the larger New River Watershed (Parece et al., 2010). The Stroubles Creek Watershed, which spans 14, 336 acres, has been divided into two parts: the Upper and Lower Stroubles Creek Watershed (Parece et al., 2010). Since the establishment of communities in the region beginning in 1740, the riparian health of the Stroubles Creek Watershed has fluctuated dramatically (Parece et al., 2010). The major land use changes that have occurred as a result of settlement over the past 270 years have impacted the watershed's capacity to endure the ecological consequences of human activity.

The Stroubles Creek Watershed has a history of impairment, as defined by the Environmental Protection Agency. In the late 1990s, the Lower Stroubles Creek Watershed was considered benthically impaired, and later TMDL calculations in 2003 indicated that sedimentation was a major cause of these impairments (Parece et al. 2010). In the past decade, restoration efforts have been implemented to help restore the riparian health of the Stroubles Creek Watershed (Parece et al. 2010).

The Virginia Tech Department of Engineering Education hosts a lab whose main function is maintaining a real-time monitoring system in Stroubles Creek. The LabView Enabled Watershed Assessment System (referred to as LEWAS henceforth) was founded in 2008 and currently operates a suite of in-situ instruments in Stroubles Creek with the goal of relaying the recorded data to the community via the Internet. The philosophy fueling the LEWAS Lab's efforts is that access to data regarding the quality and quantity of the water flowing through a system can increase public awareness of the impact that urbanization has on a local watershed. Figure 1.1 shows the location of the LEWAS Lab's sensors with respect to the boundaries of the Upper Stroubles Creek Watershed.

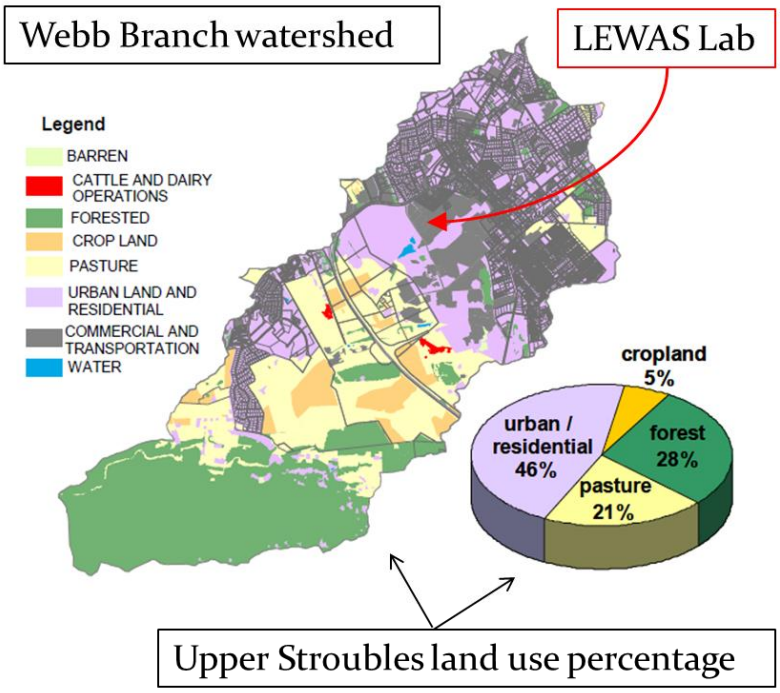

Figure 1.1 Stroubles Creek Watershed with LEWAS Lab Relative Location
(Upper Stroubles Creek Watershed TMDL Implementation Plan 2006)

Literature Review: Watershed Impairments and Real-Time Monitoring Systems

It is important to note in Figure 1.1 (above) that a significant portion of the Upper Stroubles Creek Watershed has been classified as "urban." Land that has been heavily developed with infrastructure and buildings is prone to channeling large amounts of runoff during precipitation events due to the impermeable surfaces characteristic of urban regions. This runoff can increase the amount of sediment that is displaced, resulting in erosion and excess sediment deposition. Runoff also introduces pollutants into the aquatic system in the form of chemical compounds, trash, debris, and sediment. The type of pollutants present in the runoff is dependent upon the size and location of the community generating them. Many studies suggest that deicing salts administered by communities prone to freezing temperature pose a threat to water quality in terms of chloride toxicity (Corsi et al., 2010; SEWRPC No. 316). In climates that are not as harsh, road deicing salts might not be a potential source of pollution. Other factors might be of concern in communities that are more rural: the pesticides and fertilizers used in agricultural communities can be heavy in nutrients, such as nitrates and phosphates. In excess, these compounds can cause eutrophication and can impair the system.

Research Rationale: Justification, Goals, and Objectives

Because Blacksburg, VA is considered to be more urbanized than it is agricultural, nutrient concentrations are not of investigative priority. Blacksburg experiences cold, freezing winters and has a large urban landscape, making other parameters, such as specific conductivity and turbidity of higher monitoring importance to the LEWAS Lab.

Stroubles Creek's history of impairment and the increased urbanization in Blacksburg, VA makes water quality and quantity monitoring necessary. The LEWAS Lab aims to utilize data collected in real-time to gain insight of the metabolic conditions of the Stroubles Creek Watershed, and to publicize the information for educational purposes. The ability to correlate changes in the measured values of parameters with the meteorological conditions at which they occurred has profound analytical potential: this makes it possible for the response that the watershed has to acute toxicity events to be better understood.

The LEWAS Lab has three objectives to achieve the goal of investigating the response that the Upper Stroubles Creek Watershed has to acute toxicity events: The team first must understand how a real-time monitoring system functions and how to address malfunctions of the components involved; a storm-water network map of the Upper Stroubles Creek Watershed will be delineated to verify the watershed area; and the two aforementioned objectives will be combined to produce a case study that investigates how deicing salt runoff from a storm event was transported to the field site, and the impact that it had on the water quality.

II. Research Methods:

Site Description and Experimental Set-Up

The LabView Enabled Watershed Assessment System (henceforth referred to as "LEWAS") Lab maintains a field site located in the northwestern division of the Virginia Tech campus, where Stroubles Creek emerges from a concrete culvert to flow through a weir, and onward approximately 200 feet to the Duck Pond (see Figure 2.1). Pre-specified parameters are measured and recorded by a suite of in-situ sensors at this location. An "Argonaut" Acoustic Doppler Current Profiler (henceforth ADCP), manufactured by SonTek, sits on the streambed with the sensors pointing up stream, and measures flow velocity (Figure 2.2). Meteorological conditions at the site are recorded with a solar-powered Vaisala weather station (Figure 2.3) equipped with sensors capable of measuring air temperature and pressure, relative humidity, wind speed and direction, and rainfall. Water quality parameters including temperature, pH, turbidity, specific conductivity, dissolved oxygen, oxidation reduction potential, percentage of saturated dissolved oxygen, and total dissolved solids are measured with a Hach MS 5 HydroLab Sonde (Figure 2.4). An additional "tipping-bucket" rain gauge (Figure 2.5) has also been installed to allow the rainfall readings to be compared; this is used to estimate the degree of accuracy of the rainfall tympanic membrane sensor on the weather station.

Figure 2.1 LEWAS Lab Field Site on Stroubles Creek, Virginia Tech (H. Clark June 6, 2013)

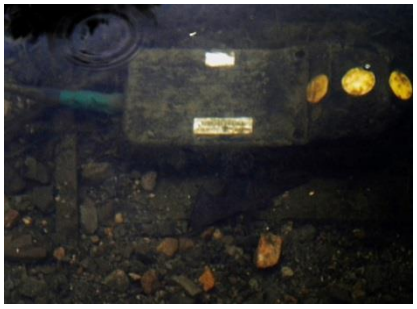

Figure 2.2 SonTek Argonaut Acoustic Doppler Current Profiler
(H. Clark June 3, 2013)

Figure 2.3 Vaisala Weather Station
(H. Clark July 9, 2013)

Figure 2.4 Hach MS 5 HydroLab Water Quality Sonde
(H. Clark July 16, 2013)

Figure 2.5 Rain Gauge
(H. Clark July 9, 2013)

The data obtained through these sensors are relayed to a Compact RIO computing device for data processing and temporary storage. The data can be retrieved through the network to LabView software, arriving at an end user interface (LEWAS Lab, 2013).

The decision to establish the LEWAS Lab monitoring station at this location (Figure 2.6) was dictated largely by the topography of the Upper Stroubles Creek Watershed. This portion of the watershed, one of two major inflows to the Duck Pond, contains part of the city of Blacksburg and much of it is covered with an impermeable infrastructure that gradually slopes towards (and ultimately drains at) the Virginia Tech Duck Pond. The urban runoff from the town flows through the weir and past the sensors at the LEWAS site to collect in the Duck Pond. Consequentially, the water quality at this location is indicative of not only how anthropogenic activity impacts water quality, but also of the watershed's functionality during flood events.

Figure 2.6 Upper Stroubles Creek Watershed Boundary Relative to the LEWAS Lab Site, Blacksburg, VA
(H. Raamanathan; M. Aguilar 2013)

Requisite Maintenance of a Real-Time Monitoring System and Laboratory Clerical Protocol

The mechanics of a real-time monitoring system and the complications associated with inoperable sensors are two fundamental components of a successful hydrology lab. Accurate, valid data is only generated by sensors which have undergone meticulous calibration and are not physically or electronically damaged. Sustaining a functioning monitoring system is a primary research method in itself, for the collection and quality of any subsequent data is dependent upon the sensors' condition.

Due to the high volume of water that the LEWAS site encounters during an event (Figure 2.7), frequent Sonde and ADCP sensor repairs and site maintenance has to be performed. These repairs must be completed as efficiently as possible to reduce the amount of time that the site is unmonitored.

Figure 2.6 Flooding from Rain Event at LEWAS Field Site
(V. Lohani July 3, 2013)

Debris in a variety of mediums—namely substantial rocks, urban garbage, and vegetal wreckage—that has been channeled through the storm-water network of Blacksburg, arrives at the site and potentially collides into the ADCP and Sonde (Figure 2.7). This disturbs the riparian ecosystem by damaging existing habitats and increasing turbidity, and requires the material to be manually removed from the structure.

Figure 2.7 Hach MS 5 HydroLab Water Quality Sonde Covered in Debris; Sedimentation Deposits on South Stream Bank
(H. Clark June 11, 2013)

Increased flow at the field site also has a negative impact on data accuracy, water quality, and riparian health in the form of substantial sediment deposition. The leading sources of sediment runoff in Blacksburg are construction sites and gravel parking lots, many of which are adjacent to the site. The accumulated sediment in the culvert (Figure 2.8) indicates the amount of sediment that will eventually be deposited into the Duck Pond in the next event. The Duck Pond has had a history of poor water quality, much of which is attributed to sediment: the pond (which functions as a large-scale storm-water catchment) was dredged three times in 1950, 1960, and 1986 because of detrimental sediment deposition (Parece et al. 2010).

Figure 2.8 Sedimentation Deposits in Culvert and Weir (looking upstream)
(W. McDonald; H. Clark 2013)

The impact of sedimentation on the water quality at the site is perceived by the Sonde registering increases in turbidity and total dissolved solids only when it is not severe; recurring acute sedimentation at the site has repeatedly resulted in sensors of the Sonde getting clogged with a combination of clay-like material and algae, resulting in bogus readings or permanent damage. To clear the openings of the sensors, a plastic handled dish brush and cotton swabs (Figure 2.9) are used to scrub off the build-up.

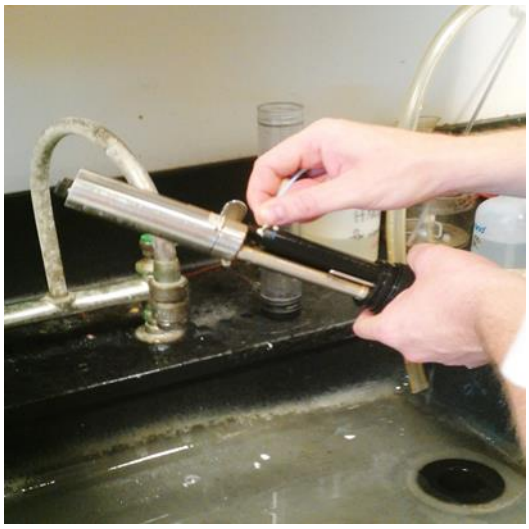

Figure 2.9 Cleaning Sonde Sensor
(H. Clark July 1, 2013)

Routine calibrations require the sensors to be removed from the stream, cleaned in a laboratory environment, and reset with standard solutions for each parameter at approximately three-week intervals. The frequency of calibration is partly determined by the "drift" measurements that have been observed since the previous procedure; if an increased margin of error is noted, then calibrations will be performed more frequently. In addition to the aforementioned maintenance performed by the LEWAS Lab, professional repairs were necessary after a particularly damaging rain event in early July: the Sonde was sent to its manufacturing company, Hach, to have the sensors replaced and recalibrated.

In July, the Acoustic Doppler Current Profiler also underwent a series of repairs following severe rain events to address internal electrical damage. The issue became apparent when measurements were not being recorded by the instrument, and it has been deduced that the connection between the conduit and the instrument had been exposed to water for a prolonged period of time. This caused water to leak into the instrument, and the pins of the male connector to become corroded (Figure 2.10.1). The lack of a functional water-tight seal could have resulted from a few hypothetical situations: the accumulation of biofilm around the port prevented a flush seal (Figure

2.10.2), debris during storm events bombarded the port and broke the seal, or the conduit was previously roughly inserted into the instrument and formed a leak.

Figure 2.10.1 ADCP Flow-Meter with Corroded Biofilm Accumulation
(H. Clark July 9, 2013)

Figure 2.10.2
Corroded ADCP Conduit Pins
(W. McDonald 2013)

To repair, the ADCP flow-meter was removed from the streambed and had the build-up of biofilm removed with a plastic handled dish brush. Electrically-conducting silicone spray was deposited into the female ports of the ADCP to create a water-tight seal. The damaged conduit wire containing the connector was spliced to a spare (to achieve the necessary length) with a soldering connection**.** The connector was then inserted into the ports of the instrument.

After the ADCP flow-meter was returned to the streambed, it was necessary for the instrument to be calibrated (R. Dymond 2013). During an event, a stream's profile is changed dramatically due to erosion and sedimentation caused by the high volume and velocity of water passing through. The ADCP calculates flow velocity based upon the principle of a stage-discharge relationship. This formulaic ratio between the height of the channel and the output of water changes when the shape of the channel is altered; the path that the water follows determines the volume of water that can physically pass through and the velocity at which it can. When this path is altered, the volume of the channel is changed, and consequently the stage-discharge relationship changes. A cross-sectional profile survey was performed (Figure 2.11) at the site in two locations—at the ADCP and downstream at a distance four to five times the stage at the original location—to generate the new profile of the stream. A repeat cross-sectional survey was performed at the same locations to record the velocities at various increments. The values were recorded using a Leica Total Station and SonTex Flow-Tracker in each procedure and were imported into Microsoft Excel to generate a graphic profile (Figure 2.12) of the stream. This allows the velocity measurements at each increment to be correlated with the respective stage measurements. An accurate estimate of the amount and velocity of water that is passing through the system is useful, in that these values directly reflect the size and speed of a load of sediment (and any debris or pollutant that it contains) that is physically able to be transported to the catchment location. Knowing the volume of water that is discharged from the LEWAS Lab field site allows projections to be made about the mass of sediment that is being deposited at the Duck Pond, and the mass (rather than concentration) of the measured pollutants passing the LEWAS field site.

It is important to note that meticulous notes and photographs were taken during each procedure performed by the LEWAS Lab: detailed records were kept of all field and laboratory visits, as well as office correspondences and meetings. These were published on an online forum accessible by all members of the lab to help streamline communications and provide continuity between projects.

Figure 2.11 Performing Cross Sectional Profile Survey Downstream of ADCP
(W. McDonald July 9, 2013)

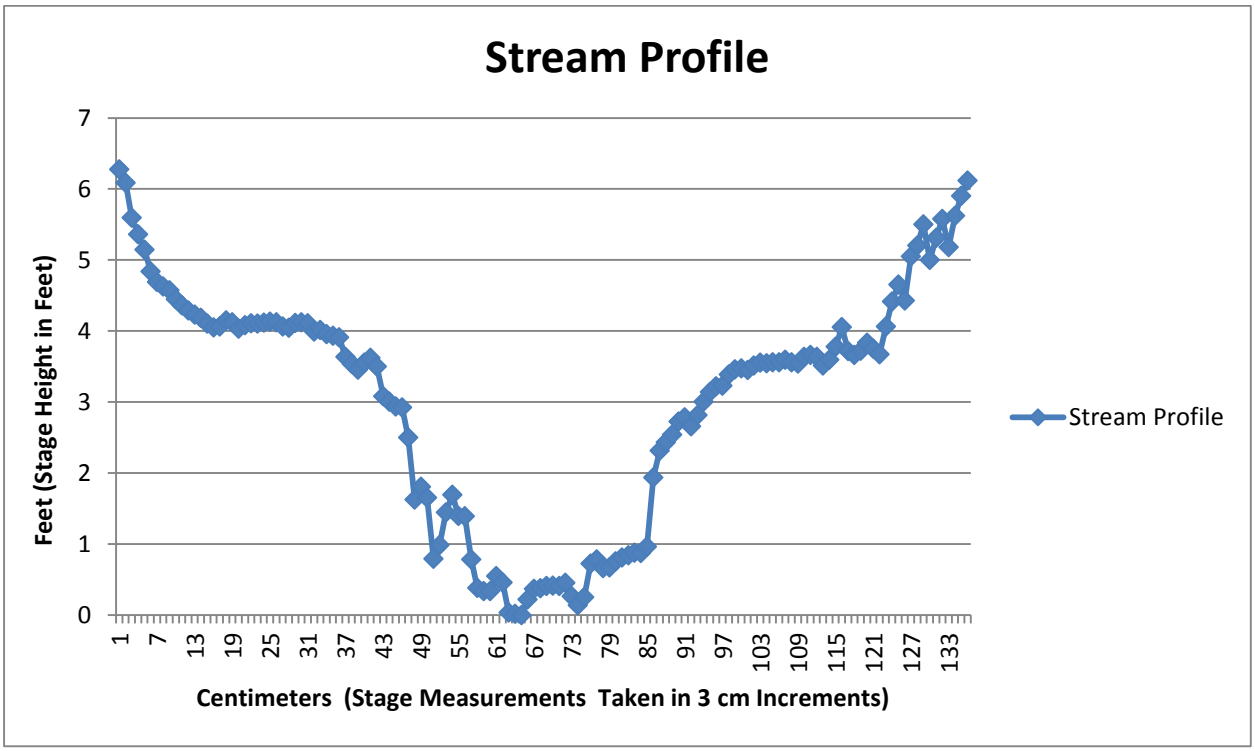

Figure 2.12 Stream Profile of Stroubles Creek as Generated by Cross-Sectional Survey
(W. McDonald; H. Raamanathan July 9, 2013)

The ADCP can be reprogrammed to account for changes in the stream profile; cross-sectional surveys have been used to indirectly "calibrate" the instrument (R. Dymond 2013).

Routine maintenance—physical cleaning, calibration, electrical rewiring, professional repairs—comprise the LEWAS Lab's fundamental methods of research. The sensors of a real-time monitoring system must be functioning properly in order for measurements to be taken at this demanding temporal resolution; maintenance and understanding the dynamic of the system are crucial to producing accurate data.

Storm-Water Network Survey: Mapping Stroubles Creek Watershed via GIS Technology

A complete storm-water network map is a necessary resource for the LEWAS Lab to determine where the sources of sedimentation are originating. An understanding of the network can also allow the routes of sediment loads to be inferred, which is useful in predicting the amount of time it takes for a pollutant to reach the site. Much of the storm-water network for the Upper Stroubles Creek Watershed had already been completed prior to June 2013.

Conducting a storm-water network survey of a portion of the McBryde neighborhood, a residential area just north of the Virginia Tech Campus, constituted the second objective of the research methods in this investigation. A map of the storm-water catchments and pipes for this portion of Blacksburg did not exist prior to this survey, making it necessary for it to be performed. The storm-water network in this neighborhood is part of the contributing watershed; understanding the "web" of conduits in this region is necessary to determine whether (and if so, how) runoff from the neighborhood reaches the field site.

To begin constructing a storm-water network map, the Google Maps aerial view feature was used to locate locations of catchments in the McBryde neighborhood that needed surveying. A marker was placed at each one to generate a rough map of the area to visit in person. While at each catchment, measurements of its attributes—pipe material, diameter, shape, azimuth, depth, type (ie.: yard inlet vs. curb inlet)—were recorded into an ArcPad instrument. A picture was taken with a GPS camera at each catchment location as well as sites with probable sources of sediment to later (upon import to ArcGIS) provide a visual relationship (Figure 2.13) between a sediment source and the means of its transportation to the site.

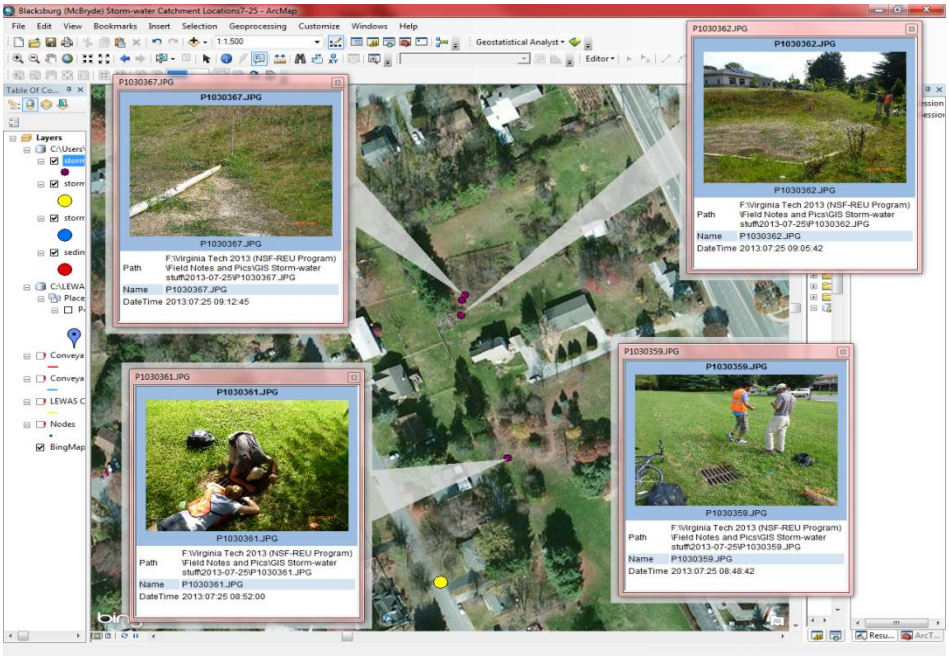

Figure 2.13 Geotagged Photographs of Catchment Locations and Sediment Sources
(H. Clark 2013)

The catchment locations and corresponding attributes stored on the portable ArcPad were imported to ArcGIS. A Python script, written prior to June 2013, was executed for each one-dimensional catchment location, generating a two-dimensional line (pipe) stub in the appropriate azimuth direction. Because the attributes of each catchment are stored in the point-feature, catchments with like attributes can be digitally connected with lines, thus representing plausible paths of the pipes in the storm-water network.

Quantifying Consequential Acute Toxicity: A Case Study in Specific Conductivity Fluctuation

The zenith of the aforementioned research methods is the production of a study that examines how the Upper Stroubles Creek Watershed responds to an acute chloride toxicity event from late March of 2013. Blacksburg experienced an early spring storm in the last few days of March, as indicated by the meteorological (specifically the drop in temperature and air pressure) data collected by the weather station (Figure 2.14). A photograph taken by one of the LEWAS Lab members (Figure 2.15) a day after the peak of the storm, shows the conditions at the LEWAS field site.

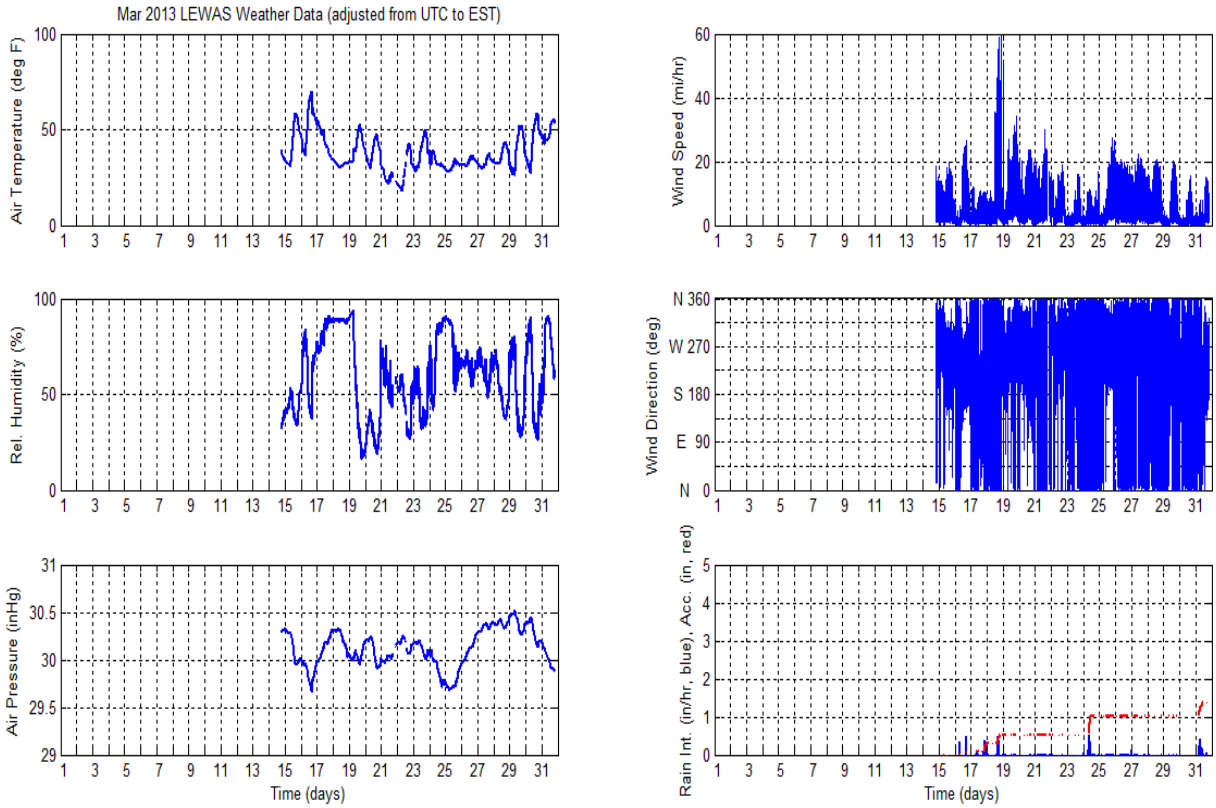

Figure 2.14 Weather Station Data for Duration of Storm Events
(A.Rai 2013)

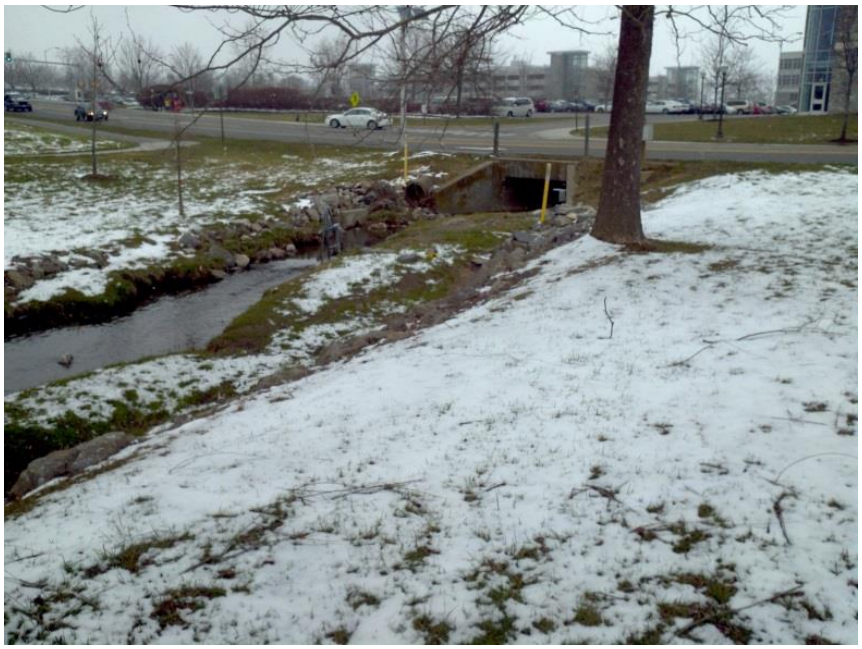

Figure 2.15 LEWAS Field Site, March 27, 2013 Storm Event
(D. Brogan March 2013)

The water quality Sonde at the field site sensed a significant "spike" in specific conductivity during this snow event (Table 2.16). Because it is known that the city of Blacksburg and Virginia Tech use a salt compound to deice the roadways around town during snow events, a connection can be drawn between the spike in specific conductivity and the road salting event. Mike Dunn, Transportation Planning Engineer, and Mark Helms, Facilities Operations Director, were contacted to confirm the chemical composition of the deicing compound Virginia Tech administers. According to the Material Safety Data Sheet provided in response to this inquiry, the deicing compound is approximately 99% NaCl. This information is necessary to find an appropriate regression equation that can show the relationship between specific conductivity and chloride concentration. The EPA has determined what concentrations of chloride are toxic in acute exposures for various freshwater organisms and indicative of stream impairment. Converting specific conductivity measurements to chloride concentrations determines whether these toxicity levels are being exceeded in a given system.

Table 2.16 Processed Sonde Data for Peak of March 25th Deicing Event

Date/Time	Temp [°C]	pH [Units]	SpCond [µS/cm]	DO [mg/l]	TurbSC [NTU]	IBatt [Volts]	EBatt [Volts]	IBatt [%Left]	EBatt [%Left]	ORP [mV]	TDS [g/l]	DO% [Sat]	Chloride µg/L	EPA Recc Cl µg/L	Average Cl Concentration
3/25/2013 02:30	6.88	7.74	6804	8.65	14.5	11.3	11.4	82	56	585	4.4	72.8	2041200	860,000	1764405
3/25/2013 02:33	6.89	7.74	6968	8.63	15.6	11.2	11.4	82	56	585	4.5	72.8	2090400	860,000	1775445
3/25/2013 02:36	6.9	7.73	7093	8.59	15.6	11.2	11.4	82	56	585	4.5	72.4	2127900	860,000	1788675
3/25/2013 02:39	6.9	7.73	7209	8.5	16	11.2	11.4	82	56	584	4.6	71.8	2162700	860,000	1804770
3/25/2013 02:42	6.92	7.73	7288	8.5	18.2	11.2	11.4	82	56	584	4.7	71.7	2186400	860,000	1823370
3/25/2013 02:45	6.93	7.73	7310	8.49	15.9	11.2	11.4	84	56	584	4.7	71.7	2193000	860,000	1843845
3/25/2013 02:48	6.94	7.72	7289	8.53	14.8	11.2	11.4	82	56	584	4.7	72.1	2186700	860,000	1866525
3/25/2013 02:51	6.95	7.72	7257	8.55	13.8	11.3	11.4	82	56	583	4.6	72.3	2177100	860,000	1891020
3/25/2013 02:54	6.94	7.73	7201	8.43	13.3	11.3	11.4	84	56	583	4.6	71.2	2160300	860,000	1916775
3/25/2013 02:57	6.94	7.73	7136	8.47	13	11.2	11.4	84	56	583	4.6	71.5	2140800	860,000	1942740
3/25/2013 03:00	6.93	7.73	7069	8.54	12.7	11.3	11.4	82	56	582	4.5	72.1	2120700	860,000	1968360
3/25/2013 03:03	6.93	7.73	7018	8.35	12.2	11.3	11.4	84	56	582	4.5	70.4	2105400	860,000	1993515
3/25/2013 03:06	6.92	7.73	6931	8.6	12.3	11.2	11.4	84	56	582	4.4	72.5	2079300	860,000	2017020
3/25/2013 03:09	6.91	7.74	6827	8.7	12.4	11.2	11.4	82	56	581	4.4	73.3	2048100	860,000	2038080
3/25/2013 03:12	6.9	7.74	6755	8.43	12.5	11.2	11.4	82	56	581	4.3	71	2026500	860,000	2056170

(W. McDonald, D. Brogan, H. Clark, July 2013)

It is also of interest to note the increase in turbidity and temperature that corresponds to the spike in specific conductivity. This indicates that there was a disturbance in the system caused by the incoming runoff from the

roadways. Runoff is often higher in temperature than the system it is entering due to the warming effect of radiated heat from impermeable surfaces.

III. Results and Discussion:

Generated Storm-Water Network Maps

A complete storm-water network map of the McBryde neighborhood was not generated in the course of this summer investigation. However, a significant portion of the neighborhood's storm-water conduits was successfully mapped out, thus improving the LEWAS Lab's understanding of the contributing Upper Stroubles Creek watershed area. Figure 3.1 shows the portion of the McBryde area that was mapped as a result of this summer's survey (highlighted), in relation to the entire known area of the Upper Stroubles Creek watershed.

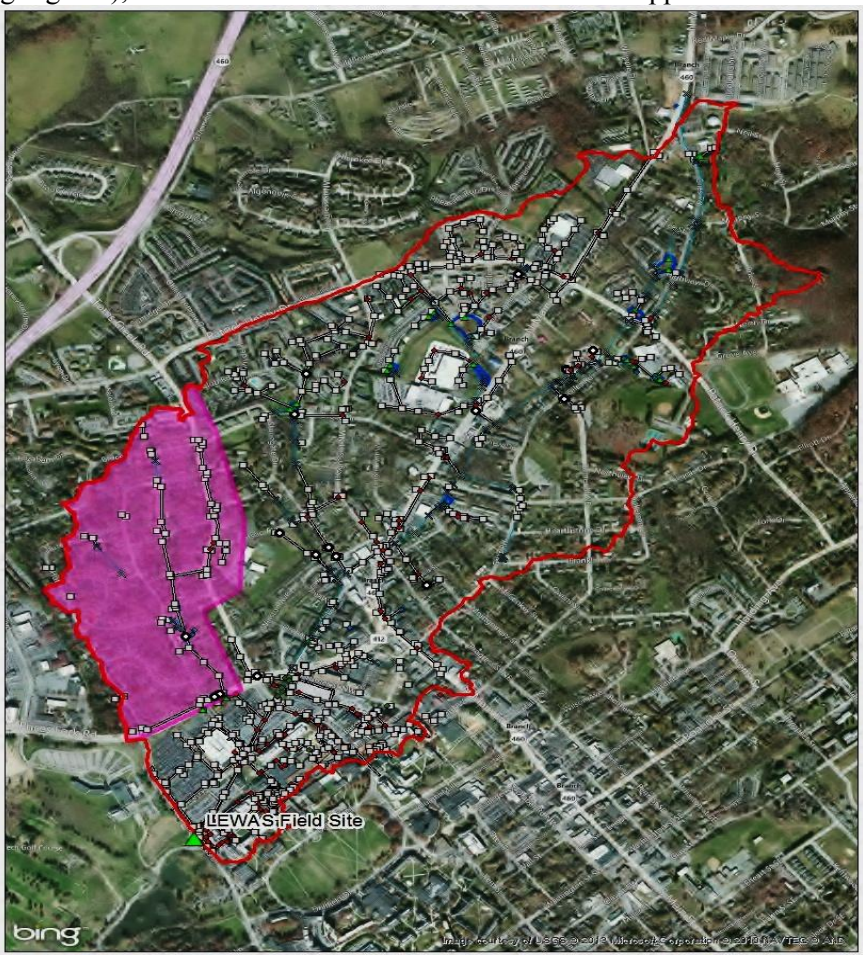

Figure 3.1 Portion of McBryde Storm-Water Network System Mapped During Summer 2013
(H. Raamanathan; M. Aguilar 2013)

The storm-water network maps created as a result of the survey process ultimately provide a visual explanation of how pollutants and sediment arrive at the LEWAS Lab field site. Knowing the path that a load travels can allow temporal estimates to be made: the amount of time that lapses between a load entering a given conduit and its presence recorded by the sensors at the monitoring site indicate how an urban watershed responds to an event. As demonstrated by the above storm-water network maps, the Upper Stroubles Creek watershed allows runoff from the vicinity of Blacksburg to be channeled to the Duck Pond. Any pollutants or sediment present in the runoff are recorded by the sensors, providing quantitative data that is used to assess water quality.

Derived Chloride Concentrations and their Implications

During the March 25, 2013 storm and accompanying deicing event, the water quality Sonde recorded the following values (Figure 3.2) for specific conductivity at the field site.

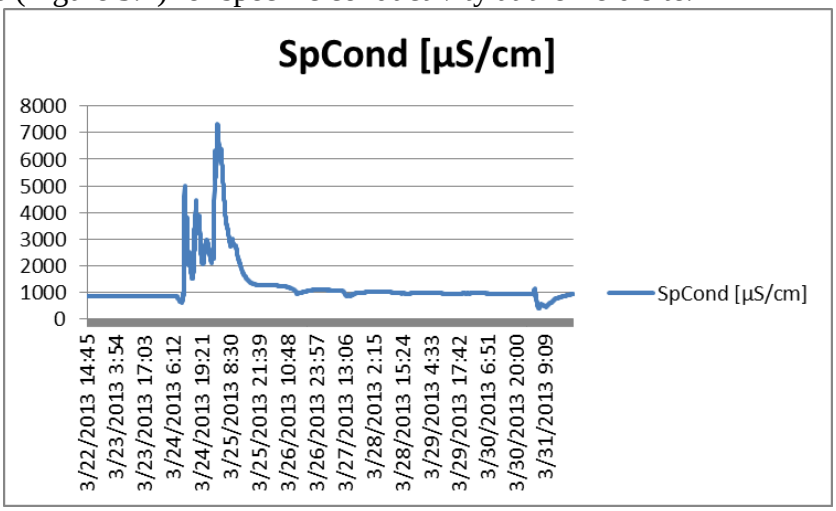

Figure 3.2 Specific Conductivity Measurements at LEWAS Lab Field Site on March 25, 2013.
(H. Clark; W. McDonald, 2013)

It is of great importance to note that the largest "spike" in specific conductivity measurements occurred in the span of approximately one hour. The Sonde was programmed to take measurements every three minutes. The following graph (Figure 3.3) focuses on the hour when the specific conductivity spiked. The value of a functioning real-time water quality monitoring system is illustrated with this graph: it is impractical to expect manual measurements to be taken at such a high temporal resolution, especially during a storm. If no measurements were taken during this critical hour, the spike in specific conductivity would obviously have occurred unnoticed. Having this data allows for a relationship to be derived between watershed response and storms that occur in urban areas.

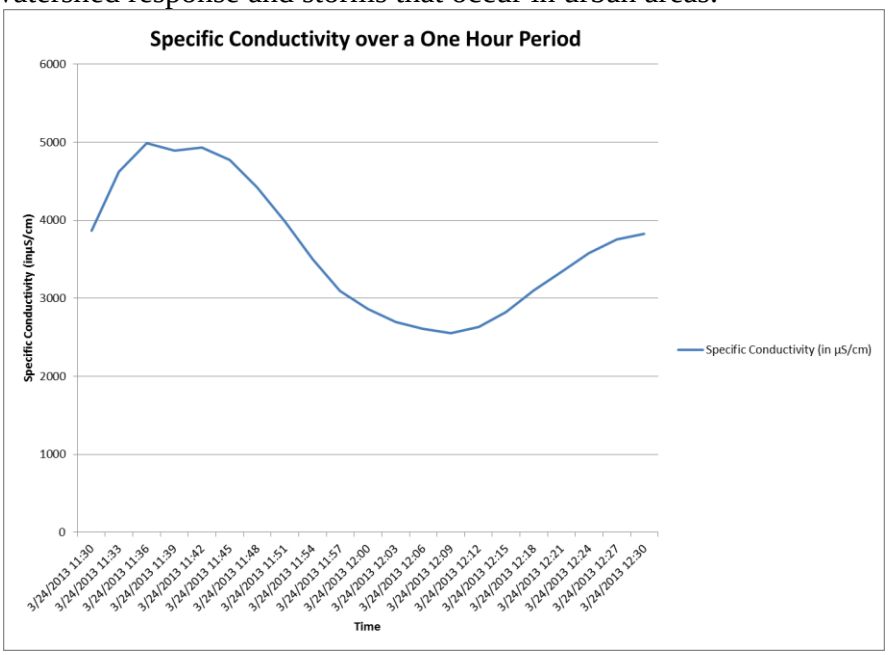

Figure 3.3 Specific Conductivity at LEWAS Field Site for a One Hour Period on March 25, 2013
(H. Clark, 2013)

As mentioned before, it was deduced that the spike in specific conductivity occurred as a result of a sodium chloride compound (specified by the Virginia Tech Facilities Operations Manager) being administered as a means of deicing the roads during the snowstorm. This overtly demonstrates how an urban practice—the act of salting roads—can impact the local watershed.

The United States Environmental Protection Agency quantifies an acute toxicity level for chloride: 860,000 µg/L (State Water Control Board, 2011). Specific conductivity is an indirect measure of chloride concentration; a regression equation can be applied to quantify the corresponding chloride concentration for a given specific conductivity measurement.

The following equation (taken from Appendix E of the 2013 SEWRPC Community Assistance Planning Report No. 316, "A Watershed Restoration Plan for the Root River Watershed") was applied to the specific conductivity data collected during the March 25, 2013 storm event:

$$\textbf{Chloride Concentration} = (0.363)SC - 271$$

where, SC equals specific conductivity amount in µS/cm, and the chloride concentration is given in mg/L.

This equation was deemed appropriate due to the similar circumstances of the environment in which it was derived: the Root River freshwater ecosystem receives sodium chloride compound deposits from road deicing runoff just as the Upper Stroubles Creek watershed does, making this regression equation applicable.

Figure 3.5 shows the derived chloride concentrations for March 25, 2013 as well as a moving average for the duration of the storm event. (The above regression equation was converted to provide chloride concentrations in µg/L instead of mg/L). The EPA recommended acute toxicity limit is plotted for comparison. As shown below, the chloride concentration exceeded 860,000 µg/L, indicating that the system was experiencing a toxic event for an acute duration.

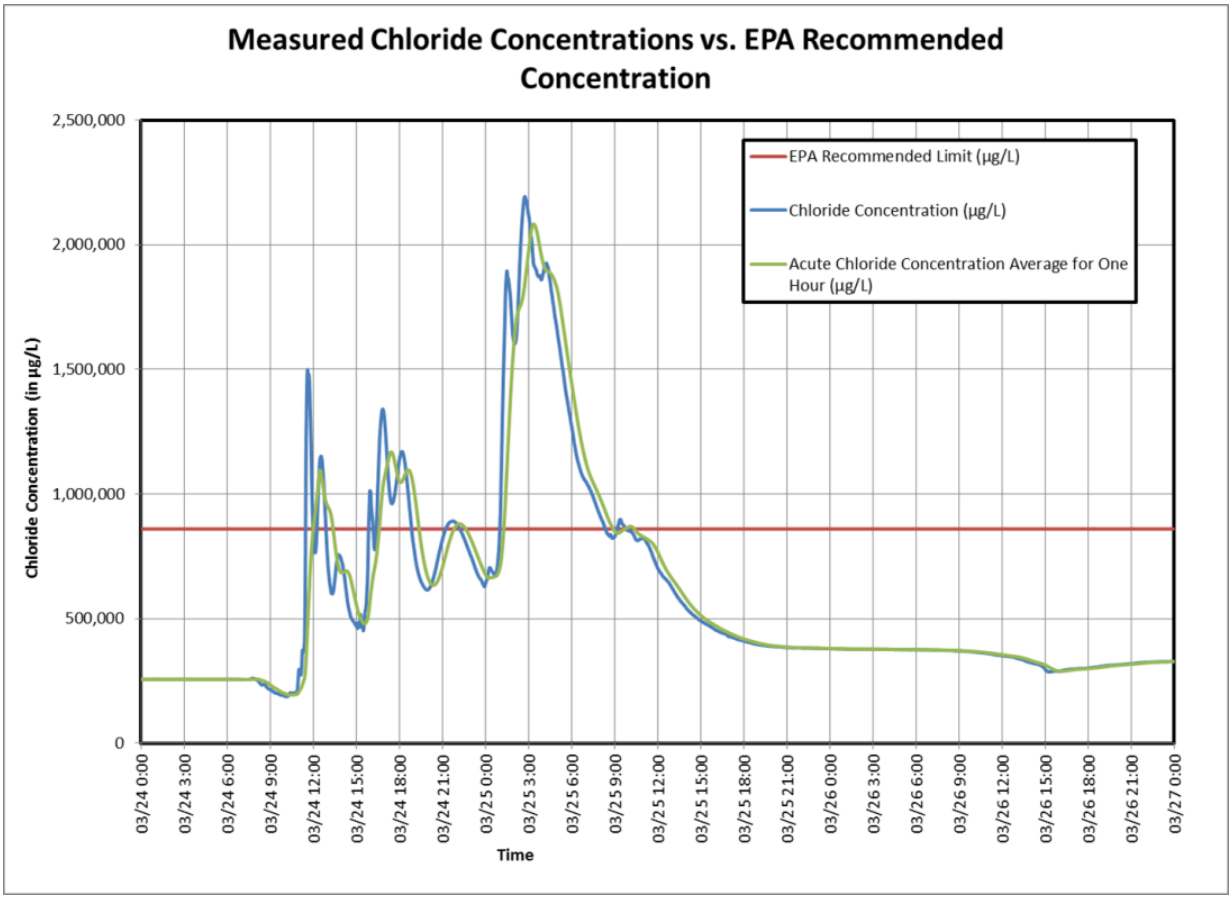

Figure 3.5 Measured Chloride Concentrations vs. EPA Recommended Concentration
(W. McDonald, H. Clark, D. Brogan July 2013)

Professor Jack Webster of the Virginia Tech Biology Department has explained that the fathead minnow (*Pimephales promelas*) is frequently used as an "indicator species" by researchers to gauge the health of a riparian ecosystem. The acute toxicity limit of a fathead minnow, as determined by the EPA, is 860-2,790 mg/L (McDonald, 2013). Because the fathead minnow is frequently found in Stroubles Creek, these high chloride concentrations are of concern and demonstrate that this event created an environment that was toxic to the fathead minnow.

For comparison, the recommended maximum concentration of chloride permitted in drinking water is 250 mg/L, or 250,000 µg/L ("Chloride and Salinity"). This amount was exceeded during the deicing event.

The runoff from a snowstorm like the March 25, 2013 event is more likely to yield higher specific conductivity measurements than runoff from a rain event. This is due to the higher probability that a sodium chloride deicing compound will be administered to the roadways. Furthermore, runoff composed of rainwater could likely have a lower specific conductivity than the system's base flow, due to the opportunity for dilution. The proximity of the LEWAS Lab field site (and ultimately the Virginia Tech Duck Pond) to a main road is also a contributing factor to the sudden spike in specific conductivity: there is very little distance between runoff that originates on the adjacent road and the monitoring system, consequently eliminating the opportunity for natural ground filtration. The phenomenon of ground filtration is essentially diminished in a subterranean conduit system, where the pipes are usually made of impermeable materials, such as corrugated plastic or steel. Understanding the characteristics of a watershed's storm-water network system allows the likelihood of processes such as

ground filtration to be determined; if the runoff does not have the opportunity to be channeled over land, it is likely to be more concentrated with pollutants and sediment. The biological implications of deicing roads with sodium chloride are negative, as perceived by the LEWAS Lab's real-time monitoring system and associated storm-water network map.

IV. Conclusion:

Monitoring Riparian Ecosystem Health in Real-Time: A Solution to Public Awareness of Watersheds

Real-time monitoring of urban watersheds can prove to be a very effective way to broadcast to a community the impact that human activity has on its water resources. The sensors that provide data in real-time require a great deal of maintenance, but providing a metabolic consensus of a given riparian ecosystem is essential to understanding the relationship between a city and its watershed.

Implementing USGS "Best Practices" in the LEWAS Lab

The LEWAS Lab also reflected upon its functionality as a research organization by studying the recommended "best practices" for a hydrology lab as presented by the United States Geological Survey. It was concluded that the LEWAS Lab could greatly benefit from improving communications: taking detailed notes during field visits or laboratory procedures not only provide a reference for later discussion, but also help make problems more apparent upon review. For example, the number of "extra trips" needed during a field visit were notated; on at least 3 occasions, an instrument needed for field work was left in the lab, making it necessary for a team member to go retrieve it. By notating this down, as recommended by the USGS, the mistake does not go unnoticed. Frequent photographs were also taken during all laboratory and field procedures. These, along with any notes taken by a team member, were complied with a word processor and uploaded to the LEWAS Lab's forum on the Internet. This made all information available, to help prevent conflicting perspectives or recollections of events. Lastly, the USGS discusses the importance of data accuracy. One of the LEWAS Lab's research methods in it of itself is to determine the actual quality of our research methods, and the data that results. Data accuracy is a form of scientific integrity, and the USGS stresses the need for data to be entirely collected and recorded immediately, at the location it was produced. Waiting to process it increases the opportunity for error. As with all of these procedures, time is valuable and should not be wasted with inefficient protocols, but the components of an investigation are not to be rushed or treated trivially.

Recommended Actions for Continued Real-Time Monitoring of Stroubles Creek

This investigation of the Upper Stroubles Creek watershed is not complete, and it is recommended that the following procedures are completed to continue the research.

The storm-water network map for the McBryde neighborhood needs to be completed. A complete map is necessary to verify the quantitative area of the contributing watershed to the LEWAS Lab field site.

The temperature sensor on the Acoustic Doppler Current Profiler should be repaired. Because the speed of sound in water is dependent upon temperature, this instrument, without an operational temperature gauge, cannot accurately calculate flow velocity. (A temporary estimate of the system's water temperature has been manually programmed into the flow-meter for temporary flow velocity measurements.)

An ultrasonic transducer should be installed on the ceiling of the culvert at the LEWAS Lab field site. This will provide a secondary flow velocity measurement without being susceptible to sedimentation deposits.

A LEWAS Lab member should visit the field site during the winter months (especially following a snowstorm) to look for fish kills (Figure 4.1). This could strengthen the hypothesis that road deicing salt is creating a hostile environment for aquatic organisms.

Figure 4.1 Fish kill at LEWAS Lab Field Site Observed July 1, 2013 following Summer Rain Event
(H. Clark, 2013)

In addition to looking for fish kills related to road deicing, the site should be photographed more frequently (several times a week) to capture evidence of fish kills associated with other causes. The photograph above, taken in July 2013, suggests that tumultuous flooding during summer storms are also hazardous to freshwater fish.

A chronic chloride toxicity analysis should be performed during the upcoming winter, to provide an idea of how the system responds to pollutants over the course of several days.

Lastly, it is recommended that a pump be installed in the large conduit pocket of the water-quality Sonde, in addition to repairing the gasket around the cavity. As shown in Figure 4.2, the conduit is prone to water seepage. The pump could help drain the cavity, and the gasket would help prevent water from entering the system. It is also recommended that the Sonde's sensors be cleaned daily of debris and calibrated more frequently (every two weeks) to ensure that the recently-repaired sensors are not subject to repeat damage.

Figure 4.2 Sonde Conduit Prone to Water and Particulate Accumulation
(H. Clark June 3, 2013)

V. Acknowledgments:

I thank Virginia Polytechnic Institute and State University for hosting this program, and the Engineering Education Department for supporting the LEWAS Lab's endeavors in real-time monitoring.

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I also thank Mr. Mike Dunn, Transportation Planning Engineer at Virginia Tech, and Mr. Mark Helms, director of Facilities Operations at Virginia Tech, for their information regarding the campus' road deicing salt protocols.

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Inactivation Of *Legionella pneumophila* Within Premise Plumbing Via Copper Ions

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ABSTRACT

Legionella pneumophila (LP), an opportunistic pathogen, is found within premise plumbing. LP can cause Legionnaire's Disease (severe pneumonia) in immunocompromised individuals, hospitalizing 8,000 to 18,000 people each year. Premise plumbing is the area of the water distribution system beyond the property line and includes buildings of businesses, schools, and private property. Premise plumbing presents a unique situation with relatively low disinfectant residuals, high surface to volume ratios and long stagnation times. To explore the possibility of combating LP by selection of plumbing materials, the effectiveness of inactivation of LP by copper is being studied with eighteen simulated water heater reactors (SWHR). All conditions have 80% of their water discarded three times a week, imitating an average family's water usage. Two plastic pipe materials, PEX and CPVC, are being utilized because past experiments have shown high levels of LP growth on PEX and there is concern of similar findings for CPVC. Copper is being dosed as previous studies indicate a possible correlation between free Cu ions and LP inactivation. pH is controlled from 7 to 9, in different reactors. A more basic pH reduces the fraction of copper present as free copper ions, which might allow LP to persist. LP levels are being analyzed via qPCR and agar plating. Total organic carbon, trace metal concentrations, ATP, and AMP Index are also being monitored. The overall goal of this study is to show the relationship between free copper ion levels and LP inactivation, with an expectation that higher levels of free copper will reduce LP.

Keywords: *Legionella pneumophila*, Copper, Premise Plumbing, PEX and CPVC Pipes, Water Distribution System, Simulated Water Heater Reactors

INTRODUCTION

Background: *Legionella pneumophila* is an opportunistic pathogen and amoeba resisting bacteria that thrives in fresh aquatic environments. LP is gram negative, meaning its cell wall is more resistant against antibiotics (Pruden et al., 2011). In fact, LP is very resistant to disinfectants. LP favors warmer fresh aquatic environments, and particularly thrives in temperatures between 25 and 45°C. The environment of most concern for LP is the drinking water distribution system, yet they do inhabit streams, rivers, and thermally polluted water (Brazeau et al, 2012). In most fresh aquatic environments, LP can become ingested by a protozoan host, which provides LP with a protective home from antibacterial disinfectants. It is here that LP is able to survive inside the digestive vacuoles of its host, feed off of the amino acids present, and reproduce. This protective environment allows LP to replicate such that eventually the host bursts open, allowing LP to re-enter the aqueous phase (Pruden et al., 2011). LP is then able to thrive in certain drinking water systems and infect human hosts. LP can be present in premise plumbing or the plumbing beyond the property line, which includes buildings of private property, schools, businesses, etc.. LP is also present in the water vapor aerosols produced by showerheads, faucets, hot tubs, pipe walls, water heaters, cooling towers, evaporative condensation, and humidifiers (AWWRF, 2009 & HHS, 2005). These water vapor aerosols can be inhaled, ingested, or enter through a cut on the host (Pruden et al., 2011). Once inhaled, LP lodges within the alveolar region of the lungs, and starts to replicate once inside the

alveolar macrophages of the lungs (Brazeau et al , 2012). Unlike other waterborne pathogens, it is unlikely that infection occurs through ingestion.

Those exposed to LP can develop Legionellosis, an illness that results in either Legionnaire's Disease or Pontiac Fever (HHS, 2005). Legionnaire's disease is a severe pneumonia that includes symptoms such as coughing, shortness of breath, high fever, muscle aches, and headaches (CDC, 2013). However, only individuals with a compromised immune system are susceptible to Legionnaire's Disease. Compromised immune system individuals include those suffering from HIV, poor lung defenses caused by smoking, alcohol abuse, and chronic pulmonary disease, and weak immune systems due to cancer, diabetes or kidney failure. Other individuals more susceptible include the elderly, and individuals going through immunosuppressive therapy (Brazeau et al, 2012 & CDC, 2013). It is important to note that only 5% of individuals exposed to LP contract Legionnaire's Disease as not all exposed have compromised immune systems. In fact, 72% of cases take place at hospital facilities as individuals there do have compromised immune systems (Brazeau et al, 2012). The majority of those affected with Legionnaire's Disease are hospitalized. In fact, each year, 8,000 to 18,000 individuals are hospitalized (CDC, 2013). Of these hospitalizations, 5 to 30% of incidences are fatal. Pontiac Fever is a much less severe form of Legionellosis. Pontiac Fever is not pneumonia and usually results in flu-like symptoms (HHS, 2005). Although less fatal than Legionnaire's disease, Pontiac Fever occurs in 95% of individuals exposed (Brazeau et al, 2012). Symptoms of Pontiac Fever only last for a few days and do not need treatment (CDC, 2013).

Legionella pneumophila was first discovered in 1976 at the American Legion Convention at The Bellevue Stratford Hotel in Philadelphia, PA. A few days after the convention had concluded, some of the men in attendance started to complain of upper respiratory infections. In fact, out of the 182 cases, 29 were fatal. The disease, Legionnaire, as well as LP, was named after the outbreak at this convention, which was discovered in the cooling tower and air conditioning system (Brazeau et al, 2012). Since then, outbreaks of LP had not been reported until 2001, when *Legionella* was added to the Waterborne Disease and Outbreak Surveillance System (WBDOSS). Since 2001, *Legionella* has been the most reported pathogen for drinking water outbreaks. In 2009, LP was added to the Environmental Protection Agency's (EPA) Contaminant Candidate List for the first time. By 2010, outbreaks taking place between 1973 and 2000 were added to the WBDOSS. During this time there were 69 outbreaks occurring in 27 states. The largest reported Legionellosis outbreak took place in New Jersey with 98 cases of Pontiac Fever reported. In fact, each year there are about 100 cases of Legionellosis reported in New Jersey (CDC, 2011). Most (66.7%) of the Legionellosis outbreaks that are reported take place in hospitals, health care facilities, and nursing homes. 16.7% of reported cases occur in hotels, motels, lodges, and inns. 12.5% occur in apartments and condominiums, with the remaining percentage in gym facilities and community water supplies (Craun et al., 2010). Essentially, all outbreaks of *Legionella* occur within premise plumbing.

Of all drinking water outbreaks, one third are associated with premise plumbing, with more than 90% of them associated with LP. Between 2007 and 2008, LP caused 12 out of 36 outbreaks and was the most frequently reported pathogen causing drinking water outbreaks. Half of these outbreaks took place in New York State, demonstrating the inconsistency in LP outbreak (CDC, 2011). During 2001 and 2006, *Legionella* was the third most reported etiology causing waterborne disease outbreaks. In fact, LP causes 70% of Legionellosis outbreaks (Buse and Ashbolt 2011). Besides premise plumbing, LP is also common among ships, including cruise and cargo ships. Between 1977 and 2001, hundreds of LP cases were reported on ships. In fact, in 1994, 50 passengers became ill from one cruise ship (World Health Organization). Since LP was added to the WBDOSS in 2001, 38 outbreaks were due to LP, which includes 25 deaths. In fact, the third most common illness from waterborne disease is acute respiratory infections, which are all caused by LP (CDC, 2011).

LP thrives within premise plumbing because of high surface area to volume ratios, water stagnation (especially in green buildings), and warmer water temperatures (Brazeau et al, 2012). In fact, water heater temperatures have been greatly associated with LP proliferation (Buse and Ashbolt 2011). Biofilm formations within premise plumbing also provide ideal environments for LP (Morvay et al., 2011). Biofilms are highly inhabited by free-living protozoa, which provide protection for LP (CDC, 2011). Essentially, all conditions associated with premise plumbing provide an ideal environment for LP growth and habitation.

Literature Review: Starting in 2008, the Centers for Disease Control and Prevention (CDC) acknowledged that the higher occurrence of waterborne disease outbreaks was due to organisms proliferating within premise plumbing, not at the treatment plant (Brazeau et al, 2012). Particularly, LP thrives within premise plumbing due to the environment provided. A significant feature of premise plumbing is warmer temperatures, which are ideal for LP proliferation. To back up this statement, a study conducted by H. Y. Buse et al. acknowledged LP's affinity for water heaters, as their temperatures (30 to 46°C) are ideal for LP proliferation. In addition to this, a study by Gunter F. Craun et al. acknowledged LP colonization in premise plumbing as well as cooling towers (Craun et al., 2010). Furthermore, a study conducted by S. L. Molloy et al. also acknowledged the fact that LP is highly prevalent in cooling towers, water heaters and plumbing systems. In fact, LP is between 6 and 61% prevalent for potable water, and has even showed up in 30% of samples taken from showers in the United States. The study does note that copper-silver ionization is successful in inactivating LP in large plumbing systems such as hospitals (Molloy et al., 2007). However, in smaller scale buildings, such as private properties and schools, this method may not be as successful or feasible.

Biofilms found within premise plumbing provide great homes for LP. In a study conducted by A. A. Morvay et al., biofilm formation was tested on copper, polyvinyl chloride (PVC), and stainless steel pipes. This study showed biofilm growth on all pipe materials, but less so on copper pipe materials. Less biofilm growth resulted on copper pipe material due to copper's antibacterial properties. Specifically, copper is known to damage cell membranes and nucleic acid properties. Essentially, copper disrupts cellular functions. The study also discussed the possibility of biofilm formation as a cause of LP growth within premise plumbing. However, biofilm cannot be eradicated at the water treatment plant, as it is only present in premise plumbing. The study advises the use of copper pipe material for household plumbing to battle biofilm formation and thus LP proliferation within premise plumbing (Morvay et al., 2011).

If lack of biofilm formation is correlated to LP proliferation within premise plumbing, then lack of biofilm should also mean lack of LP or at least less LP than there would be with biofilm colonies. The study conducted by Markku J. Lehtola et al. found that copper pipes had slower biofilm formation than polyethylene (PE) pipes. Copper pipes even exhibited less microbial growth compared to PE pipes. The study further supported the finding that more biofilm growth correlated with more LP growth. However, these results were only shown for the first 200 days of the study. After that, microbial growth and biofilm formation were similar to PE pipes (Lehtola et al., 2004). To support this finding, a study by Hong Wang et al. examined the influence of various pipe materials, water ages, and disinfectant type on the occurrence of opportunistic pathogens such as LP. Their findings showed that copper pipes inhibited LP, but only for two years. After that, copper pipes were not effective in inactivating LP because they started to corrode (Wang et al., 2012). A study conducted by Dick van der Kooij et al. tested the effect of pipe material on biofilm formation as well. Their study resulted in copper pipes having less overall ATP concentrations, biofilm formations, and LP. Again, this study showed that LP growth was low for two years, but after that, levels were similar to other pipe materials. They also hypothesize that copper's ineffectiveness after two years is due to corrosion (Van Der Kooji, 2005). Furthermore, a different study conducted by Markku J. Lehtola et al. demonstrated that biofilm formation was much lower in copper pipes than in PE pipes. Yet, they also noted that biofilm formation on copper pipes aided in protecting copper from corrosion, which leads to more copper ions in the effluent water (Lehtola et al., 2005).

The study being conducted for this research paper is also testing the effect of different pH levels on LP inactivation with the addition of copper ions. It is hypothesized that a higher pH will correlate with steady LP levels, as copper dosages will not affect LP growth. This hypothesis is backed by the study conducted by Yu-sen E. Lin et al., which demonstrated higher pH conditions less effective in inactivating LP. In an Ohio Hospital, the Cu-Ag ionization method (doses of copper and silver ions within the water distribution system of many hospitals, is successful at inactivating LP) was oddly unsuccessful. Upon inspection, the water in the hospital was found to have a pH as high as 9. Copper ions found in neutral pH environments are positively charged, thus able to bind to the negative charge of the bacterial cell wall, causing cell lysis and death. With more basic pH's, the copper ion species present within the water are predominantly negatively charged, which allows them to not be attracted to the negative charge of LP's cell wall. The repulsive forces of the two negative charges allow copper to be ineffective at inactivating LP in more basic pH levels. As for silver, pH level differences did not change the

species present. Copper doses that are successful in inactivating LP are between 0.2 and 0.4 mg/L (Lin et al., 2002). In another study conducted by Yu-Sen E. Lin et al., they demonstrated the effectiveness of copper and silver ions in inactivating LP. Lin et al. discussed how copper ions are faster than silver ions in inactivating LP. Again, the minimum and maximum requirement for LP cell death is between 0.2 and 0.4 mg/L as more than this amount was not shown to have an effect (Lin et al., 1996).

Statement: *Legionella pneumophila* (LP) is an opportunistic pathogen and amoeba resisting bacteria (Pruden et al., 2011). It can be found within premise plumbing, which is the area of the water distribution system beyond the property line. This includes buildings of businesses, schools, and private property. Premise plumbing provides the ideal environment for LP as it has a low disinfectant residual, high surface to volume ratios, long stagnation times, and warm temperatures (AWWRF 2009). LP can cause Legionellosis, which can either result in Legionnaire's Disease or Pontiac Fever. Legionnaire's Disease is a severe pneumonia that infects 5% of individuals exposed, whereas Pontiac Fever only exhibits flu-like symptoms and infects 95% of exposed individuals (Brazeau et al, 2012). Each year, LP hospitalizes 8,000 to 18,000 people (Legionella 2013). In order to combat LP, selection of copper plumbing material may be an effective inactivation of the bacteria. To study this, 18 simulated water heater reactors are being tested for the inactivation of LP via copper ions. Copper is being dosed because studies indicate a possible correlation between free Cu ions and LP inactivation. All SWHR have 80% of their water discarded three times a week, imitating an average family's water usage. Two plastic pipe materials, PEX and CPVC, are being utilized because past experiments have shown high levels of LP growth on PEX and there is concern of similar findings for CPVC. Four conditions have a neutral pH of 7, one condition has pH 8, and the last has a pH of 9. A more basic pH reduces the fraction of copper present as free copper ions, as the positive charge of the copper ions are binding to the negative charge of the OH^- ions. At a more neutral pH, less OH^- ions are floating in solution and copper has fewer opportunities for form complexes (Zhang 2008). Instead, copper can bind to the negative charge of the cell wall of LP, causing cell lysis and death. It is hypothesized that with a more basic pH, LP levels will stay constant as copper is added. At a more neutral pH, LP levels will drop with copper dosage. LP levels are being analyzed via qPCR and agar plating. Total organic carbon, trace metal concentration, ATP, and AMP Index are also being monitored. The overall goal of this study is to show the relationship between free copper ion levels and LP inactivation, with an expectation that higher levels of free copper will reduce LP.

Justification: With the high infection rates of LP previously stated it is clear that research is required to combat LP within premise plumbing. LP is a huge public health issue affecting thousands of individuals within the United States each year. If action is not taken against LP, the financial stress of combating LP will continue to grow. However, combating LP will be a challenge, as it is only present in premise plumbing. This is plumbing beyond the property line, meaning plumbing not under the responsibility of the water treatment plant. It will then be the home/business owner's responsibility to combat LP. An easy and inexpensive solution is thus required. In 2008, the CDC even stressed the importance of combating pathogens found within premise plumbing, and not at the treatment plant (Brazeau et al, 2012).

In order to address this problem, an easy solution needs to be found for the private property owner. Essentially, the purpose of this study is to see the effectiveness of the addition of copper pipes in premise plumbing to combat LP proliferation. Most buildings have copper pipes, and simple installation of them for buildings that lack copper pipes could solve a huge public health issue. Another possible solution is the mandating of all future buildings constructed to have copper pipes installed as the primary plumbing material. Although copper pipes may be more costly than other alternatives, in the long run, installation of copper pipes will be cost effective. Less immunocompromised individuals will be hospitalized, lowering hospital care costs, and individual hospital bill costs.

While installation of copper pipes is an easy solution, it is important to consider research about the harmful health effects of drinking water contaminated with copper ions. The EPA maximum standard for copper ions within drinking water is 1.3 mg/L, which is equivalent to parts per million (ppm). The European Union (EU) and World Health Organization (WHO) maximum standards are 2.0 mg/L. Amounts that have been found to cause toxicity are between 30 to 300 mg/L for alcohol and tea, and less than 10 mg/L for drinking water. Some studies have shown toxicity at levels of 3 and 5 mg/L. Clearly, there is a lack of evidence for current maximum standards.

However, the level that is set now is below levels that have been reported and have shown toxicity (Fewtrell et al., 2001). In a study conducted by Markku J. Lehtola et al., they found that more copper ions were present within the water system after longer periods of time. Yet, the maximum amounts of copper ions present was only after 16 hours, and from this time, copper ion levels started to decrease again, and then stay at a steady level. Essentially, the maximum amount of copper ions present within the water system occurred at 16 hours. This maximum amount was below the maximum standard set by the EPA (Lehtola et al., 2007). Clearly, even with the installation of copper pipes, the maximum amount of copper ions leeched into the effluent water is still not above or near the EPA standards.

RESEARCH METHODS

Simulated Water Heater Reactors Set Up and Operation: Eighteen simulated water heater reactors (SWHR), with six conditions run in triplicate, are being studied. The SWHR are glass 125 mL bottles kept in an incubator of 32°C. Each condition is testing the inactivation of *Legionella pneumophila* (LP) with the addition of $CuSO_4$. Each reactor was acid washed and baked at 550°C for a total of five hours before the start of the experiment.

¾ inch nominal diameter PEX and CPVC pipes were cut into 1.25 inches segments for insertion into reactors. Some were also cut in half lengthwise. All coupons were soaked for one day in a bleach and Thermo Scientific Barnstead Nanopure® water mixture for disinfection. Subsequently, all coupons were soaked for 6 days with Thermo Scientific Barnstead Nanopure® water with periodic replacement of rinse water in order to reduce organic carbon leaching from new pipe materials. Coupons were inserted under sterile conditions and kept in place with friction.

Reactor Conditions: The names of each condition are 0PEX, Control, 7CPVC, 7PEX, 8PEX, and 9PEX. All conditions except 7CPVC contain cross-linked polyethylene (PEX) pipe material. One condition contains chlorinated polyvinyl chloride (CPVC) instead of PEX to control for a different plastic pipe material. 0PEX, Control, 7CPVC, and 7PEX will be kept at a pH of 6.9 to 7.1 on water change dates after 6.21.13. A pH of 7 allows the SWHR to have a neutral pH setting, similar to an average water heater. 0PEX and Control will not have $CuSO_4$ added during any water change for the duration of the experiment. 0PEX and Control differ in that 0PEX is further filtered through an ion exchange resin filter. The ion exchange resin filter absorbs cations, allowing copper ions to be further filtered out of the influent water (FWI, 2013). 8PEX and 9PEX have more basic conditions, allowing for more OH^- ions to free float within solution. With the addition of $CuSO_4$, the positive copper ions should bind to the negative OH^- ions and thus be removed from solution.

Water Filtration Process: The influent water is Blacksburg, VA tap water after a ten-minute flush period from the ICTAS II building at Virginia Tech. After ten minutes, a 10,000 mL KIMAX® Kimble media storage bottle (No. 14395) is filled to a 10 L fill line. The water is then breakpoint chlorinated with Sodium Hypochlorite stock solution. The DR 2700™ Portable Spectrometer HACH is utilized by selection of the 80 Chlorine F&T PP program and usage of DPD Total Chlorine Reagent. The water is then separated into three 4,000 mL KIMAX® flasks (No. 26500, Stopper No. 10) and heated to 90˚C on a stirring hot plate in order to remove excess residual. After heating, the water is filtered through a point of use granular activated carbon (GAC) filter. GAC is composed of raw organic materials that have a high carbon content. The carbon is able to remove certain chemicals in the water as it passes through the filter (MDH, 2013). The purpose of this filter is to lower the total organic carbon value, and remove organic chemicals from the water. After GAC filtration, the water is vacuum filtered with a 0.45 μm Hydrophilic Durapore® Membrane Filter, and then transferred into either a 1,000 mL PYREX® media storage bottle (No. 1395), or a 500 mL PYREX® flask (No. 4980). The 0PEX condition's influent water goes through the same described process. However, it is further ion exchange resin filtered, which is first GAC filtered, and lastly filtered through a 0.45 μm Hydrophilic Durapore® Membrane Filter.

pH Calibration, Storage, and Water Change: After the water filtration process is complete, all water is adjusted to between 0.1 higher or lower than the target pH using an OakTon® pH Meter with RS232 (pH 110 Series, Serial No. 1318634). The SWHR are stored in a Precision Incubator set to 32°C. Water is allowed to heat in the incubators for a minimum of 30 minutes before use. All water changes take place in a Biological Safety Cabinet (Thermo Scientific 1300 Series A2, EHSS ID #009170). 80% (approximately 90 mL) of each SWHR's

water is wasted into a bleach solution and replaced with the water further described below. Each reactor is placed back into the 32˚C Precision Incubator after the influent water is added. Water changes are conducted three times a week (MWF), simulating an average family's water usage.

Influent Water: The first water change took place on May 30, 2013. Filtered and adjusted water described in the "Water Filtration Process" section is added to each reactor after 80% of the water is dumped. On water change dates 5.30.13, 6.1.13, and 6.3.13, each reactor was inoculated with 34 mL of water from other similar established SWHR. The inoculation water consists of effluent from three conditions: "PEX 700" SWHR (contains PEX pipe material, stored in a 32˚C Precision Incubator, is GAC filtered, and has 700 ppb of carbon added with acetate and glucose in each water change), the "PEX Low" SWHR (contains PEX pipe material, stored in a 32˚C Precision Incubator, and is GAC filtered), and the "Low Control" SWHR (contains no pipe material, 32˚C Precision Incubator, and is GAC filtered). 34 mL total combined were added to each SWHR with the addition of the influent water. Table 1 shows changes made to the influent water added to the SWHRs during the study. Spike indicates the inoculation process previously described.

Table 1. This table demonstrates each phase of the study in regards to the influent water added to each condition during the water changes.

<table>
<tr><th>Phase</th><th>Dates</th><th>0-PEX</th><th>Control</th><th>7-CPVC</th><th>7-PEX</th><th>8-PEX</th><th>9-PEX</th></tr>
<tr><td rowspan="3">I</td><td>5.30</td><td colspan="6" rowspan="3">Influent is GAC filtered, pH adjusted to 7.5, and spike is added from other reactors.</td></tr>
<tr><td>6.1</td></tr>
<tr><td>6.3</td></tr>
<tr><td rowspan="8">II</td><td>6.5</td><td colspan="6" rowspan="8">Influent is GAC filtered, and pH is adjusted to 7.5</td></tr>
<tr><td>6.7</td></tr>
<tr><td>6.10</td></tr>
<tr><td>6.12</td></tr>
<tr><td>6.14</td></tr>
<tr><td>6.17</td></tr>
<tr><td>6.19</td></tr>
<tr><td>6.21</td></tr>
<tr><td rowspan="6">III</td><td>6.23</td><td rowspan="13">Influent is GAC and Ion Exchange Resin filtered, and pH is adjusted to 7</td><td colspan="2" rowspan="6">Influent is GAC filtered, and pH is adjusted to 7</td><td rowspan="6">Influent is GAC filtered, and pH is adjusted to 8</td><td rowspan="6">Influent is GAC filtered, and pH is adjusted to 9</td></tr>
<tr><td>6.25</td></tr>
<tr><td>6.27</td></tr>
<tr><td>7.1</td></tr>
<tr><td>7.3</td></tr>
<tr><td>7.5</td></tr>
<tr><td rowspan="7">IV</td><td>7.8</td><td rowspan="7">Influent is GAC filtered, and pH is adjusted to 7</td><td rowspan="7">Influent is GAC filtered, pH is adjusted to 7, and 5ppb of $CuSO_4$ is added.</td><td rowspan="7">Influent is GAC filtered, pH is adjusted to 8, and 5ppb of $CuSO_4$ is added.</td><td rowspan="7">Influent is GAC filtered, pH is adjusted to 9, and 5ppb of $CuSO_4$ is added.</td></tr>
<tr><td>7.10</td></tr>
<tr><td>7.12</td></tr>
<tr><td>7.15</td></tr>
<tr><td>7.17</td></tr>
<tr><td>7.19</td></tr>
<tr><td></td></tr>
<tr><td rowspan="6">V</td><td>7.21</td><td rowspan="6">Influent is GAC filtered, pH is adjusted to 7, and 30ppb of $CuSO_4$ is added.</td><td rowspan="6">Influent is GAC filtered, pH is adjusted to 8, and 30ppb of $CuSO_4$ is added.</td><td rowspan="6">Influent is GAC filtered, pH is adjusted to 9, and 30ppb of $CuSO_4$ is added.</td></tr>
<tr><td>7.23</td></tr>
<tr><td>7.25</td></tr>
<tr><td>7.28</td></tr>
<tr><td>7.30</td></tr>
<tr><td>8.1</td></tr>
</table>

Chemical Analysis: Trace metal concentrations, total organic carbon, ATP, and AMP Index are measured every two weeks during water changes. Trace metal concentrations are measured using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Two samples for each SWHR are collected for trace metal concentration data, one total and one soluble, 36 samples total. 10 mL of sample from each SWHR is transferred into sterile tubes for the total sample. 20 mL of samples from each SWHR are vacuum filtered with a 0.45 μm Hydrophilic Durapore® Membrane Filter. 10 mL from this is then transferred into a sterile tube for the soluble sample. Each of these samples have 200 μl of Nitric Acid added before analysis. The purpose of this measurement is to show the total concentration of Cu present within each SWHR as well as concentrations of other metals of interest.

Total organic carbon measurements are taken for each condition, 6 samples total. 10 mL from each SWHR are taken and stored in combined condition TOC-MS vials, for a total sample size of 30mL. Each sample has 100 μl of Phosphoric acid added and N_2 gas bubbled through for 3 minutes. Samples are then placed in the Sievers 5310 C Laboratory TOC-MS Analyzer using the Data Pro 5310 C Computer Program. The purpose of total organic carbon data is to analyze the amount of total organic carbon present within each condition. The carbon present represents food and energy available to the cells in each condition.

ATP and AMP Index measurements are taken using LUMINULTRA® Quench-Gone™ Aqueous Test Kit (Product #: QGA-25/QGA-100). 20 mL samples of each SWHR are transferred into a BD 60 mL Syringe (fresh one per condition) and a total volume of 60 mL is filtered through a Quench-Gone syringe filter. 1 mL of UltraLyse is transferred into the syringe and completely filtered through into a clear sterile tube. The remaining process follows the LUMINULTRA® Quench-Gone™ Aqueous Test Kit (Product #: QGA-25/QGA-100) steps for both ATP and AMP Index data. The purpose of ATP and AMP Index are to show the energy levels, viable conditions, and stress levels of cells within the SWHR.

Biological Analysis: DNA extraction, qPCR, and agar plating are utilized to analyze concentration of LP. For DNA extraction, 90 mL of each reactor is filtered through a 0.22 μm Millipore filter during a water change. With sterilized tweezers, each filter is folded, ripped, and placed within Lysing Matrix tubes from the FastDNA® SPIN Kit. The Lysing Matrix tubes are then transferred to a -20˚C freezer. These samples are then thawed and have their DNA extracted according to the procedure outlined in the FastDNA® SPIN Kit. Final samples are stored in Microcentrifuge Tubes and 1:10 dilutions are also made for analysis.

Diluted samples go through Quantitative Polymerase Chain Reaction for 16S rRNA and LP. LmipR, LmipF, Lmip Probe, H_2O, and Probe Supermix are used for LP. For 16S rRNA: 1369F, 1492R, H_2O, and Evagreen are used. Plates are analyzed using the Bio-Rad CFX96™ Real-Time System C1000™ Thermal Cycler using the Bio-Rad CFX Manager 3.0 computer program.

Agar plating is performed with 1 mL samples heated to 50°C for thermal shock to select for LP. 100 μl of each sample is transferred to a plate with L-cysteine hydrochloride and one without. The sample is spread on the plates using a sterilized glass rod. Plates are then parafilm sealed, labeled, and stored in a 37˚C incubator to allow for LP growth. After at least three days of incubation, plates are taken out of the 37˚C incubator and placed within a 4°C cold room to stop growth. Colony counts of LP are performed using Quantity One computer program on the Bio-Rad Machine.

Data Analysis: All data collections allow for results to be analyzed on Microsoft Excel. Data on Microsoft Excel is converted and charted for easy analysis.

RESULTS AND DISCUSSION

Throughout this study, qPCR, and LP culture plates have been conducted in order to determine LP concentrations in each SWHR. qPCR determines levels in gene copies/mL, while LP culture plates determine LP levels in colony forming unit (CFU)/mL. qPCR was conducted on samples taken on 6.17.13, and 7.1.13. qPCR results from 6.17.13 showed the influent water sample to have $1.8 \cdot 10^4$ gene copies/mL, while five out of six conditions had less than $1.3 \cdot 10^4$ gene copies/mL. The 9PEX condition had more than $2.5 \cdot 10^4$ gene copies/mL. Since influent

water samples contained higher gene copies/mL than the majority of SWHRs, data from this sample date will not be used in analysis. However, qPCR from sample date 7.1.13 can be accurately analyzed. This data, shown in Figure 2, demonstrated gene copies/mL of LP present in all conditions after the pH's had been adjusted to 7, 8, and 9. All conditions had gene copies/mL amounts between $1.0 \cdot 10^3$ and $4.0 \cdot 10^3$. From this information, it can be concluded that LP was present within all conditions. It is important to note that conditions with more basic pH values, 8 and 9, had less than $3.0 \cdot 10^3$ gene copies/mL than three of the conditions with a pH of 7. This is consistent with our hypothesis that more basic pH levels will have less LP levels present, as LP does not favor basic conditions. It is expected that with the addition of copper, basic LP levels will stay constant, while neutral pH LP levels will drop.

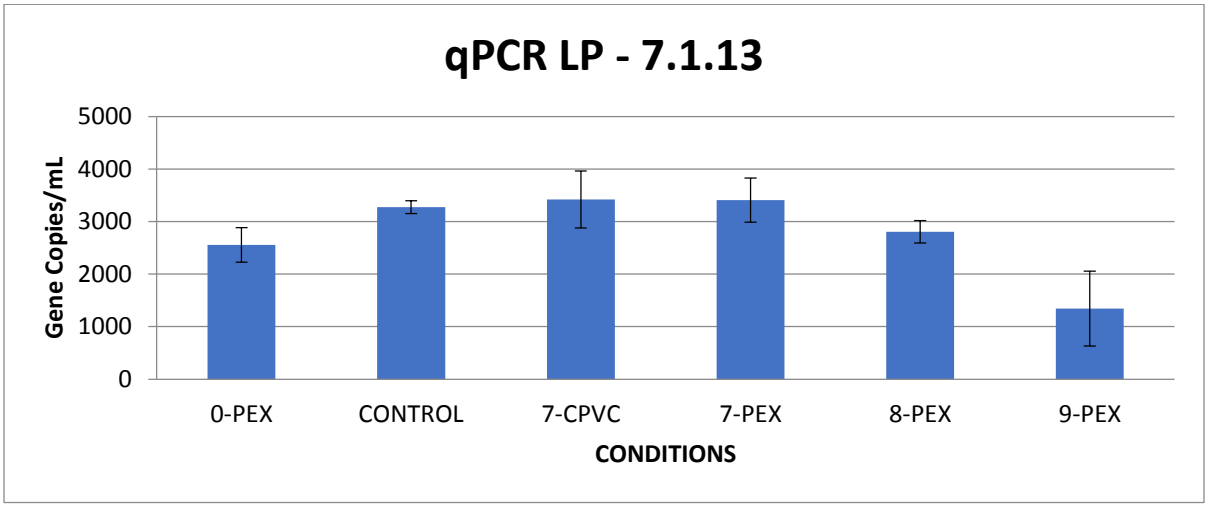

Figure 2. The x-axis shows the SWHR conditions. The y-axis represents LP levels via gene copies/mL. At more basic pH levels, LP levels dropped.

LP culture plates allow us to know if there is viable and culturable LP present within each SWHR. Samples from the SWHRs have been taken on dates 6.19.13, 7.3.13, and 7.17.13. Figure 3 shows colony counts for all three dates. When all conditions had a neutral pH level of 7.5, LP levels were between $3.0 \cdot 10^3$ and $9.0 \cdot 10^4$ CFU/mL. When pH was adjusted, LP colony numbers were between $5.0 \cdot 10^2$ and $5.0 \cdot 10^3$ CFU/mL, with the highest value for 0PEX. It is possible that LP levels did not drop for this condition because of the ion exchange resin filter, which further filtered copper ions out of the influent water. Even though copper ions were not being added to the other SWHR during this time, the further filtration of the influent water through the ion exchange resin filter could have possibly removed all copper ions, allowing there to not be an effect of LP levels. When $CuSO_4$ was added, LP colony counts stayed between $2.0 \cdot 10^2$ and $6.0 \cdot 10^2$ CFU/mL, clearly a significant drop from the start of the experiment. It should be noted that colony forming unit counts are not accurate measurements of LP levels within each SWHR, as they only represent LP that is viable and culturable. There could still be more LP present within each SWHR that may be viable, but not culturable, therefore not accurately representing the total amount of LP present within each SWHR. Nevertheless, it is important to note that as pH is adjusted, and copper ions are added to solutions, LP levels started to drop. However, all LP levels dropped regardless of pH and copper addition. This could be due to the fact that the inoculum used was pH 7.5, which was most similar to the first sampling.

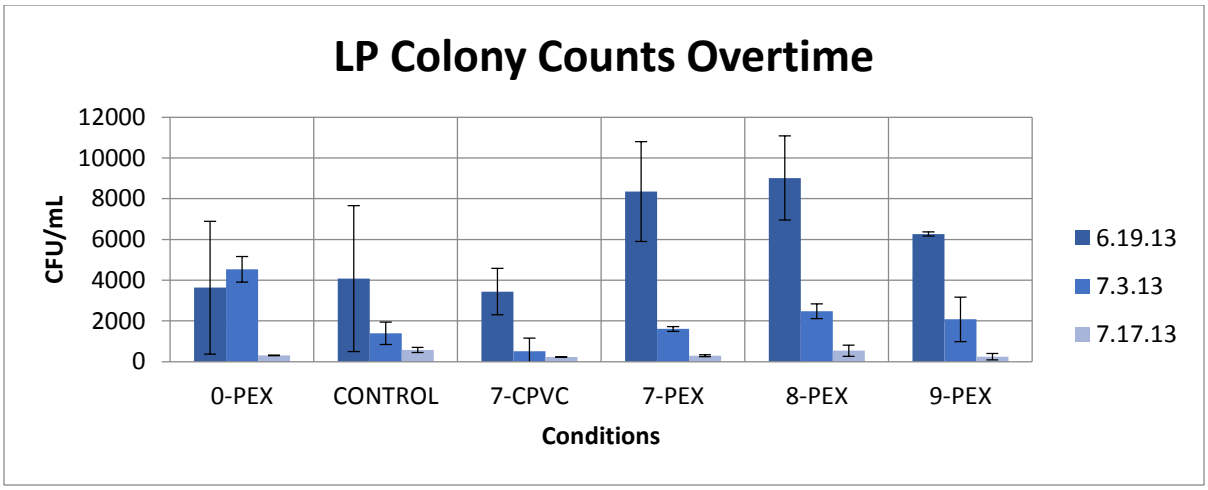

Figure 3. The x-axis shows the simulated hot water heater reactor conditions. The y-axis represents LP levels via colony counts. As is shown in the figure, pH adjustments and the addition of copper ions dropped LP levels for all conditions.

Total organic carbon data has also been recorded throughout this study. The TOC-MS machine measures the amount of total organic carbon present within each condition. Organic carbon is the food source for bacteria as well as LP in each condition. With high levels of total organic carbon present, it can be shown that LP has food available to thrive within the SWHR. Figure 4 shows levels of total organic carbon to be between 0.5 and 3 ppm for all conditions on sample dates 6.19.13 and 7.3.13. It is interesting to note that total organic carbon values are below 1 ppm for only 7CPVC SWHR. This could be due to the fact that PEX pipe material leaches more carbon into the water than CPVC does. On sample date 7.17.30, total organic carbon levels for four out of five conditions drop to between 0.5 and 1.5 ppm. Again, the only condition to increase is 7CPVC. Sample date 7.30.13 shows total organic carbon levels dropping for only conditions Control, 7PEX, 8PEX, and 9PEX. Total organic carbon levels dropped most dramatically for 7PEX and 9PEX, with a drop between 0.1 and 0.3 ppm. However, these drops are so small, that conclusions cannot be made from them. It should be noted that similar trends are seen throughout all PEX conditions, while CPVC doesn't seem to be leaching any total organic carbon.

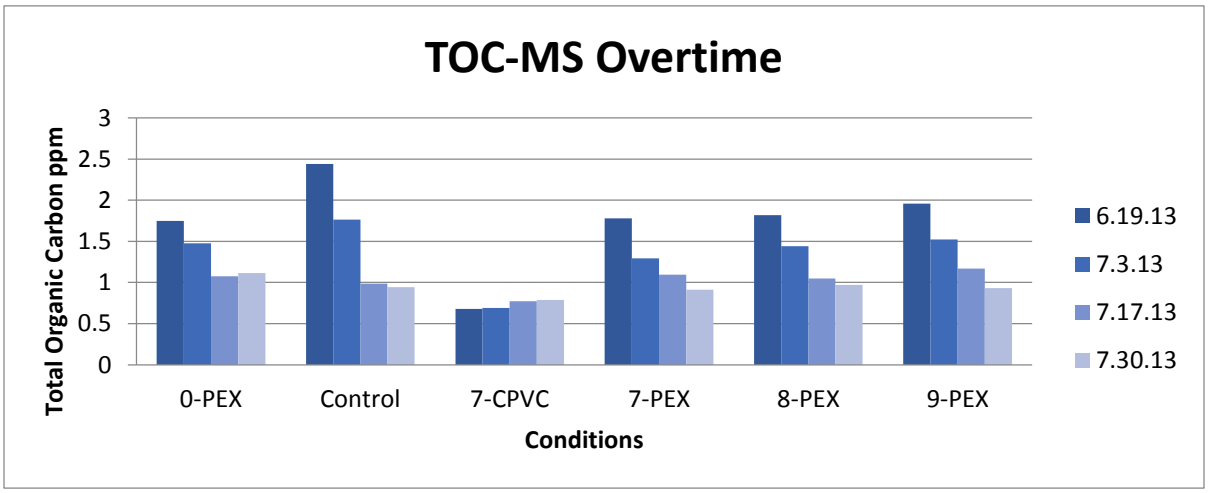

Figure 4. The x-axis shows the SWHR conditions. The y-axis represents total organic carbon levels in ppm. It is important to note that total organic carbon ppm levels are high and constant throughout conditions.

Soluble and total measurements of trace metal concentrations, with specific attention to 65Cu, are also being analyzed using ICP-MS. Figures 5 & 6 show soluble levels to be, on average, higher than total levels. This may be due to human error. Not all copper levels being transferred evenly during sample extraction processes could

also cause error. However, it is important to note that all copper levels for soluble values did increase significantly, between 2 and 6 ppb, for conditions that copper was added to during the third sampling date.

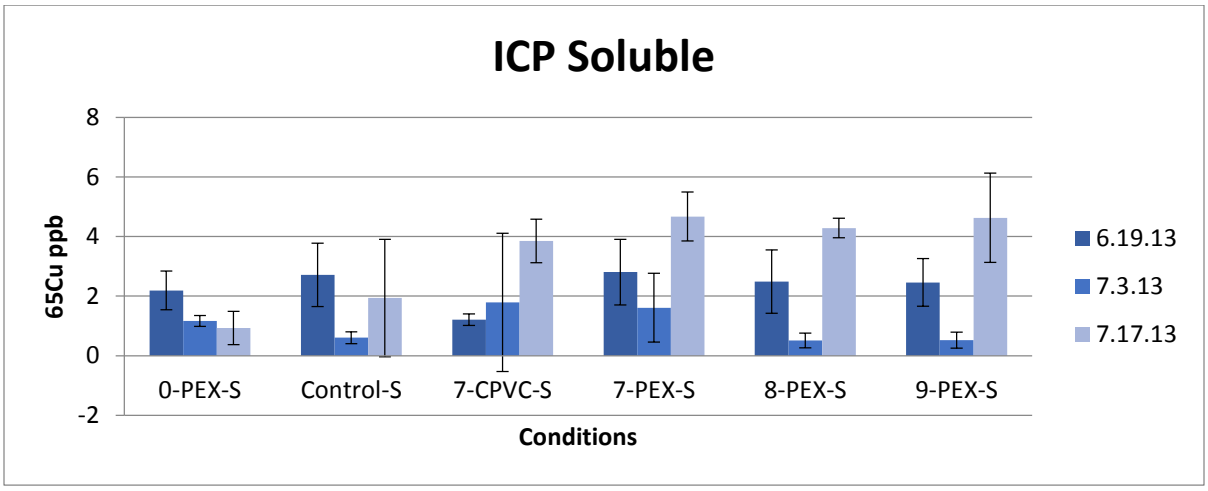

Figure 5. The x-axis shows the SWHR conditions. The y-axis represents 65Cu levels in ppb. It is important to note that copper levels increased during the third sample date.

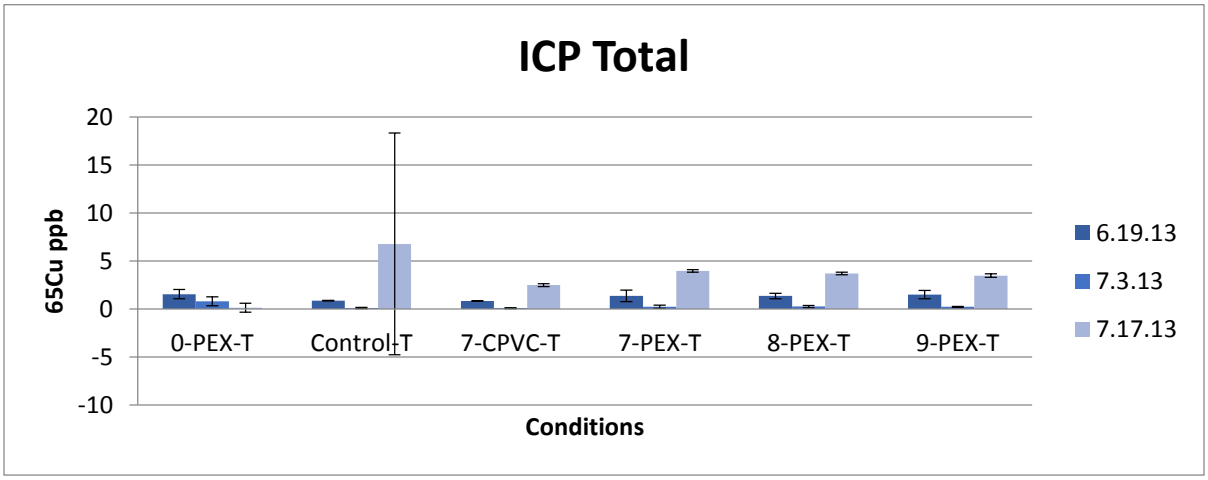

Figure 6. The x-axis shows the SWHR conditions. The y-axis represents 65Cu levels in ppb. It is important to note that copper levels increased during the third sample date.

The last samples were taken to determine ATP concentrations and AMP Index. ATP stands for Adenosine Triphosphate, which transports energy throughout the cell. ATP can be used as a measure of activity within cells. AMP Index measures the stress levels of cells. Cells with an AMP Index of less than 0.1 will have no stress. Cells with stress levels between 1.0 and 3.0 will have moderate stress, and cells with stress levels above 3.0 will die. Figures 7 & 8 show ATP concentrations and AMP Index stress levels during three sample periods: 6.19.13, 7.3.13, and 7.17.13. ATP concentrations during the first sample period were between $2.0 \cdot 10^2$ and $4.0 \cdot 10^2$ pg/mL across all conditions. Once pH was adjusted, ATP concentrations were between $1.5 \cdot 10^2$ and $2.0 \cdot 10^2$ pg/mL, demonstrating a significant drop in ATP concentration levels. During the addition of copper ions, ATP concentration levels dropped to between $5.0 \cdot 10^1$ and $1.0 \cdot 10^2$ pg/mL for conditions 7CPVC, 7PEX, 8PEX, and 9PEX. The 0PEX and Control condition levels increased or were about the same. This could be due to copper's effect on LP, yet this cannot be concluded from just these data points. AMP Index had an inverse effect. As pH was adjusted and $CuSO_4$ was added, stress levels of the cells increased from between -1 to 0, to 0 to 1. This could also help us conclude that the cells are more stressed due to the addition of copper ions or the change in pH levels. However, all conditions demonstrated increased stress levels, and not just those with additional copper added. Yet, the 8PEX and 9PEX conditions did exhibit higher stress levels than all other conditions.

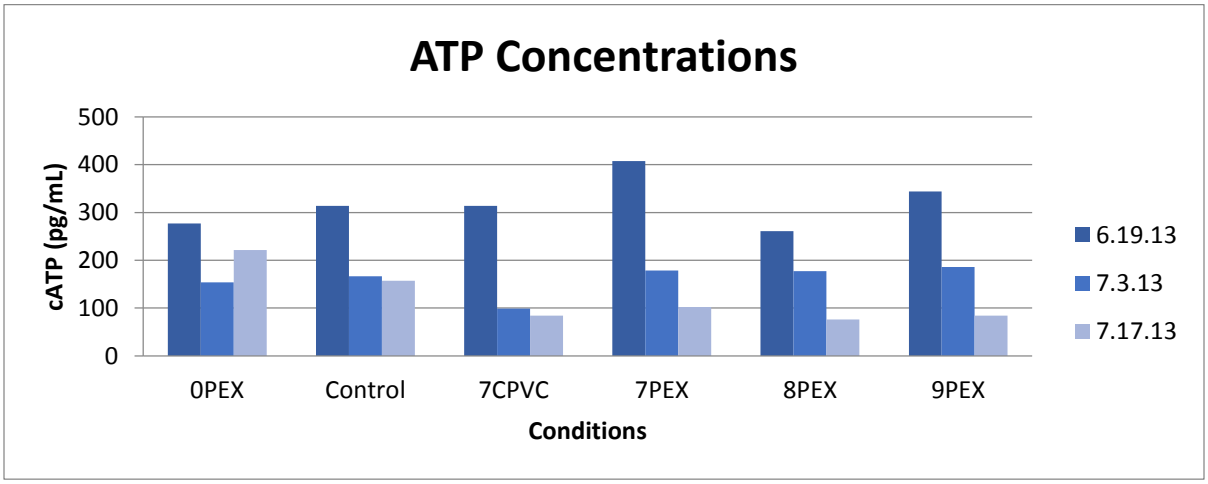

Figure 7. The x-axis shows the SWHR conditions. The y-axis represents ATP concentration in pg/mL. It is important to note that the addition of copper ions decreased ATP concentrations.

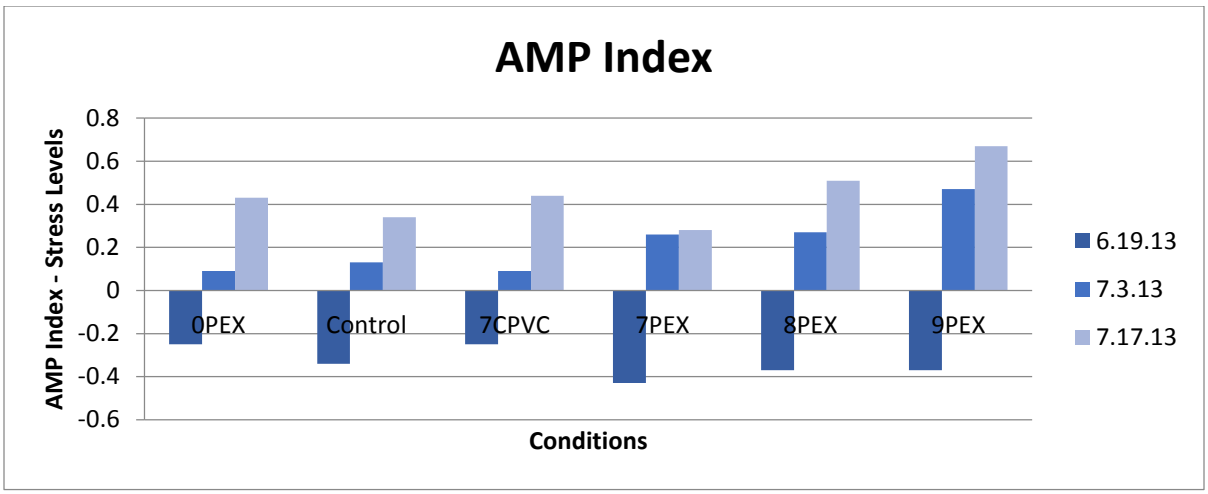

Figure 8. The x-axis shows the SWHR conditions. The y-axis represents AMP Index stress levels. It should be noted that adjusted pH levels and the addition of copper ions increased stress levels.

CONCLUSION

The only major findings that are conclusive at this point in the research are ATP concentration values and AMP Index. While qPCR and LP culture plates are good indicators of LP levels within the SWHR, there is currently no data with the addition of $CuSO_4$ to draw conclusive results as to whether or not copper inactivates LP. Total organic carbon, and trace metal concentration data does not fully explain the effects of copper on LP. ATP concentrations and AMP Index are good indicators of the effect of copper. During the first sample date before pH was adjusted or copper was added, all ATP concentrations were high and all AMP Index stress levels were negative. This means that bacteria as well as LP were viable. As pH was adjusted, ATP concentrations dropped, showing how pH changes (becoming more acidic and more basic) affected the bacteria and LP levels. AMP Index stress levels became positive, further emphasizing bacteria and LP's stress during pH changes. Stress levels are also higher for more basic conditions. When $CuSO_4$ was added to the four conditions, ATP concentrations greatly decreased for those conditions. The two conditions that did not have $CuSO_4$ added, had higher or constant ATP concentrations. This could be due to the fact that $CuSO_4$ did in fact affect bacterial viability, including that of LP. For AMP Index, stress levels increased greatly for all conditions besides CPVC. Conclusions are inadequate at this time since even conditions with $CuSO_4$ added had higher stress levels. However, stress levels for pH 8 and 9 were much higher than that for other conditions.

Although there are not conclusive results at this time, it is expected that the addition of copper ions within the four conditions will inactivate LP. This hypothesis is due to previous research mentioned within the literature review section that has shown copper ions to inactivate LP.

Future Research Needs: Future research needs include collecting data for qPCR when $CuSO_4$ is added to the four conditions. Consistent sampling with $CuSO_4$ added over time would allow for conclusions to be made as to whether or not copper has an effect on LP. It is hoped that this research will continue in order to determine this.

ACKNOWLEDGMENTS

We acknowledge the support of the National Science Foundation through NSF/REU Site Grant EEC-1062860. Any opinions, findings, and conclusions or recommendations expressed in this paper are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. I would like to personally thank Caitlin R. Proctor for aiding in the formation of this paper, and the research project overall. She has been extremely helpful in guiding me throughout this research project, and for that, I am truly grateful. I would also like to thank Dr. Marc A. Edwards for assisting in the creation of the research project, and his guidance throughout. I would like to acknowledge Dr. Amy Pruden, and William Rhoads support as well. Lastly, a huge thank you to Dr. Vinod Lohani, and the NSF/REU Interdisciplinary Water Sciences and Engineering program.

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A Tale of Two Reservoirs: Unraveling the Manganese Mystery Downstream of Smith Mountain and Leesville Lakes

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ABSTRACT

Manganese (Mn) is an aesthetic hazard in drinking water often found in elevated concentrations downstream of stratified reservoirs. This project continues an effort to characterize Mn behavioral dynamics in the tailrace (i.e. downstream) of Smith Mountain and Leesville Lakes, a dual reservoir hydroelectric system created by the impoundment of the Roanoke River in Southwest Virginia. Results show that hydrologic condition (i.e. stage) controlled Mn behavior from a major tributary (Goose Creek) whereas a complex set of parameters ('reservoir dynamics') controlled Mn behavior from Leesville Lake. When reservoir dynamics favored high-Mn releases from Leesville Dam and river stage was low, the reservoir operated as primary Mn source; when river stage was high and dam releases were low in Mn, most Mn came from Goose Creek. In all situations, most Mn was particulate. Results suggest that the dual reservoir system, never previously studied with relation to Mn, complicates Mn behavior downstream.

Keywords: Manganese, Reservoirs, Attenuation, Tailrace, Mass Balance

Introduction:

Manganese (Mn) is an aesthetic hazard in drinking water that can cause staining and is difficult to treat. For this reason, the U.S. Environmental Protection Agency (EPA) maintains a secondary standard of 50 total parts per billion (ppb) (EPA). Though groundwater drinkers are frequently confronted with Mn in excess of this standard, oxygen-rich streams and rivers typically immobilize Mn, which limits concentrations. However, large reservoirs can reduce oxygen levels, dissolving and mobilizing Mn for downstream transport.

During summer months, reservoirs undergo thermal stratification. Warmer, sun-exposed waters in the epilimnion (upper layer) host photosynthesizing organisms and are generally oxygen-rich; the cooler hypolimnion (lower layer) will generally be oxygen-poor. The anoxic conditions in the hypolimnion dissolve constituents like Mn, Iron (Fe) and Sulfur (S) that are typically insoluble in oxidizing environments, such as the rivers and creeks feeding the reservoir. Their dissolution mobilizes them for downstream transport if dam intakes are located in the hypolimnion.

As a result, Mn levels immediately downstream of reservoirs can be elevated, and that Mn is typically dissolved. Ashby et al. (1999), for instance, found primarily dissolved Mn in the Lake Texoma tailrace (downstream), at concentrations up to and exceeding 1000 ppb. Nix et al. (1991) showed dissolved and particulate (oxidized) components nearly equal over their downstream reach. Gordon (1984) saw Mn up to 2000 ppb immediately downstream of Normandy Dam in Tennesse, 400 times the EPA secondary standard!

Once Mn-rich water from the reservoir is released into the oxygen-rich tailrace, however, the thermodynamic equilibrium shifts in favor of oxidized, insoluble Mn and dissolved Mn is exposed to adsorption sites (Munger 2012). As a result, Mn is frequently said to 'attenuate' in reservoir tailraces,

following a particular kinetic pattern – namely, ‘first-order’ removal. Figure 1 (from Gordon 1984) provides an example of this characteristic behavior: high Mn levels near the dam rapidly decrease away from it. While in laboratory settings oxidation of Mn takes decades – 20 to 30 years for 25% to be attenuated (Wilson 1980) – field observations find swifter removal [(1984), (1991) (Dortch 1995), (1999)]. Gordon, for instance, observed that Mn attenuated 25% in around five hours below Normandy Dam in Tennessee.

Bio-accelerated oxidation and adsorption are typically attributed for expediting attenuation of dissolved Mn in the field [(1984), (1995), (1999)]. Dissolved Mn is thought to adsorb onto particulate matter (1980) that, like oxidized Mn, will remain in suspension until precipitated out of the water column. However, “sorption onto manganese-coated surfaces” (1984) in the substrate takes Mn directly out of the water column, and has been considered the “primary removal mechanism” (1995). Substrate adsorption is a mass-limited reaction, meaning faster flows yield faster removal rates. Locations with “higher stream slope,” and thus higher “shear velocity,” experienced more rapid attenuation for Dortch (1995). Mn attenuation, similarly, was positively correlated with discharges in the Normandy Dam tailrace (1984).

Notably, these observations, and all previous studies, have focused on single-reservoir tailraces.

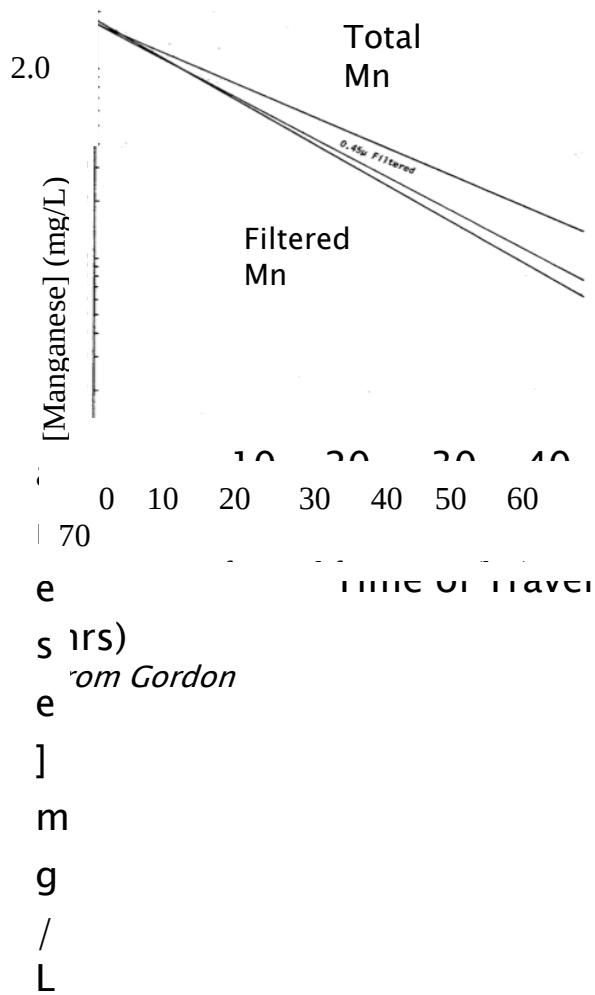


Figure 1: First-Order Attenuation of Total and Filtered (Dissolved) Mn with respect to time of travel downstream from Normandy Dam. *From Gordon (1984)*

This project studied Mn behavior in the tailrace of the Smith Mountain Project, a reservoir system along the Roanoke River in Southwest Virginia.

Two reservoirs comprise the Smith Mountain Project, located in Virginia's Bedford, Campbell, Franklin and Pittsylvania counties. They are a hydroelectric apparatus created in the 1960s by impoundment of the Roanoke River and run by Appalachian Electric Power (Figure 2).

Smith Mountain Lake is the furthest upstream and largest reservoir, with a surface area of 20,600 acres, a storage volume of 1,142,000 acre-feet and a typical elevation of 795 ft (Kleinschmidt 2008). Previous studies show the lake heavily stratified during the summer, yielding a positive correlation between Mn levels and depth (Figure 3). Five release intakes with different discharge capacities and elevations characterize Smith Mountain Dam (Table 1). Notably, three units can pump water from downstream back into the reservoir (when demand is low), and two of these are located in the hypolimnion.

Immediately downstream of Smith Mountain Lake is the smaller Leesville Lake, with 3,270 acres of surface area and a storage volume of 94,900 acre-feet (2008). Maximum discharge from Smith Mountain Lake can raise the Leesville Lake surface level from 600 to 613 feet, while lowering the former's just two feet, making Leesville Lake a far more dynamic and therefore difficult to characterize reservoir. Leesville Dam, which has a minimum hydraulic capacity of 3,750 cfs and maximum of 4,500 cfs, releases for approximately 20 minutes each hour into the Roanoke River downstream.

Smith Mountain Lake is connected to North Carolina's Kerr Reservoir by a 113-mile stretch of the Roanoke River. Several small towns and power plants are situated along the reach, and mining of low-grade Mn ores was common in the area several decades ago.

This study is part of an ongoing effort to characterize Mn in the Roanoke River downstream of Smith Mountain Lake and Leesville Lake. Within this descriptive framework, we broadly ask: how does a dual-reservoir system affect Mn behavior downstream? Special emphasis was put on comparing and contrasting Mn behavior (i.e. whether or not it attenuated), form (particulate or dissolved), magnitude (concentration) and apparent path-determining mechanisms (hydrologic, riverine, bio-controlled) with previous observations about tailraces. Previously, only single-reservoir systems have been studied.

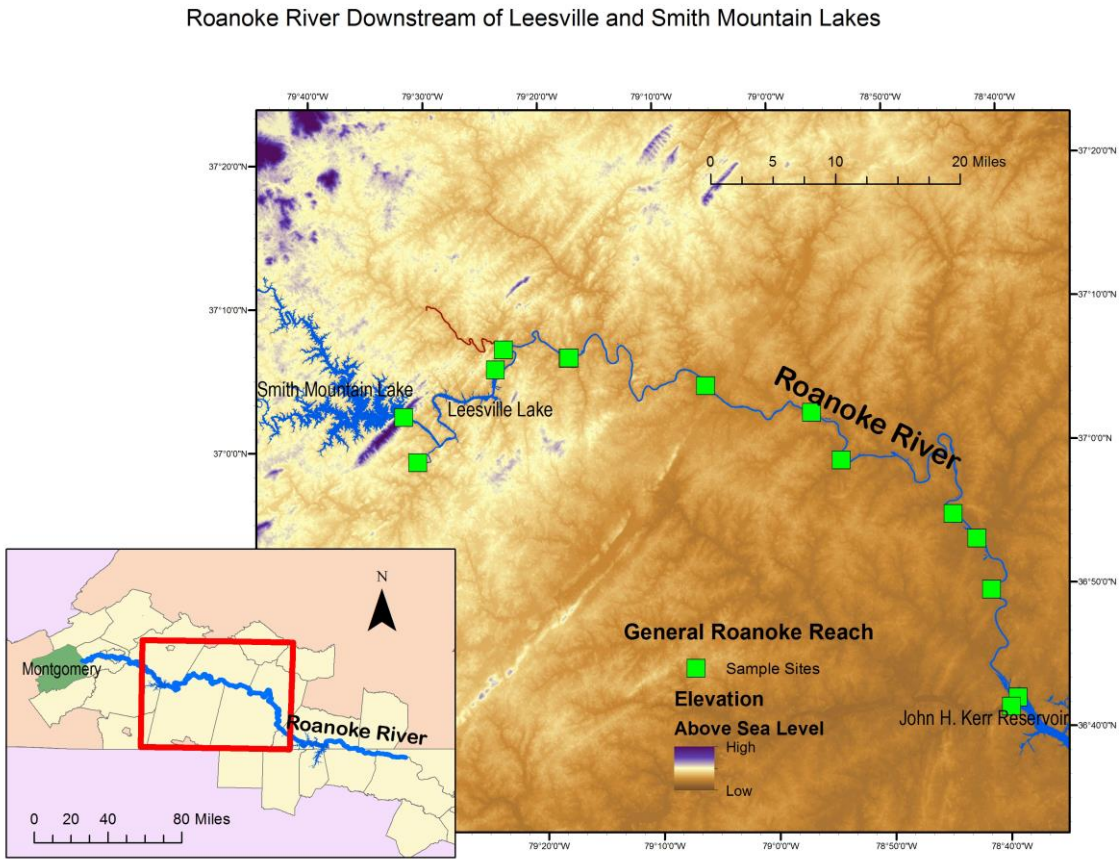
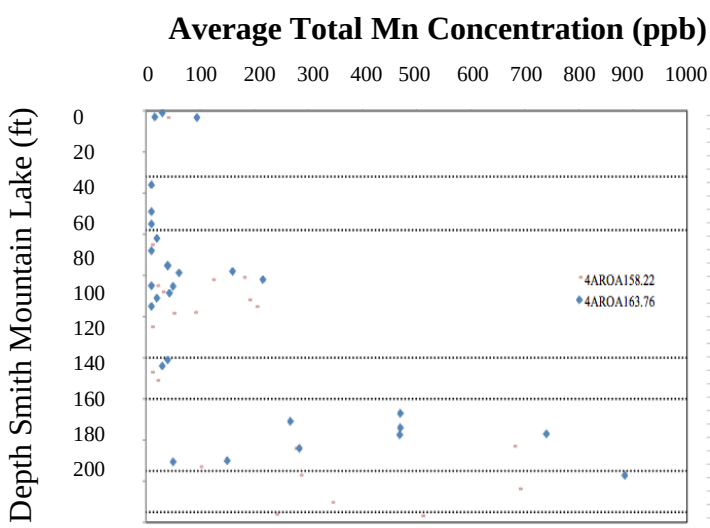


Figure 2: General Study Reach. Smith Mountain Lake, Leesville Lake and Kerr Reservoir buffet a 113-mile stretch of the Roanoke River.
Data Sources: NHDH and EarthExplorer



Unit No.	Hydraulic Range (cfs)	Pump Back Capacity (cfs)	Intake Invert Elevation (ft)	Depth at Normal Reservoir Elevation (795 ft)	Intake Diameter (ft)
1	2,700 - 5,200	4,490	655	140	20
2	10,000 - 14,000	--	737	58	26
3	5,700 - 9,000	6,830	737	58	26
4	10,000 - 14,000	--	737	58	26
5	2,700 - 5,400	4,490	600	195	20

Source: Smith Mountain Project (FERC No. 2210) Pre-Application Document

Table 1: Smith Mountain Dam unit hydraulic capacities, pump back capabilities, intake elevations and penstock diameters

Figure 3: Smith Mountain Lake depth with relation to Mn concentration. *Source:*

Methods:

Samples along the 113-mile reach of the Roanoke River between Smith Mountain Lake and Kerr Reservoir were collected regularly over the last year and analyzed for Mn and related parameters like Fe, dissolved oxygen, alkalinity and total suspended solids (TSS). This project, however, focused its study on a condensed 10-mile stretch immediately downstream of Leesville Lake, the second (downstream), smaller reservoir. This stretch is characterized by relatively large fluctuations in discharge (due to the episodic nature of dam releases), woodland surroundings and the confluence of a major tributary, Goose Creek (Figure 4a). Because preliminary sampling showed Mn attenuating rapidly along this reach, it made an excellent space to investigate Mn.

As proposed by Munger (2012), and following the model of House and Warwick (1998), a mass balance approach was used to estimate amounts and causes of Mn and TSS loss and/or gain in the condensed study reach. Mass Balance is predicated on the law of conservation of mass, i.e. that matter can always be accounted for. The expression of mass balance for this study is:

$$\text{Equation 1: } M_T = M_U + M_R + M_{NET}$$

Munger (2012)

Where M_T is total downstream mass, M_U is total upstream mass, M_R is total mass entering the system through tributary inputs and M_{NET} is a catchall for all mass entering or leaving the study area otherwise unquantified. Mnet accounts for all biogeochemical, depositional/erosional and groundwater-related effects. 'M' at any given location 'i' is expressed by Munger:

$$\text{Equation 2: } M_i = \int C_i(t)Q_i(t)dt$$

Munger 2012

Where Ci is the concentration of mass at location 'i,' Qi is the location's discharge and 't' is time. More succinctly, M_i is a mass flow (unit mass per unit time), the product of the concentration 'C' of studied parameter and discharge 'Q' of water at that location. Mn and TSS were studied using mass balance — the latter with the intention of shedding light on the former.

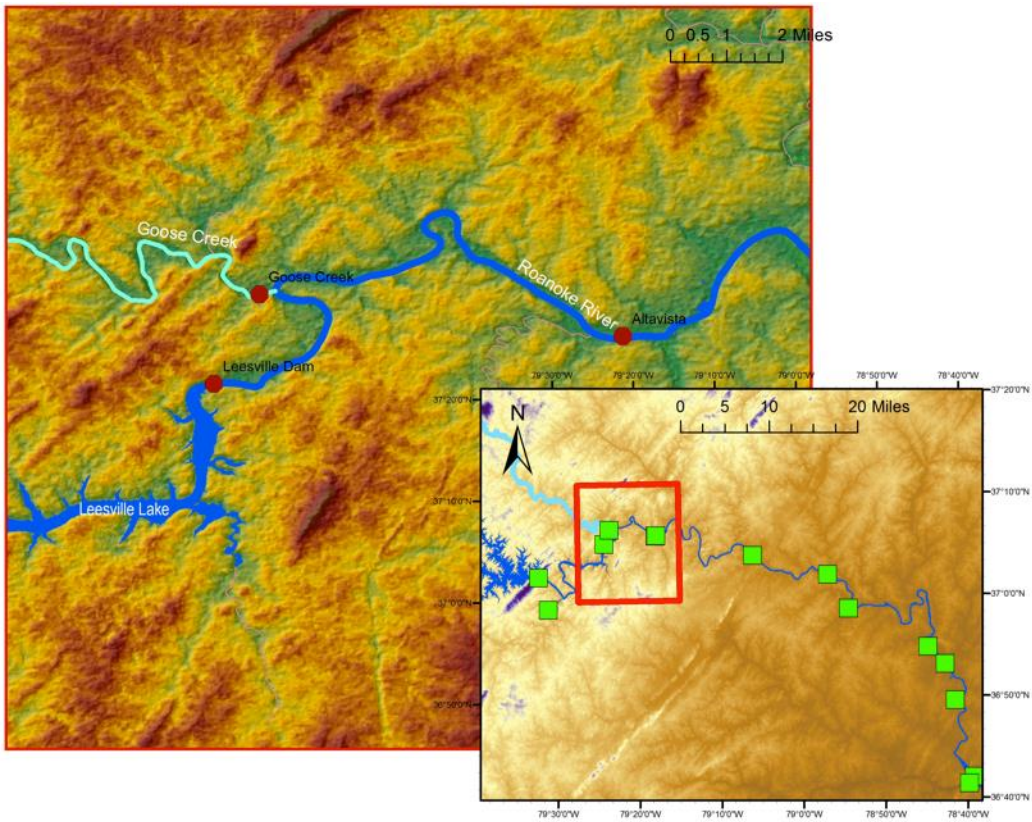
M_T, or 'downstream' mass, was the mass flow at Altavista, a country town 10.3 miles downstream of Leesville Dam. M_U, or 'upstream' mass, was the mass flow meters downstream from Leesville Dam. M_R, or 'tributary' mass, came from Goose Creek, the only major tributary in that reach. M_{NET} was merely the difference between the sum of M_R and M_U and M_T. M_{NET} values were negative if the Roanoke reach experienced a sediment deficit ($M_T > M_R + M_U$); positive if the inverse. (Figure 4b)

A Nalgene Depth-Integrated sampler was used at each site, as close to the thalweg as possible. If necessary, a small yellow inflatable banana boat was utilized. However due to the considerable discharge of the Roanoke during high flow, raft use wasn't always possible. A transect at the M_U site was taken to test the soundness of integrating the sample across water body depth but not width. Little variation was found between samples collected at 20-foot intervals.

As cation samples were processed off-site, data from May, June and July are incomplete at the time of writing. Later Mn and Fe particulate (un-filtered) samples were microwave digested before being tested. A standard, vacuum-catalyzed .45 micron filtration method was used to discern TSS.

Qi values were calculated from USGS gaging station data. Data from a gage immediately upstream of Altavista were used in the calculation of M_T. Discharge data from a gage upstream of the confluence of Goose Creek with the Roanoke River were used to compute M_R. An ADCP acoustic Doppler Radar Raft ferried its way across Goose Creek adjacent to its confluence with the Roanoke, confirming the accuracy of the gage data. The difference between the discharge measurements at the two

gaging stations for any given sampling event was used as defacto M_U discharge. Discharge immediately downstream of Leesville Dam is difficult to quantify due to the erratic nature of releases.



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Results:

Mn in the Leesville Lake tailrace was mostly in particulate form. Figure 5 shows relative concentrations of total and dissolved Mn at sampling stations in the Smith Mountain Lake tailrace during an early fall (November 2012) sampling event. At all locations, very little Mn was dissolved, including the sampling sites immediately downstream of Smith Mountain Dam and immediately downstream of Leesville Dam. Using Gordon's finding downstream of Normandy Dam that very little Mn is colloidal (1984), the particulate Mn at each site can be inferred as the difference between total and

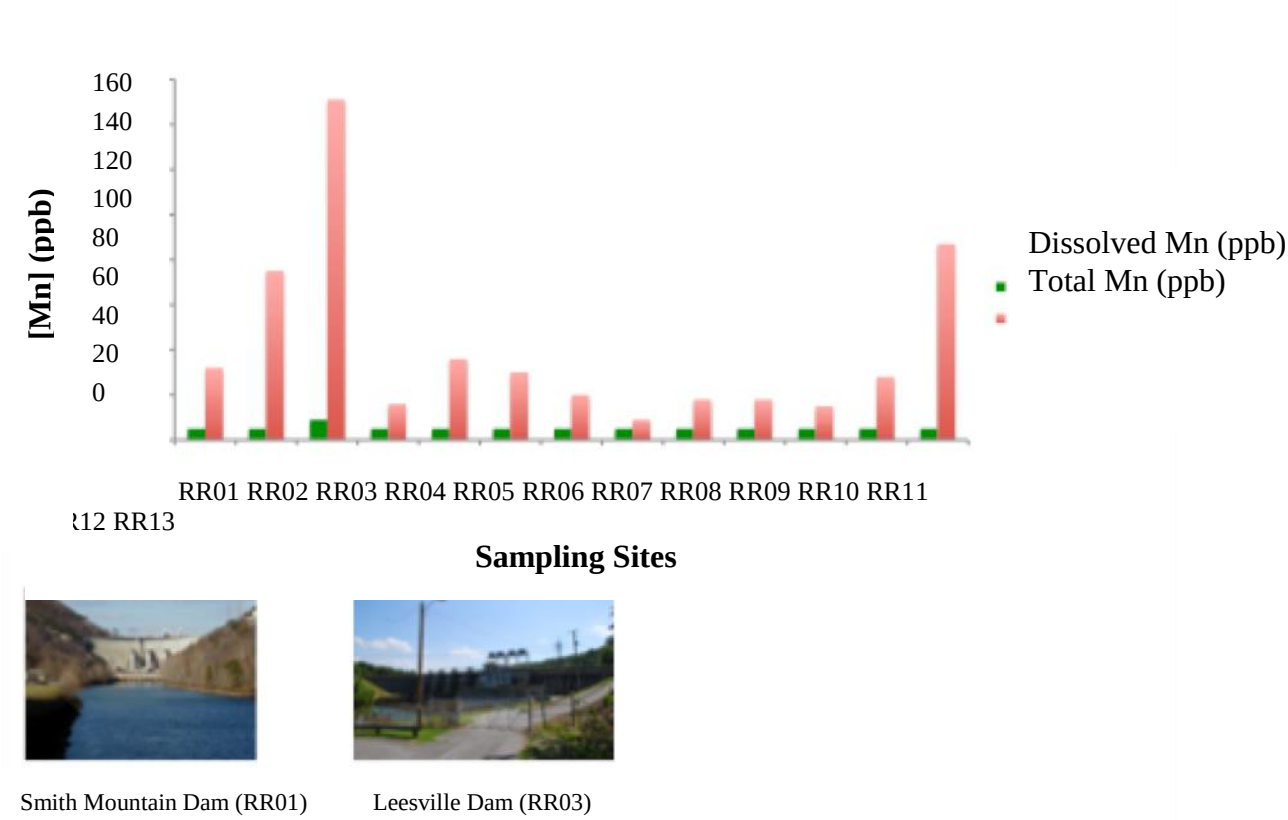


Figure 5: Total and dissolved Mn along the Roanoke River in Nov. 2013. Most Mn was particulate (total – dissolved), even at sampling locations immediately downstream of Smith Mountain Dam (RR01) and Leesville Dam (RR03)

dissolved components.

The particular behavior was consistent across sampling events from all seasons and under all hydrologic conditions. This contrasted sharply with previous studies' findings of mostly dissolved Mn in dam tailraces. Because the first-order kinetic attenuation observed in those pieces was contingent on there being significant dissolved/reduced Mn in the water (i.e. adsorption and oxidation occur only for Mn in dissolved/reduced form), this data suggested other mechanisms controlled Mn in our study reach.

Specifically, impacts of river-processes (namely, dilution) and hydrologic condition (i.e. stage) on the mostly-particulate Mn were more closely examined using the mass balance method outlined in Equations (1) and (2). Because particulate Mn would presumably exhibit behavior related to total particulate material, a mass balance for TSS was also computed.

Mn mass balance results varied between sampling events, with no appreciable relationship to hydrologic condition (Fig 6). During winter (Feb. 2013) and early summer (Jun. 2013) mass was 'balanced' (i.e. mass exiting the system (M_T) could be accounted for by upstream and tributary (M_U + M_R) inputs into the system). However, an early fall (Nov. 2012) sampling event showed mass 'unbalanced': less mass left the system than entered it and M_{Net} was negative. The 'balanced' mass results from early summer and winter suggest that river processes were determinant of Mn behavior during those sampling events. In other words, water from Goose Creek diluted Mn coming out of Leesville Dam, or vice versa. Alternatively, the fall sampling event suggested that some Mn was 'left behind' in the system. While this 'Mn deficit' was characteristic of attenuation, substrate adsorption – often characterized as an attenuation driver – was an unlikely cause. The predominance of particulate Mn over dissolved Mn in the study reach makes deposition of suspended sediments more likely.

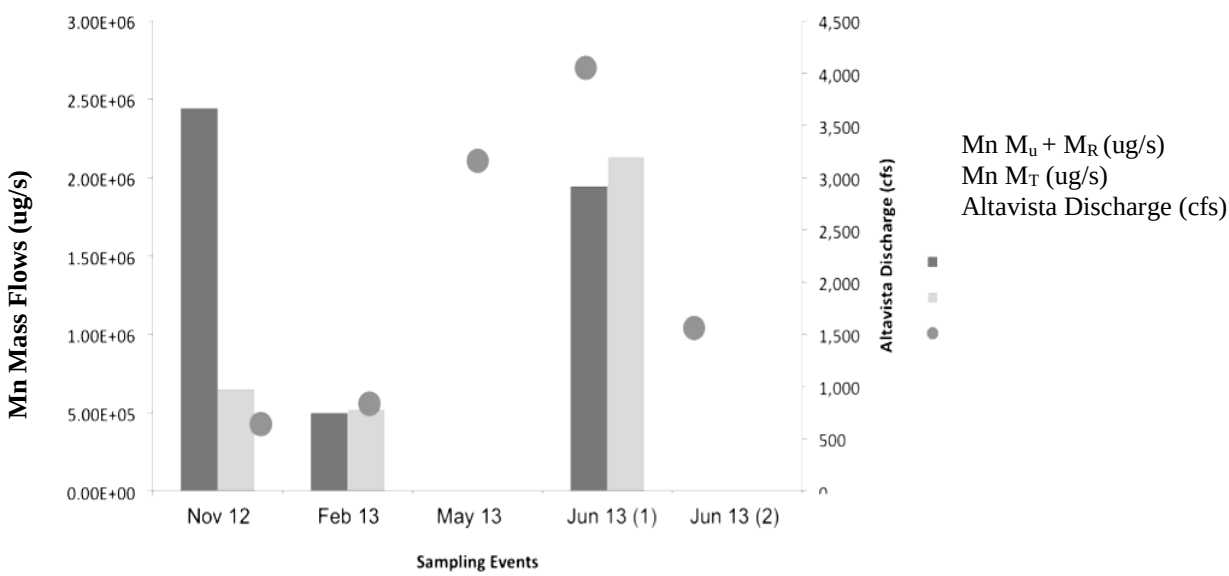


Figure 6: Mn mass balance results in study reach. Mass was accounted for in winter and early-spring but not in early-fall, with no relationship to hydrologic condition.

While TSS mass balance results also varied between sampling events, mass flows were obviously related to Altavista discharge (Figure 7). During baseflow, the hydrologic condition characterizing the Nov. 2012 and Feb. 2013 sampling events, slightly less TSS left the system (M_T) than entered (M_U + M_R), characteristic of a mildly depositional environment. During high flow, the hydrologic condition characterizing the last three sampling events, more mass left the system than entered it, characteristic of an erosional environment. Plainly, whether the Roanoke River deposited sediment or brought it into suspension over the study reach was controlled by how much and how quickly the water flowed.

The results of the mass balance analysis show that TSS mass flows experienced a pointed hydrologic control, while the relationship between discharge and Mn mass flows was more dubious. While this outcome suggests that Mn in the study reach faces a different set of behavioral determinants, it doesn't necessarily undermine the possibility of *some* interrelationship between Mn and TSS, and Mn and hydrologic condition. The effects of Leesville Dam (M_U) and Goose Creek (M_R) were summed for

the purposes of the mass balance, mixing together the impacts of reservoir dynamics (from Leesville and Smith Mountain Lakes) with 'natural' stream dynamics (from Goose Creek and the Roanoke River) on downstream behavior of TSS and Mn. In order to parse more closely the differing effects of Goose Creek and Leesville Dam, concentrations of TSS and Mn were isolated in Figures 8 and 9.

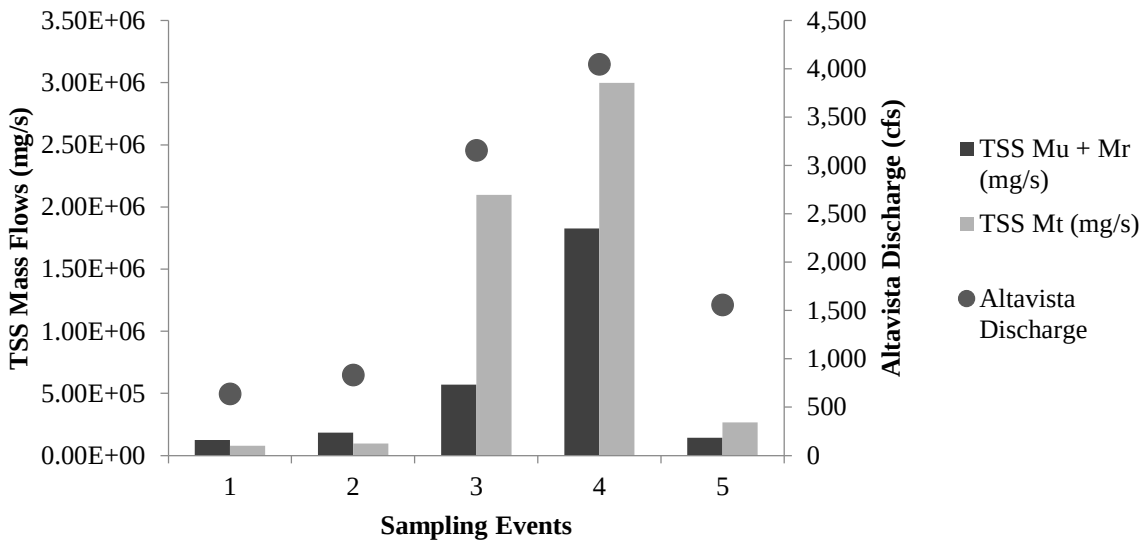


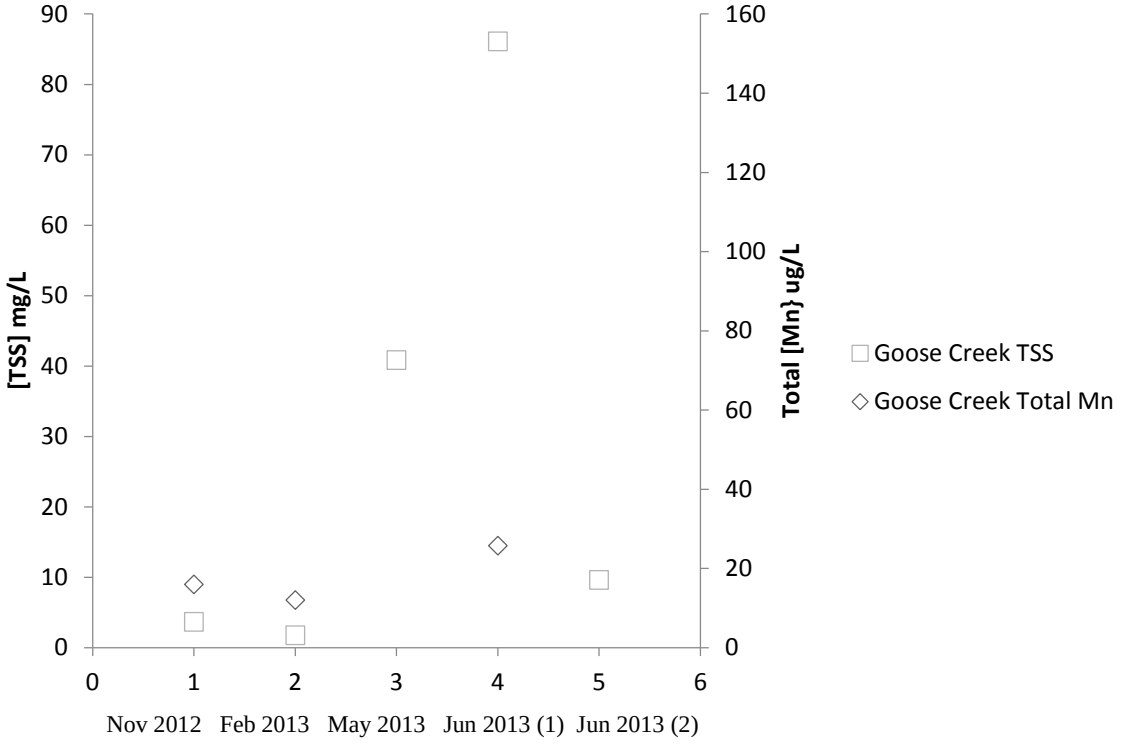
Figure 7: TSS mass balance shows that mass flows in the study reach have a hydrologic control.

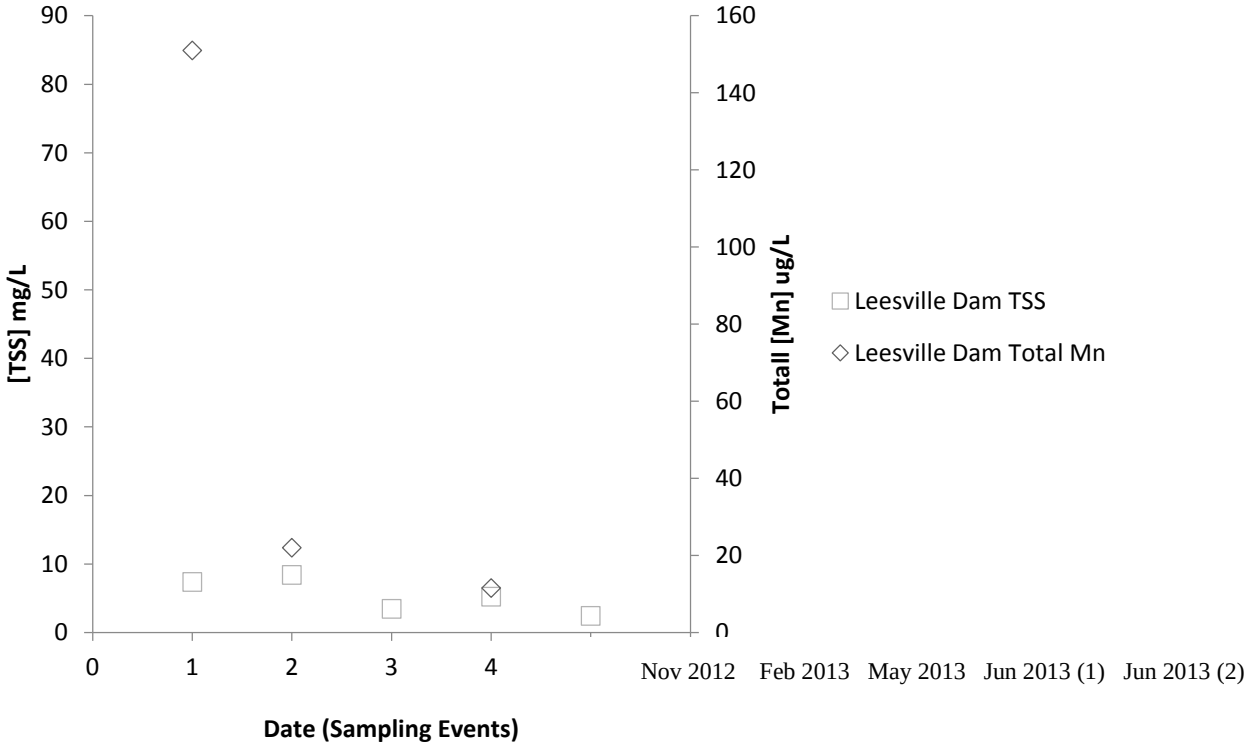
TSS concentrations were more erratic at Goose Creek (Figure 8) than at Leesville Dam (Figure 9), and were on average higher. High flow was a good predictor of high TSS concentrations; the contour of Altavista discharges (Figures 6 and 7) closely mimicked the contour of TSS concentrations at Goose Creek, with the highest TSS concentrations (86 mg/L) coming when discharge at Altavista was highest (4050 cfs). TSS levels out of Leesville Dam were lower, less variable and unrelated to downstream discharge, following previous observations about reservoir effects on suspended particulate [(Dai and Lu 2013), (Erwin et al., 2011)].

Mn concentrations coming out of Leesville Dam were more variable and, usually, higher than Mn levels from Goose Creek, with Mn levels as high as 151 ppb for Leesville Dam but no greater than 26 ppb for Goose Creek. The thrust of this trend was predicted by the literature [(1995), (1984)]. Notably, variation in Mn at Goose Creek positively correlated with variation in TSS, whereas variation in Mn at Leesville Dam did not. As a result, TSS, Mn and discharge (not charted) seem coupled at Goose Creek. Meanwhile, Mn concentrations and TSS concentrations at Leesville Dam appeared unrelated to each other and to downstream discharge.

Though Goose Creek and Leesville Lake are the fundamental inputs of Mn into the Roanoke River, Figs. 8 and 9 show that how *much* Mn each releases is unrelated. They experience differing controlling conditions. At Goose Creek, the observed inter-relationship between Mn, TSS and downstream discharge suggests that 'hydrologic condition' determines how much Mn enters the Roanoke River from the tributary (and, probably, downstream tributaries). However, because Mn, TSS and downstream discharge appeared unrelated out of Leesville Dam, an alternative model is needed to describe the effect of 'reservoir dynamics' on Mn entering the Roanoke River there.

How do these very different inputs into the study area, one ostensibly controlled by 'hydrologic condition' and the other by more mysterious 'reservoir dynamics,' affect the distribution of downstream Mn concentrations?





Figures 8 and 9: Mn and TSS concentrations for sampling events were plotted together for Goose Creek (Figure 8) and Leesville Dam (Figure 9). Mn and TSS concentrations were correlated at Goose Creek but not at Leesville Dam.

A very simple conceptual model highlights how Mn behavior in the study area was impacted by two previously-identified parameters: 'hydrologic condition' (for Goose Creek) and 'reservoir dynamics' (for Leesville Dam). The model, which looks a bit like a game-theory problem (Varian 2010), identifies two possible 'states' for each variable. 'Hydrologic condition' was determined as either 'high Q' (discharge at the Altavista gaging station greater than the 50 year median of 875 cfs) or 'low Q' (less than the median). Mn concentrations at Leesville Dam were a proxy for the parameter 'reservoir dynamics' – 'high dam' for relatively high Mn concentrations; 'low dam' for relatively low Mn concentrations. The two possible 'states' for each parameter yielded four possible outcomes for Mn behavior. Although at the time of writing not all possible outcomes had been observed, striking relationships between Mn behavior in the study reach and the interaction of these two parameters were found.

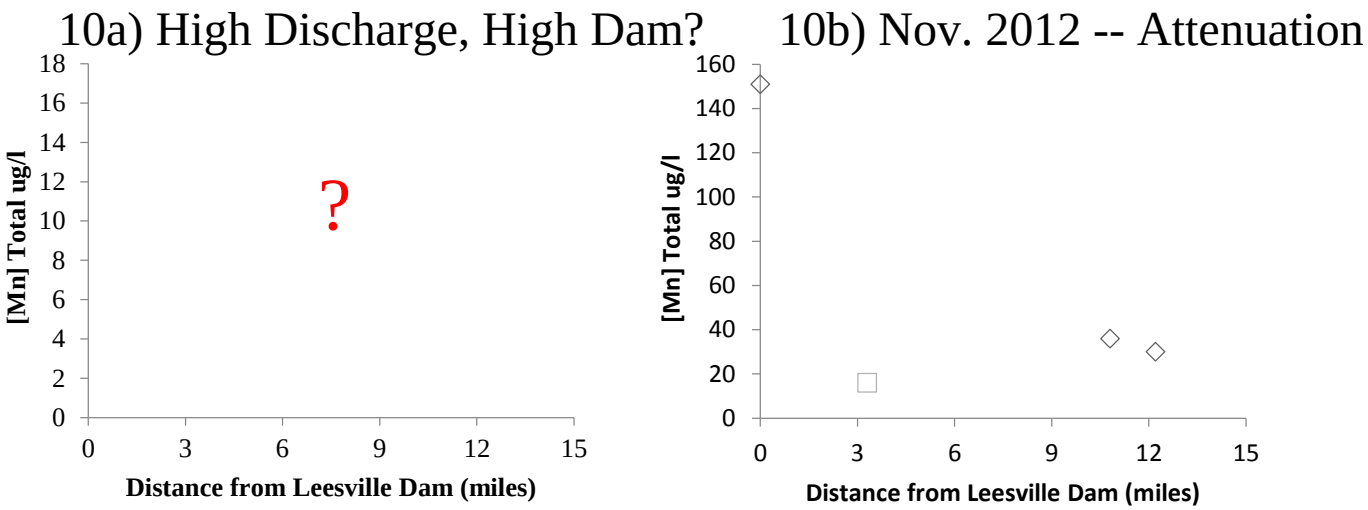
A 'Low Q, Hi Dam' case study came from the November 2012 sampling event (Figure 10b). Smith Mountain and Leesville Lake were the primary source for Mn (concentrations from Leesville Lake were nearly eight times greater than concentrations from Goose Creek) and the cumulative effect along the Roanoke River was one of 'attenuation.' Mn removal was reminiscent of the first-order kinetic pattern observed in the literature [(1984), (1995), (1999)]. The Nov. 2012 event represented the only time in nine months of sampling that Mn behavior resembled this predicted pattern.

When Mn releases from Leesville Dam and discharge were 'low,' the condition that characterized the February 2012 sampling event, Mn concentrations in the study area were unchanging and low – or 'stagnant' (Figure 10d). Mn concentrations were flat at about 22 ppb along the study reach, with Goose Creek contributing a negligibly different 13 ppb, suggesting that neither Goose Creek nor Leesville Dam served as a primary source for Mn.

The 'Hi Q, Low Dam' condition, from the June 2013 sampling event, yielded *increasing* Mn concentrations downstream (Figure 10c). Goose Creek appeared to be primary source for Mn, with concentrations more than twice those at Leesville Dam.

The June 2013 and November 2012 sampling events were influenced by diametrically opposed conditions for the parameters ('hydrologic condition' and 'reservoir dynamics'), which yielded diametrically opposed Mn behaviors. However, the degree to which these parameters effaced these changes in behavior was not equal. For instance, Mn attenuated rapidly for the 'Low Q, Hi Dam' condition, but increased only marginally for the 'Low Dam, Hi Q' condition. Additionally, variation in reservoir dynamics yielded higher highs (151 ppb) and lower lows (11.5 ppb) for Mn from Leesville Dam than variations in hydrologic condition did for Mn from Goose Creek (26 ppb maximum; 12 ppb minimum). From the data at hand, it appears that hydrologic condition and reservoir dynamics both play vital roles in shaping Mn behavior downstream, but reservoir dynamics are more important.

To illustrate this, one could predict the behavior of Mn in the study reach during a 'Hi Q, Hi Dam' event, which hadn't been observed at the time of writing (Figure 10a). Our hypothesis would be for Mn to attenuate over the study reach, though at a slower rate than under the 'Low Q, Hi Dam' condition. The reason for this is that, while Mn concentrations at Goose Creek and Leesville Dam would both elevate, Leesville Dam Mn would elevate to a greater extent. While Goose Creek would dilute Mn at its confluence with the Roanoke River, it wouldn't dilute it by nearly as much as it does under the 'Low Q, Hi Dam' condition, when its Mn concentrations are even lower. Hence, we might also infer that Mn behavior is flow dependent – higher flows yield slower attenuation, and perhaps even an increase, of Mn downstream of Leesville Dam.



Reservoir Dynamics	Hydrologic Condition		
		'High Flow' (Q at Altavista > 875 cfs)	'Low Flow' (Q at Altavista < 875 cfs)
	High Leesville Dam Mn Concentrations	Hi Q, Hi Dam =?	Low Q, Hi Dam = Attenuation
	Low Leesville Dam Mn Concentrations	Hi Q, Low Dam = Increase	Low Q, Low Dam = Stagnation

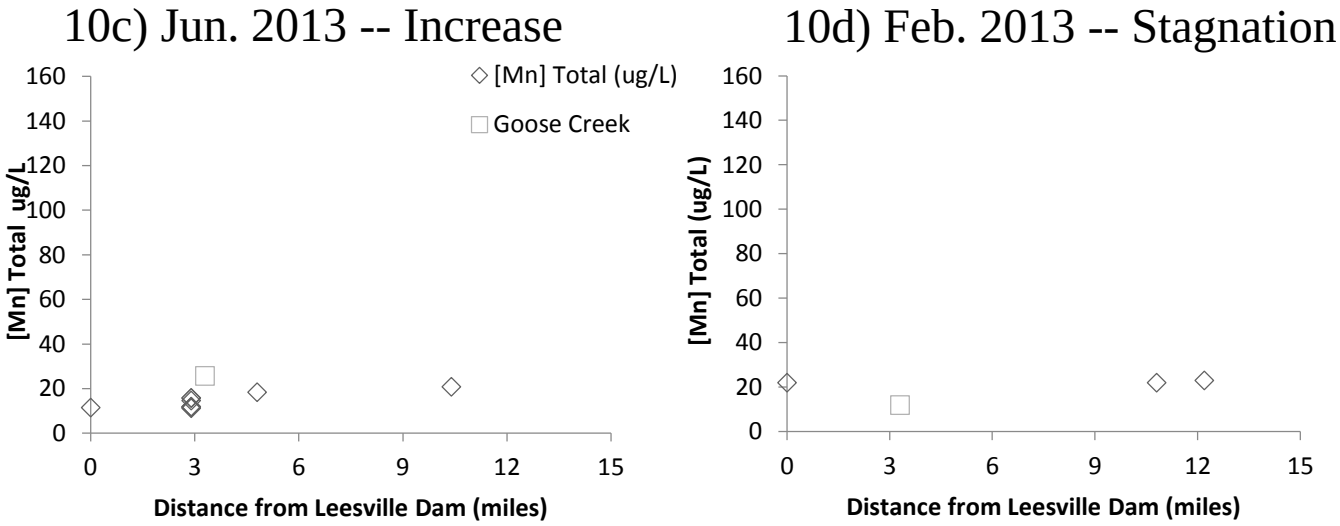


Figure 10: Two parameters – hydrologic condition (which effects Mn levels from Goose Creek) and reservoir dynamics (which effects Mn levels at Leesville Dam) – with two possible conditions combine to form four possible behavioral outcomes for Mn in the study reach. Hi Q and Hi Dam (a) theoretically would lead to slow attenuation. Low Q and HI Dam yielded faster attenuation (b). When discharge was Hi and Mn from Leesville Dam was low, Mn increased over the reach (c). When both conditions were low, there was little variation in Mn concentrations downstream (d).

A final thought. Although this paper treats ‘reservoir dynamics’ like a single, quantifiable element, the parameter is really a proxy for a set of parameters, many known but not closely examined, that act on Leesville Lake and Smith Mountain Lake. Unraveling the nature of their interaction is probably the key to unraveling the nature of Mn behavior in the Roanoke River. They are: 1) Seasonal. Until a full year of data is collected, the seasonal impacts on the reservoirs and Roanoke River will remain unknown. The most obvious of these seasonal impacts is stratification, which has been shown to occur in the Smith Mountain Lake during late-summer and early-autumn (Figure 3). A June 2013 depth profile of Smith Mountain Lake showed that stratification with respect to Mn had not yet occurred (Figure 11), and comprehensive data on Leesville Lake stratification were not collected and could not be found. 2) Operational. Smith Mountain and Leesville Dams are coy about how much, when, and in the case of Smith Mountain Dam, from where in the depth profile, they release. This study cannot account for whether or not releases come from the hypolimnion, where Mn concentrations are highest. 3) Structural. Most importantly, no studies to date have traced Mn behavior in the tailrace of a dual reservoir system like that of the Smith Mountain Project. The interaction between the two might completely alter Mn behavior from what is predicted, and to a certain extent this seems to be the case. Characterizing the effect of dual reservoir system will be a crucial next step.

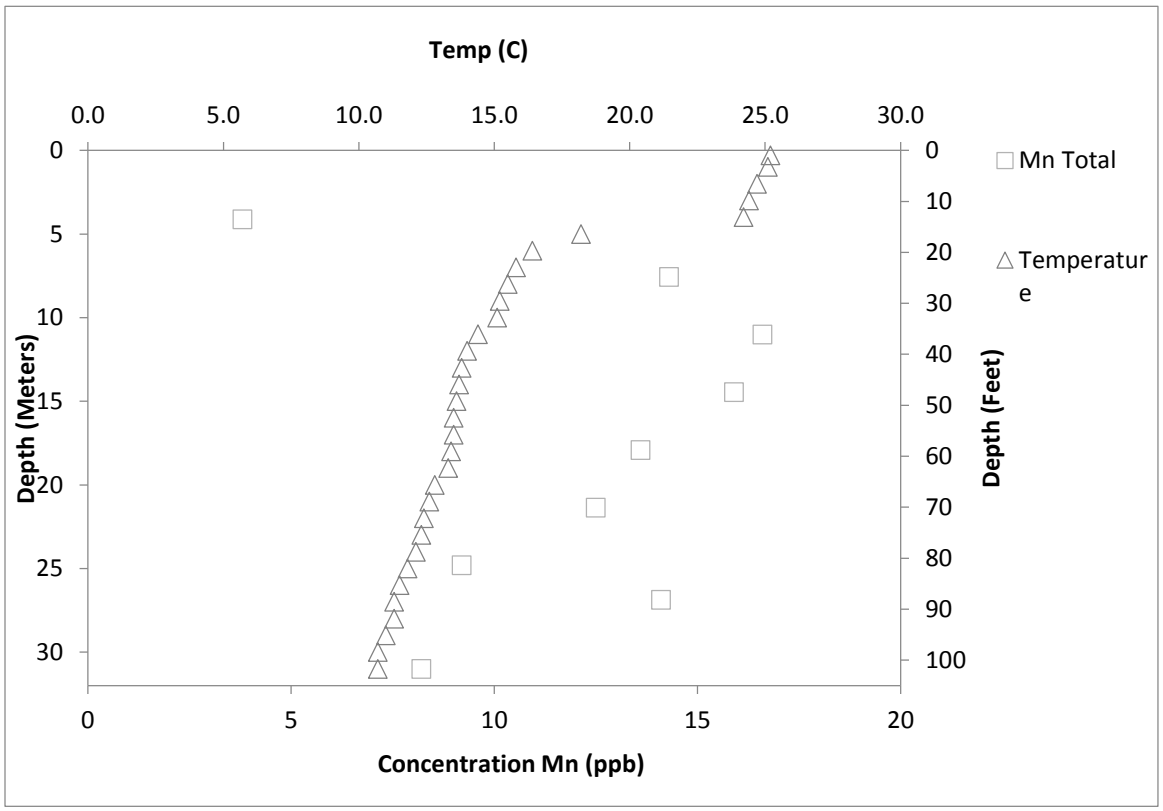


Figure 11: Thermal stratification but not Mn stratification in Smith Mountain Lake during mid-June

Conclusions

A comparison between dissolved and total Mn downstream of Leesville Dam showed that most Mn was in particulate, not dissolved, form. This contrasted with previous observations about reservoir tailraces, which found most Mn to be in dissolved form.

Mass balance analysis showed that Mn exiting the study area could sometimes be accounted for by inputs from Leesville Dam and Goose Creek, but other times mass exiting the system was less than

mass entering the system. This variability in Mn mass balance results was not linked to hydrologic condition (discharge). This differed from the results of a TSS mass balance, which clearly showed mass ‘gains’ (re-suspension) during high flow events and mass ‘loss’ (deposition) during low flow events.

When TSS and Mn concentrations at Goose Creek and Leesville Dam, respectively, were isolated and compared, the messiness of their downstream relationship was somewhat clarified. TSS, Mn and hydrologic condition (discharge) were positively correlated at Goose Creek, but the three seemed unrelated at Leesville Dam. As a result, two primary drivers of downstream Mn behavior were identified: 1) ‘hydrologic condition’ (impetus for Mn behavior in Goose Creek) and 2) ‘reservoir dynamics' (impetus for Mn behavior at Leesville Dam).

A game-theory conceptual model identified ‘reservior dynamics’ as the most important driver of Mn behavior downstream, although both parameters played crucial roles. Mn attenuated when the Roanoke River was at base flow and Mn levels from the dam were high, and was predicted to attenuate when the Roanoke is at high flow and the reservoirs release high Mn concentrations. During high flow events when the Mn concentrations at Leesville Lake were low, Mn levels actually increased slightly over the reach.

The conceptual model brought to light a few interesting contrasts between the study reach and other dam tailraces: 1) Attenuation was observed less frequently than increase and/or stagnation of Mn concentrations over the study reach, suggesting that the dam isn’t the only, or even primary, Mn source in the study area. Previous observations about dam tailraces found Mn nearly always attenuating away from the dam. 2) In part due to the hydrologic control for Goose Creek Mn and the form (particulate) of most Mn in the stretch, rate of attenuation was inversely correlated with discharge; ‘negative’ attenuation (increase downstream) of Mn characterized high flow events whereas steep, ‘positive’ attenuation (decrease downstream) characterized low flow events. This complemented and contrasted with an observation by Dortch (1995) that flow rate drove Mn attenuation. Dortch’s primarily-dissolved Mn, however, attenuated more quickly with higher flows – the opposite of the behavior observed in the Leesville Lake tailrace.

While Mn behavior in the Roanoke River is primarily driven by ‘reservoir dynamics,’ as previously observed in dam tailraces, the dual reservoir system of the Smith Mountain Project make characterization of those dynamics difficult. Understanding the nuances of Smith Mountain Lake, Leesville Lake and their relationship is crucial to understanding the nuance of Mn behavior downstream, but that’s the topic of another paper, another study.

Recommendations:

In order to strengthen and elaborate upon the conclusions of this paper, a full year of sampling must be completed. Particularly, effort should be made to characterize Mn behavior over the reach when flows are high and Leesville Dam releases concentrated Mn (the missing piece in our conceptual model).

Downstream tributaries should be sampled for during high and low flow events to see if a correlation exists between TSS, Mn and discharge similar to the one found at Goose Creek.
Multiple mass balances should be computed for the entire river, as downstream stretches of the Roanoke River may experience a very different set of controls than those immediately downstream of the Leesville dam.

Leesville Lake should be fully characterized. Does it stratify? Is this stratification prolonged or ephemeral? How long is residence time? Does Mn type and concentration change appreciably during residence (i.e., away from Smith Mountain Dam)?

Because most Mn is particulate, characterization of the particulate matter might indicate whether most Mn is adsorbed to particulate matter or part of its mineralogical composition. This should probably be done in conjunction with an in-depth investigation of the surrounding geology. Early Scanning Electron Microscope (SEM) analysis of particulate matter as per Pierce (1991) and Syvitski (1991) yielded little in the way of insight. An assortment of phyllosilicates and clays predominated, with no

discernable assemblage characterizing Goose Creek or Roanoke River. We did get some cool pictures, like this muscovite (Figure 12).

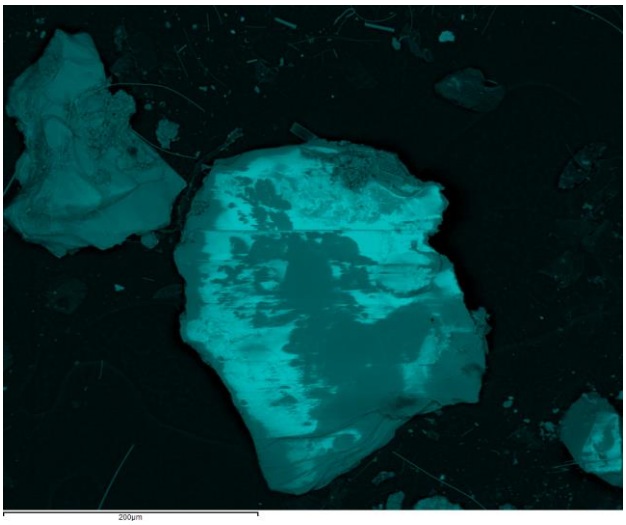


Figure 12: Muscovite from the Roanoke River under Scanning Electron Microscope.

Acknowledgements:

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Determining the Longevity of Carbon in Aquifer Sediment Samples

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ABSTRACT

To treat soil contaminated with tetrachloroethene (PCE) and trichloroethene (TCE), an emulsified vegetable oil biodegrades the compounds. However, it is not known for how long the carbon from the oil remains in the sediment. An experiment was set up to estimate the amount of time for carbon levels to decrease by 90% in aquifer sediment after injection with an emulsified soybean oil. The control, inactive microbial sediment, and active microbial sediment TOC removal rates were 0.099% per day, 0.221% per day, and 0.067% per day, respectively. Using these rates, it would take 374 days for inactive sediment, 913 days for the control, and 1256 days until 90% of TOC to be removed from the active sediment.

Keywords: Total Organic Carbon, Potential Bio-available Organic Carbon, emulsified vegetable oil, groundwater sediment

Introduction and Background

Across the United States, industrial chemicals enter groundwater supplies through spills, leakages, and inadequate or unregulated environmental safety precautions. Since contaminants threaten local and regional population centers, relevant researchers and agencies developed methods to clean these areas in a fast, efficient, and cost effective manner. A popular choice is bioremediation. Bioremediation uses micro-organisms to consume and break down hazardous organic material (Wilson, 1992). This method is popular due to its non-invasive strategy; contaminated soils, sediments, and water do not have to be physically removed and treated elsewhere, which reduces the price. Monitored Natural Attenuation (MNA) uses natural processes such as oxidation reduction reactions to break chemicals into less potent daughter products. This tactic can be assisted through biostimulation and bioaugmentation.

Whereas MNA monitors but does not interfere with natural processes, biostimulation and bioaugmentation facilitate change by spurring growth in micro-organism populations, thereby decreasing the time for adequate biodegradation (Bento, 2004). Biostimulation is the injection of micronutrients to help micro-organisms grow. Bioaugmentation is the direct application of specific organisms to increase population effectiveness.

Key pollutants that can be treated by these processes are tetrachloroethene (PCE) and trichloroethene (TCE) in groundwater supplies. As powerful degreasers, these chemicals commonly pollute groundwater below military instillations and dry cleaning establishments. Over half of the US Environmental Protection Agency (EPA) National Priorities List (NPL) sites contain these compounds (Mattson, 2004). NPL sites are treated through Superfund, or the Comprehenisve Environmental Response, Compensation, and Liability Act of 1980 (CERCLA) (Environmental Protection Agency, 2011). A Superfund is a site that has been contaminated by an industry or government agency and is slated for cleaning, and over a thousand of them exist throughout the United States.

PCE and TCE are important pollutants due to their public health effects and the amount of contamination throughout the United States. Health effects of chronic exposure to TCE and PCE include enhanced risks of Leukemia, Rectal Cancer, Bladder Cancer, Breast Cancer, Lung Cancer, and Non-Hodgkins lymphoma (Agency for Toxic Substances and Disease Registry, 2010). The EPA recommends a maximum contaminant limit (MCL) at 0.005 mg/L, or 5 parts per billion (ppb).

Despite the complicated health effects and great number of toxic sites, TCE and PCE contamination is treated through a straightforward biostimulation process. Emulsified vegetable oil injected into the contaminated site spur bacteria growth. *Dehalococcoides ethenogenes* strain 195 dechlorinates PCE and *Dehalococcoides* special strain FL2 dechlorinates TCE (Lendvay, 2003). The increased numbers of bacteria feed primarily on oxygen, causing the location to become anaerobic. As oxygen is depleted, bacteria start releasing protons, which bond to carbon molecules and replace chlorine.

The process starts as PCE, Cl_4C_2, gains a hydrogen and loses a chlorine molecule, becoming TCE, C_2HCl_3 (Oak Ridge National Laboratory, 2008). Furthuremore, TCE degrades into Cis - 1, 2 dichloroethene, $C_2H_2Cl_2$, vinyl chloride (VC), C_2H_3Cl, and finally to chloride, Cl_2 and ethane, C_2H_4. A major drawback to biostimulation concerns the production of daughter product VC. VC is a known human carcinogen, and chronic ingestion increases the risk of lung, brain, and liver cancer (Delaware Health and Social Services, 2009). It has an MCL of 2 ppb in drinking water.

Dover Air Force Base, Delaware, presents a perfect case study for TCE and PCE degradation by emulsified oil. Activated in 1942, it has a history of chlorinated toxins present in groundwater sediment due to aircraft engine degreasers and local drycleaners. In 1996, the Air Force conducted a field study to determine the amount of TCE and PCE contamination, Figures 1 and 2 (Witt, 2002). Both TCE and PCE levels exceeded the MCL, 5 ppb, or 5 µg/L. Therefore, in June 2006, the Air Force injected emulsified soybean oil into the area, Figure 3.

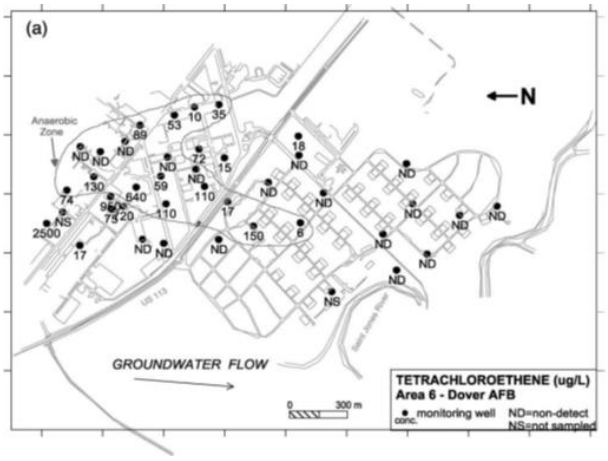

Figure 1: PCE concentration at Dover AFB, 1996

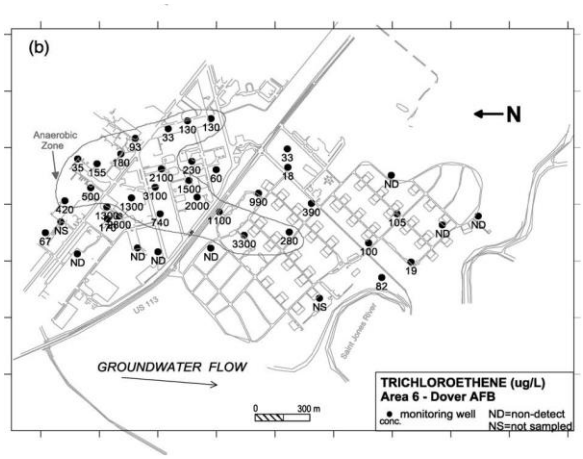

Figure 2: TCE contamination at Dover AFB, 1996

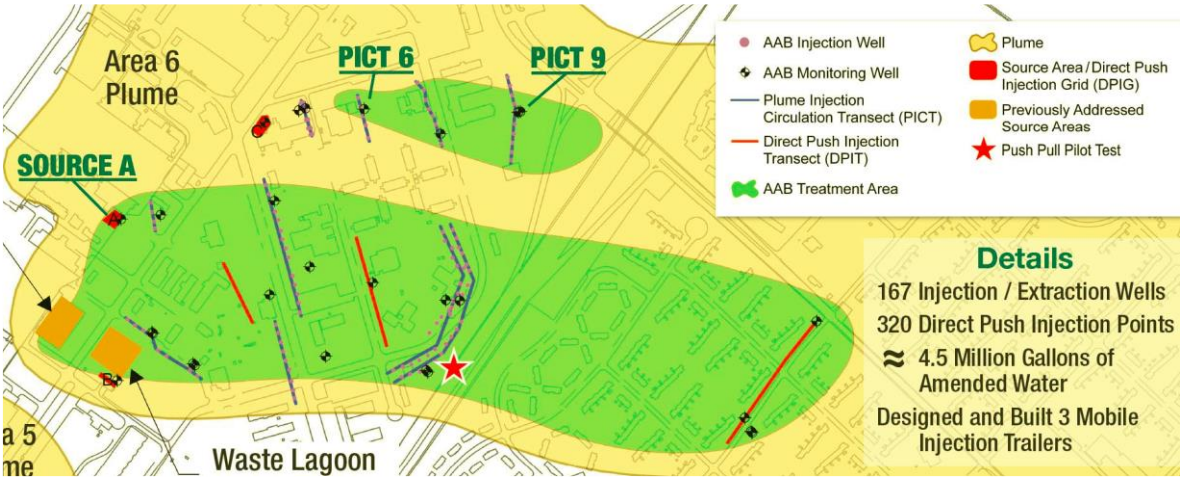

Figure 3: Location of injection points at Dover AFB, 2006

Every six months for the next year and a half, samples showed the volatile organic compound (VOC) concentrations for the locations Source A and PICT 9, Figures 4 and 5. As the main contaminant concentrations decreased, the amount of daughter products, Cis 1,2-dichloroethene and VC, increased. Thus, the major drawback to bioremediation with emulsified oil injections is VC production. Steps were taken to ensure public welfare during this critical stage, as the amount of VC exceeded its MCL. The treatment must continue for some time after injection to safely degrade the VOCs. Normal biostimulation treatment often includes one dose of emulsified vegetable oil. However, often times, one dose is not enough as contaminate concentrations start to increase after a period of time. More doses are sometimes needed, wasting time and money while putting the local population at risk. Being able to estimate how long the carbon will remain in the sediment is important to properly reduce dangerous compounds.

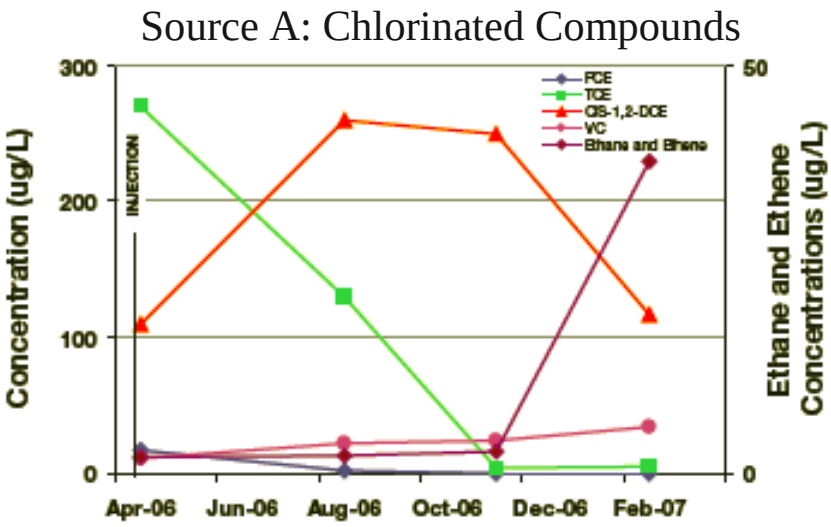

Figure 4: TCE and PCE daughter product concentrations at Source A

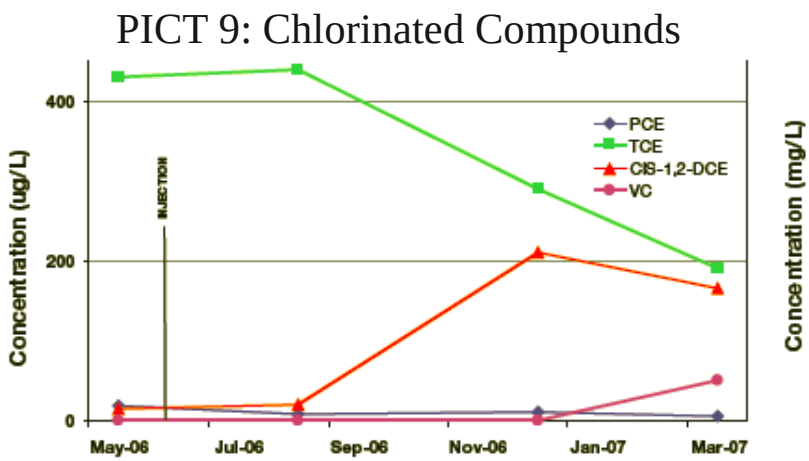

Figure 5: TCE and PCE daughter product concentrations at PICT 9

An experiment was set up to estimate the amount of time for carbon levels to decrease by 90% in aquifer sediment after injection with an emulsified soybean oil. Two groundwater sediment samples were taken from a sight contaminated with Methyl Ethyl Ketone (MEK), mapped in Figure 6. MEK is an organic carbon compound commonly used in industrial applications (Journal of Chemical Education, 2004). A groundwater sediment sample, labeled I1, was taken from an uncontaminated area at the top of the map, highlighted in green. This sample was used as a background control. Another groundwater sediment sample, MW K18, was taken from the lower right area of the map. The sediment was lightly contaminated with MEK, so TOC levels are expected to be higher than those of the control.

Figure 6: The location of sediment samples relative to each other and their contamination with MEK

The experiment had five concurrent stages: measuring initial potential bio-available organic carbon (PBOC), adding a known amount of carbon into sediment samples via soybean oil, depleting the carbon from these samples with daily injections and extractions of water, measuring the final PBOC, and calculating linear equations to determine the time until 90% carbon is removed. Water was injected and extracted daily to simulate natural field conditions.

PBOC is the amount of carbon available for bacterial consumption. As bacteria can choose not to utilize available carbon, PBOC provides an adequate standard for biodegradation capability, but not a certainty. Both background and final PBOC calculations followed a special procedure, as all of the carbon must be extracted from void spaces in the sediment pores (Rectanus, 2006).

Three sediment types were developed for this experiment, a control, active, and inactive. Active and inactive refer to the status of micro-organisms at the beginning before oil injection, and both were from the MW K18 area. The inactive sediment was dried in an oven at 100^0 C for 24 hours and sieved through 2 mm holes to destroy micro-organism populations. Neither the active nor the control sediments were dried. The control sediment came from the uncontaminated I1 area.

Sediment samples were stored in burettes and injected with a known volume and concentration of emulsified soybean oil. All water used for these experiments was 18Ω nanopure. On day 9 of the test a soil probe took samples from each burette, and the PBOC concentration measurement procedure was repeated. However, the experiment continued after day 9 without the top layer of sediment. To find the mass of carbon extracted each day, the TOC concentration was measured and multiplied by the sample volume. This was then compared with remaining TOC mass in the sediment to determine the rate of change. These rates estimated when 90% of the total carbon would be removed.

Methods and Materials

PBOC Extraction

In order to model the decrease in organic carbon over time, the original and final PBOC concentrations were needed. This was done through a five phase extraction process. Both the original

and final concentrations were found using the same procedure. 10 grams of sediment were dried at 100 ^{o} C for 24 hours and filtered through a 2 mm sieve. The sediment was mixed in centrifuge tubes with 0.1% sodium pyrophosphate solute and rotated by an end-over-end tumbler for 24 hours. After this rotation, a centrifuge separated the liquid and sediment for 1.5 hours at 3000 rpm. The solute was extracted and stored in a freezer at 4° C.

Fresh sodium pyrophosphate solution was injected and extracted using the above steps 3 times. For the fourth extraction, 5 N sodium hydroixide removed carbon in sediment pore spaces. 0.1% Sodium pyrophosphate was used again for the fifth and final extraction. Each sample went through a total organic carbon (TOC) analysis to find PBOC using a TOC-VCSN (Shimadzu, Santa Clara, California). To find background PBOC concentrations, two groups of triplicates were used for both sediment types and a control group of dry sand, for a total of 18 separate samples. Four groups, one from each of three burettes and a negative of dry sand, in duplicate, for a total of 8 samples, comprised the sediment for final PBOC concentration measurement.

Carbon Depletion

Three burettes, shown in Figure 7, held the sediment samples. Burette 1, left, held the control sediment. Burette 2, middle, held the inactive sediment, while Burette 3, right, held the active sediment.

Figure 7: Carbon Depletion set up

Fluid was injected into and extracted from each burette in a 24 hour cycle. Before sediment addition, the burettes were cleaned with methanol and water. Water was drained through each burette to determine background TOC concentrations. Following this, 130 mL sediment was added, along with the first pure water injection for saturation purposes. 24 hours later, the fluid was extracted and stored. The emulsified vegetable oil underwent a 4 parts water, one part oil dilution and was injected into the active and inactive sediment. After oil, only water was used. No oil was injected into the control sediment for comparison purposes.

The injection process entailed carefully measuring 30 mL of water into clean pipettes. The pipettes slowly released the water as close to the sediment as possible. The major concern during injection was having the fluid forge a channel through the sediment. If channels developed, then TOC removal through extraction would be less efficient, thereby skewing the results. During extraction, stopcocks at the base of each burette released fluid into centrifuge tubes. Once the burettes were drained, the centrifuge tubes were labeled and stored in a walk-in refrigerator at 4° C.

Following injection 6 on Day 9, a soil probe sampled the top sediment from each burette for another PBOC analysis. The fluid collected from each extraction was placed into a centrifuge tube and rotated for 20 minutes at 3000 rpm to separate any sediment from the fluid. The fluid volume was measured and prepared for TOC measurement.

Results and Discussion

PBOC Analysis

Figures 7 and 8 show the background and day 9 PBOC, respectively. The sum of these extractions multiplied by the mass of sediment provides the total PBOC, presented in Table 1. The active and inactive samples are of the same sediment, so one test was performed for both. As the MW K18 sediment was lightly contaminated with MEK, it was expected to have a higher PBOC concentration than the control. PBOC concentrations increased dramatically with oil addition, while the control concentration decreased as TOC was removed. Both of these results were expected and compared with TOC removal after nine days.

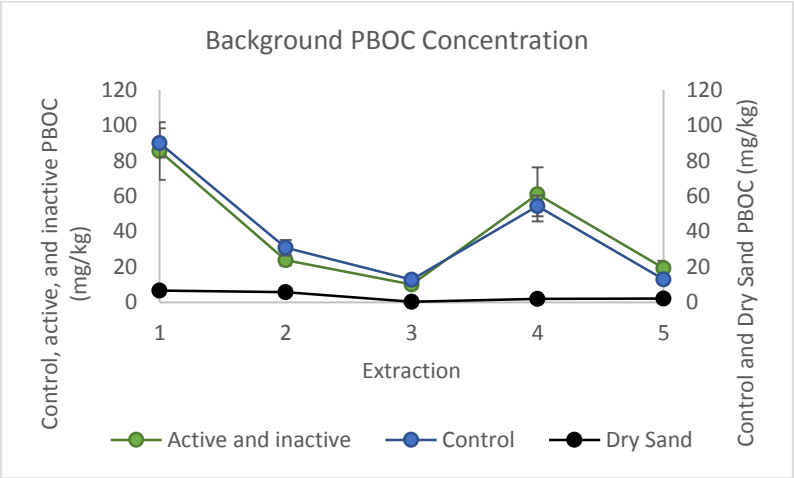

Figure 8: Background PBOC concentrations per extraction

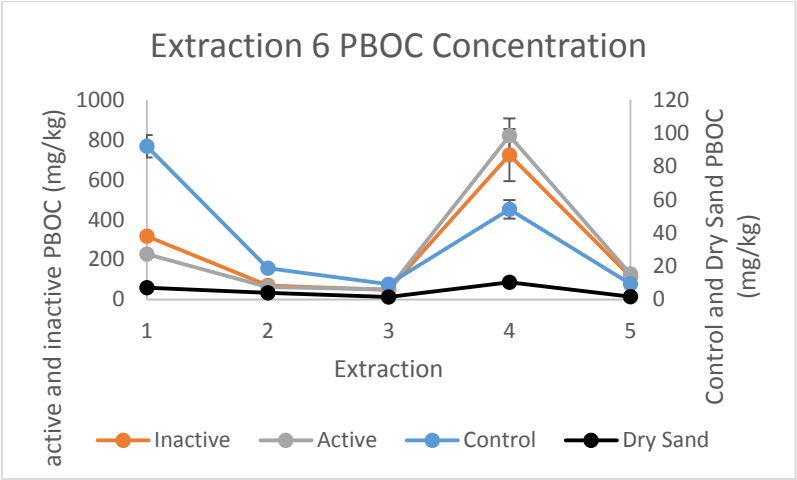

Figure 9: Day 9 PBOC concentrations per extraction

Table 1: Total PBOC in each sediment sample

Sediment	Mass of PBOC (mg)		Concentrations of PBOC (mg/kg)	
	Total	Day 9	Background	Day 9
Control	28.70	26.21	201.25	183.78
Inactive	32.65	208.51	199.70	1275.32
Active	31.43	203.07	199.70	1290.14

TOC Analysis

Figure 10 shows the TOC remaining in each sediment sample, calculated by the difference of TOC removed from TOC added. From days 1 – 3, it decreased more rapidly. After day 4, it decreased in the inactive sediment more rapidly. However, release was almost horizontal in both sediment samples. TOC removal when no oil was added followed a negative linear trend, although a smaller fraction was depleted with each extraction than the active and inactive sediments. At Day 9, when samples were taken for PBOC measurement, the amount of TOC in the control, inactive, and active sediments were 26.0 mg, 1,124.9 mg, and 1354.7 mg, respectively. The difference between the TOC and PBOC values could be because of an un-uniform distribution of carbon throughout the sample. Only the top layer of sediment was taken for PBOC. If the soybean oil percolated throughout the entire column, then layers would have varied concentrations.

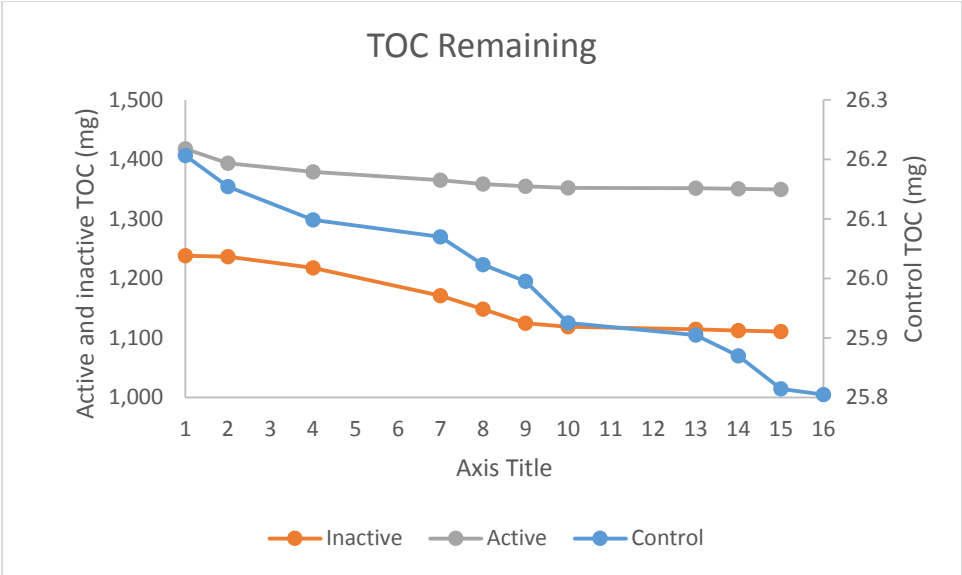

Figure 10: The amount of TOC remaining in each burette

Time to TOC Depletion

Figure 11 shows the cumulative percentage of TOC removed. The majority of TOC extracted from the active sediment during this experiment happened in the first 9 days. Starting on day 10, active and inactive sediment removal rates showed a strong linear relationship. Therefore, the four data points between Days 10 – 15 were used to create linear equations, displayed in Table 2. As the entire control data set showed a strong linear relationship, every point was used to make an equation. The control, inactive, and active sediment TOC removal rates were 0.099% per day, 0.221% per day, and 0.067% per day, respectively. R^2 values indicated the correlation between each sample. The control, inactive, and active R^2 values were 0.978, 0.952, and 0.998 respectively. As these values were close to 1, there was a strong correlation between the estimated equation and data points.

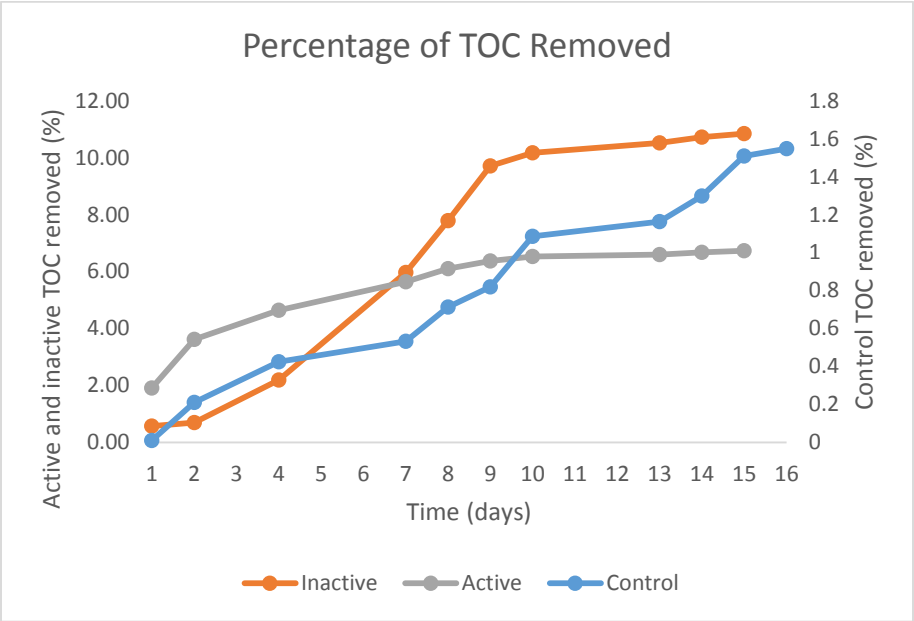

Figure 11: The cumulative percentage of TOC removed

Table 2: the linear relationships between TOC removal and time for each sediment sample

Linear Equations		
Sediment	Equation	R^2
Control	TOC Removal = 0.099(days)-0.0422	0.9782
Inactive	TOC Removal = 0.221(days)+7.3538	0.9523
Active	TOC Removal = 0.067(days)+5.6562	0.9984

The equations calculated from Figure 11 and shown in Table 2 were used to estimate Figure 12. Figure 12 calculates when each sediment sample will reach a 90% TOC depletion. It would take 374 days for inactive sediment, 913 days for the control, and 1256 days until 90% of TOC to be removed from the active sediment.

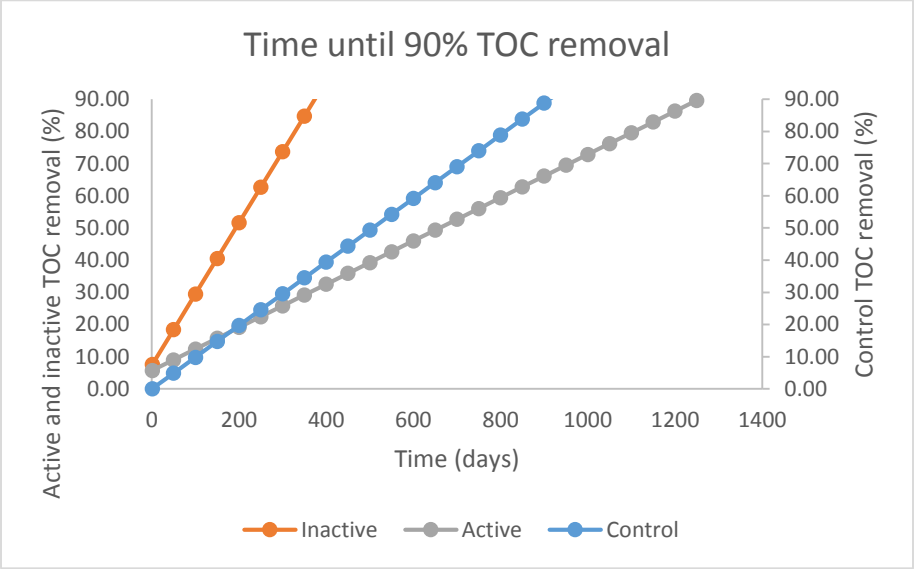

Figure 12: The estimated time until the TOC from each carbon sample is 90% removed

Conclusion

Even though biostimulation with an emulsified vegetable oil is a common way to treat TCE and PCE contamination, very little research has been done to explore the longevity of carbon in groundwater sediment. An experiment was done to estimate TOC removal rates in groundwater sediment samples. An emulsified soybean oil was injected into active and inactive groundwater samples with simulated aquifer conditions. For 15 days after oil injection, the amount of TOC extracted was measured, and the PBOC concentration of the top layer of sediment was analyzed. The carbon depletion data developed a linear relationship, which was used to estimate the time until 90% removal. It was projected to take 374 days for inactive sediment, 913 days for the control, and 1256 days for active sediment to deplete 90% of TOC. As active sediment more closely resembled field conditions than the inactive sediment because its native micro-organism populations remained intact it carbon depletion was estimated to take 3.44 years.

This experiment has helped answer questions about the biostimulation process and will lead to further investigation to help make PCE and TCE treatment more efficient.

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Evaluation of Mineral Content on the Performance of Point-of-Use Filters for Improving Drinking Water Quality

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ABSTRACT

Although point-of-use filters are commonly used by consumers to improve drinking water quality, few data exist concerning impacts of minerals, as measured by hardness and total dissolved solids (TDS), on filter performance for removing metal contaminants. This study examines filter performance in removing manganese and lead from drinking water containing varying mineral levels. Three water types, one with low hardness and TDS, one with high TDS, and one with high hardness, were augmented with 1 mg/L Mn(II) and 0.15 mg/L Pb(II) and two types of filters were used to treat each. Manganese, lead, and other parameters were measured before and after filtration in ten-liter increments as well as intervals between 0 and 200% capacity. Results indicate that filter efficiency decreases as each filter reaches capacity and that there is high variability in performance between filters.

Keywords: Point-of-use filters, manganese, lead, drinking water quality

Introduction

Throughout more than 40% of American households, home water treatment systems are utilized to improve the aesthetic or health qualities of tap water (US-EPA, 2005). From small point-of-use filters employing filter medias such as granulated activated carbon (GAC) and an ion-exchange resin to large reverse-osmosis systems that treat water at the point of its entry, there are multiple filtration options commercially available. This study will focus on point-of-use filters containing GAC and ion-exchange resin. The aims of this study are to examine the impact of mineral content in water, as measured by hardness and total dissolved solids (TDS) on point-of-use filter performance for removing lead and manganese.

Manganese is considered an important nutrient, but overexposure can lead to toxicity. "[Manganese] toxicity has been reported through occupational (e.g. welder) and dietary overexposure and is evidenced primarily in the central nervous system, although lung, cardiac, liver, reproductive and fetal toxicity have been noted" (Crossgrove & Zheng, 2004). The United States Environmental Protection Agency has established secondary drinking water regulations that set forth non-enforceable guidelines for contaminants in water whose primary effects are aesthetic. Manganese, which can cause black-brown discoloration of water, has a secondary standard of 0.05 mg/L (US-EPA, 2002). Despite this secondary standard, "[d]ata collected by the U.S. Geological Survey have shown that 65% of domestic wells tested have manganese concentrations higher than 300 ug/L" (Sharma, 2006). Furthermore, recent studies have linked long-term exposure of manganese in drinking water to intellectual impairment in children (Bouchard, et al., 2011). Figure 1 illustrates the concentrations of manganese in groundwater sampled across the United States.

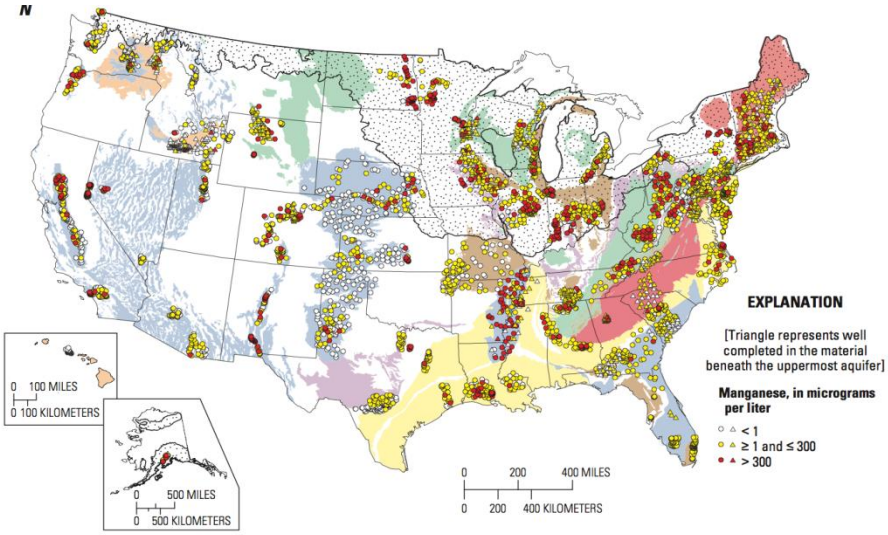

Figure 1: Concentrations of manganese in sampled groundwater (Ayotte 2011). Red symbolsindicate concentrations above 0.3 mg/L, yellow symbols indicate concentrations between 0.001 and 0.3 mg/L, and white symbols indicate concentrations below 0.001 mg/L.

Unlike manganese, the United States Environmental Protection Agency regulates lead as a primary contaminant due to its adverse health effects. The "…EPA estimates that 10 to 20 percent of human exposure to lead may come from lead in drinking water. Infants who consume mostly mixed formula can receive 40 to 60 percent of their exposure to lead from drinking water" (US-EPA, 2012). Lead can inhibit mental and physical development in children and result in kidney damage or high blood pressure in adults (US-EPA, 2012). As set forth by the US EPA's Lead and Copper Rule, "[i]f lead concentrations exceed an action level of 15 ppb or copper concentrations exceed an action level of 1.3 ppm in more than 10% of customer taps sampled, the system must undertake a number of additional actions to control corrosion" (US EPA, 2012). Furthermore, studies have "observed evidence of synergism between lead and manganese, whereby lead toxicity was increased among children with high manganese coexposure" (Henn, et al., 2012). Lead is found in smaller concentrations in groundwater than manganese (see **Figure 2**).

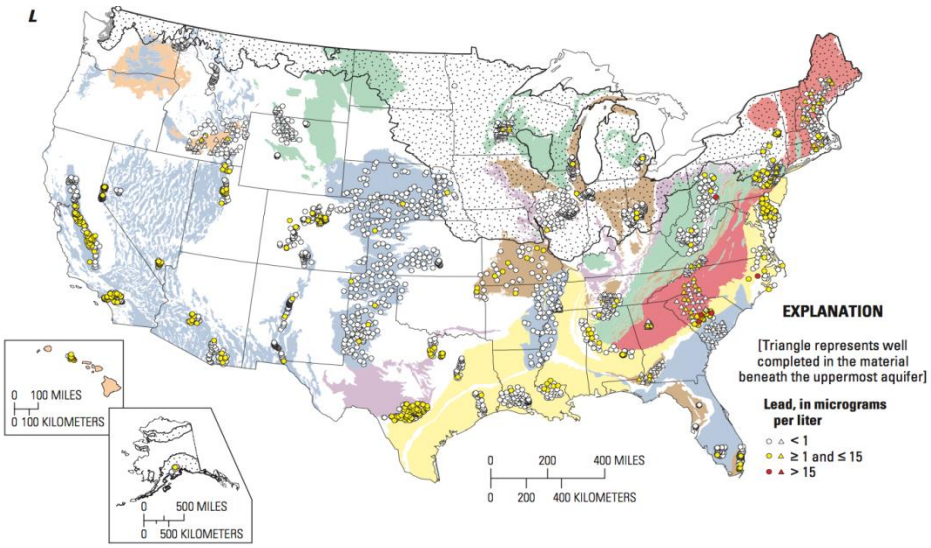

Figure 2: Concentrations of lead in sampled groundwater(Ayotte 2011). Red symbols indicate concentrations above 0.015 mg/L, yellow symbols indicate concentrations between 0.001 and 0.015 mg/L, and white symbols indicate concentrations below 0.001 mg/L.

While the calcium and magnesium in hard water are beneficial to health (Calderone and Hunter, 2009), hardness is a concern in many areas in the United States (Figure 3) as it can cause a buildup of calcium carbonate

on boilers and other equipment in addition to requiring extra soap and detergent for washing. As shown in Figure 4 hard water can leave residue on surfaces such as the plastic ones employed in many filters. Calcium and magnesium are the principle contributors to water hardness, although strontium may contribute slightly (Sawyer, McCarty, & Parkin, 2003). Hard water with large concentrations of calcium has the potential to negatively impact the efficiency of filters containing ion exchange resins. Calcium has a high selectivity coefficient (see Table 1) and will thus be more attracted and more likely to adhere to the ion exchange resin than other cations, including manganese, which has a lower selectivity coefficient. High concentrations of calcium, such as those found in hard water, increase the potential of the ion exchange resin to release cations with lower selectivity coefficients back into the water once the ion exchange resin's capacity has been reached.

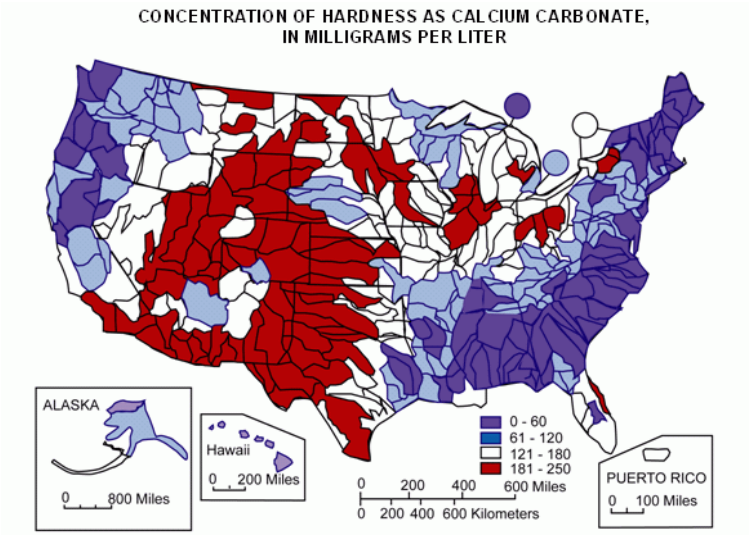

Figure 3: Distribution of hard water in the United States (adapted from Briggs & Ficke, 1997 by the United States Geological Survey).

Figure 4: Residue left by hard water in the pitcher reservoir (A. Nasser 2013)

Ion	**Selectivity Coefficient**
Li^+	1.0

H^+	1.3
Na^+	2.0
K	2.9
Mg^{2+}	3.3
Cu^{2+}	3.8
Mn_2^+	4.1
Pb^{2+}	5.0
Ca^{2+}	5.2
Sr^{2+}	6.5
Ra^{2+}	13.0

Table 1: Ion selectivity coefficients (Crittenden et al., 2005) for strong acid cation (SAC) and strong-base cation (SBC) resins. Ions with a higher selectivity coefficient are more attracted and more likely to adhere to the ion exchange resin in the filters.

Additionally, the level of total dissolved solids (TDS) in water is another measure of water quality and has the potential to impact filter efficiency by rapidly depleting the filter media's ability to remove contaminants from the water. Water high in TDS may contain high levels of potassium, sodium, calcium, magnesium, and carbonates, as well as other compounds. Bottled and tap water often contain clinically important amounts of calcium and magnesium that can contribute to dietary intake (Azoulay, Garzon, & Eisenberg, 2001), so high TDS waters are not uncommon. TDS levels are regulated as a secondary standard by the EPA at 500 mg/L. Previous studies have not investigated the impact that TDS or hardness levels have on point-of-use filter efficiency at removing lead and manganese.

Research Methods

Filters

All tested filters used granulated activated carbon and an ion exchange resin to remove contaminants from water. These were the only two filter medias used in each filter as confirmed by a representative from each production company. Each filter cartridge has a rated capacity of 150 liters. For each experiment, each filter cartridge was prepared according to the manufacturer's standards, which included soaking the filter in its respective water type for 15 minutes and then filtering water of the same water type for another 15 seconds.

Challenge Water

Table 2: Challenge water types and their characteristics; mean + standard deviation are provided.

Water Type	Total Dissolved Solids (Average) in mg/L	Hardness (Average) in mg/L as $CaCO_3$	pH (Average)
Blacksburg Tap	88.0 ±11.8	43.8 ±8.4	8.08 ±0.19
High Hardness Water	491.0 ±11.1	224.1 ±14.0	8.36 ±0.12
High TDS Water	498.8 ±49.2	39.3 ±4.3	8.57 ±.22

Three water types were used for this experiment: a representative tap water from a surface water source (Blacksburg Tap Water), a high hardness water, and a water with a high level of TDS (see **Table 2: Challenge water types and their characteristics; mean + standard deviation are provided.** for a list of the water characteristics). For each of the three water types, water was prepared in 10-liter batches inside glass jugs. Each batch was augmented with 1.0 mg/L manganese, from a 1000 mg/L Mn stock solution of 50% $MnCl_2$ (Fisher Scientific, NJ, CAS 13446-34-9) and 50% $MnSO_4$ (Spectrum, CA, CAS 10034-96-5), and 0.15 mg/L lead, from a stock solution of 1.5 g/L lead (II) sulfate. Hard water was prepared according to the EPA's recipe for synthetic hard freshwater (US-EPA, 2007) by the addition of 384 mg/L $NaHCO_3$ (Spectrum, CA, CAS 144-55-8), 205.5 mg/L $CaSO_4$ (Spectrum, CA, CAS 10101-41-4), 215.5 mg/L food grade $MgSO_4$ (Spectrum, CA, CAS 10034-99-8), and 16 mg/L KCl (Fisher Scientific, NJ, CAS 7447-40-7). Water with a high level of TDS was prepared with 384 mg/L $NaHCO_3$

(Spectrum, CA, CAS 144-55-8), 309.38 mg/L $Na2SO_4$ (Fisher Scientific, NJ, CAS 7757-82-6), 42.17 mg/L K_2SO_4 (Fisher Scientific, NJ, CAS 7778-80-5), and 16 mg/L KCl (Fisher Scientific, NJ, CAS 7447-40-7). The temperature of the water was measured to ensure that it was within the required parameter of 20 +/- 2.5° C as set forth by the National Sanitation Foundation/American National Standards Institute (NSF/ANSI) Standard 53 for the health effects of drinking water treatment units as an acceptable test water temperature (NSF/ANSI, 2012).

Experimental Procedure

A volume of 500 mL of water was collected from each of the 10-liter batches of the challenge water and set aside for water quality testing. The remaining 9.5 liters of water were poured through each filter by hand. Prior to filtration, the 500 mL of challenge water was tested for pH, temperature, TDS, and conductivity measurements of mS/cm, ms/cm[a], and MQ*cm using a HANNA Multiparameter Meter HI 9829. Results were recorded in an excel spreadsheet. 30 mL of the pre-filtered water were collected from each of the 500 mL samples and stored in a HDPE acid-washed bottle and acidified to 2% HNO_3. At 0, 25, 50, 75, 100, 120, 150, 180, and 200% of capacity, additional samples of 125 mL were collected for more extensive tests, including alkalinity as well as analysis by ICP-MS for a more comprehensive measure of the metals present in each sample including Ag, Al, As, Ca, Ce, Cd, Cl, Co, Cr, Cu, Fe, K, Mg, Mo, Na, Ni, P, Pb, S, Si, Sn, V, and Zn. After each water type had been sampled and tested, the pitcher reservoirs were emptied and rinsed, and the unfiltered 500 mL sample of water was poured through each filter to provide the post-filtration samples. The filtered water was then collected and tested for the aforementioned parameters. This procedure was repeated for every batch of 10 liters prepared and at each percentage benchmark. At the end of each day of testing, 50 mL of the pre-filtered water sample taken at percentage benchmarks were titrated to determine alkalinity according to Method 2310 B in Standard Methods for the Examination of Water and Wastewater (American Public Health Association, American Water Works Association, and the Water Environment Federation, 1997). The remainder of the percentage benchmark samples as well as each 30-mL sample underwent acid digestion via the addition of trace metal grade HNO_3 (Fisher Scientific, NJ, CAS 7697-37-2), with a resultant concentration of 2% nitric acid.

Experiment 1

Three Filter A cartridges were prepared as described in the filters section. Each filter was assigned one of the three challenge water types that were prepared as mentioned in the challenge water section. Initially, experimental procedure was followed as above. However, due to slow filtration rate, a peristaltic pump (Cole Parmer) was used and the filters were run continuously over a period of seven weeks with sampling of water pre- and post-filtration as described in the experimental procedure.

Experiment 2

Three Filter B cartridges were prepared as described in the filters section. Only the representative tap water from the surface water source as mentioned above was used in this experiment to produce results in triplicate. The filters were suspended over a sink (see Figure 3) and the experiment followed the procedures as described in the experimental procedure section. Following the filtration of the 200th liter, an additional liter of Blacksburg Tap water that had not been augmented with manganese or lead was filtered, with samples collected before and after filtration.

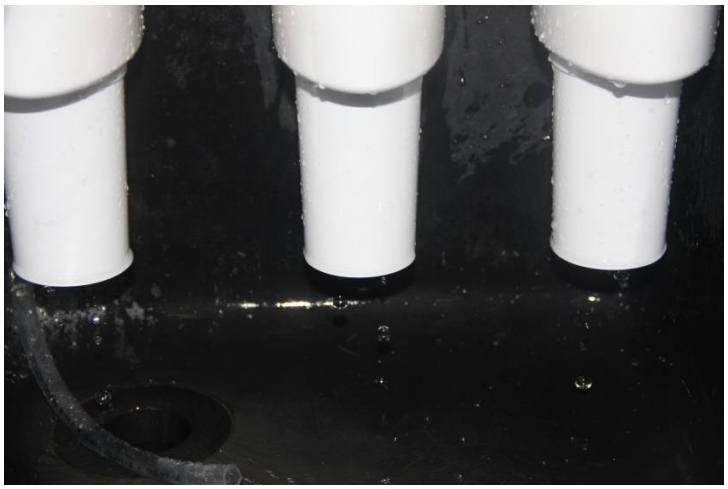

Figure 3: Filters suspended over the sink (A. Nasser 2013). Samples of the effluent were collected every 10 liters and at the percentage benchmarks .

Experiment 3

Three Filter B cartridges were prepared as described in the filters section. Only the high hardness challenge water was used in this experiment to produce results in triplicate. The filters were suspended over a sink and the experiment followed the procedures as described in the experimental procedures. Following the filtration of the 200th liter, an additional liter of the high hardness challenge water that had not been augmented with manganese or lead was filtered, with samples collected before and after. The same procedure was followed with a liter of Blacksburg Tap water and a liter of Nanopure water.

Results

The results of the study for Filter A are still in progress due to its slow and variable filtration rates. This did not allow for filtration to 200% capacity as intended. The full course of testing and sampling was undergone only with Filter B in Blacksburg Tap and hard water.

Manganese

Manganese removal was observed in all water and filter types. During Experiment 2 in Blacksburg Tap Water (see Figure 4), all three filters maintained a certain level of manganese removal. Manganese removal began at an average of 76.1% during the first 10 liters. The filters then peaked between 0 and 20 liters at an average of 80.7 ±5.2% removal of manganese. During the last 10 liters, the filters ended with an average of 59.1 ±1.5% removal of manganese. The lowest level of removal occurred at different points for each filter. The lowest levels of removal were 49.7, 58.1, and 51.3% at 170, 100, and 190 liters respectively.

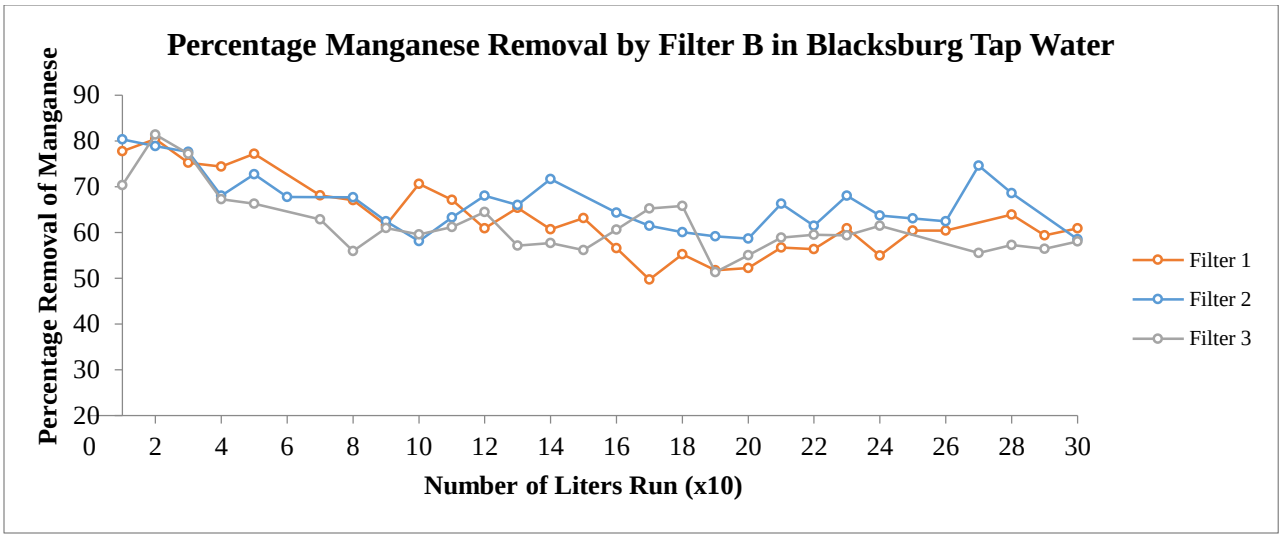

Figure 4: Graphical representation of the percentage of manganese removed by Filter B in Blacksburg Tap water.

With the utilization of hard water as the challenge water in Experiment 3 there was a change in the percentage of manganese removed (see Figure 5). Manganese removal began at an average of 72.8 ±9.3% during the first 10 liters. Filter 1 and Filter 2 then peaked within the first 20 liters, removing 72.5 and 83.0% of the manganese present in the challenge water. Filter 3 removed its highest percentage of manganese, 71.9%, between 240 and 250 liters. During the final10 liters, the filters ended with an average of 67.7 ±2.1% removal of manganese. The lowest level of removal occurred at different points for each filter. The lowest levels of removal were 55.8, 63.9, and 53.9% at 190, 170, and 90 liters respectively.

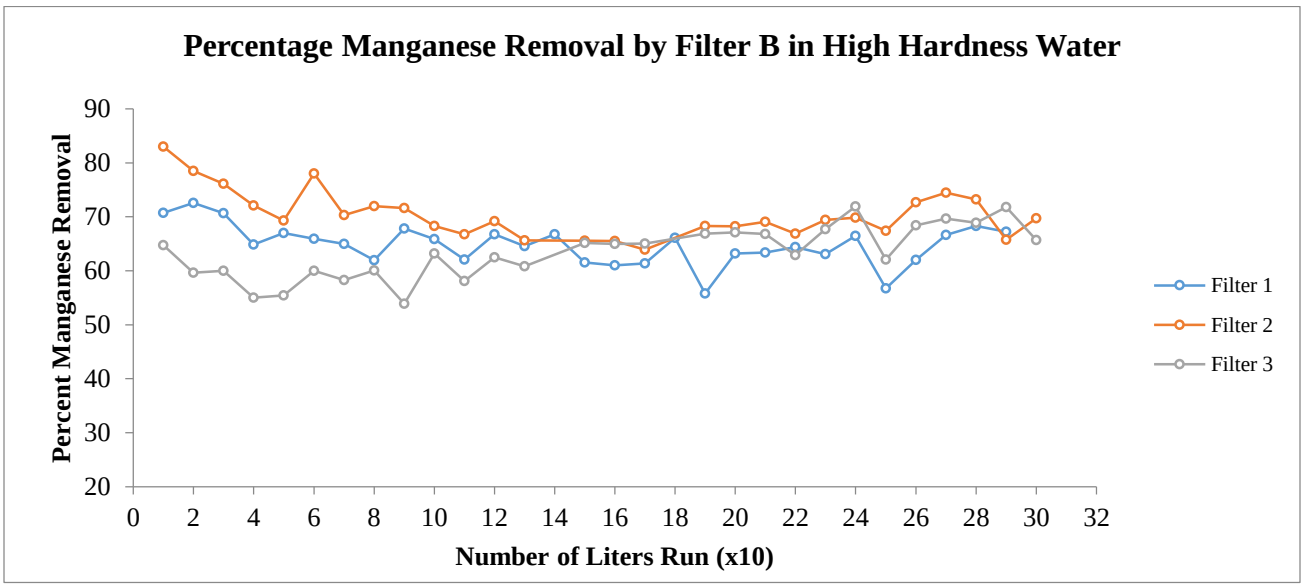

Figure 5: Graphical representation of the percentage of manganese removed by Filter B in hard water.

Manganese removal in Filter A from Experiment 1 exhibited a different pattern between water types (see Figure 6). Due to slower filtration by Filter A, not all data has been collected. Filter A consistently removed over 97.5% of the manganese in the presence of both Blacksburg Tap Water and high TDS water. In the presence of high TDS water, filter efficiency increased from its starting manganese removal percentage of 97.5 to a high of 99.7% manganese removal at 100% of capacity. With high hardness challenge water, filter efficiency began at 96% manganese removal before falling to 69.1% manganese removal at 120% of capacity and 77.3% manganese removal at 150% capacity.

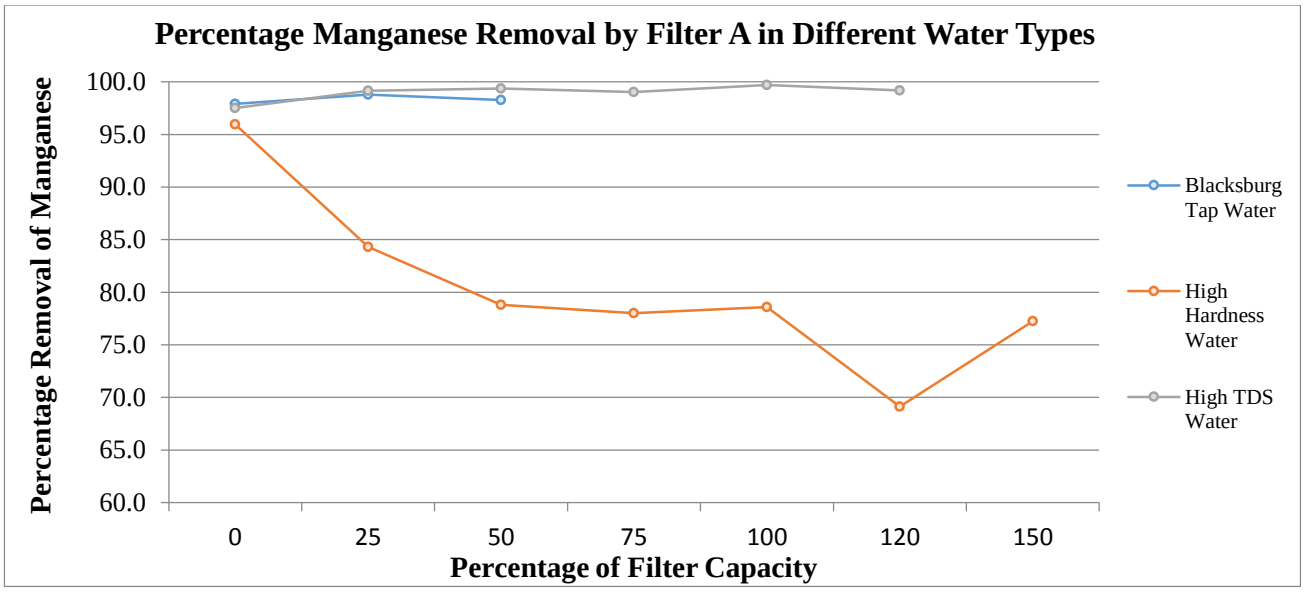

Figure 6: Graphical representation of the percentage of manganese removed by Filter A in three different water types versus the percentage of filter capacity.

Lead

Lead removal was observed across all water and filter types. During Experiment 2 in Blacksburg Tap Water (see Figure 8), all three filters maintained a particular level of lead removal. Lead removal began at an average of 41.4 ±9.0% and declined to end at 200% capacity with an average of 39.8 ±7.5% lead removal. The highest level of lead removal occurred at 75% of capacity for Filters 1 and 3 with 97.6 and 89.2 percent removal of lead respectively. Following this peak, lead removal declined for both filters with the exception of an increase over the last 20% of capacity, with Filter 1 increasing from 16.5% removal to 33.6% removal, and Filter 3 increasing from 25 to 48.1% removal of lead. Filter 2's percentage of lead removal increased between 120 and 150% capacity to 71.6% lead removal, but declined thereafter. Filter 2 is missing data points due to error for 75 and 100% of capacity.

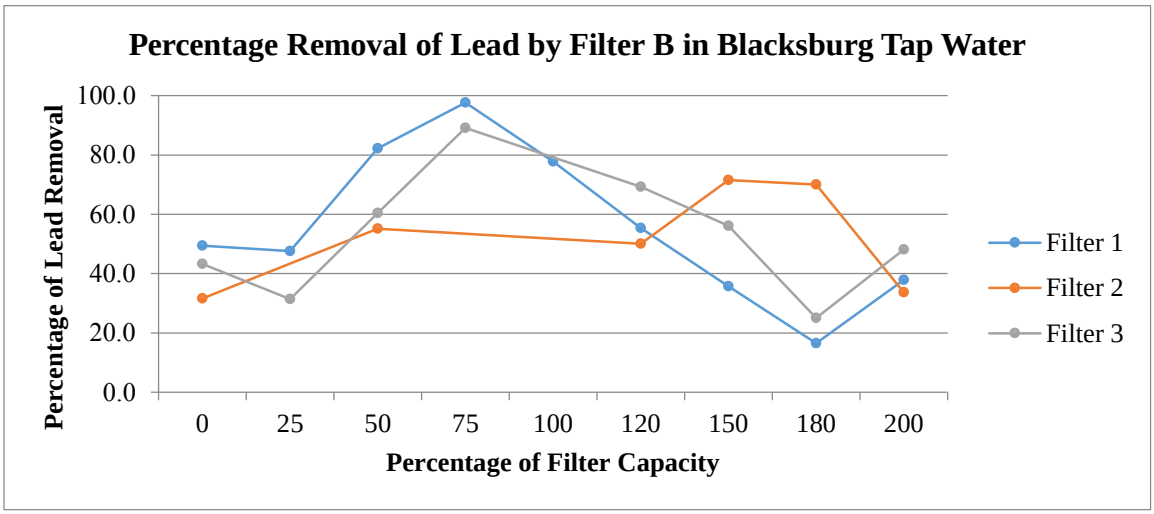

Figure 7: Graphical Representation of the percentage of lead removal by Filter B in the presence of Blacksburg Tap water versus the percentage of filter capacity.

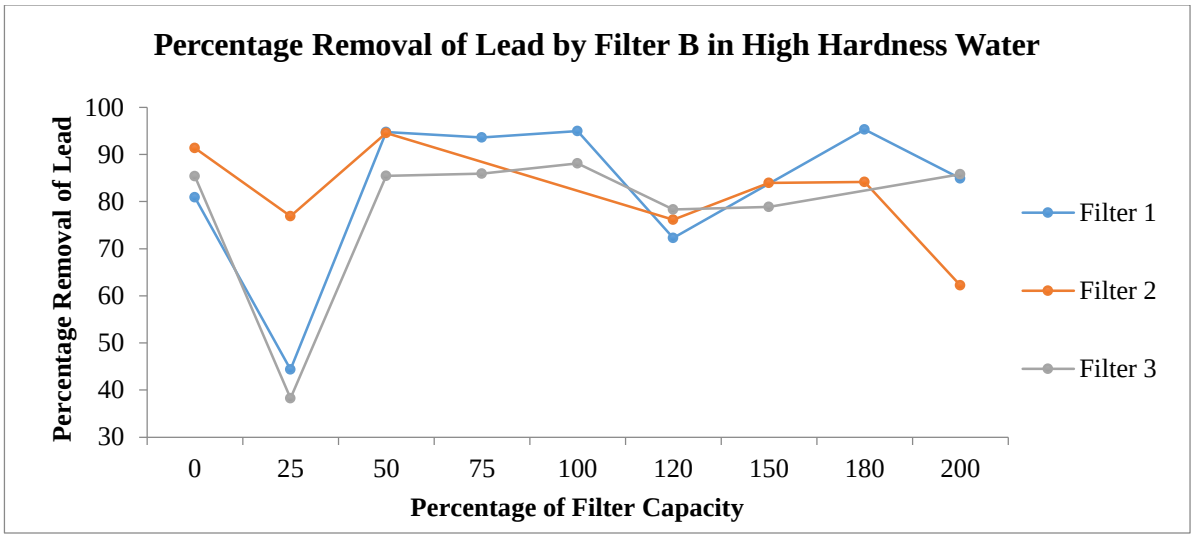

Figure 8: Graphical Representation of the percentage of lead removal by Filter B in the presence of the high hardness challenge water versus the percentage of filter capacity.

During Experiment 3, in which high hardness water was utilized, changes were observed in the percentage of lead removed (see Figure 8). Lead removal began between 80 and 92% for all three filters but declined sharply at 25% capacity for all three filters. Filter 1 dropped to 44.3% lead removal, Filter 2 dropped to 76.9% lead removal, and Filter 3 dropped to 38.3% of lead removal. Following the 25% capacity mark, lead removal increased to in between 85.5 and 94.6% removal. Due to error, the challenge water was not augmented with lead for several trials and thus the data for Filter 1 at 150% capacity, for Filter 2 at 75 and 100% of capacity, and for Filter 3 at 180% of capacity has been excluded.

Lead removal in Filter A from Experiment 1 was significantly different between water types (see Figure 10). Due to slower filtration by Filter A, not all data has been collected. At 0% capacity, Filter A removed 95.7% of lead in Blacksburg Tap water, 89.9% of lead in the high hardness challenge water, and 87.8% of lead in the high TDS challenge water. These values decreased and, between 25 and 50% of capacity, the percentage of lead removal was at its lowest. Following these initial decreases in lead removal, however, the filters in the high TDS and high hardness challenge waters increased the amount of lead removed. At 100% capacity, the filter in the high hardness challenge water removed 97.0% of lead and the filter in the high TDS challenge water removed 91.5% of lead.

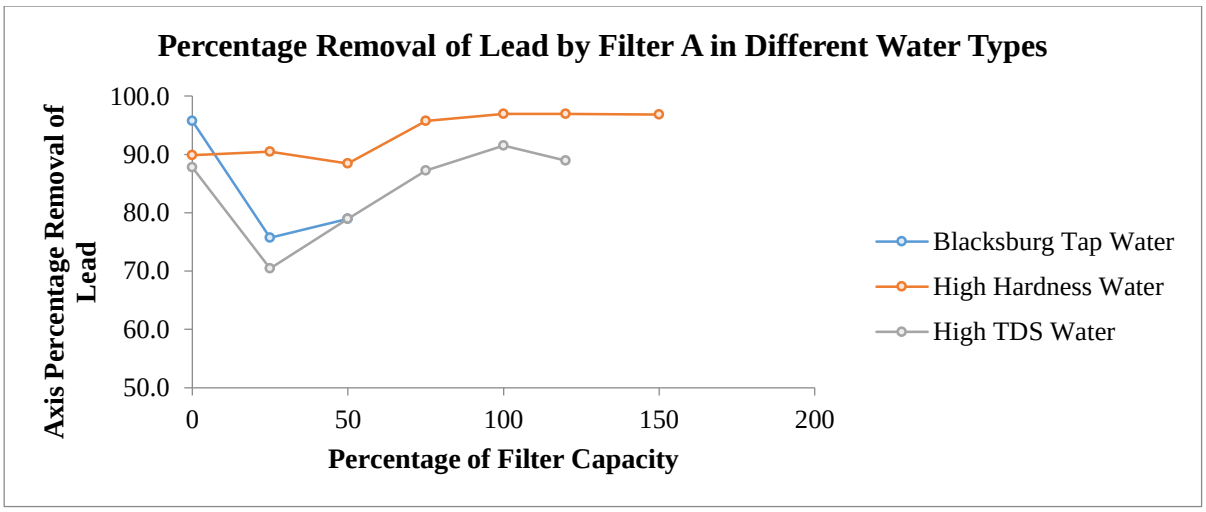

Figure 9: Graphical Representation of the percentage of lead removal by Filter A in the presence different challenge waters versus the percentage of filter capacity.

Calcium & Magnesium

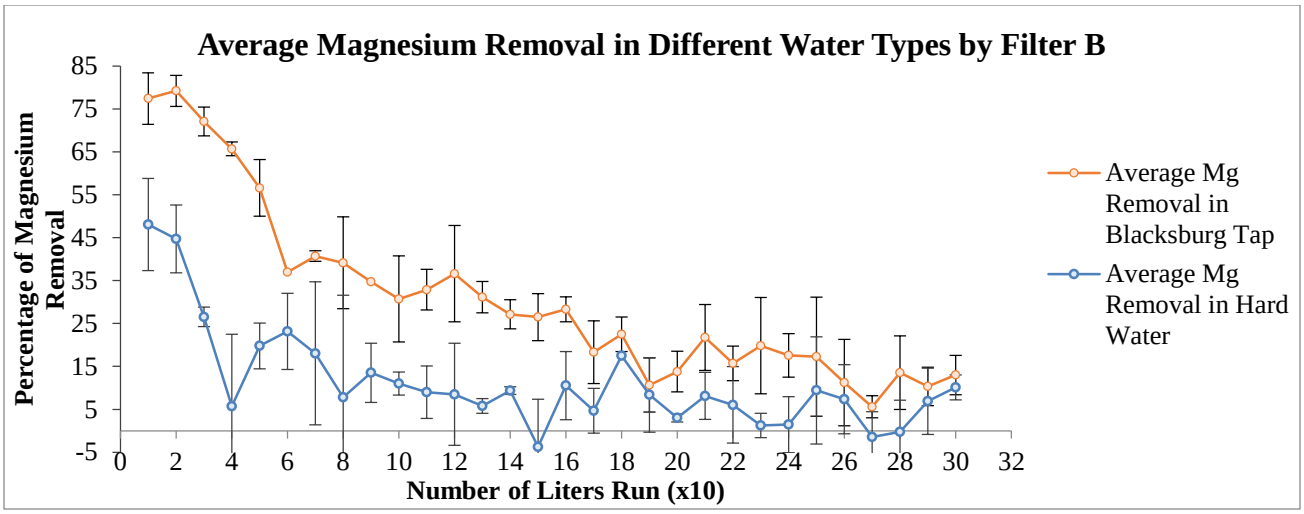

Figure 10: Graphical Representation of the average magnesium removal in two different water types by Filter B. Error bars represent standard deviation.

Magnesium removal decreased the longer the filters remained in use (see Figure 10). In Experiment 2 with Blacksburg tap water, Filter B removed an average of 77.4 ±6.0% of the magnesium present in the challenge water during the first 10 liters and 79.2 ±3.6% of the magnesium present in the challenge water in between 10 and 20 liters. Between 20 and 300 liters, the percentage of magnesium removal declined, reaching an average 5.6 ±2.6% removal of magnesium at the 270 liter point. At the end of 300 liters, Filter B was removing an average of 13.0 ±4.6% of the magnesium. In Experiment 3 with the high hardness challenge water, Filter B removed an average of 48.1 ±10.7% of magnesium present in the challenge water in the first 10 liters. Following the first 10 liters, the percentage of magnesium removal declined rapidly, reaching its lowest average of -3.7 ±11.1% at 150 liters, during which effluent concentration of magnesium was higher than the magnesium concentration of the challenge water.

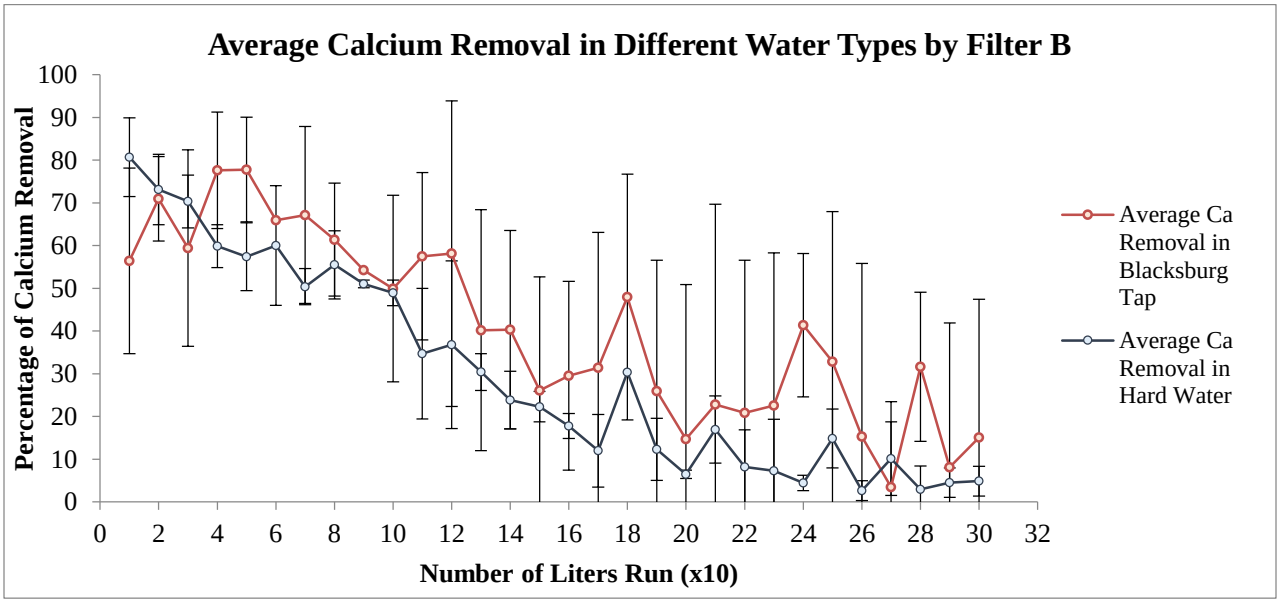

Figure 11: Graphical Representation of the average magnesium removal in two different water types by Filter B.

Calcium removal decreased as the number of liters increased for both water types. In Experiment 2, calcium removal began at an average of 56.4 ±21.7% in Blacksburg Tap water in the first ten liters. At 50 liters, calcium removal in Blacksburg Tap water peaked at 77.8 ±12.2%. The lowest level of calcium removal, 3.4 ±20.0%, occurred at 270 liters. At 200% capacity, the filters removed an average of 15.1 ±32.3% of calcium present in the challenge water. In Experiment 3, calcium removal declined in the presence of hard water. In the

first ten liters, the filters removed 80.7 ±9.2% of calcium present in the challenge water. Calcium removal never increased above this initial removal level, but declined to an average removal of 2.6 ±2.3% of calcium. After the filtration of 300 liters, the filters removed an average of 4.8 ±3.5% of calcium present in the high hardness challenge water.

Total Dissolved Solids

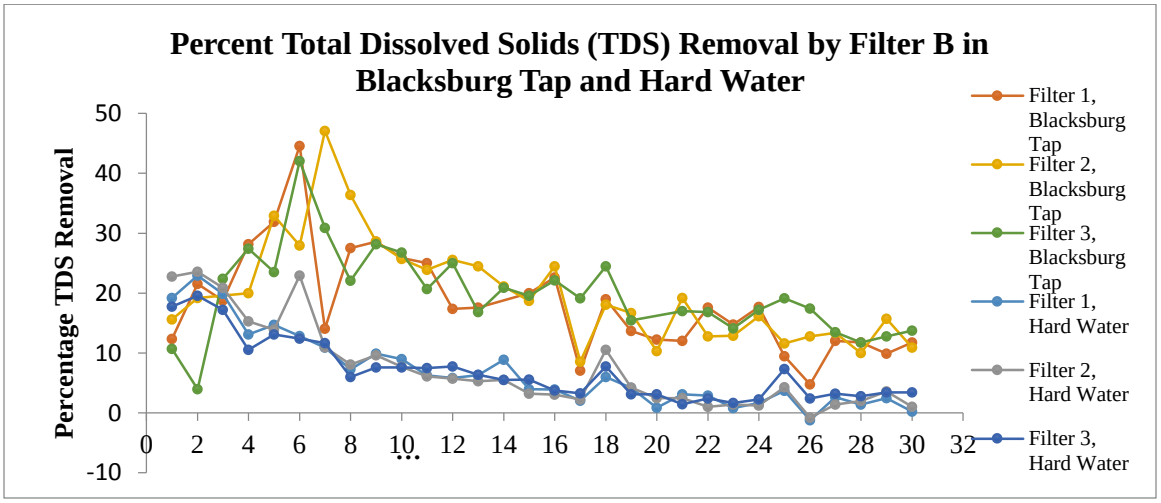

Figure 12: Graphical Representation of the percentage of TDS removal by Filter B in the high hardness and Blacksburg Tap challenge waters.

Percent removal of total dissolved solids (TDS) varied between water types but remained fairly consistent between each set of triplicate filters. With Blacksburg Tap as the challenge water, percent TDS removal started in between 10 and 15% removal and escalated to remove an average of 44.5 ±2.5% of TDS between 60 and 70 liters. Following this peak, TDS removal in Blacksburg Tap water decreased as the number of liters run increased, ending at an average of 12.1 ±1.5% removal of TDS. The percent total dissolved solids removed by Filter B with the high hardness water as the challenge water followed a different trend. Filter B removed a higher percentage of TDS initially—an average of 19.9 ±2.6% removal in the first 10 liters—but declined as the number of liters run through the filter increased. Between 260 and 270 liters, Filters 1 and 2 deposited TDS into the water, making the effluent concentrations higher than the challenge water concentration of TDS.

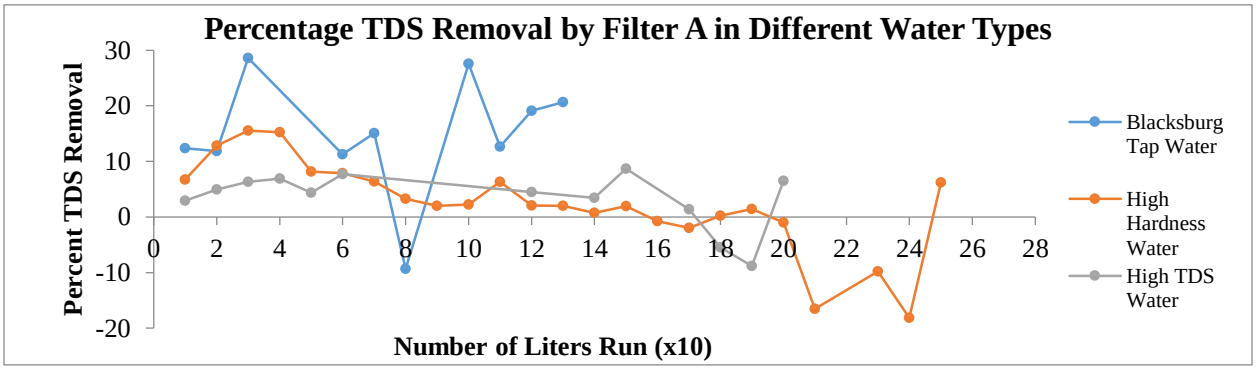

Figure 13: Graphical Representation of the percentage of TDS removal by Filter A in three different water types.

Percent removal of total dissolved solids (TDS) by Filter A varied widely between water types. In the presence of Blacksburg Tap water, Filter A initially removed 12.4% of TDS present in the challenge water before increasing to 28.6% removal at 30 liters and then falling to -9.3% removal at 80 liters (indicating a higher concentration of TDS in the effluent). Following 80 liters, percent TDS removal increased to 20.7% at 130 liters. With the high hardness challenge water, Filter A removed 6.7% of TDS initially before declining steadily as the number of liters run increased. At 170 liters, Filter A deposited an additional 2.0% of TDS into the effluent. Following 170 liters, percent TDS removal never exceeded 1.4% and reached a low of -18.1%. For the high TDS

challenge water, Filter A performed similarly as with the high hardness water. Initially, Filter A removed 2.9% of TDS present in the challenge water. Following the filtration of the 170th liter, percent TDS removal decreased to -5.5% and subsequently to -8.8% in the next 20 liters. Following the filtration of 200 liters, percent TDS removal increased to 6.5%.

Post 200 Liter Tests

For the liters of water run following the 200% capacity point Experiments 2 and 3, results were obtained on the ICP-MS (see Table 3).

Initial Water Type & Filter Number	Challenge Water Type (no Pb or Mn added)	Challenge Water Pb Concentration (ppb)	Effluent Pb Concentration (ppb)	Challenge Water Mn Concentration (ppb)	Effluent Mn Concentration (ppb)
Blacksburg Tap, Filter 1	Blacksburg Tap Water	13.9	1.9	2.5	275.8
Blacksburg Tap, Filter 2	Blacksburg Tap Water	1.4	20.8	2	272.4
Blacksburg Tap, Filter 3	Blacksburg Tap Water	1.4	29.7	2.3	287.5
High Hardness Water, Filter 1	High Hardness Water	5.9	6.2	9.2	224.5
High Hardness Water, Filter 1	Blacksburg Tap Water	1	4.5	2	33.7
High Hardness Water, Filter 1	Nanopure Water	.1	15.6	.5	24.2
High Hardness Water, Filter 2	High Hardness Water	5.9	10.8	9.2	228.6
High Hardness Water, Filter 2	Blacksburg Tap Water	1	20.5	2	44.6
High Hardness Water, Filter 2	Nanopure Water	.1	36.8	.5	34.8
High Hardness Water, Filter 3	High Hardness Water	5.9	6	9.2	253.2
High Hardness Water, Filter 3	Blacksburg Tap Water	1	6	2	35.8
High Hardness Water, Filter 3	Nanopure Water	.1	13.8	.5	36.3

Table 3: Results from the post 200-liter tests. During these tests, the challenge water was not augmented with lead or manganese, but the water was measured before and after to determine if any manganese or lead had been added by the filter.

All effluent concentrations were higher than those present in the challenge water. All filters added some amount of lead and manganese into the effluent. When Nanopure water was run through each filter that had been tested with high hardness water, it produced the highest concentrations of lead in the effluent with 15.6, 36.8, and 13.8 ppb respectively. Concentrations of manganese present in the effluent were highest when the high hardness water was used with the filters. The effluent had concentrations of manganese measuring 224.5, 228.6, and 253.2 ppb respectively. Following the use of high hardness water, the cartridges were used to filter Blacksburg Tap and Nanopure water. Manganese concentrations in the effluent for both of these waters was much lower—33.7, 44.6, and 35.8 ppb for Blacksburg Tap and 24.2, 34.8, and 36.3 ppb for Nanopure Water.

Discussion and Recommendations

While further data collection is needed to obtain a better insight into the ways in which mineral content impacts the efficiency of point-of-use filters at removing contaminants of water, the data obtained through this study suggests that a filter's ability to remove contaminants is impacted by mineral content in water. It also indicates that filter performance is variable between and within filter types. Based on the limited results from Filter A and the results from Filter B, it can be seen that each filter performs differently. Manganese removal by Filter B in each of the challenge waters did not decline as markedly as the percentage removal of calcium and magnesium. Despite calcium's lower selectivity coefficient, it was filtered less readily throughout the duration of the tests while manganese removal never dropped below 49%.

The data obtained in this study also indicate the potential for filters to deposit previously filtered contaminants back into the drinking water. Both Filter A and Filter B produced effluents with higher concentrations of magnesium or TDS at a point past their rated capacity. Although neither filter deposited lead or manganese into the filtered water during the course of testing within the first 200 liters, it can be seen by the results in Table 3 that, in the presence of water not augmented with lead or manganese, the filters deposit some level of manganese and lead back into the effluent. This presents a risk for turning acute exposure into chronic exposure. Temporary increases in lead or manganese concentrations may lead to accumulation of each metal on the filter media. Following the temporary increase, and with the filter's continued use, there remains the potential (as indicated in the post-200 liter tests) for metal concentrations to be higher in the effluent than they are in the challenge water.

Results for lead removal present concerns based on the NSF/ANSI Standard 53. Both filter types were certified according to the standard for lead removal. Although the high hardness challenge water did not fit within the NSF/ANSI Standard 53's requirements for challenge water, Blacksburg Tap water fit within the parameters. With the addition of 0.15 mg/L lead, the filters have been certified to reduce the lead concentration to a maximum of 0.015 mg/L lead, or 90% removal. However, the filters only performed at this level during several of the sampled points that had also been set forth by the NSF/ANSI Standard 53.

Furthermore, it is unclear how the rated capacity of each filter is determined. There did not appear to be a marked decline following 100% capacity (150 liters) in any of the performed tests. In fact, Filter A's ability to remove lead from the challenge water increased as 100% capacity was reached.

Recommendations for future research include performing tests on Filter A with each water type, running these tests in triplicate, and testing Filter B with the third challenge water (high TDS water). Future tests may also include running the filters past 200% capacity to determine the point at which the effluent concentrations are the same as the challenge water concentrations.

Acknowledgements

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Examining the Biological Effects Of 4-Nonylphenol on Freshwater Mussels (Medionidus *conradicus*)

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ABSTRACT

Nonylphenol ethoxylates are anthropogenically-produced substances utilized as stabilizers in plastics, as surfactants in detergents, agricultural sprays, and personal care products, and as spermicide in contraceptives (Jennifer Diehl et. al, 2012). Nonylphenol ethoxylates (NPEs) are generally discharged in large quantities into aquatic environments either directly from untreated effluents or indirectly from sewage treatment plants (Riccardo et. al, 2008). The major transformation product of NPEs is 4-nonylphenol (4-NP), which is an endocrine disruptor in many organisms. Water and sediment samples taken upstream and downstream of the Tazewell Wastewater Treatment Plant and the Richlands Regional Wastewater Treatment Facility (both located in Tazewell County, Virginia) along the Clinch River were analyzed for 4-NP using a GC/MS/MS. 4-NP was detected in the water and sediment samples at a maximum of 0.2µg/L and 160µg/kg (dry weight basis) respectively. The effects of 4-NP on one freshwater mussel species inhabiting the Clinch River, Medionidus Conradicus (Cumberland moccasinshell) were also investigated. After acclimating the mussels for a week in natural pond water, they were exposed to 4-NP for two weeks at a concentration of 0.25µg/L. To achieve continuous 4-NP exposure at this concentration, 4-NP was spiked into an algal solution used to feed the mussels, and this solution was slowly released into 18.2 L of natural pond water within one hour. The mussels were fed and the water was replaced daily. Water samples were collected during the feeding period and were analyzed for 4-NP. After two weeks of exposure, the mussel tissues were analyzed to estimate 4-NP uptake and accumulation. The activities of glutathione-S-transferase (GST; a detoxification enzyme) and Na+/K+-ATPases in the tissues were also analyzed. The effect of 4-NP exposure on the activities in the mussels will be discussed.

Keywords: 4-Nonylphenol; Medionidus *conradicus*; Clinch River and Gas Chromatography Tandem mass spectrometer (GC/MS/MS)

Introduction

Nonylphenol Ethoxylate and 4-Nonylphenol

Nonylphenol ethoxylates are anthropogenically-produced substances utilized as stabilizers in plastics, as surfactants in detergents, agricultural sprays, and personal care products, and as spermicide in contraceptives (Jennifer Diehl et. al, 2012).It is a xenobiotic compound classified as an endocrine disruptor capable of interfering with the hormone system of numerous organisms (Soares et. al, 2008). The fate of nonylphenol in different environmental compartments (surface water, sediment, ground water, soil and air) is controlled predominantly by its physical-chemical properties and those in turn influence its degradation (Soares et. al, 2008). Among nonylphenols, 4-nonylphenol has been identified as the most critical metabolite because of its high resistance to biodegradation, toxicity and strong estrogenic activities (Riva et al, 2010). 4-Nonylphenol (4-NP) originates principally from the

degradation of nonylphenol ethoxylates (Soares et. al, 2008). It occurs frequently as a stable intermediate in effluents and sewage sludge with a higher incidence in those works treating wastewaters of an industrial character or with high population densities indicative of urban areas (Soares et al, 2008). Due to its physical-chemical characteristics, such as low solubility and high hydrophobicity, nonylphenol accumulate sin environmental compartments that are characterized by high organic content, typically sewage, sludge and river sediments where it persists (Soares et. al, 2008). Since regulation began, average levels in surface waters tend to diminish below 1μg/L in most receiving waters(Riva et. al, 2008).Yet several hundreds of μg/L have been recorded close to pollution sources when treatment plants are inefficient or absent (Riva et. al, 2008). The impacts of nonylphenol in the environment include feminization of aquatic organisms, decrease in male fertility and survival of juveniles at concentrations as low as 8.2μg/L (Soares et. al, 2008). In a report released by the EPA in 2010, fresh water quality criteria for nonylphenol are 28μg/L for acute and 6.6μg/L for chronic exposure (U.S. Environmental Protection Agency, 2012).

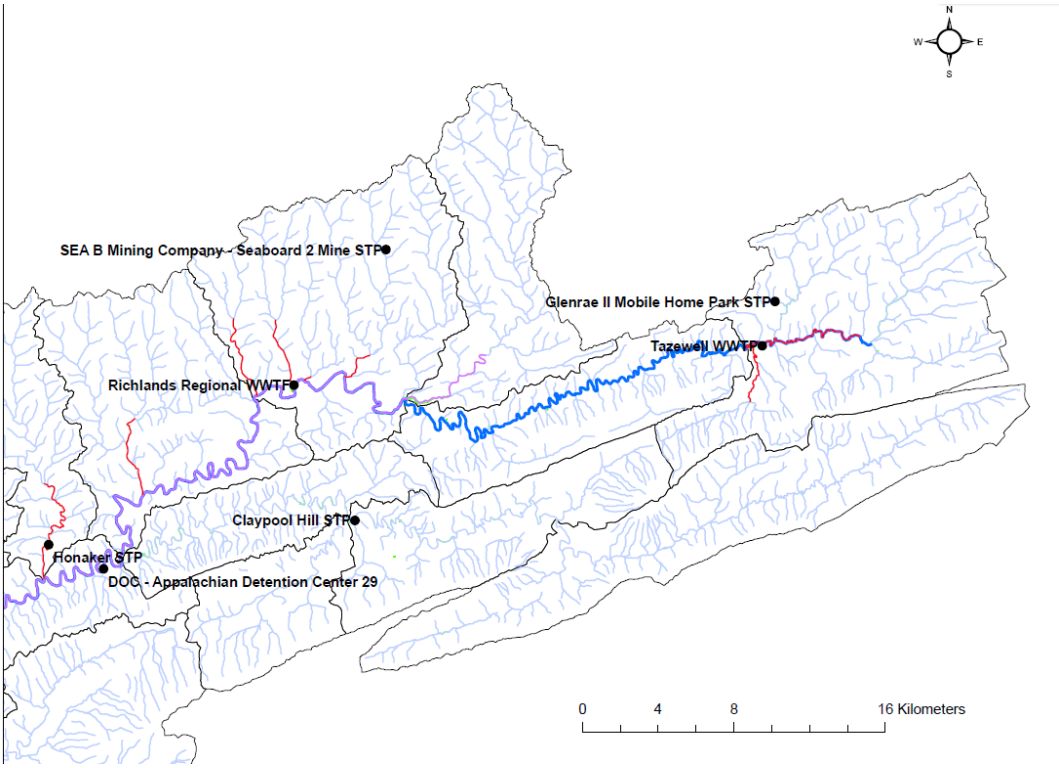
Medionidus conradicus

They occur in small creeks to large rivers and are restricted to shoal and run habitat. They are usually found in substrates composed of mixtures of sand and gravel often with cobble and boulders. They frequently occur under flat rocks. They are long term brooders and the sexes mature by the age of 3. They are a species of concern and are a small stream species uncommon in the Tennessee River (James et al, 2008).

Clinch and Powell River

It contains the highest concentration of rare and endangered aquatic species in the United States. Due to their ancient and stable geology, these watersheds have been called a “cradle of diversity” for aquatic life in the southern Appalachians. Of the 222 native fish species in the Tennessee River basin, the Clinch and Powell rivers alone are home to 118, including five threatened or endangered species (Illustrative Case Study…, 2013).

Figure 1 Sites for Water and Sediment Collection



Glutathione-S-transferase and Na+K+-ATPase

Glutathione-S-transferases (GSTs) are a group of enzymes that are important in the detoxification of many different xenobiotics by catalyzing the conjugation of the tripepetide glutathione (GSH) with the xenobiotic in the phase II of the biotransformation process promoting its elimination from the organism (Frasco and Guilhermino, 2002). This allows for the organism to defend its cells against the mutagenic, carcinogenic and toxic effects of the compounds. Na+K+-ATPase is an ion-translocating enzyme present in all animal cells (McCormick, 1993). The activity of Na+, K+-ATPase functions are to maintain ionic regulation in eukaryotes by transporting sodium ions out of and potassium ions into cell membranes (Mosher et. al, 2010). In chloride cells, Na+K+-ATPase creates ionic and electrical gradients that are used for salt secretion and possibly for ion uptake in fresh water (McCormick, 1993).

Overall Goal

Examine the biological effects of 4-nonylphenol on *Medionidus conradicus* by analyzing Na+/K+ATPase activity and gluthathione-S-transferase.

Materials and Methods

Water and Sediment Collection from site

The site for our study of 4-nonylphenol was the Clinch River. We visited two treatment plants along the Clinch River to collect our water and sediment samples which were the Richlands Regional Water Treatment Facility and the Tazewell Wastewater Treatment Plant both located in Tazewell County Virginia. We collected the water in 750 ml amber glass bottles. We first rinsed the bottles with the river water and then collected two samples from each site, one upstream of the main discharge pipe and the other downstream of it at both locations. We also collected a field blank at the first site which was at the Tazewell Wastewater Treatment Plant. We collected the sediment in mason jars. We collected various samples along the river at each site in a metal pan and mixed the samples together to get an even distribution of samples along the river. After properly mixing, we then transferred the sediment into the mason jars ensuring we collected mostly sediment and not water. We placed all the samples in a cooler filled with ice to preserve the samples and froze them for future analysis.

Classifying the mussels

The freshwater mussel selected for the experiment was the Medionidus Conradicus (Cumberland moccasin shell). Sixty of the mussels were collected from Tazewell in the Clinch River watershed. We measured each mussel's length using calipers. The mussels were then separated into categories based on their lengths. The ranges were less than 30 mm, 30-34mm, 35-40mm, 41-45mm and greater than 45mm.The majority of the mussels fell in the range between 35-40mm. As the endocrine system in the mussels function quite differently from most organisms, we did not seek to classify the mussels according to gender as 4-nonylphenol according to literature does not cause feminization of male mussels.

Establishing Feeding Clearance Rate

We selected 12 mussels from the 35-40 mm range as this was the most common range. We separated the mussels into 6 jars, with 2 each jar selected at random. We took the measurements of the mussels using the calipers. We made replicates of 3 concentrations of algal solution that we sought to use for the experiment. The concentrations of algae were 10E6 cells/ml, 150,000 cells/ml and 10,000cells/ml. The instant algae shellfish diet was obtained from Reed Mariculture Inc (871 Hamilton Ave Ste D, Campbell, CA). To make the solution, we added 1ml of the algae (2E9 cells/ml) into 49 ml of pond water to make 4E7cells/ml. We took 10 ml of this and added it to 390ml of pond water. To make the 150,000 cells/ml we took 0.030ml of 2E9 cells/ml stock solution and added it to 400 ml of pond water. For the 10,000 cells/ml, we took 0.1 ml of 4E7 cells/ml and added it to 400 ml of pond water. We allowed a 24 hr period after each feeding and then took a sample of the water which we examined under a light microscope with a hemacytometer. We counted the top two outside squares on the hemacytometer. For a high density square (average of 2 top squares contained more than 5 algal cells), we multiplied the average of the two squares by 10,000 cells/ml to determine the amount of cells/ml cleared. For a lower density square (average of all 4 squares < 4), we multiplied the average by 2,500 cells/ml to obtain the total cells/ml cleared. The following formula was used to determine the clearance rate of algae by the mussels:

$$m = \frac{M}{nt} \ln \frac{C_0}{C}$$

Where m is the clearance rate (ml/mussel/hr), M is volume of solvent, pond water (ml), n is the # of mussels per jar, t is amount of time from feeding to water collection (hrs), C_0 is initial concentration of cells and C is final concentration of cells.

The final clearance rate of 15,000 cells/ml used for the experiment was later established by performing chlorophyll analysis on the water collected after a 24 hr feeding period. At this rate, the mussels were clearing all the algae as evident by the lack of Chlorophyll A during analysis.

Chlorophyll Analysis

24 hours after the mussels were fed, a water sample was collected and analyzed to determine the amount of algea required for the feeding experiment. As a hemacytometer count proved inefficient, we resolved to use chlorophyll analysis in establishing a suitable concentration. For Chlorophyll A analysis, 750 ml of water was filtered through a glass fiber filter (GF/F) using a vacuum pump. The filters were extracted with 90% $MgCO_3$-buffered acetone. The Chlorophyll A was then analyzed using a spectrometer as specified (Steinman et. Al, 2006).

Feeding Experiment

Feeding experiments were conducted in 5 gallon paint buckets. The mussels were collected from along the Clinch River of which there were 60 of them. The mussels were suspended in the water with another bucket with a mesh bottom placed inside the bucket which allowed for feces and pseudo-feces to

settle to the bottom. The algae used for feeding was purchased from Reed Mariculture Inc (Campbell, CA) and the nonylphenol for the spiking was purchased from (insert here). The 16oz glass bottles for feeding were chew proof glass by Lixit products. The mussels were fed with the spike algae which were allowed to drip into the bucket over a period of an hour. Aerators in each buckets kept the algae suspended in the bucket. Care was taken to avoid cross contamination by demarcating each bucket-mesh-bottle per sample. Water was changed after every 24 hours and refilled with pond water during which mussels were also fed.

Water Testing Procedure

After defrosting the water samples from the night before, measure 200 ml of each water sample to us in extraction. Measure out 200 ml of MilliQ DI water for use as blank. For the spike, measure out 200 ml of ultrapure water spiked to 1µg/L (To make the spike, take 10 µl of 1.016E6 standard of 4-NP stock solution and dilute in 990 µL of acetone. This gives you 10.16 ppm solution. Take 500µL of the 10.16 ppm solution and add 4.5 ml of acetone to it to get a final concentration of 1.016 ppm solution of 4-NP). Add 196.9 µL of the 1.016ppm solution to the 200 ml of ultrapure water to create your spike.

Condition each 3cc, 60 mg Oasis HLB SPE Extraction cartridge (Waters) with the following: 3ml of methanol at a flow rate of 5ml/min, then 3 ml of ultrapure water at a flow rate of 5ml/min. Ensure that the cartridges do not run dry.

Transfer the 200 mL water sample to the conditioned Oasis HLB Cartridge using a Pasteur pipette at a flow rate of 2ml per minute. Take care not to cross contaminate the water samples while pipetting as well as ensuring that none of the cartridges dry up during this process.

When done, wash the cartridge s with 5 ml of 5% methanol in water, then 5 mL of MilliQ DI Water. Dry the cartridges by drawing air through it with a vacuum for 30 minutes. Elute the 4-nonylphenol (4-NP) from the cartridge into test tubes using two 3ml washes of dichloromethane/acetone (7:3, v/v) at a flow rate of 2ml/min.

Concentrate the eluent under a stream of Nitrogen in the Rapid Turbo-Vap until completely dry. Reconstitute the dried eluent by adding 0.5 ml of acetone in each vial and vortexing it for 30 seconds. Transfer into GC vials for analysis with the gas chromatotography tandem mass spectrometer (GC/MS/MS).

Prepare the following standards for the analysis with the GC/MS/MS as follows:

- 20.32 ppb – from the 1.016 ppm solution of 4-NP, add 20 µL of 4-NP to 980 ml of acetone.
- 101.6 ppb- from the 1.016 ppm solution of 4-NP, add 100µL of 4-NP to 900 ml of acetone.
- 406.4 ppb- from the 1.016 ppm solution of 4-NP, add 400µL of 4-NP to 600 ml of acetone.
- 812.8ppb- from the 1.016 ppm solution of 4-NP, add 800µL of 4-NP to 200 ml of acetone.

Transfer all the standards into gas chromatography (GC) vials and make two acetone blank samples by adding 1 ml of acetone into each GC vial.

Sediment Testing Procedure

Freeze dry the sediment sample prior to testing. Condition appropriate amount the silica gel (60-200 um,60Å) @ 150ºC for 24h and the anhydrous Na_2SO_4 @ 400ºC for 4 h the day before using them. After conditioning both, immediately store them in a dessicator until usage the following day.

Prepare Silica Gel cartridges using cleaned 3 ml cartridges by adding in the following order:

- 1 gram of silica gel
- 1 gram of sodium sulfate
- Store the cartridges in a dessicator until use (no longer than 2 days maximum).

Weigh 2 grams of each freeze-dried sediment sample, transfer to 20 mL glass vial. Add 10 mL of acetone/hexane (1:1, v/v) solution to each vial. Spike an additional 2 gram sample to 1 µg/L. (for

percent recovery determination)This is done by adding 20 µL of 10.16 ppm solution of 4-NP to the sediment.
Sonicate the samples for 20 minutes at room temperature in the sonication bath. Centrifuge the samples at 3500rpm for 10minutes at room temperature.

Set up Silica Gel Cartridges in the SPE Manifold. Condition the silica gel cartridge with:
3ml of methanol followed by
3ml of hexane
Do not let the cartridge run dry

Pass extract from the sediment through the cartridge and collect in the test tubes. Concentrate the extract under a stream of nitrogen in the Rapid turbo-vap until completely dry.
Reconstitute with 0.5ml of acetone. Vortex the test tubes for 30 seconds.
Transfer the concentrate to a GC vial.

For most of sediment samples, the following dilution procedure is needed.
Take 0.1 mL from the 0.5 mL sample and transfer into 0.9 mL acetone in a GC vial, cap immediately and mixing by shaking the vial end to end by hand.

GC/MS/MS Analytical Procedure

GC-MS/MS analysis was performed using a gas chromatograph (7890A, Agilent, USA) coupled with an Agilent 7000 series triple quadrupole GC/MS detector and a 7693 autosampler. The GC/MS/MS conditions were as follows: The initial column was held at 60ºC for 0.5 min, increased to 100ºC at 15ºC min^{-1} ramp rate, further increased to 200ºC at 5ºC min^{-1} ramp rate, and finally increased to 280ºC at 25ºC min^{-1} ramp rate. The backflush time is 3.0188min making the whole time for a cycle 26.867min. The inlet temperature, transfer line temperature, and the ion source temperature were set at 200ºC, 250ºC, and 200ºC, respectively. The analytical column is HP-5MS (30 m ×0.250 mm i.d., 0.25um film thickness, Agilent, USA) and with a backflush column of Agilent res.(0.78m×150 um ×0 um). Splitless mode was used at a Helium gas flow rate of 2.25 mL min^{-1}. The injection volume was 1 uL and the collision gas used was nitrogen. The x compound was qualified by electron impact at 70 eV using multiple reactions monitoring (MRM) mode. The MS/MS quantification and confirmation ions are m/z 107+121+135+149).

Bioassay

ATP-ase hydrolyzes adenosine triphosphate (ATP) to adenosine diphosphate (ADP), coupled with the oxidation of NADH to NAD+. The oxidation of NADH changes the absorbance over time and this change can be measured to determine ATPase activity. We utilized a plate reader with an absorbance of 340nm to test for ADP-ase activity in the gill tissue. The bioassay used for ATPase was concurrent with the one described in McCormick, 1993 which was modified for freshwater mussels by Mosher et. al, 2010. The samples were then read on a plate reader. The bioassay for the GST was done using the method in Frasco and Guilhermino, 2002. An albumin assay was used to normalize for enzyme activity of both Na+K+-ATPase as well as glutathione-S-transferase (GST).

Results

Table 1 Initial Lengths of mussels used for establishing clearance Rate

Concentration (cells/ml)	1	2
10E6 (Jar 1)	40	35
10E6 (Jar 2)	40	39
150,000 (Jar 1)	38	38
150,000(Jar 2)	36	36

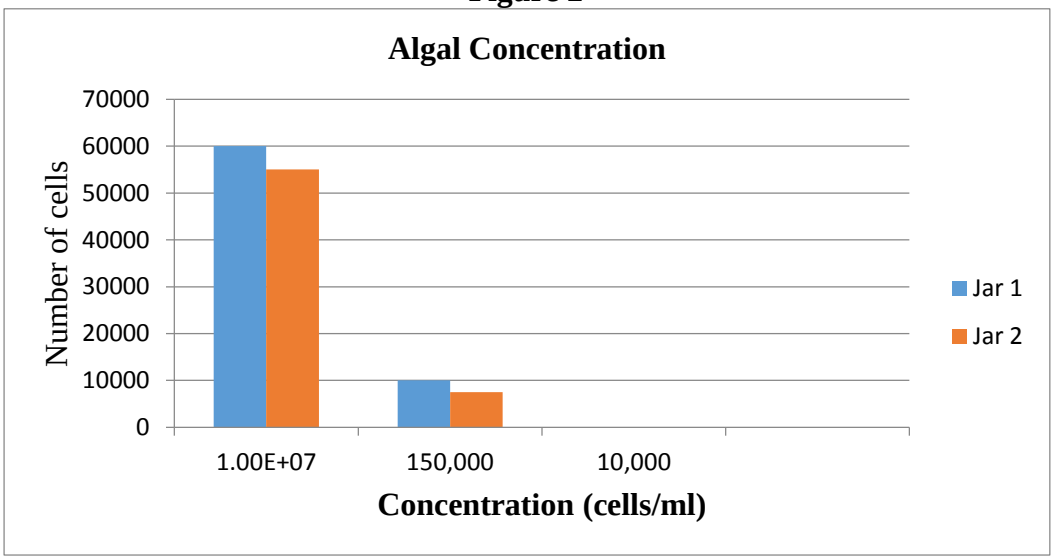
10,000 (Jar 1)	38	40
10,000 (Jar 2)	40	37

After the mussels were measured, they were placed into their individual jars and then the algae l solution was pippeted into the glass jars. The process was concluded at 4:00p.m on 06/11/2013.

Table 2 Algal count based on hemacytometer readings

Concentration (cells/ml)	Time of sample collection	Jar 1	Jar 2
10E6	9:20a.m	60,000	55,000
150,000	10:11a.m	10,000	7,500
10,000	10:52 a.m	0	0

Figure 2



The times of collection represents when we took the sample from the jar to analyze using the hemacytometer and light microscope.

*The 150,000 cells/ml both had small densities of algae and as such all four squares were counted as opposed to only the top two squares. The 10,000 cells/ml were entirely cleared and this proved unusable to calculate feeding clearance rate.

Table 3 Water Test using GC/MS/MS of Field Sites

Water Analysis of Field Sites	
Sites	Concentration (µg/L)
Tazewell Wastewater Treatment Plant Upstream	0.257
Tazewell Wastewater Treatment Plant Downstream	0.228
Richlands Regional Water Treatment Facility Upstream	0.190
Richlands Regional Water Treatment Facility Downstream	0.294

*Upstream and Downstream refer to upstream and downstream of the major discharge pipe from the water treatment facilities into the Clinch River.

Table 4 Sediment Test using GC/MS/MS of Field Sites

Sediment Analysis of Field Sites	
Sites	Concentration (µg/kg)
Tazewell Wastewater Treatment Plant Upstream	164.3
Tazewell Wastewater Treatment Plant Downstream	166.8
Richlands Regional Water Treatment Facility Upstream	164.7
Richlands Regional Water Treatment Facility Downstream	165.4

Figure 3 GST activity analyzed using plate reader

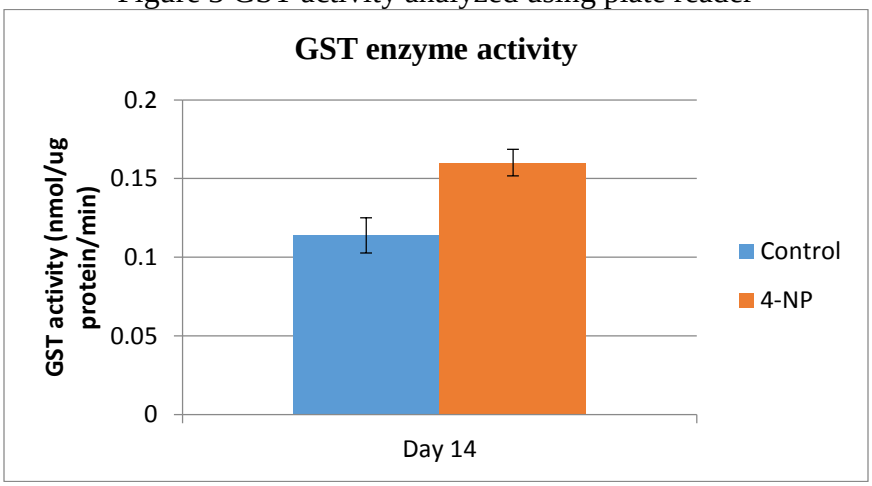


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Figure 4 ADPase Activity analyzed using plate reader.

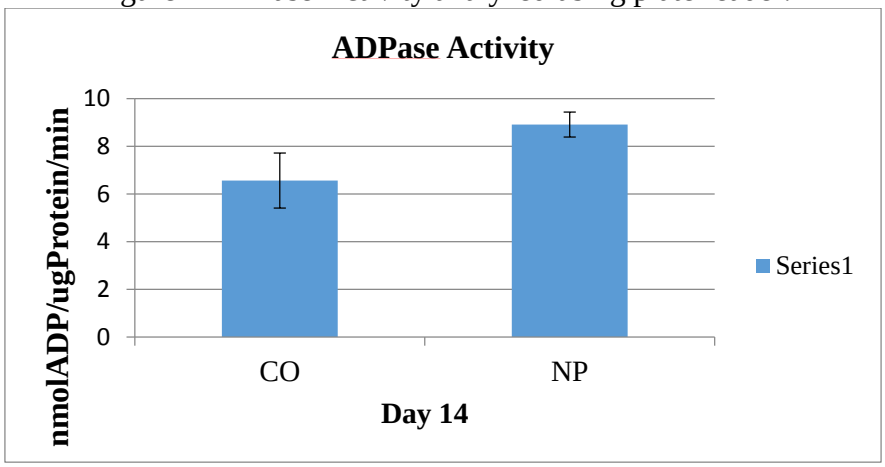


Figure 5 Chlorophyll A

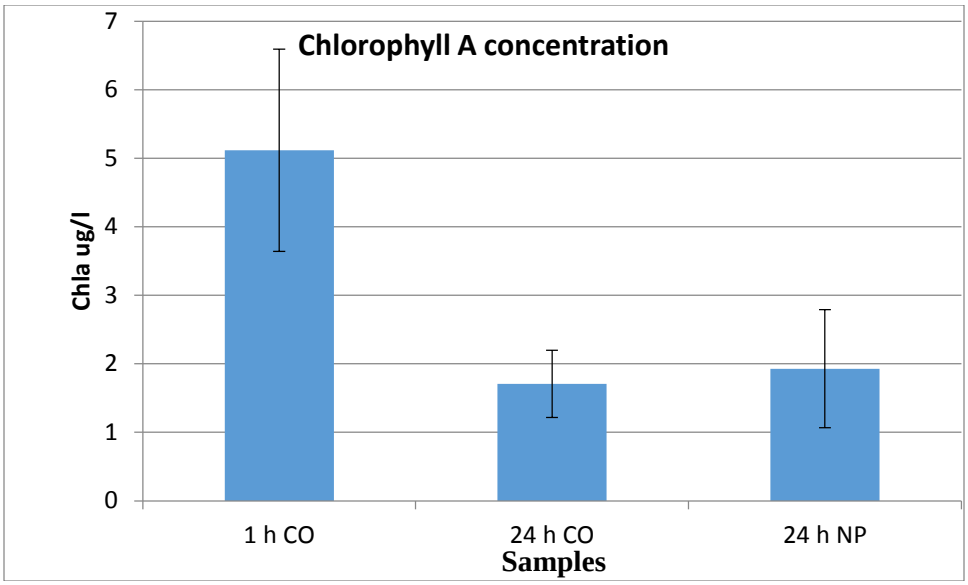
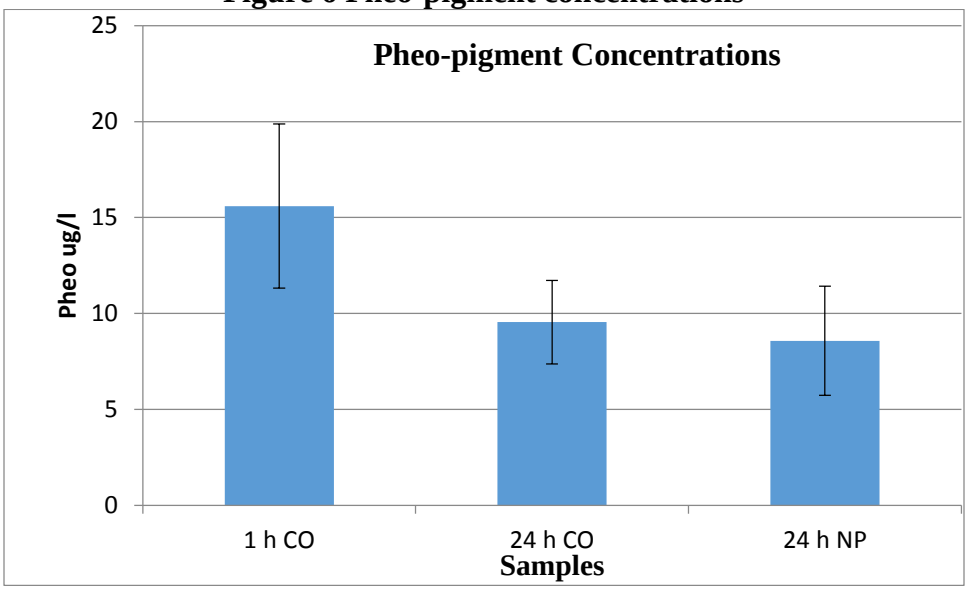


Figure 6 Pheo-pigment concentrations



Discussion

Nonylphenol ethoxylate and its metabolites are well known endocrine disruptors with regards to most vertebrates. However, the effect of 4-NP on the endocrine system of mussel is still shrouded in mystery as even the hormones native to the species do not appear to bind to the receptors as evident in other animals. As such for the purpose of our experiment we sought to view the effect on Na+K+-ADPase
activity as well as glutathione-S-tranferase (GST) activity. The ADP-ase enzyme is located in the gills of the species while the GST enzyme is located in the digestive glands of the species. Upon completion of the bioassay, we found that there was a significant increase in GST activity after exposure to 4-NP. The control group mean measured at 0.11 nmol/µg Protein/min ranging from a low of 0.08 nmol/µg Protein/min to a high of 0.179 nmol/µg Protein/min. The standard error of the sample was 0.033, a standard error of 0.011. The mean for the 4-NP samples was 0.16 nmol/µg Protein/min with a low of

0.130 nmol/μg Protein/min and a high of 0.183 nmol/μg Protein/min. The standard error for the 4-NP samples 0.0252 nmol/ μg Protein/min. Using a p-test, we ended up with a value of 0.01 which is statistically significant. This tells us that there is definitely a clear impact of 4-NP on GST activity.

For the ADP-ase bioassay, the mean value for the control was 6.56 nmol ADP/μg Protein/min with a low of 1.91 nmol ADP/μg Protein/min and a high of 11.86 nmol ADP/μg Protein/min. For the 4-NP the mean value was 8.91 nmol ADP/μg Protein/min with a low of 7.20 nmol ADP/μg Protein/min and a high of 11.10 nmol ADP/μg Protein/min. A t-test of the samples yielded an alpha of 0.07 not statistically significant.

Conclusion

While there is still relatively little information known about 4-NP's estrogenic effects on mussels, based on our results, we can conclude that 4-NP still has a biological effect on mussels. As evident from the increased activity of glutathione-S-tranferase between the control sample and test samples, we can conclude that the compound is toxic to mussels who then produce more GST to combat exposure to it. In addition from the analysis of the ADP activity in the mussel tissue, we can conclude that there is also an increase in activity of mussels exposed to 4-NP. Overall, we can conclude that 4-NP causes biological effects to mussels exposed to it. Also as mussels are sediment dwellers as well as filter feeders, they serve as a good indicator species for the compound and can be used as such.

Future Recommendations

Based on my completion of the project, I would suggest a couple of things for successfully running this project in the future. Running the water and sediment analysis in a two day period as opposed to a one day period allows for proper execution of the analysis as it allows adequate time to run the water samples through the SPE cartridges as well as dry them gradually in the Rapid Trubovap. Doing this reduces the chances of cross contamination which can severely alter accuracy of results. Also I would recommend paying close attention during the feeding experiment as well as possibly washing the bottles and buckets before each feeding to prevent any cross contamination with the 4-NP. This also reduces the chances of accidentally over spiking with 4-NP or spiking the control. In addition, running multiple feeding experiments with possibly another species of mussel would also clarify any discrepancies about biological effects of 4-NP on mussels.

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A LabVIEW Enabled Weather Monitoring System with an Interactive Database

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ABSTRACT

Since 2008, a real-time weather and water monitoring system - LabVIEW Enabled Watershed Assessment System (LEWAS) - has been developed on the Virginia Tech campus. This system monitors rainfall-runoff events in a small urban stream flowing through the campus by recording water quality and quantity and weather data. The current research automates the analysis of the weather data by adding database storage and retrieval stages. This study consists of three major components, i.e. creating a database specific to the parameters measured by the LEWAS Lab, creating a connection between the real-time LabVIEW system and the database, and designing a data-retrieval web-interface. The challenges encountered in completing the study and future work are described.

Keywords: LabVIEW, remote sensing, real-time, database, web-interface, watershed assessment

[1] Introduction

Development of a LabVIEW Enabled Watershed Monitoring System (LEWAS) at Virginia Tech has been underway since 2008. The main goal of developing this system is to be able to collect, analyze and store real-time stream water and weather data for water sustainability research and education (Delgoshaei, et al., 2012; Dymond, et al., 2013)). In order to achieve this goal, the LEWAS LAB uses three main instruments: a Hydrolab MS-5 Sonde (Sonde), which measures water temperature, pH, conductivity, turbidity and dissolved oxygen, a Sontek Flow Meter (flowmeter), which measures the flow and stage of the water, and a Vaisala Weather Station, which measures barometric pressure, air temperature, relative humidity, speed and direction of wind, and the amount of precipitation (Martinez, et al., 2012; Rogers, 2012; Welch, et al., 2011). Both the Sonde and the flowmeter have been deployed in the LEWAS field site stream as it is seen in the Figure 1, and both are currently operational. The weather station has been installed on the electric power pole on the east side of the stream right across the West Campus Drive.

Figure 1. LEWAS Lab Field Site.

The LEWAS uses LabVIEW, a graphical programming language developed by National Instruments (NI), to develop the watershed monitoring system. LabVIEW has a capability of real-time data acquisition and analysis through hardware-software integration. Unlike any scripting programming language, LabVIEW has built-in graphical controls and indicators that make it easier and more convenient for programmers to produce programs using the LabVIEW platform. A cRIO is an embedded hardware system developed by National Instruments, which can be programmed using LabVIEW. In this application, the c-RIO-9072 model has been chosen. This cRIO is setup at the field site where it collects data from the respective instruments (Sonde, flowmeter and weather station) and sends data wirelessly across a LAN set up by Virginia Tech Communications Network Services to the computer in the lab where real-time data are analyzed and plotted on charts via LabVIEW VIs (virtual instruments). Previously, LabVIEW version 8.6 was used for the system and the system was operational until May 2013. As part of the current research, the system was stopped at the end of May to update LEWAS with the newest LabVIEW version, 2012 Service pack 1 (SP1).

The goal of this project was to develop a weather monitoring system with an interactive web-based user interface using the historical and real-time weather data recorded at the LEWAS site. This goal was achieved by adding database management capability and a user friendly web-interface to the existing weather monitoring system (Nguyen, 2012; Seo, Lee, et al. 2008). A database is a data storage and retrieval device and a web-interface is a platform where a user can have access to the data stored in the database. Later on, the system will be developed further to monitor the stream water data as well. Figure 2 illustrates the operation of the LabVIEW enabled real-time weather monitoring system. The weathe sensor collects data and sends it to the cRIO. The cRIO converts data from text strings to numeric form and sends it to a MySQL database. Via a web-interface, a user can then access the data stored in the database.

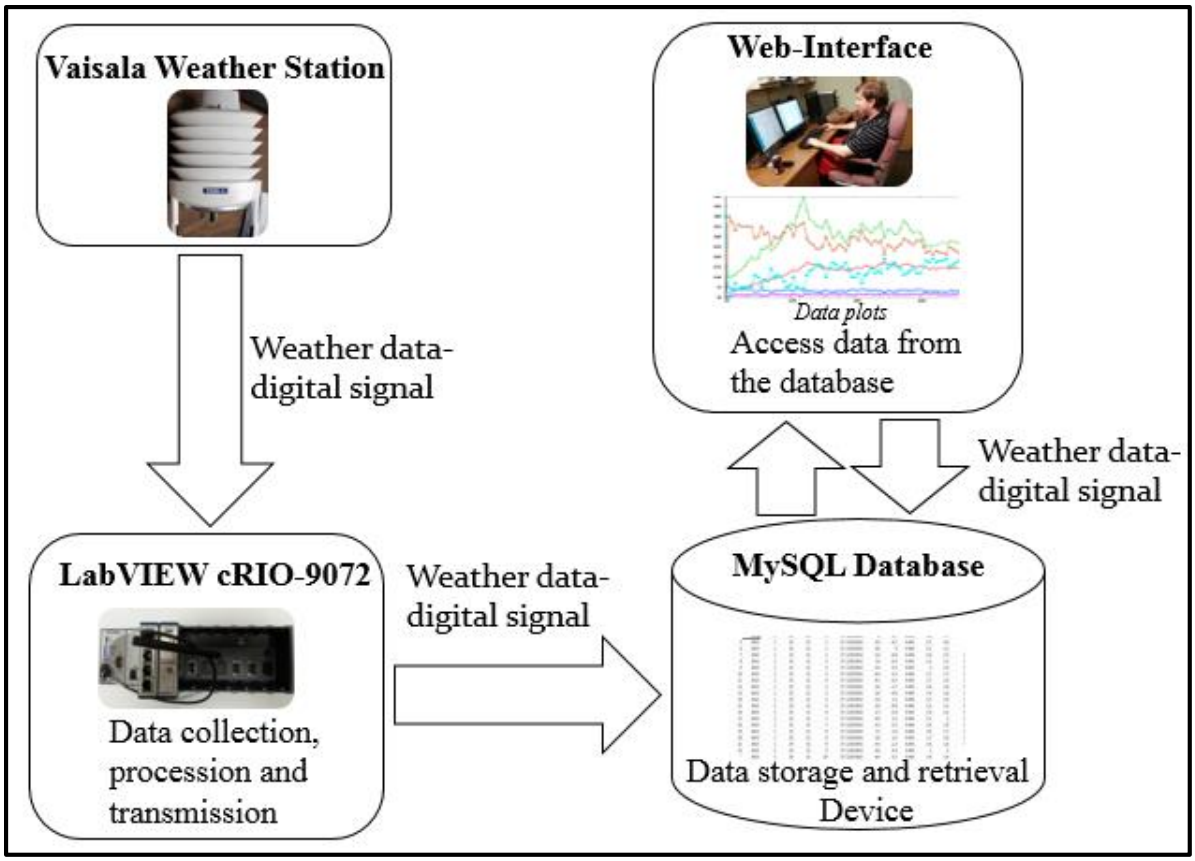

Figure 2: A visual schematic of the LabVIEW Enabled Real-time Weather Monitoring System

A Brief Overview of LEWAS LAB

The LEWAS LAB's office space is located in room 335 of McBryde Hall at Virginia Tech. The LAB team currently consists of Faculty members, Graduate and Undergraduate students from Engineering Education, Civil & Environmental Engineering, Electrical/Computer Engineering and Chemical Engineering. Although all lab and faculty members work as a single team but for the purpose of day-to-day operation, the lab members are divided into two sub-teams: the Civil & Environmental Engineering team, primarily responsible civil engineering related works such as field site maintenance, and instrument deployment and repair, and the Electrical/Computer Engineering team, primarily responsible for electric equipment installation and repair, software development, database management and web-interface development. Some areas such as data collection and analysis require the integrated efforts of members from both sub-teams. Figure 3 shows the inside view of the LEWAS LAB. The picture on the left shows a part of the civil & environmental engineering work station and the picture on the right shows a part of the electoral & computer engineering work station.

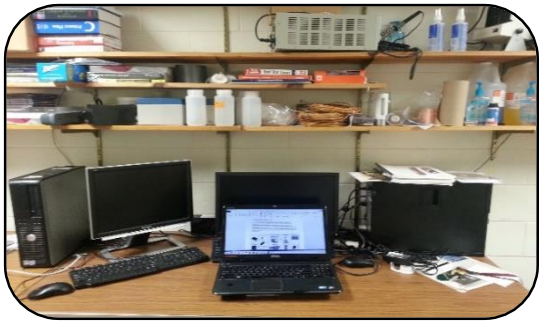

Figure 3: Inside views of LEWAS LAB Office space. Pictures taken on 07/23/2013

Figure 4 below illustrates how the LEWAS LAB instruments operate. The solar panel generates 24V electric power that charges two 12V batteries in series. The weather station, flow meter and MS-5 Sonde are operated by the batteries. These three instruments send data to a LabVIEW programmed cRIO. The cRIO sends data wirelessly to database, and via a web-interface a user can access the data from the database on a computer.

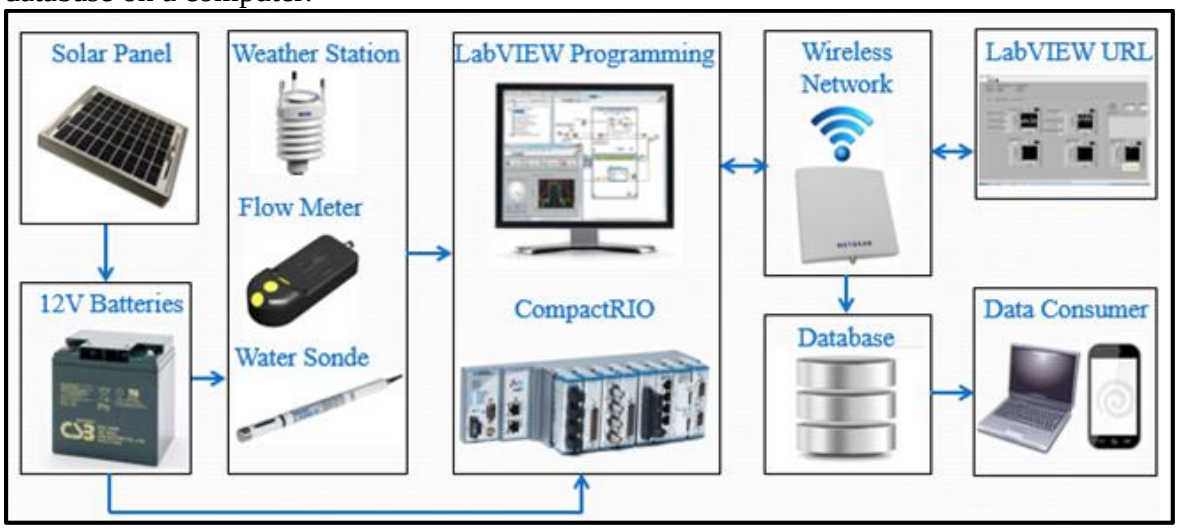

Figure 4: LEWAS LAB Operational Diagram Prepared by Walter McDonald.

LEWAS Field Site

The LEWAS field site is located on the Webb branch of Stroubles Creek in Blacksburg, Virginia, directly across West Campus Drive from Hahn Hall. The stream originates in the north part of Blacksburg, flows through the parking garage of Virginia Tech into the Duck Pond downstream the LEWAS field site and meets Stroubles Creek at its outlet. The satellite view on the Figure 5 shows the location of LEWAS field site.

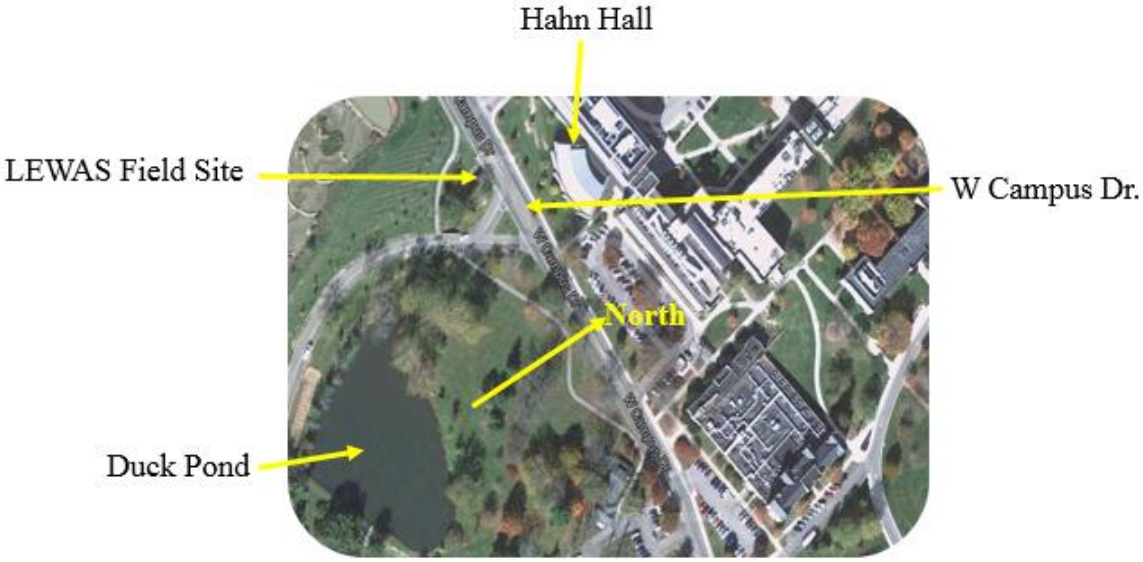

Figure 5: The Satellite view of the LEWAS field site retrieved by the lead author using "Google maps" on 07/23/2013

The picture on the top left corner of the diagram in Figure 6 is the weather station. The MS-5 Sonde is on the bottom left corner and the flowmeter is between the weather station and the MS-5 Sonde. These instruments send data to the cRIOon the right side of the diagram. The router connected to the cRIO acts as a wireless bridge that connects to Virginia Tech wireless network through a dedicated IP address.

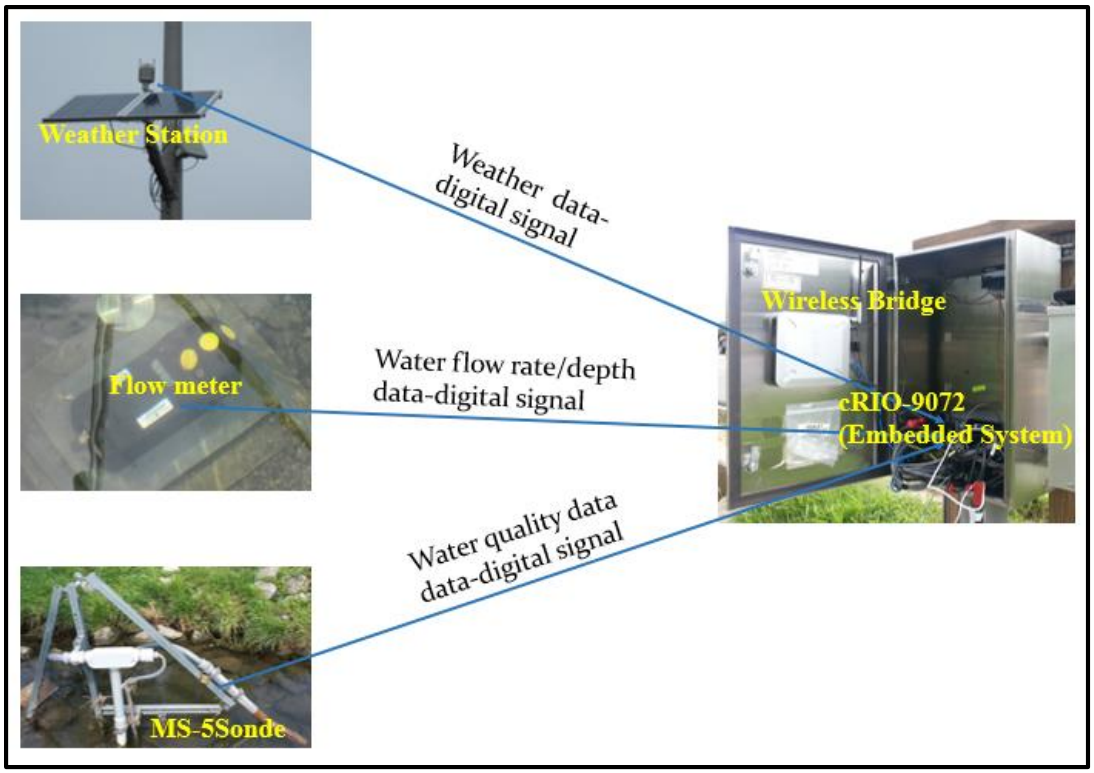

Figure 6: System Connection at the field site. Pictures taken on 07/17/2013.

[2] Literature Review: Real-time Weather Monitoring Systems

Real-time weather monitoring systems similar to the one being developed at the LEWAS LAB have been previously developed in different parts of the world (Garg, et al. 2013; Ibrahim, 2011; Jin, Zhao, Yuanpeng, 2012; Simon, Wartosfsky, 2013). In a study in Switzerland, a weather monitoring system was developed by utilizing MEMS (micro-electro-mechanical system) technology (Ma, et al. 2007). The system has a MEMS-based weather sensor that collects and sends data through an amplifying circuit to an Octopus II transmitter. The hardware consists of the MSP430F61 core processing chips produced by Texas Instruments. It converts the analog signals to digital ones and sends data in digital forms wirelessly (WSN technology) to an Octopus II receiver. The Octopus II receiver communicates wirelessly to a computer at a remote location where data is received via an antenna, and analyzed and displayed on the computer screen using. Beside this, a data logger is attached to the transmitter for saving the data for future uses.

Another weather monitoring system was developed in Sao Paulo, Brazil by two researchers Aisla Foina and Ahmed EL-deeb based on pervasive computing and application technology in 2008. The pervasive computing and application is a method of collecting and processing information (data) by devices embedded in our daily activities (Ahmed, 2008). This weather monitoring system consists of three components; WeBo (weather box) – a microcontroller equipped with weather sensors and GPS (Global Positioning System), TeCo (terminal computer) – a receiver and SysCo (system computer) – the main server. The WeBos are placed on the roofs of city area buses. Each Webo on each bus collects data throughout the journey of the bus and the data are collected by TeCo as soon as the bus arrives at the bus station. The data is then transmitted to SysCo where the data is processed and analyzed. The buses arrive at the bus station at 3–5 minute intervals. Thus the data delivery is fast, which minimizes the data gaps. The visual schematic of the weather system is given below.

The LEWAS is different from the ones described above. Table 1 compares key features of the LEWAS and two weather monitoring systems discussed earlier. The database management capability and web-interface make the LEWAS a unique system implemented on a small urbanized watershed and a detailed review of literature is needed to further validate this claim.

Table 1. Differences between three Real-time Weather Monitoring Systems

Hardware/Features	**LEWAS LAB at Virginia Tech**	**Sao Paulo, Brazil**	**Basil, Switzerland**
Embedded System	cRIO-9072	WeBo / TeCo / SysCo	Octopus II
Embedded System Manufacturer	NI	Unknown	Texas Instruments
Wireless medium	LAN	None	WSN
Data logger	Yes	Yes	Yes
Database Management	Yes	No	No
Web - interface	Yes	No	No
Data available to public on the internet?	Yes	No	No

[3] Research Methods

Developing the LabVIEW enabled weather monitoring system included three key components, i.e., (i) Creating a database (ii) Creating cRIO-database link and (ii) Designing a data retrieval web-interface. Before initiating the first component, i.e. creating a database, connecting the cRIOsetup in the field with the desktop computer in the lab and updating the old VIs on the existing system was essential (B O 'Flynn, et al. 2010).

cRIO connection and LabVIEW VIs update

The cRIO has to be connected to the computer in the lab in order to create LabVIEW VIs on the cRIO. To accomplish this task, CNS (Virginia Tech Campus Network Services) has provided a dedicated IP address. A wireless router has been setup with the cRIO in the field and a sensor on Hahn Hall right across West campus drive from the LEWAS field site (Figure 9). The router connects to the Virginia Tech network wirelessly through the dedicated IP address (Breed, 2013; Beccali, et al. 2008).

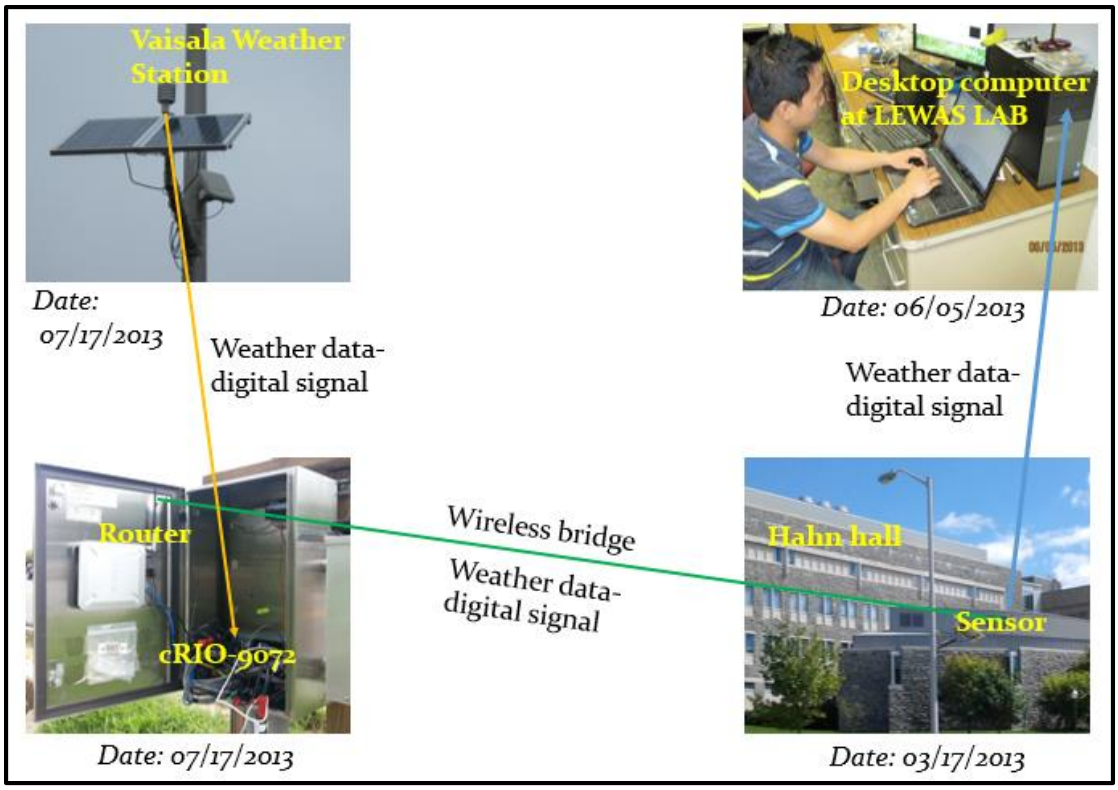

Figure 7: A diagram illustrating the communication between the cRIO-9072 and the Desktop computer at LEWAS LAB.

Several steps were required in order to update the system. Firstly, the cRIO was disconnected from the weather station and reformatted. The new versions of FPGA (Field Programmable Gate Array) and RT (Real-time) modules and other software were installed on the cRIO through NI MAX (National Instruments Measurement and Automation Explorer) in the desktop computer. In order to establish the connection between the cRIO and the desktop computer, the cRIO was rebooted and the dedicated IP address of the LAN was configured on the cRIO through MAX. The next step involved updating the VIs. A new project was created and the VIs were recreated using the old VIs in the old computer (LabVIEW 8.6) as references. The appearance of most of the controls and indicators were the same and they were under same functions except for a few ones that were updated in LabVIEW 2012 SP1. LabVIEW is a graphical programming environment. Instead of typing codes in a text editor as in many scripting languages such as C/C++, JavaScript, C# etc., there are built-in graphical controls and indicators in LabVIEW. The appropriate controls and indicators are wired together using visual lines, i.e. *wires*, to create a LabVIEW program. A file containing a LabVIEW program is called a VI and the main file containing many VIs is called a LabVIEW project. A VI comprises two main components, i.e. a front panel and a block diagram. A front panel is a graphical user interface where a user has access to input and outputs of the LabVIEW program, and a block diagram is the platform where LabVIEW codes are written. Both the front panel and the block diagram are automatically created when a new VI is created. An example VI's front panel and block diagram are shown in Figure 8. It shows a simple code on the Block Diagram and user interface for inputs on the Front Panel of a LabVIEW VI to multiply two numbers.

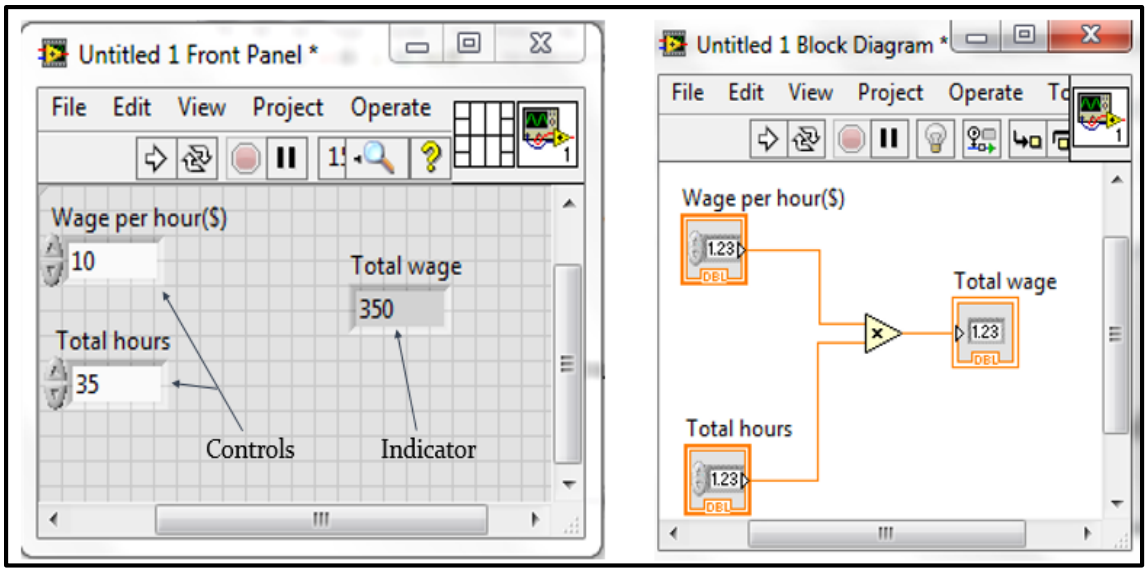

Figure 8: A simple LabVIEW VI

Debugging the LabVIEW VIs involves fixing broken and misplaced wires. If different data types of controls and indicators are wired, the broken wire error is shown in the block diagram. If any control or indicator is left unwired, a broken wire run error is shown. The errors can be fixed by wiring only controls and indicators of the same data types.

Building VIs for the cRIO is slightly different from building VIs without a hardware platform. Instead of creating VIs directly under a project heading, VIs for a cRIO specific project are created under the hardware device inside the Project Explorer. In order to create VIs for the cRIO, the correct version of FPGA and RT module must be installed on the host computer. After all VIs and Sub VIs have been created, the VIs need to be compiled through a compile server before the programs in the VIs can be burnt into the FPGA in the cRIO.

Figure 9 below is a LabVIEW VI taken from the LabVIEW project of the LEWAS weather monitoring system. The block diagram of the VI contains LabVIEW program. In this figure, the "FPGA VI reference" function on the top right corner of the block diagram gives reference to the FPGA on the cRIO. There is a function outside the case structure (lower right, the innermost rectangular box) that receives string data from the cRIO, parses the string data and sent it into the case structures. There are different case structures based on the types of data such as temperature, pressure, rain intensity, humidity, wind speed and direction etc. Some case structures contain multiple parameters. The codes inside each case structure process the data and convert it to appropriate numeric value. The data are plotted and displayed on charts on the front panel (left side).

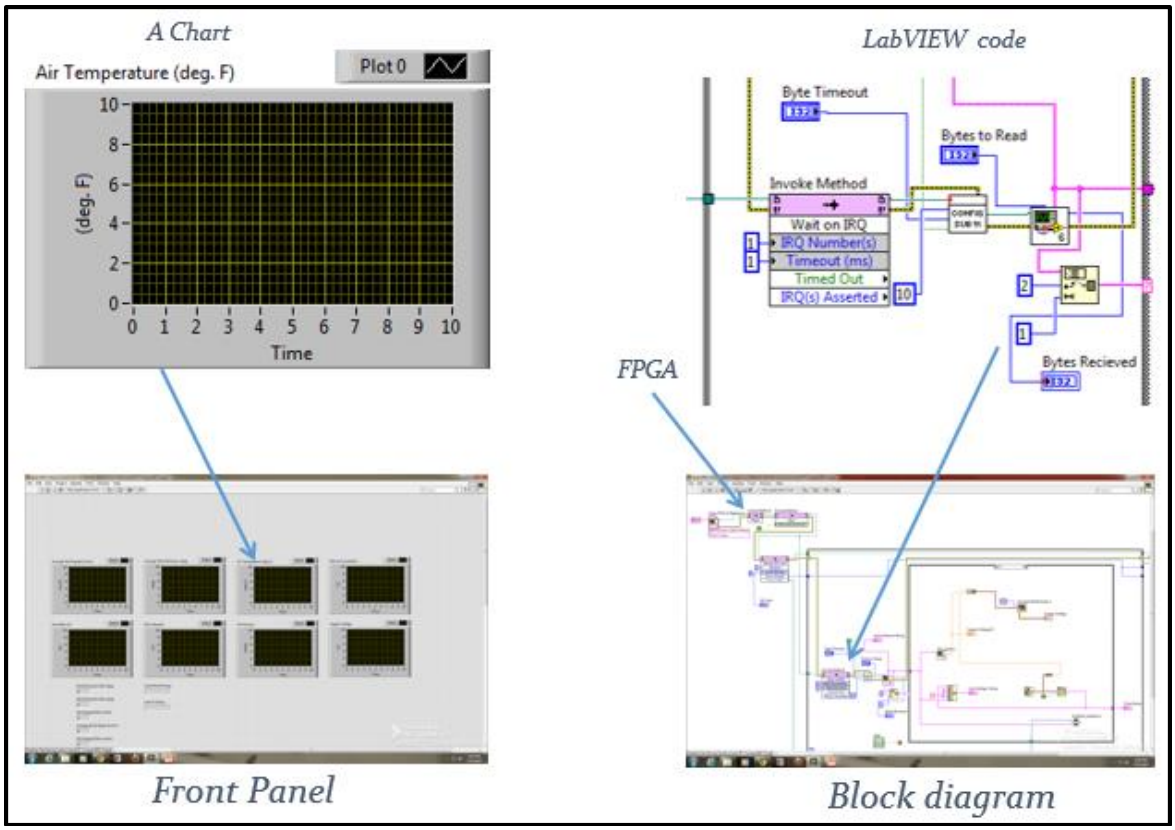

Figure 9: DMA (host).vi

After the VIs are successfully compiled. The LabVIEW programs (FPGA VIs) are burned into the FPGA in the cRIO. As the DMA(host).vi is run, the real-time data of the selected parameters are plotted on charts on the front panel of the user interface VI. Next, implementation details of the three components are given.

[3.1] Construction of a Database Management System

A database is a collection of data in an organized form (What is MySQL, 2013). Data are stored in tabular forms in a database. MySQL database system is popular open source database system. The word "MySQL" comes from SQL. SQL is a structured query language used to communicate with database. The SQL commands are used to update data in the database and retrieve data from the database. The main SQL commands are "Select", "Insert", "Update", "Delete", and "Create". These command are used for storing, retrieving and manipulating data stored in the MySQL database. Constructing a MySQL database involves the following steps (Beavers, 2005).

Creating a new database

The first step is to download an open source software tool, e.g. PhPMyAdmin fromwww.myphpadmin.net and create an account. When logging into the PhPMyAdmin, it prompts to name the new database. A MySQL database is named with a format; username_databasename. The naming format allows the database server to know the username and the database associated with that particular username. It only allows the particular username to access the database.

Creating tables

The next step involves creating tables in the database in order to store data. The newly created database is selected from the drop box which then prompts the user to name the table(s). The tables are named to reflect the data being stored, e.g. weather parameters. The tables are filled with a number of fields as required. For storing weather data in the database, the fields are named with parameter names such as air pressure, air temperature, relative humidity, wind speed, wind direction and precipitation.

Defining fields

In order to store data correctly, the fields must be defined by appropriate data types such as INT (an integer value), FLOAT (a floating point number), DOUBLE (a double precision floating number), etc. VARCHAR allows to store character data type of up to 255 character in length. DATE is the data type to store dates whereas DATETIME is the data type to store the combination of date and time together. FlOAT data type stores decimal numbers. Weather data can be integer or decimal numbers, so FLOAT is used to store weather data because it keeps the integers after the decimal places. For example; temperature on a particular time may be 80.4° C. Using integer data type truncates 0.4 and stores only the integer value 80, but using FLOAT data stores the whole decimal value 80.4 which is more precise than the integer value 80.

The LEWAS Database

For the current research, four tables were needed for the database, i.e. wind (speed and direction), air (temperature, relative humidity and air pressure), precipitation (rain and hail) and system (supply voltage, etc). The reason that four separate tables were needed was that these four sets of data are measured by the weather station at different intervals. Furthermore, the precipitation data was collected at irregular intervals depending on the current precipitation rate. Figure 10 shows an example database table.

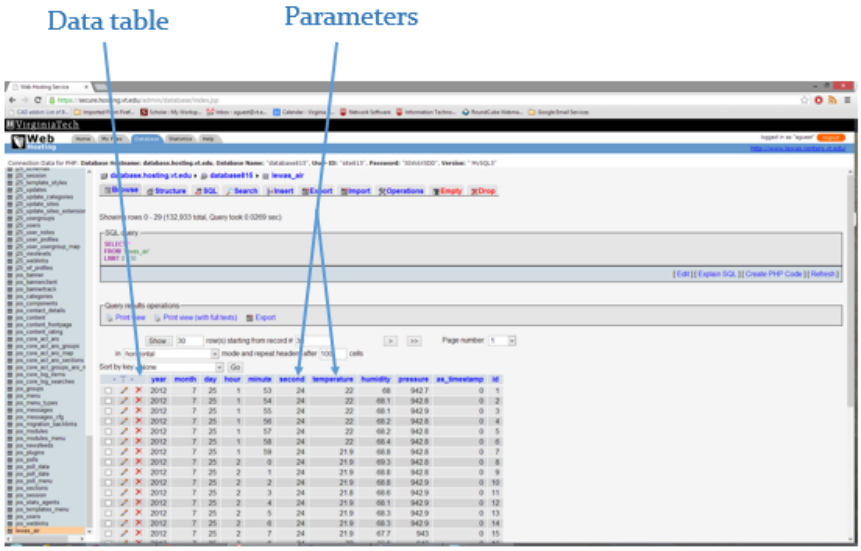

Figure 10: An example of a MySQL database

[3.2] cRIO and MySQL database Interface

After the database is created, it needs to be linked to the LabVIEW program in order to populate the database with live weather data. A software application development company-SAPHIR Signal Physics & Instrumentation -has designed a software called SmartSQLVIEW that connects a LabVIEW program to a MySQL database. The SmartSQLVIEW is compatible with the Real-time module and it executes SQL queries (SAPHIR Signal Physics & Instrumentation, 2013). This software is available for download on the website of National Instruments. SmartSQLVIEW is sufficient in order to accomplish the cRIO-MySQL database interface task (Using MySQL with the Database Connectivity Toolkit on Windows, 2013). Figure 11 shows a diagram illustrating the MySQL database-LabVIEW connection through SmartSQLVIEW software. The cRIO connects to the MySQL database and sends data to the database. From a computer, the data can be accessed using LabVIEW. These processes are made feasible by installing the SmartSQLVIEW software toolkit on the host computer. Because of limited time and also a system power failure issue, this task could not be completed within this ten-week program. When the power issue is resolved, this task will be continued.

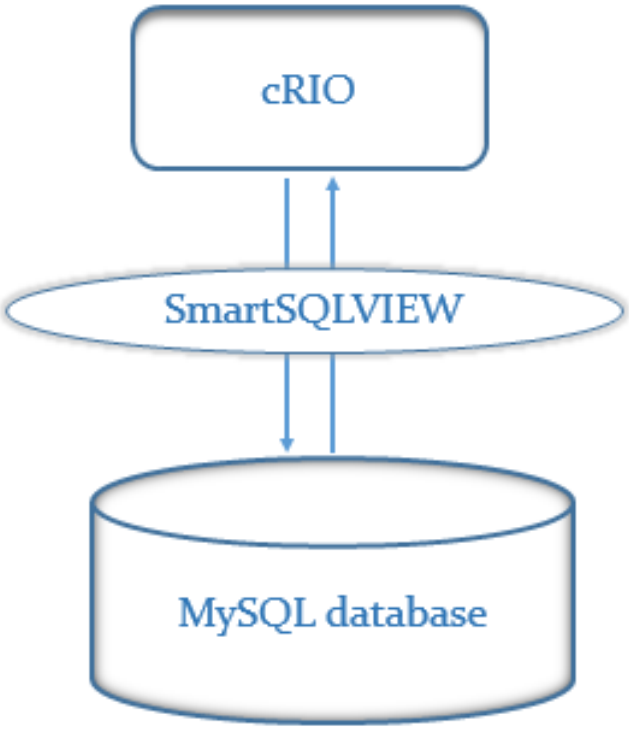

Figure 11. A cRIO-MySQL connection diagram

[3.3] Design and creation of a Web-interface

A web-interface is a platform that provides a user with an ability to have access to the information (data) stored in a database. In order to design a web-interface, selecting the parameters of the information to be accessed by a user and sketching the layout of the web page are essential steps. Creating a web-interface requires proficiency in a programming language used for web-development. Out of many programming languages such as C++, C#, Java, PHP etc., PHP was used in creating the LEWAS web-interface.

PHP (hypertext preprocessor) is a scripting programming language used to control and query data in the database through a web interface (Bringing MySQL to the web, 2013). Syntactically, the PHP codes are similar to C/C++.It supports HTML, SQL and JavaScript commands. Programming well in PHP requires knowledge in HTML, SQL and JavaScript. Other object-oriented languages such as C++ or C# may be used in place of PHP. However, the reasons behind the choice of PHP over other languages are, firstly, that it is an open source language that it is available for free and, secondly, that it is convenient and flexible for use in web development. C++ and C# are great tools in developing web sites, but the web sites developed with these languages can be controlled from the hosting computer only where the programs have been written. In order to access the web development environment of a website from another computer at another location, the original program must be copied to that computer which makes these languages inconvenient and inflexible for web development from multiple locations. However, web sites developed with PHP languages are accessible from multiple computers, so web developers do not need to have the copy of the original codes for web development. Also, C++ and C# generate executable files upon compiling the program and the executable files run in the computer. Unlike C++ and C#, PHP can run directly on the web server to control the web site. Thus, PHP is an excellent web development tool in terms of flexibility and convenience (MacIntyre, 2010). For this LEWAS system, the selected parameters are air pressure, air temperature, humidity, wind speed and direction and the amount of precipitation. The layout diagram is shown in Figure 12. In the box on the top right corner of the weather web page, current weather conditions are displayed. In the box on the top left corner of the weather web page, historical data is displayed. There are dropdown boxes for the

start and end date of the timeframe for the weather data. As a user selects the parameter, and the start and end date, the data are displayed on a table directly below the dropdown boxes. There is a chart under the historical data box that by default plots the current data. When user selects his/her desired parameter and the timeframe from the historical data box, the default graph is replaced by the graph of the selected parameter. Also, there is a button on the lower right corner of the box containing graph exports data to a file in .csv format. The box on the bottom right corner is for displaying camera pictures. The purpose of displaying pictures is to provide the user with access to view both data and the picture simultaneously.

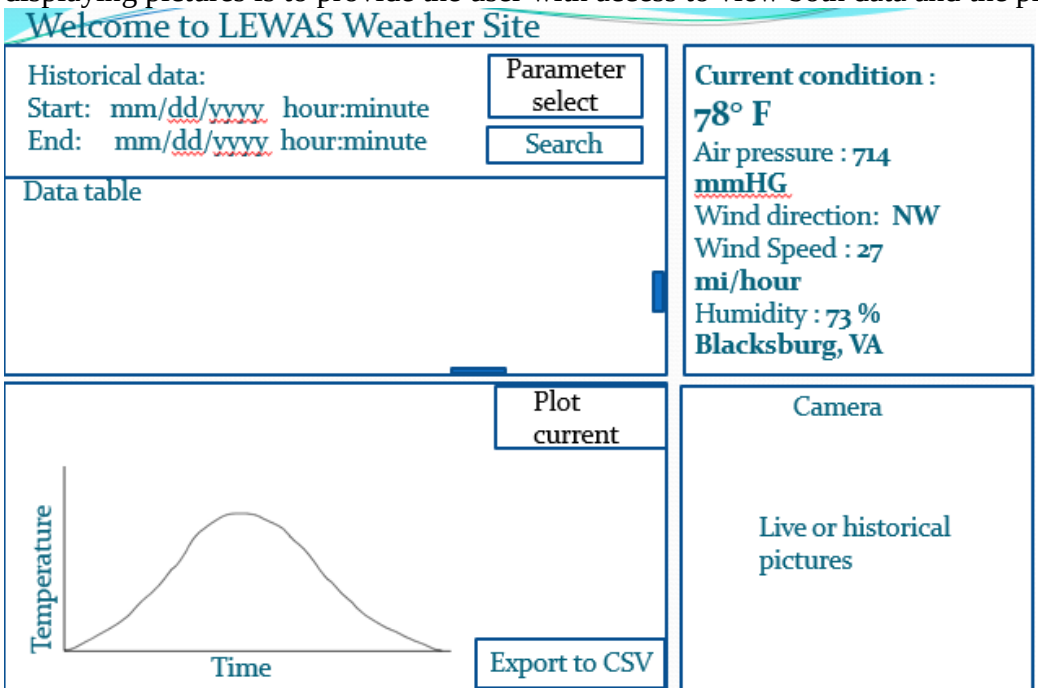

Figure 12: LEWAS LAB Web-interface layout

[4] Implementation Challenges

Three main challenges were encountered over the course of this LabVIEW enabled real-time weather monitoring system development process. The first challenge was encountered in connecting the cRIOwith the desktop computer in the lab. Even though the cRIO was reformatted and the correct versions of FPGA and RT modules were installed on the cRIO, the cRIO did not appear in NI MAX on the desktop computer. It took more than three weeks to be able to successfully connect the cRIO and the desktop computer. The efforts for overcoming the challenge involved following each cRIO setup instruction in the manual to consulting with NI technical support team.

The second challenge was to compile the LabVIEW VIs. There are a few ways to compile the LabVIEW VIs. The most common one is to compile the VIs on the host computer. The next way is to compile the program into a dedicated remote server computer through the "compile cloud" developed by National Instruments. Initially, the "compile cloud" was chosen as it did not require any software to be installed. The only requirement was to register the cRIO on which the LabVIEW programs are to be burnt into. However, the programs did not compile. After contacting the NI technical support team for assistance, they recommended installing "xilinx" (a software) developed by NI to compile the program to the local host computer.

The third challenge was to design and create the web-interface. The challenge was resolved with the help of the third author.

[5] Results and discussions

Despite the technical challenges, the final component of the project – design and creation of a web-interface – was implemented. Figure 13 below shows the web page of the current LEWAS Weather Site. It displays the current temperature, pressure, wind direction and speed, and humidity on the top

right corner box. It plots the data since the last hour on the graph below. A user can select the start and end date from the dropdown box provided on the top left corner box. The user can view both the data on the table and the graph simultaneously. The "export to CSV" bottom will allow the user to export the data to a csv file. The graph on the figure shows the temperature vs. time graph.

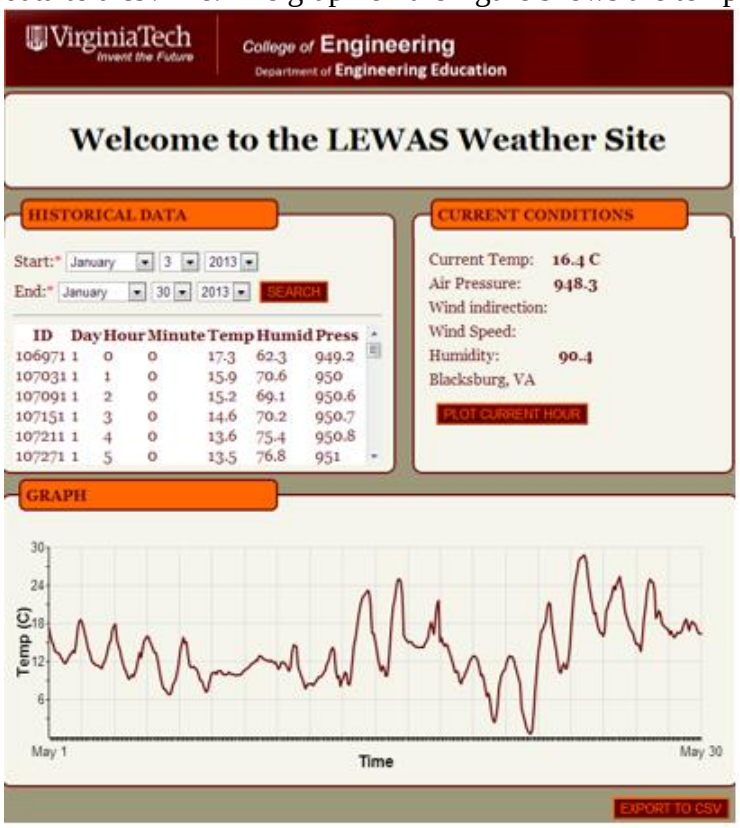

Figure 13: The outlook of LEWAS Weather Site

There are three remaining features of the web interface yet to be implemented, i.e. "Parameter select", "Plot current" and "Camera pictures" on the interface and this work is continuing at the time of this writing.

[6] Conclusion

The LEWAS at Virginia Tech campus has been an ongoing, long-term project since 2008. Although power failure issues did not allow to finish up the final stages of the LabVIEW implementation in developing the LEWAS weather monitoring system, the majority of the work necessary to achieve the goals of the current research, i.e., building a database management system and a user friendly web-interface for the system, was completed within the ten week project limit. A preliminary web-interface using historical data has been successfully completed. The future tasks include the remaining tasks necessary to fully achieve the project goals. The first task will be to complete the deployment of LabVIEW measurement VIs. The second task will be to establish the connection between the cRIO and the MySQL database, and the third task will be to modify and implement the remaining web-interface components. Furthermore, adding water quality and quantity measurement data and adding webcam capability to the existing database system are included in future work tasks. Adding the water quality and quantity, and imagery data to the system will further increase the research and educational capabilities of the system in the near future.

[7] Acknowledgements

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Surface Water and Groundwater Exchange during Floodplain Inundation

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ABSTRACT

Surface water-groundwater (SW-GW) exchange occurs naturally below a stream and in a stream's floodplain during periods of inundation. This exchange has become increasingly of interest to stream restoration research as it allows for a steep gradient in redox (reduction-oxidation) conditions that enable biogeochemical reactions, buffer stream temperatures and impact water quality. For stream restoration projects attempting to improve stream water quality through removal or retention of nutrients, increasing SW-GW exchange can improve water quality under certain circumstances. The objective of this study was to examine the effects of seasons and soil moisture on SW-GW exchange during controlled floodplain inundation in Stroubles Creek, Blacksburg, VA. This was achieved through monitoring water elevations, temperature, and electrical conductivity in the surface and subsurface the floodplain during natural and artificial flood events. Results show that no significant difference of SW-GW exchange was observed between spring and summer flood events when antecedent moisture conditions were similar.

Keywords: Surface water and groundwater exchange; Floodplain; Water quality

Introduction

Stream restoration has become increasingly common throughout the United States. It is currently a billion dollar industry and is performed for a variety of reasons including aesthetics, erosion reduction, and water quality improvement (Bernhardt & Palmer, 2005). A common restoration practice is floodplain reconnection; although few studies have examined the effectiveness of the efforts for water quality improvement. Water quality is affected by many parameters; however, excess nutrient loading is especially widespread. Increased human population and lifestyle has led to doubling natural nitrogen loading from enhanced fertilizer use and urban drainage (Vitousek et al., 1997). Excess nitrogen as well as other nutrients in streams can lead to eutrophication and dead zones in large bodies of water.

Floodplains can be classified as either a nutrient sink or source depending on their specific characteristics, and often transform nutrients between inorganic and organic forms. In several cases, floodplains with long inundation periods have been found to be significant nutrient transformers in several cases. This is often because significant SW-GW exchange can occur and remove or retain nutrients in the floodplain (Tockner et al., 1999). Increased water residence time achieved by periods of inundation allow for nutrients to be cycled into the groundwater. Groundwater containing areas of high organic carbon and anoxic conditions can permanently convert nitrate (NO_3) into dinitrogen gas (N_2) through nitrogen cycling (Roley et al., 2012). Short duration floodplain inundation in small streams is more common than long inundation periods, yet less research has been conducted on their effectiveness as nutrient transformers. In a study by Noe and Hupp a short-hydroperiod floodplain was examined and proven to have significant nutrient transforming properties by exporting inorganic nutrients and importing organic nutrients (Noe & Hupp, 2007). By further examining the hydraulics in a short-period inundated floodplain, a greater understanding of SW-GW exchange and its potential benefits for water quality can be determined.

This study examined the effects of seasonal change and varied antecedent moisture conditions on SW-GW exchange during artificially induced overbank flood events. The floodplain reach examined was on a recently restored reach of Stroubles Creek, located downstream of the main campus of Virginia Tech in Blacksburg, VA.

The restored reach had its bank reshaped along with added natural vegetation. The floodplain reach of Stroubles Creek was inundated for short periods and monitored with a network of piezometers and moisture probes. This paper presents data from two of the flood events taking place in the Spring 2013 and Summer 2013. This study is part of a larger study that will examine the floodplain's reaction to these artificial events in all seasons.

Research Methods

Site Description

This study examined a recently restored stream floodplain of Stroubles Creek downstream from Virginia Tech main campus in Blacksburg, VA. This reach of Stroubles Creek was listed as impaired by the Virginia Department of Environmental Quality (VDEQ) and has since under gone a Total Maximum Daily Load (TMDL) implementation study (Yagow et al., 2006). Stroubles Creek is a second order urban stream that empties into the New River and ultimately the Gulf of Mexico. Stroubles has a watershed of approximately 17.1 km^2 and contains urban, forested, and agricultural land. The floodplain reach studied is surrounded by crop fields and cow pastures (Resop & Hession, 2010). There is little vegetation on the floodplain during winter with rapid growth during summer. The floodplain selected for the study was an abandoned meander that would allow easy flow management and return to the creek (Figure 1).

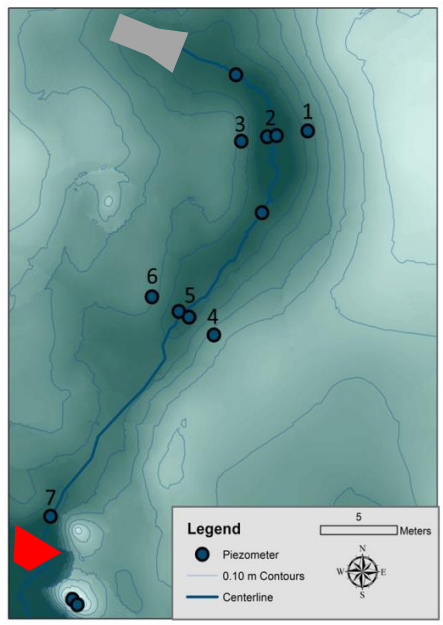

Figure 1. Stroubles floodplain topographic map with piezometer locations

Piezometer Network

In order to monitor the floodplain for both artificial and natural flood events, a network of piezometers was placed in the floodplain. A natural low in the surface topography was determined as an artificially induced centerline flow. Figure 1 shows the elevation of the floodplain, where darker colors indicate lower elevations, and the location of the piezometers are small blue circles. Piezometers are numbered in stations from one to seven and form three cross sections. Cross-section 1 (XS1) is made up of stations 1-3, Cross-section 2 (XS2) includes stations 4-6, and Cross-section 3 (XS3) includes station 7. Stations 8 and 9 were not included on the map, but were also monitored for background behavior of floodplain hydrology. Station 8 is located higher on the floodplain and station 9 is located near Stroubles Creek. The green outlet near the top of the Figure 1 indicates were the flow was released from, while the red rhombus outlines the flume where the water reenters the creek.

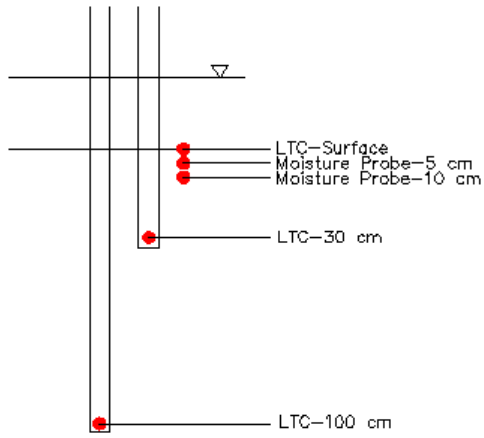

Figure 2. Centerline piezometer and moisture probe arrangement

Three different types of probes were used to monitor the floodplain – Solinst LTC levelogger junior, Onset HOBO pressure transducer, and Decagon GS3 moisture probes. LTCs record temperature, pressure, and electrical conductivity. HOBOs record both temperature and pressure. LTCs and HOBOs were placed in piezometers, generally within the area inundated by the artificial flood. The moisture probes were placed directly in shallow soil and recorded temperature, electrical conductivity, and volumetric moisture content. Stations 2, 5, and 7 were located on the centerline of the assumed flow path and were monitored more frequently than the stations not on the direct flow path. Figure 2 shows the arrangement of piezometers and probes at a typical centerline station. Probes were set at the bottom of the surface water column and at depths of 5 cm, 10 cm, 30 cm, and 100 cm below ground surface.

Spring Flood

The spring flood events began on April 8th, 2013 at 12:18 PM and April 9th, 2013 at 12:00PM. Both floods ran for three hours. The floodplain was inundated with a Berkeley B3-ZRMS pump with a Briggs and Stratton Vanguard gas motor that pumped an average flow of 0.825 cfs from the creek channel with a standard deviation below 0.005. The flow rate of the pump was monitored using a Fuji Electric Ultrasonic Flowmeter M-Flow PW and was recorded every five minutes. Stroubles Creek was dammed with sand bags previous to each artificial flood to create the necessary head for the pump. An EchoWP-1000 pump was used to prime the Berkeley B3-ZRMS pump to induce the flood. The Berkeley B3-ZRMS pump sent water through PVC pipe into a fire hose, and released into a larger black pipe. The outlet of the pipe released the water onto a tarp, which contained several cinderblocks that disrupted the flow to reduce velocity and distribute the water evenly over the floodplain. The pipe and hose were also reinforced with sandbags. The outlet can be seen in Figure 3.

Figure 3. Pump outlet at start of spring flood reinforced with sandbags

Each flood event is actually a pair of floods performed approximately twenty-four hours apart. Before the first day of flooding there were at least three days without precipitation. This allowed for a comparison of the antecedent moisture conditions of the floodplains. The first flood would represent a drier floodplain, typical of a situation without recent rain. The second flood would represent a wetter floodplain, typical of a situation with recent rain. The pump was run for exactly three hours. After two hours a 1 kg NaCl tracer diluted in 10 gallons of water was added to the outlet of the pump in an attempt to create a spike in the specific conductance to examine the flow path. A 3" Parshall flume was set at the end of the floodplain and measured the return flow of water to Stroubles Creek. A HOBO probe monitored the elevation of the water in the flume, which was converted to flow by a curve provided by the manufacturer. This allowed a water balance to be performed and the storage of the floodplain to be calculated.

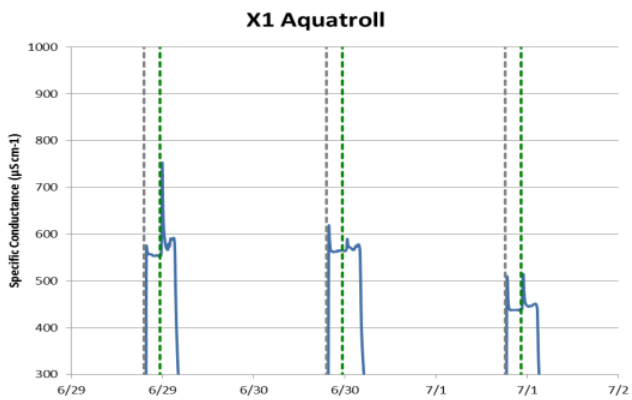

Figure 4. X1 Aqua TROLL®s showing spikes in specific conductance from NaCl injections

Summer Flood

The summer flood event took place June 29th, 2013 at 9:42 AM and June 30th, 2013 at 9:42 AM. Both floods were run for three hours. However during the last hour of the June 30th flood the hose detached from the outlet. Due to the discrepancy a third flood was performed on July 1st, 2013 at 9:13 AM to make sure that the same procedure was followed. Again the floodplain was inundated using the Berkeley B3-ZRMS pump this time with a slightly lower flow rate of 0.770 cfs with a standard deviation below 0.008. All three floods took place approximately 24 hours apart and the antecedent moisture conditions present in the floodplain were comparable to the spring flood during each of the events. The NaCl injection was also increased to a concentration of 2 kg in 10 gallons to ensure that the probes would pick up the injection from the background electrical conductivity. In order to test for the NaCl tracer Aqua TROLL®s were used and placed where the preferential surface water path location was estimated. These Aqua TROLL®s determined temperature and electrical conductivity and were placed between the sections and in line with XS2 and XS3. X1 Aqua TROLL®, placed between XS1 and XS2 probe measured a clear spike in specific conductance with the NaCl injection as seen in Figure 4. An LTC at surface station 7 was switched with the HOBO prior to the summer flood. The water elevation leaving the floodplain was again measured to determine the water balance in the floodplain.

Results and Discussion

Site Data

In order to better understand the permeability of the soil and water movement in the floodplain, rising head tests (or bail tests) were performed on all piezometers to determine the hydraulic conductivity at each station in the floodplain. These tests were performed between the spring and summer flood events and provided the results tabulated below. The rising head tests drained the water out of the piezometers and monitored the rate at which the water level returned to its original level. There is a clay layer in the floodplain, which accounts for the large variation in permeability. Some of the tests were unsuccessful as the water level in the piezometers never returned to a significant height to be used to calculate their permeability and are listed as N/A in Table 1.

Table 1. Permeability of soil surrounding piezometers stations in the floodplain

Station	K (ms^{-1})
1	3.8E-07
2s	3.2E-06
2d	2.3E-06
3	1.2E-07
4	3.4E-08
5s	5.0E-08
5d	2.5E-07
6	3.4E-08
7s	N/A
7d	N/A
8	N/A
9	N/A

Table 1 shows that soil permeability at XS1 was higher than that of the other two cross-sections. The high permeability allowed for water to penetrate into the subsurface of the soil at a faster rate. At XS2, the permeability was slightly lower than at XS1. The soil at XS3 had such low permeability that after 28 days the water levels in the soil had not returned to their original water level previous to the rising head tests.

Spring Flood

The water expelled from the outlet was expected to travel through the centerline of the cross-sections and exit out through the flume. Table 5 shows the water elevations of each of the cross-sections. The surface water elevation of all the sections in green shows a clear increase and then decrease during the flood. However, the water elevation in the floodplain subsurface reacted very differently depending on location. Figure 5 shows an instantaneous response at XS1 to the pump flow. It is likely that there is preferential flow occurring at or near XS1 causing the rapid response. Another explanation could be that a pressure wave is occurring from the flood start. Vidon articulated this concept as increased water pressure induces a rapid pressure lift from the groundwater in close proximately (Vidon, 2012). The artificially induced flood could have caused a pressure wave generating the groundwater pressure seen in Figure 5. XS2 shows that there is an impact of the flood in the deep well (100cm) but not at the shallow well (30cm). The deep well does have a slightly higher permeability but not so high as to suggest a preferential flow path. It is possible that a preferential flow paths exists that bypasses the clay and deposits water in the subsurface and then Darcy flow allows the water to move to the 100cm well of XS2. At XS3 there is little to no effect from the flood on the water elevation in the subsurface. This agrees with the low observed permeability.

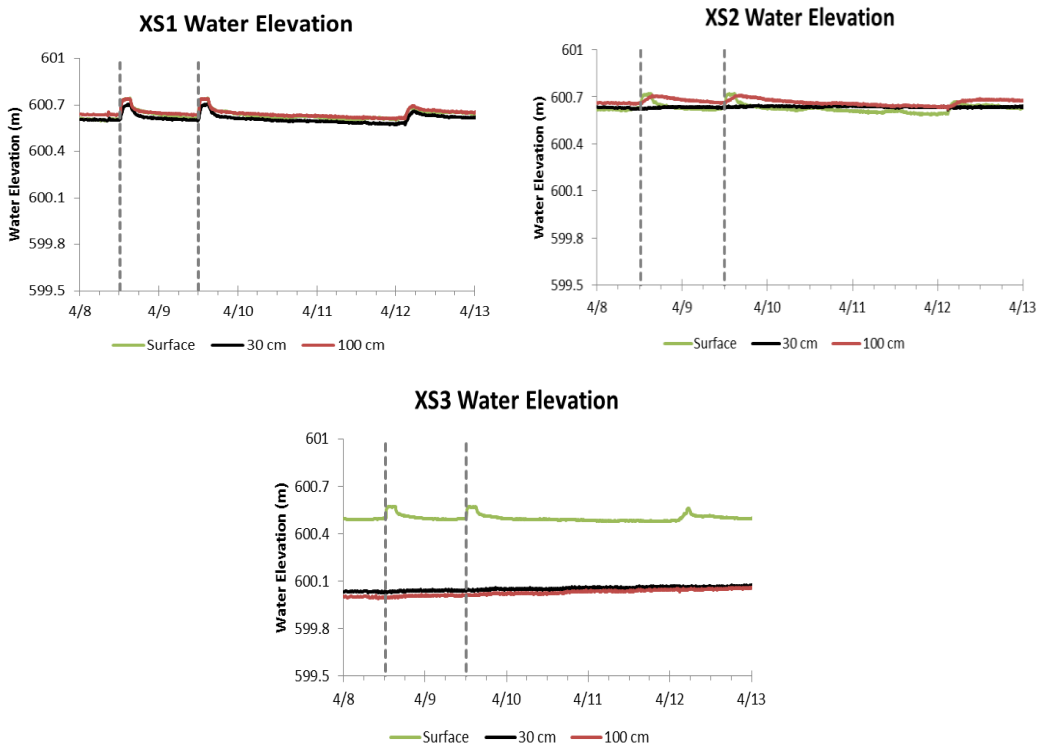

Figure 5. Water elevations of spring floods with dashed lines indicating flood start times

Although the flow from the outlet did inundate the floodplain and pass through the cross-sections, the preferential surface flow did not follow the assumed centerline. When the NaCl tracer was added at hour 2 on the first day a green dye was added. The dye followed a path closer to Stroubles Creek and appeared to effectively bypass both XS1 and XS2. The path of the tracer (green dye) can be seen in Figure 6 This bypass occurs at a natural topographic low and could have been enhanced by wheel tracks. Thus the water did not flow through the floodplain's lowest elevation as assumed.

Figure 6. Spring flood showing green dye NaCl injection on preferential surface flow path

The electrical conductivity of water in the floodplain was monitored and is shown in the Figure 7. The surface water stations as seen in green shown clear peaks after the injections were added in the surface electrical conductivity of floodplain. This can be only at XS1 and XS2 as there was not a LTC placed at XS3 during the spring flood. However, apart from the surface probes, no other probes showed the injections. This was likely due to the high background electrical conductivity of the floodplain. The moisture probes at 5 cm and 10 cm were not able to yield any useable data for any of the flood events. During every flood the soil was always saturated. Since the antecedent moisture conditions did not change the moisture probes could not be used to track the saturation of the floodplain. This will be further examined in the future and different results may be seen when the floodplain is not saturated. The electrical conductivity of the stream water was approximately 750 µS/cm and should have

shifted the conductance of the floodplain closer to that number. Since there was no reaction to the flood event in terms of conductance at XS1 in the deep well it seems more likely that a pressure wave occurred then preferential flow. If the surface water had penetrated the groundwater that quickly a change in conductance should have been seen. This can be seen in the shallow well of XS1, which shows clear peaks in reaction to the flood showing that SW-GW exchange occurred.

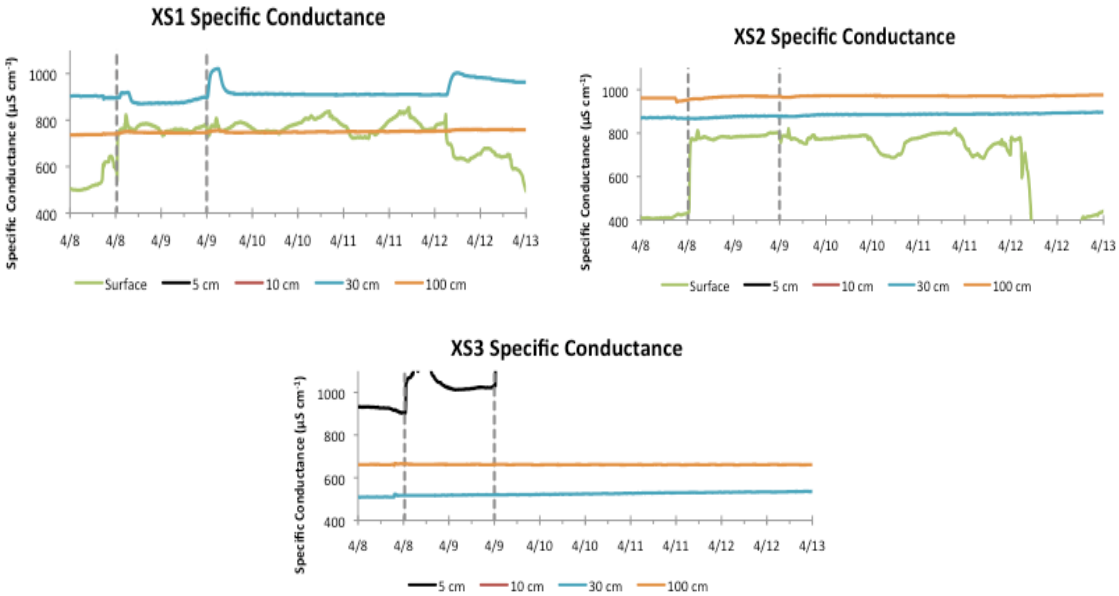

Figure 7. Specific conductance of spring flood with dashed lines indicating flood start times

The vertical head gradients of each cross-section are graphed in Figure 8. This graph compares the head gradients in each of piezometers on the centerline to see if losing conditions occurred. Any positive relationship between stations indicates gaining conditions. A negative gradient indicates losing conditions between the two stations. At XS1 there are both gaining and losing conditions between the shallow well and the surface and deep wells. The deviations at XS2 were larger than XS1 and show losing conditions from the surface to shallow well. Station XS3 shows a downward relationship between the surface and both depths in the subsurface. The downward or losing relationships seen in XS1 and XS2 along with are water elevation data and the electrical conductivity data indicates that the floodwater is exchanging with the subsurface of the floodplain. Although the vertical head gradients show a downward relationship, due to the low hydraulic permeability it is significant that any SW-GW exchange is occurring between the piezometers.

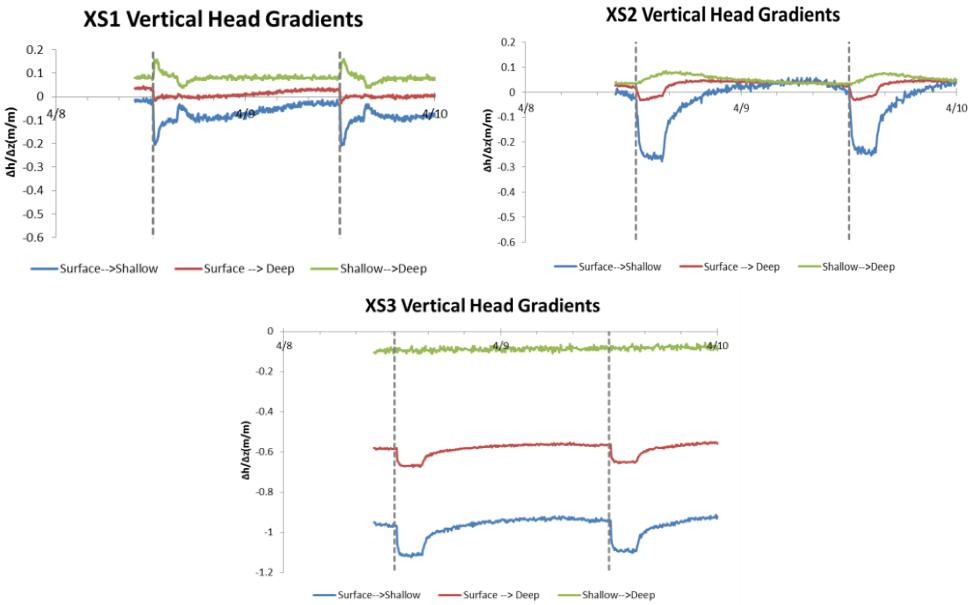

Figure 8. Vertical head gradients of spring flood with dashed lines indicating floods

The storage volume was estimated by measuring the flow from the pump entering the floodplain and then monitoring the height of water in the flume as it re-entered Stroubles. By taking the difference between the two, a water loss of 5.03% was found. This means that a maximum of 5% of the stream water was stored in the floodplain. However, some of the water might have bypassed the flume and returned to the stream without being measured. As seen in Figure 9, the storage volume during the spring flood reached a maximum around 55 m^3 and levels out at about 12 m^3. The graph of the storage volume has a steep slope that declines as it reaches the maximum volume. The surface flow moved across the floodplain initially penetrating the soil subsurface quickly, and then more gradually as the floodplain became more saturated. The peak indicates where the water entering the floodplain was less than the water leaving the floodplain through the flume.

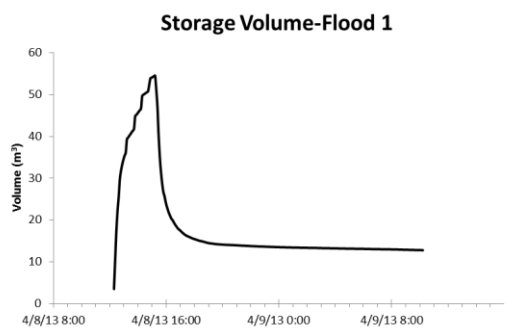

Figure 9. Transient storage achieved during the spring flood

Summer Flood

The water elevations from the summer flood event were remarkably similar to the spring flood. As seen in Figure 10, each of the cross-sections responded similarly to their behavior in the spring. The subsurface also generally remained in agreement with the spring flood. The slight increase between the second and third summer floods was a natural rain event and can be seen in the surface elevations. Also the water elevations in the subsurface piezometers at XS3 appear to have fluctuations and a general increase. The amount is minimal, and likely due to a natural daily fluctuation than an effect of the flood.

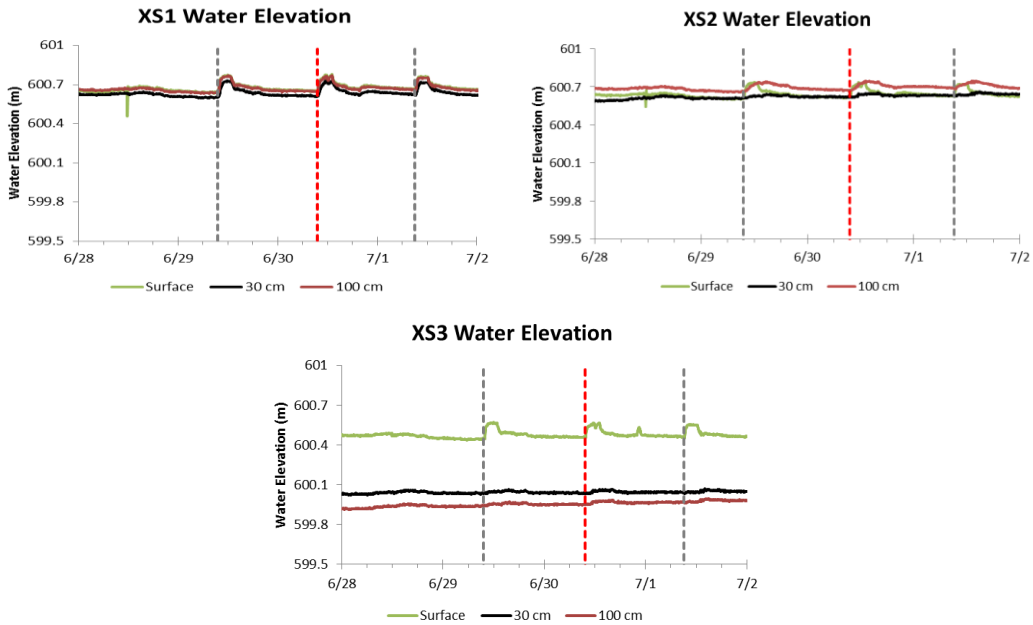

Figure 10. Water elevations of summer floods with dashed lines indicating flood start times

The electrical conductivity in the summer floodwater was measured by an LTC at the surface of XS3 and through the use of Aqua TROLL®s. The LTC data can be seen in Figure 11, while the data from the Aqua TROLL®s are presented in Figure 12. The surface probes at XS1 and XS3 rapidly approach 600 µS/cm, the approximate background electrical conductivity present in the stream. However, XS2 surface does not react to the flood in the same manner, and instead dropped lower and then steadily climbed. The impact of the NaCl injection can be clearly seen at hour two in XS3 surface as a peak. The electrical conductivity in surface water at XS1 and XS2 increased simultaneously with the NaCl injection. However the specific conductance of XS1 increased rapidly and then had a level slope. This can be seen in the surface specific conductance clearly in XS1 of Figure 11. This is likely because of significant ponding occurred around XS1 and XS2 that did not wash the water through, but instead kept the highly conductive water. This ponding did not abate until another flood event occurred, clearing the stagnant water.

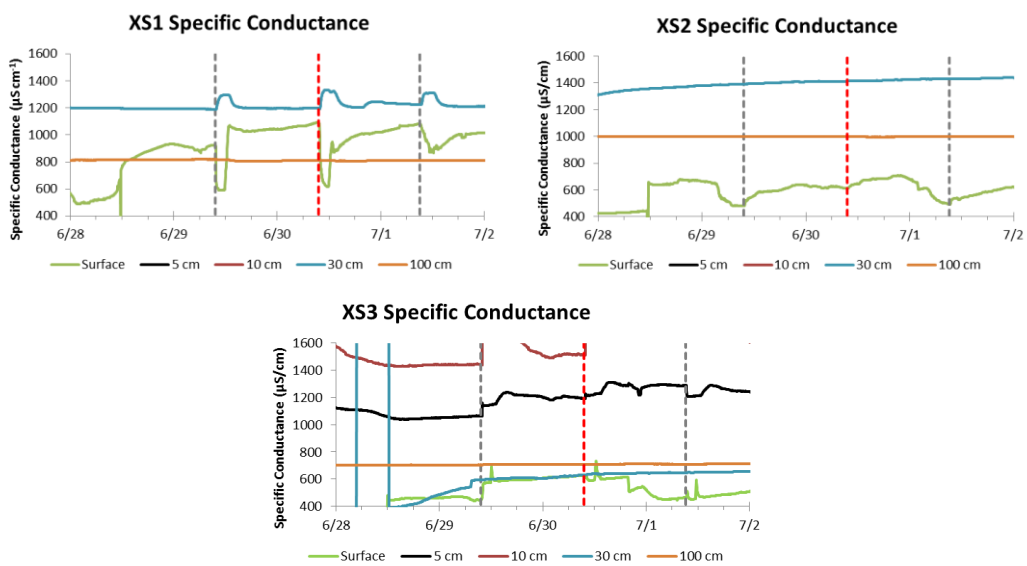

Figure 11. Specific conductance of summer flood with dashed lines indicating flood start times

The ponding was likely due to the increased vegetation that grew during the summer and reduced the overland speed of flow. The vegetation likely diverted the quicker flow away from the centerline and into the bypass where there was less thick vegetation. The Aqua TROLL®s were placed in the bypass and gave clear indication of the injections, confirming this hypothesis (Figure 12). The Aqua TROLL®s read only electrical conductivity and gave no reading when they were not submerged. The green line represents the injection and each of the days show a clear spike at the 2-hour mark. The one exception could be day two where a mishap with the outlet pipe caused the pump to shut off for 20 minutes and did not allow for the larger injection to travel through the floodplain. The small hump seen in the XS2 Aqua TROLL® was a natural storm event.

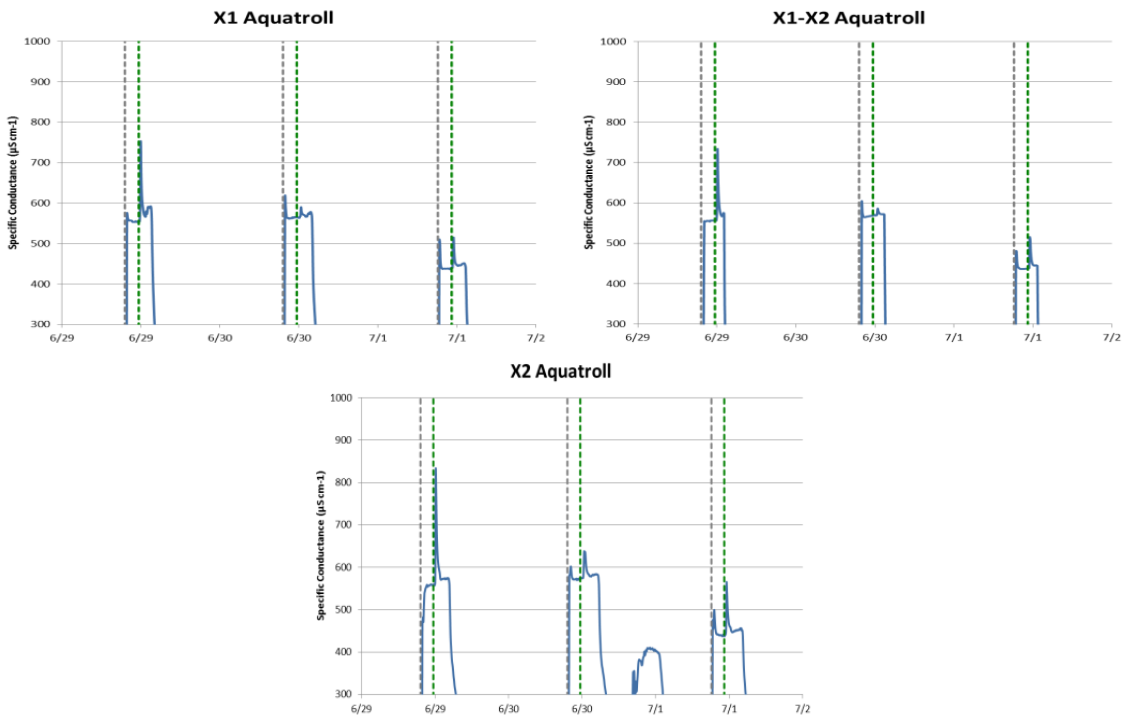

Figure 12. Specific conductance of Aqua TROLL®s during the summer flood

The vertical head gradients from the summer flood as seen in Figure 13 are very similar to the gradients occurring during the spring flood. This further reinforces that downward conditions are present at the cross sections. Along with the rest of the summer data it appears downward flow is occurring because the hydraulic conductivity is high at these locations. XS3 however does have losing conditions between the depths. The low hydraulic conductivity likely prevents flow from traveling downward near XS3. This data does suggest that SW-GW exchange is occurring in sections of the floodplains during both seasons.

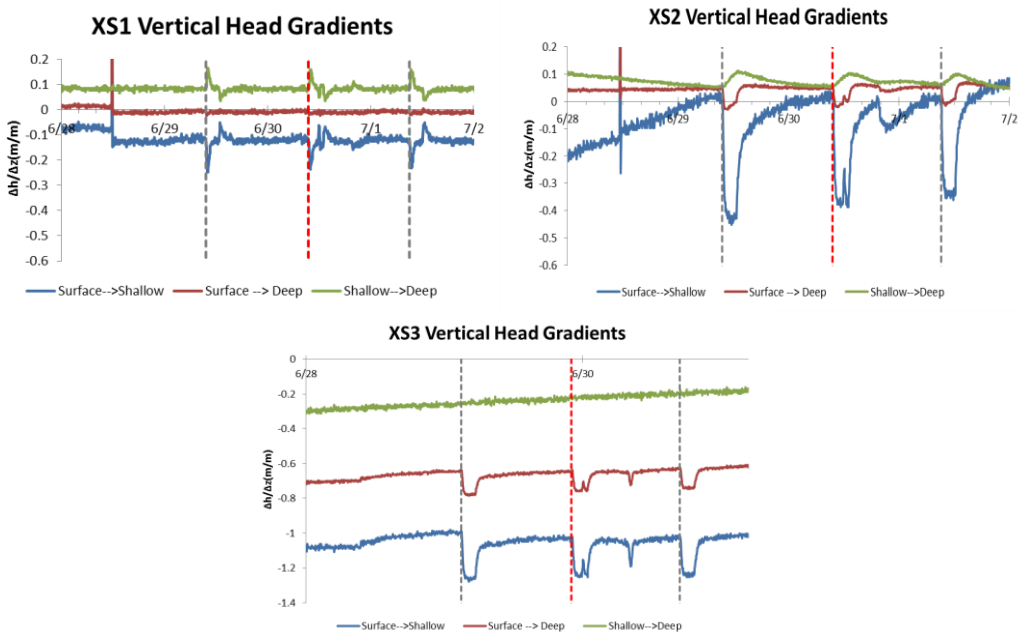

Figure 13. Vertical head gradients of during the summer flood with dashed lines indicating flood events

The storage volume that was achieved by the summer flood was quite close to the spring flood. The water loss was found to be approximately 4.8% between the water entering and leaving the floodplain. The storage volume peaked around 45 m^3, slightly lower than the spring flood. This is likely because of the lower inflow rate of the summer flood. After the flood the storage volume approached approximately 12 m^3. The water balance showed a percent difference of 4.8% compared to the 5.03% difference from the spring flood. The only major difference between the seasons is the sharp clear slope of the storage curve indicating that the surface water moved slower in the summer than during the spring, which can be attributed the increased vegetation. The steeper slope and low water velocity meant there was increased time for water to penetrate into the floodplain before exiting through the flume.

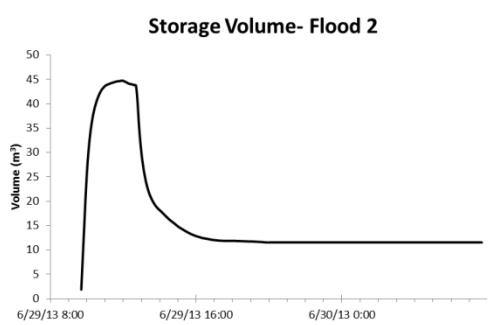

Figure 14. Transient storage achieved during the summer flood

Hydraulic Behavior

Surface water movement through the floodplain varied greatly at different locations. During flood events, water ponded at XS1 and XS2. This allowed for increased residence time and greater surface area inundation. XS3 consistently had moving flow, which allowed for decreased residence time in that part of the floodplain. The bypass allowed for some of the water to have reduced travel time over the surface and reach flume much quicker than water that travelled along the assumed centerline. The water covered the area shown in the Figure 14 at its peak. As seen below, some of the water travelled in overland flow and dispersed throughout the floodplain and was not recorded by the flume. Any of this water that did not pass through the flume was recorded as loss. This loss represented the surface water that did not travel through the flume or water that entered the groundwater.

The groundwater movement occurred in several flows in the floodplain. At XS1 groundwater flow rose in the form of a pressure wave increasing and decreasing. This was seen with all flood events, and does not indicate SW-GW exchange. There does appear to be sections of preferential flow occurring in the floodplain were peaks of electrical conductivity were seen at the shallow well in XS1. Once these preferential flows allowed the water to penetrate the subsurface it appears that the flows slowed and move with Darcy flow through the floodplain.

Figure 15. Outline of peak inundation during spring flood event

Both flood events yielded similar water elevations and electrical conductivity behavior. Water elevation responded almost the same to both the spring and summer, while electrical conductivity only showed slight differences. All of the cross-sections had a general trend in increasing temperature at all depths below the surface. This was expected but did not seem to influence the other factors between seasons. The major difference was the addition of vegetation on the floodplain. During the spring the floodplain was barren and had very little vegetation, which allowed the water to flow quicker over the floodplain with no impediments. During the summer flood the vegetation grew in thick throughout the floodplain and reached heights of approximately two meters. Figure 16 shows a comparison in vegetation surrounding the pump outlet during the spring and summer.

Figure 16. Spring vegetation (left) and summer vegetation (right) near pump outlet

The thick vegetation during the summer flood increased the manning's coefficient of the floodplain creating more friction to reduce the overland surface water velocity. This led to ponding in low-lying areas of the floodplain and longer residence time. Even though the ponding occurred, there was no increase in head loss between the seasons. As the antecedent moisture conditions of both floods were closely comparable this study gives strong evidence that seasonal change between spring and summer on this floodplain has little to no effect on hydraulics.

Conclusions

These preliminary results here suggest that SW-GW exchange is occurring in the shallow sub-surface of the Stroubles Creek floodplain during these artificial flood events. The data proves that there is a clear relationship between the floods and the subsurface of the floodplain. This can be seen in the specific conductance signal observed after the flood events in the shallow well at XS1 and the Aqua TROLL® and other surface LTCs. There is also a clear relationship between the change in groundwater water elevation and flood events in some of the stations. Variation in groundwater hydraulic response is likely due to the low permeability near XS3 and more permeable soil near XS1 and XS2. At this point all testing has been performed while the floodplain was in a saturated condition. Given different antecedent moisture conditions, hydraulic movement through the floodplain may provide different results.

Currently the seasonality does not appear to have as significant an impact on the SW-GW exchange as the antecedent moisture conditions. However, more data collection and further analysis is necessary to better classify the Stroubles Creek floodplain behavior and any kind of temporal variation.

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Estimating Coastal Flood Inundation Distances Using Digital Cameras

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ABSTRACT

After a coastal storm, inundation distance is commonly measured as an indicator of storm intensity. Our goal was to estimate this distance using rectified photographs instead of using survey equipment. Several camera properties were tested to determine their effect on horizontal distances estimated from the images. Lens distortion caused a 3% maximum error at the lowest zoom, whereas, autofocus and center of image had a negligible effect. The method was tested at Duck, NC, where coastal features were measured using the images with an accuracy of about 75%, indicating its usefulness during site visits.

Keywords: Digital Cameras, Inundation Distance, Rectification

Introduction

When a coastal storm event occurs, storm surge and waves with high energy cause water to inundate, or flood, the coastal area. The inundation distance is measured from the shoreline to the inland-most flooded point. This point can be found after a storm using the wrack line, which is a line of debris that has been pushed onto shore by the water. Inundation distance is a common measurement taken after a storm event because it can reveal the intensity of the storm and the energy carried by the waves. However, bulky survey equipment is needed to obtain these measurements, and there are often areas that are difficult or dangerous to access by foot. One way to overcome this problem is to collect data using remote sensing equipment such as video imagery.

Long-term video imaging and time lapses have been used in coastal areas to assess shoreline changes (Del Rio and Gracia, 2013), predict waves and currents (Allard et al., 2008), measure longshore currents (Chickadel et al., 2003), observe wave attenuation (Pereira et al., 2011), and assess post-storm shoreline recovery (Splinter et al., 2011). After using video imagery to assess wave attenuation due to muddy waters, Pereira et al., 2011 concluded that video imagery is an inexpensive and practical way to obtain near shore data about waves without using bulky, expensive survey equipment. However, video imagery cannot always be used due to the difficulty of permanently mounting a video camera in a coastal area. Also, in the event of a storm, a mounted camera is not likely to obtain useful images or it may not survive.

The use of digital cameras on the shoreline after a storm event could greatly reduce the need for bulky survey equipment to be lugged from place to place along the shore as well as decrease the time and cost of data collection. However, there are many parameters that must be considered when using digital cameras to obtain data. Extrinsic parameters include the location and orientation of the camera whereas intrinsic parameters include the camera's focal length, center of image, and optical distortion caused by the camera lens (Pawlowicz, 2003 and Holland et al., 1997). Holland et al., 1997 presents a camera calibration model that requires knowledge of camera position, focal length, optical distortion, image center, and as few as two surveyed points in an image. The model can then be used to rectify images, or to determine relative real-world locations of every pixel in the image.

In this paper, we hypothesize that images taken with a common digital camera and survey data collected by a handheld Global Positioning System (GPS) device can be used to estimate horizontal distances, such as inundation distance, observed within the images. Research methods are presented in in the following section and describe the methodology for testing camera properties such as center of image, optical distortion and autofocus as well as camera orientation and target layout. Results and Discussion describes the errors associated with the different camera properties as well as the limitations and usefulness of this method. Finally, the last section

includes concluding remarks about the usefulness and applications of this method as well as suggested further research.

Research Methods

To determine the effects of camera settings and parameters on object locations within an image, photographs were rectified, or converted from an image in pixel coordinates to an image in real-world coordinates with units of meters. First, Direct Linear Transformation (DLT) coefficients, which are a set of 11 idealized coefficients that relate pixel coordinates in an image to their real world locations, were calculated. This was done using the Field Calibration method described in Holland et al., 1997, which uses intrinsic camera properties and real-world locations of the camera position and ground control points. Then, the DLT coefficients were applied to the original image to obtain the real-world values of each pixel. To do this, a spatial constraint was implemented in the real-world coordinates, as described in Holland et al., 1997, so that the underdetermined system became one that is easily solvable.

The DLT coefficients were determined using targets made out of colored construction paper arranged in a rectangular formation or a semi-linear formation aligned with the camera's line of view. The rectangular formation is shown in Figure 14 (a). Although two is the minimum number of points required to use this method, six targets were used to improve the spatial accuracy of the rectified images. Next, target and camera locations were measured from a control point on the ground and pixel coordinates were obtained for the center of each target. Using this data, the DLT coefficients were calculated and applied to the original image to obtain the rectified image in Figure 14 (b). From this image, the dimensions of the targets can be measured and compared to their actual dimensions.

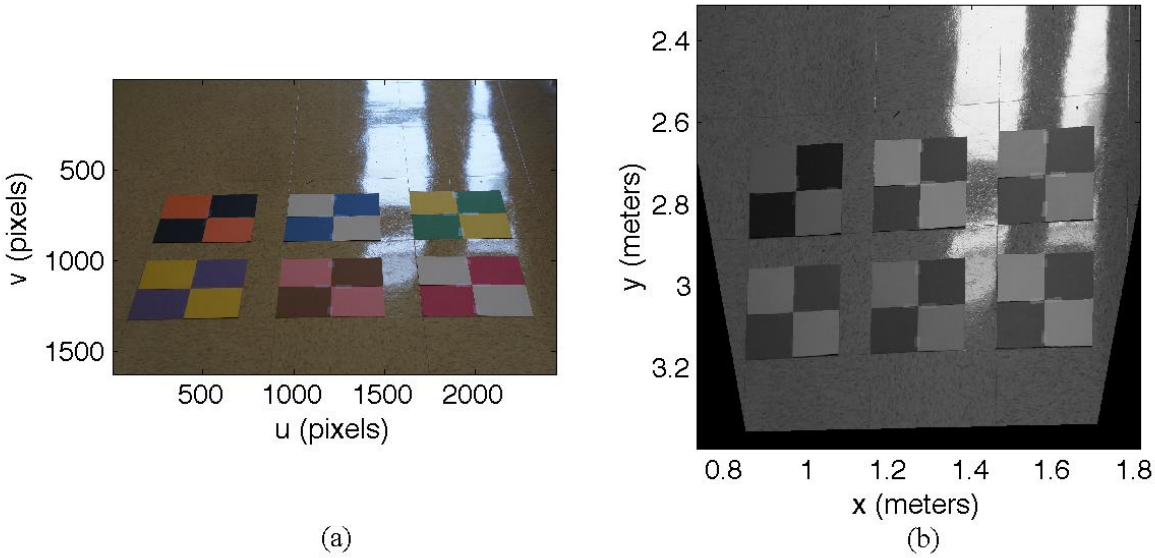


Figure 14. Six targets arranged in a rectangular formation (a) and the corresponding rectified image (b).

By comparing the estimated locations of objects within the rectified image to their actual locations, the effects of center of image shifts, distortion corrections, autofocus, camera position, and target layout on object locations were determined. All experiments were performed using a Sony NEX-5N handheld digital camera.

Center of Image Shifts

The center of image, which is not usually the center pixel of an image, is determined experimentally (Willson and Shafer, 1994). First, the camera, which was set to 55mm zoom, was mounted on a balanced tripod so that the camera's image sensor was approximately parallel to a flat surface. Secondly, string was strung from one corner of the image to the opposite corner as shown in Figure 15. Finally, the center of image was the pixel coordinate at the center of the "X".

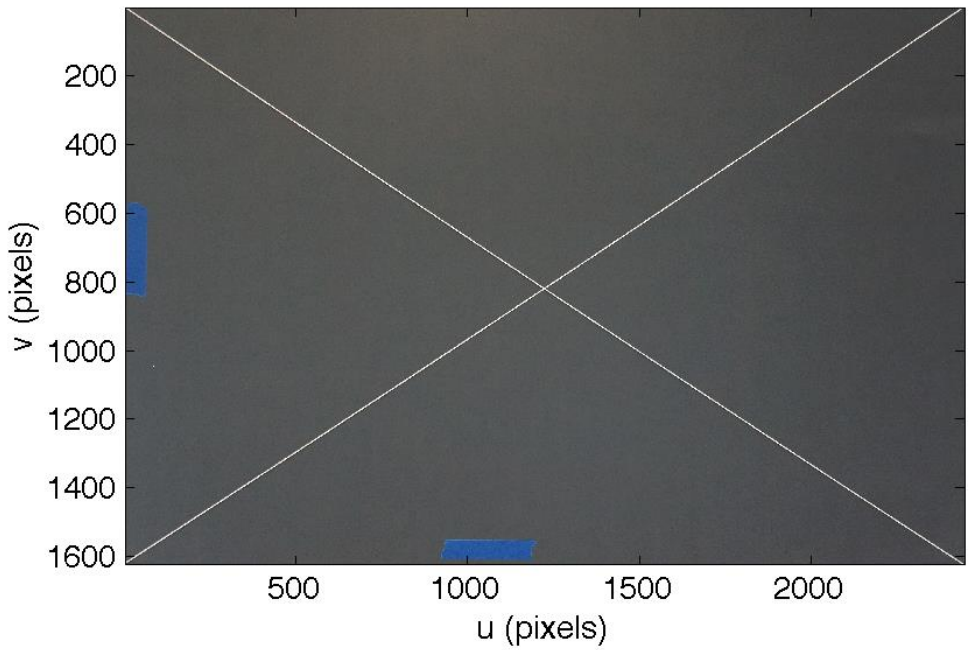


Figure 15. "X" formed by intersecting string at image center to find the center of image.

Due to the limited size of the camera's viewfinder, we used a small (2448 x 1624 pixels) image size because it was almost entirely viewable on the display. The strings were then adjusted by looking at the image in playback mode on the camera and zooming in on the corners. Based on the zoomed in image, the strings were adjusted at the corners. Since the strings could not be adjusted to intersect the image corners perfectly, the corners were classified as "on", "slightly off", or "off", where "on" indicates a visually perfect intersection (offset by less than 3 pixels), "slightly off" indicates a nearly perfect intersection (offset by 3 to 6 pixels), and "off" indicates a visually imperfect intersection (offset by more than 6 pixels). Table 4 lists the center of image of those images with no corners classified as off and two or more corners classified as on. The values for the horizontal (u) and vertical (v) pixel coordinates at the center of the most accurate images were averaged to be 1225 x 820 pixels from the top left corner of the image.

Table 4. Center of images with no corners classified as off and two or more corners classified as on, and the average of theses center of images.

Image Number	u Center of Image	v Center of Image	Top Left Corner	Bottom Left Corner	Bottom Right Corner	Top Right Corner
2706	1223.5	819.4	on	slightly off	on	slightly off
2708	1223.3	819.0	on	slightly off	on	slightly off
2709	1223.7	820.2	on	slightly off	on	slightly off
2711	1227.7	821.1	on	on	slightly off	slightly off
Average	1224.6	819.9				

Optical Distortion

Optical distortion is caused by the camera lens and is most prevalent along the outer edges of an image. Two types of optical distortion are pincushion distortion and barrel distortion. Pincushion distortion causes the image to be pinched toward the center and commonly occurs at high levels of zoom (Weng et al., 1992). Barrel distortion causes the image to be inflated and commonly occurs at low levels of zoom (Weng et al., 1992).

The method used to determine lens distortion was developed by Holland et al., 1997. We used an image taken of regularly spaced circles positioned in front of the camera, as shown in Figure 16, such that the focal

plane was approximately parallel to the paper. The positions of the circle centers in the picture were then compared to the circle centers on the paper. The distortion, Δr, was calculated using

$$\Delta r = k_1 r^3 + k_2 r \tag{1}$$

where, r is the distance of each pixel from the image center and the distortion coefficients, k_1 and k_2 are determined by fitting Equation (1) to r and Δr data. The picture was then corrected using distortion coefficients to eliminate pincushion and barrel distortion.

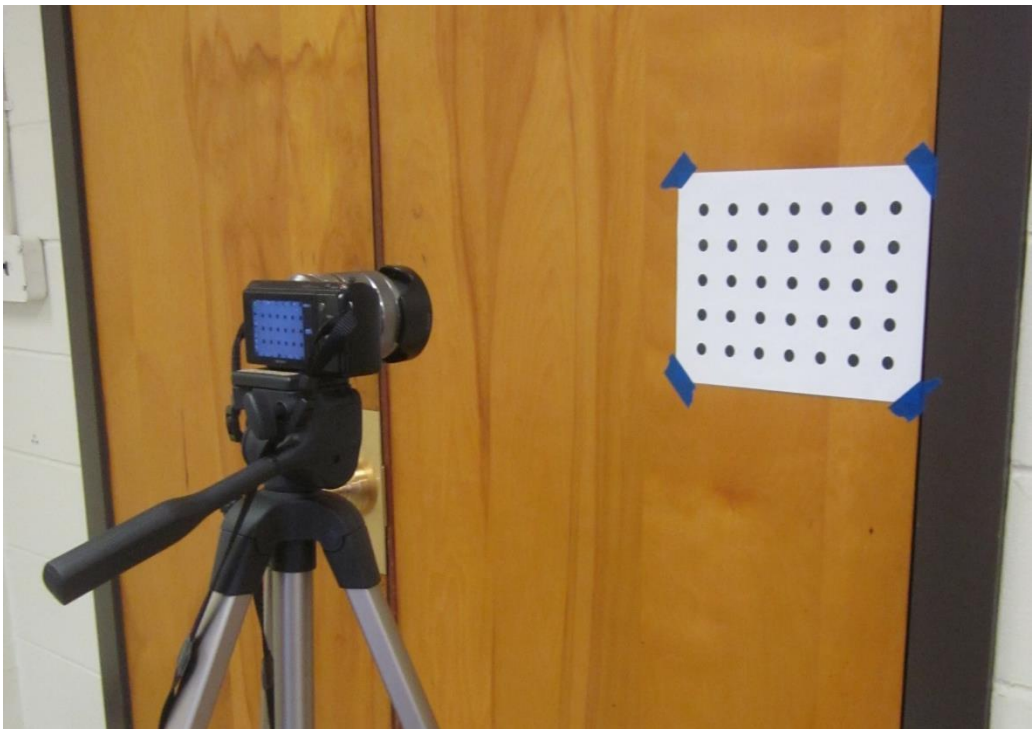
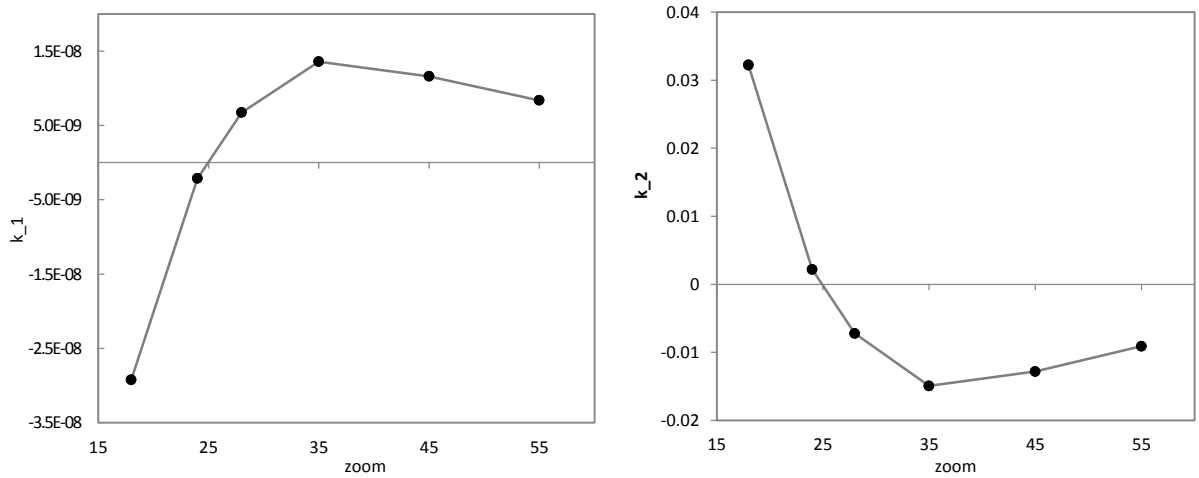


Figure 16. Camera set up with focal plane parallel to test paper containing regularly spaced black circles on a white background.

Different distortion coefficients were produced at different levels of zoom. To account for this without testing every zoom value, we obtained the distortion coefficients for six different zoom values on the camera (Table 5) and linearly interpolated between them to get the intermediate values (Figure 17).

Table 5. Distortion coefficient values at six different zoom levels.

Image Number	Zoom (mm)	k_1	k_2
3335	55	8.37E-09	-0.0091
3341	45	1.16E-08	-0.0128
3343	35	1.36E-08	-0.0149
3349	28	6.74E-09	-0.0072
3351	24	-2.12E-09	0.0022
3359	18	-2.92E-08	0.0322



(a) (b)

Figure 17. Interpolation of (a) distortion coefficient k_1 and (b) distortion coefficient k_2 at six different zoom values: 18, 24, 28, 35, 45, and 55 mm.

The Sony NEX-5N has a built-in setting that will automatically correct for distortion. Although this setting decreased the amount of distortion in the image, it did not correct for all of it. Based on this, we decided that the use of the distortion correction setting within the camera was not accurate enough for the purpose of this experiment.

Auto Focus

The effects of auto focus on object locations within the image were tested in the context of the distortion coefficient tests. All tests were zoomed to 55mm and used auto focus or manual focus. Two different camera settings were used: intelligent auto which automatically identifies characteristics of the scene, and program auto which allows the user to manually adjust certain settings, such as auto focus location. As shown in Table 6, the distortion coefficients were nearly identical, with standard deviations of 2.15E-11 and 1.81E-18 for k_1 and k_2, respectively.

Table 6. Distortion coefficients testing auto focus at full zoom under several different camera settings.

Image Number	k_1	k_2	Zoom	Focus	Focus Location	Camera Setting
2744	8.7865E-09	-0.0094	55mm	auto		intelligent auto
2746	8.7500E-09	-0.0094	55mm	manual	center	intelligent auto
2748	8.7661E-09	-0.0094	55mm	auto	center	program auto
2750	8.7755E-09	-0.0094	55mm	auto	multi	program auto
2752	8.7330E-09	-0.0094	55mm	auto	flex (top right)	program auto
2754	8.8036E-09	-0.0094	55mm	manual	center	program auto
Standard Deviation	2.15E-11	1.81E-18				

Camera Position and Target Layout

Spatial Resolution and Camera Orientation. On July 3, 2013, Blacksburg, VA was hit with a flash flood event causing the Duck Pond and Stroubles Creek to flood. A wrack line was observed on the south side of Stroubles Creek leaving the Duck Pond. On July 9, 2013 pictures were taken and analyzed using a gridded twelve-foot by six-foot area with one foot spacing. We also placed two targets at 8 feet and 16 feet from the end of the gridded area to test the spatial resolution. To test camera orientation's effect on rectified images, the camera was oriented in the direction of the grid, Figure 18 (a), and slightly counterclockwise of the grid. To test the accuracy of the rectification algorithm, we analyzed the dimensions of a close one-foot by one-foot square and a far one-foot by one-foot square of the grid, as well as the distance between the two targets and between the targets and the grid edge as shown in **Error! Reference source not found.** (b).

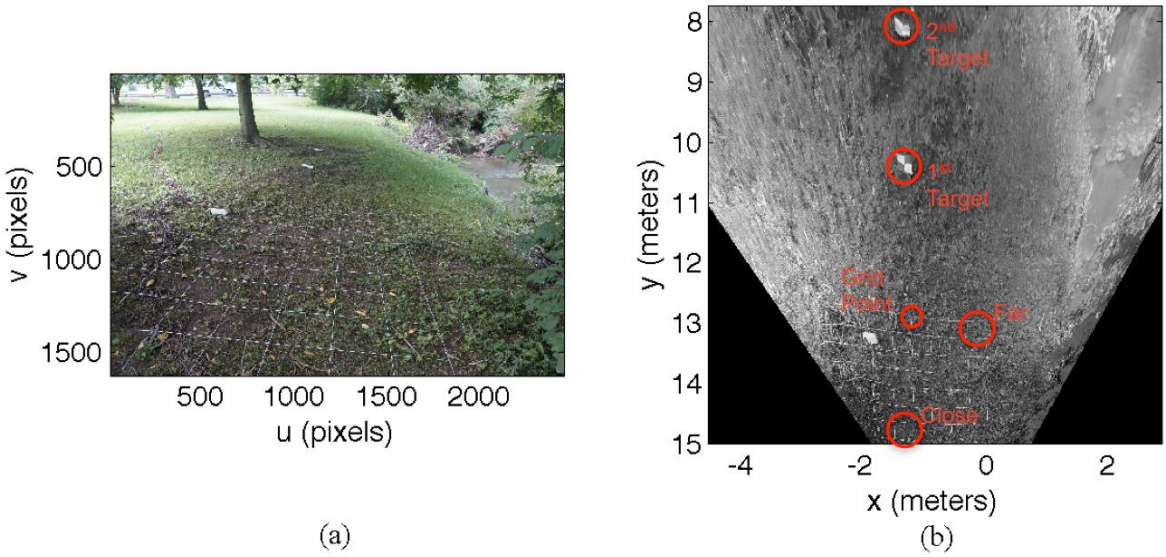


Figure 18. Picture taken near Stroubles Creek looking directly at the grid (a) and the corresponding rectified image (b) with points used for error calculations circled.

Target Layout. To evaluate the effects of target layout, we used a handheld GPS in the Drill Field at the Virginia Tech campus in Blacksburg, VA. The handheld GPS used is a Garmin eTrex 30, which generally had an accuracy of 3 m. We set up the camera at the north end of the Drill Field, across the street from Patton Hall, facing the sidewalk path intersection in the middle of the Drill Field. In the pictures taken, 4 to 6 light poles, a manhole, and 3 corners of the sidewalk path intersection were visible, as indicated by the red dots in Figure 19, all of which were used as known locations measured by the GPS.

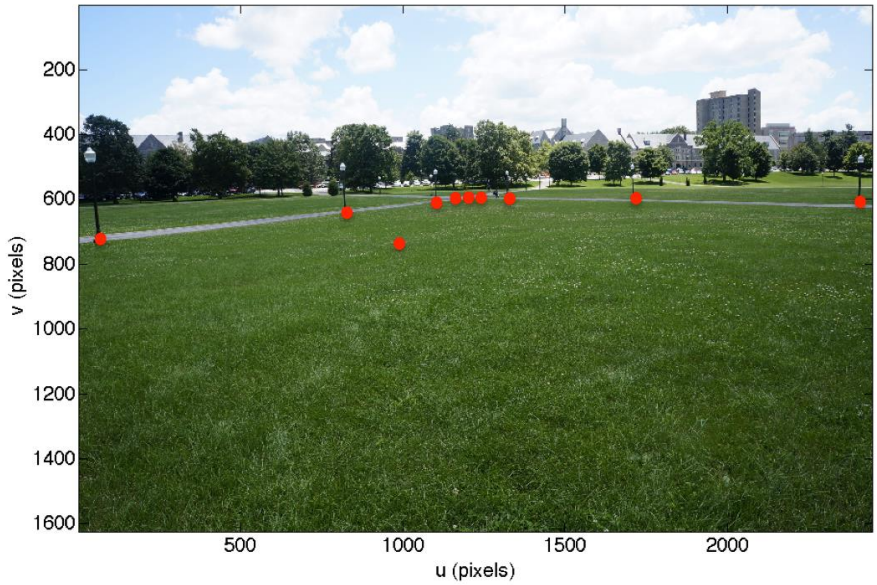


Figure 19. Original picture of drill field with ground control points marked with red dots.

Usefulness of Method

In order to determine the usefulness of the method for site visits, we collected data at The US Army Corps of Engineers Field Research Facility (FRF) in Duck, NC on July 17, 2013. Pictures and GPS data were taken at three different locations within the facility. These locations include: i) the front of the gazebo on the north side of the entrance drive where we used 9 reflective circles on the south side of the gazebo as our known data points, ii) the FRF tower looking east down the pier where we used five signs and two life preservers on the side of the pier as our known data points, and iii) the FRF tower looking north down the shoreline where we used two scraps of wood paneling on the beach, a small red flag at the base of the dune face, three wooden circular signs at the top of the dune, and a white building at the top of the dune as our known data points. The accuracy of the rectified images was analyzed by comparing the distances between points in the rectified images to GPS data.

Results and Discussion

To test the accuracy of the rectified images, we compared the horizontal distances estimated from the rectified images to known measured distances. Percent error was calculated as

$$Percent\ Error = \frac{Rectified\ Dimension - Real\ World\ Dimension}{Real\ World\ Dimension} * 100\% \qquad (2)$$

to compare all dimensions in rectified images with their real-world dimensions. The effects of autofocus, camera position, and target layout on object locations were determined.

Center of Image and Distortion

To test the effects of distortion and center of image on object dimensions in rectified images, four sets of images were rectified and compared: i) no distortion correction and no center of image shift (1224 x 812 pixels), ii) distortion correction, but no center of image shift, iii) no distortion correction but with the center of image set as 1225 x 820 pixels, and iv) distortion correction and the center of image set as 1225 x 820 pixels.

The center of image had little to no effect on object dimensions in the rectified images, but was found to vary slightly at different zoom levels. Table 7 shows the percent errors calculated at two zooms (18mm and 55mm) using the width (x) and depth (y) of two targets (Front and Back), which are circled in Figure 20. At no zoom, which is 18mm, the center of image induced a decrease in error of 0.2% in the x direction, but had no error difference in the y direction. At full zoom, which is 55mm, the center of image induced an increase in error of 0.2% in the x direction, but had no error difference in the y direction. This is most likely because the center of

image is only one pixel different in the x direction and 8 pixels different in the y direction from the center pixel of the image. These are both less than a 0.1% difference in position.

Table 7. Error differences from the use of the center pixel versus the center of image for DLT and rectification. Negative percent error indicates that the distance in the rectified image was smaller than the real-world distance.

Center	Zoom	Back x	Back y	Front x	Front y
Center Pixel	18	7.84%	5.66%	7.40%	3.90%
Center of Image	18	7.62%	5.66%	7.19%	3.90%
Center Pixel	55	-0.80%	-3.74%	-0.38%	-2.00%
Center of Image	55	-1.01%	-3.74%	-0.60%	-2.00%

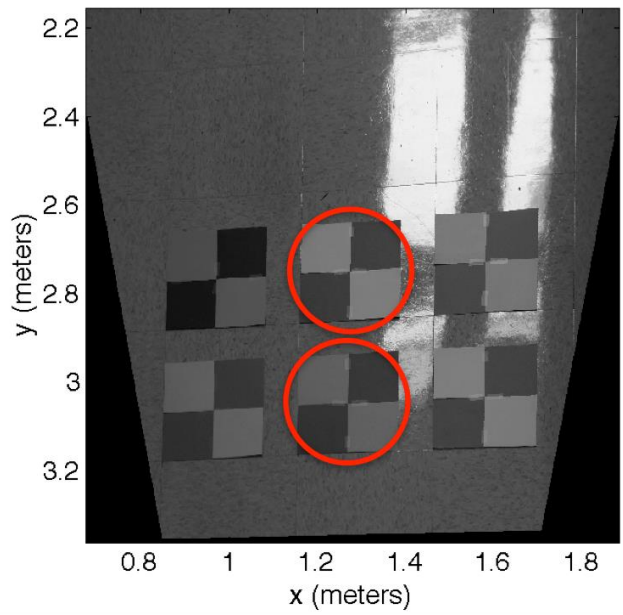


Figure 20. Rectified image used for center of image with front and back canter targets circled.

The distortion coefficients had a large effect on the accuracy of the image rectification at certain levels of zoom as shown in Figure 21. The distortion coefficients reduced the error in the rectified images by an average of 2.8% at 18mm zoom (corresponding to 0.256 inches), 0.4% at 23mm zoom (corresponding to 0.034 inches), 0.3% at 45mm zoom (corresponding to 0.030 inches), and 0.5% at 55mm zoom (corresponding to 0.040 inches). However, the distortion coefficients at 27mm and 35mm zoom had a negative effect on the accuracy in the rectified image, resulting in an average increased error of 0.3% (corresponding to 0.034 inches) and 0.8% (corresponding to 0.024 inches), respectively.

Wang et al., 2008 report that there are many other types of distortion, such as decentering and thin prism distortion, that our method does not correct. These additional sources of distortion may have a greater effect at 23, 27, and 55 mm zooms, since the percent error is relatively large (greater than 2%) even after distortion has been corrected. At 35 and 45 mm zoom, the percent errors are relatively small (less than 2%) prior to distortion correction, so the changes in errors taken at these zooms are negligible. Although the error in images at 18 mm zoom is decreased significantly after distortion correction, the remaining error could also be associated with additional sources of distortion.

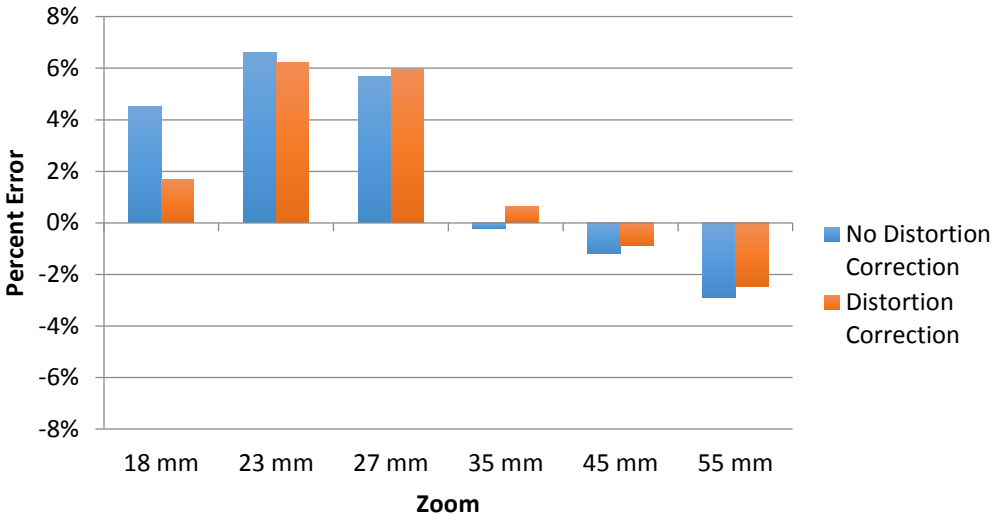


Figure 21. Errors associated with the use and non-use of distortion coefficients in the rectification of images at different zoom values. Negative percent error indicates that the distance in the rectified image was smaller than the real-world distance.

Spatial Resolution and Camera Orientation

Two sets of images using 18mm zoom were taken at Stroubles Creek. Errors were measured in the x direction looking at the close grid box and the far grid box, as circled in Figure 18. Errors were measured in the Y direction looking at the close grid box, the far grid box, as well as the distance from the grid point to the first target, between the two targets, and from the grid point to the second target, as circled in Figure 18. For the first set, the camera was angled so that the view was nearly inline with the grid. The percent errors for this view were all less than 10%, as shown in blue in Figure 22. The second set of images was taken with the camera's azimuth oriented slightly counterclockwise from the grid, causing the view to be nearly inline with the wrack line. The red bars in Figure 22 show that this camera orientation produced errors ranging from about 3% to 18%. Also, the errors are generally negative indicating the rectified images are under-estimating the distances.

These differences in accuracy may be associated with the algorithm's ability to predict real-world locations along the edges of the photographs, causing greater error in the distance at larger camera azimuth. Also, Holland et al., 1997 states that incorrect elevation in the real-world coordinates can cause errors in horizontal positions. These errors scale proportional to the difference between the correct and incorrect elevations times the tangent of the camera tilt angle. In the case of Stroubles Creek, all elevations were set to zero in the DLT and rectification algorithms. However, the ground sloped down from the left side of the image to the right, as seen in Figure 18. The errors shown in Figure 22 are most likely induced by incorrect elevation values. It is more pronounced when the camera is rotated toward the wrack line because the slope of the ground in the picture becomes more pronounced.

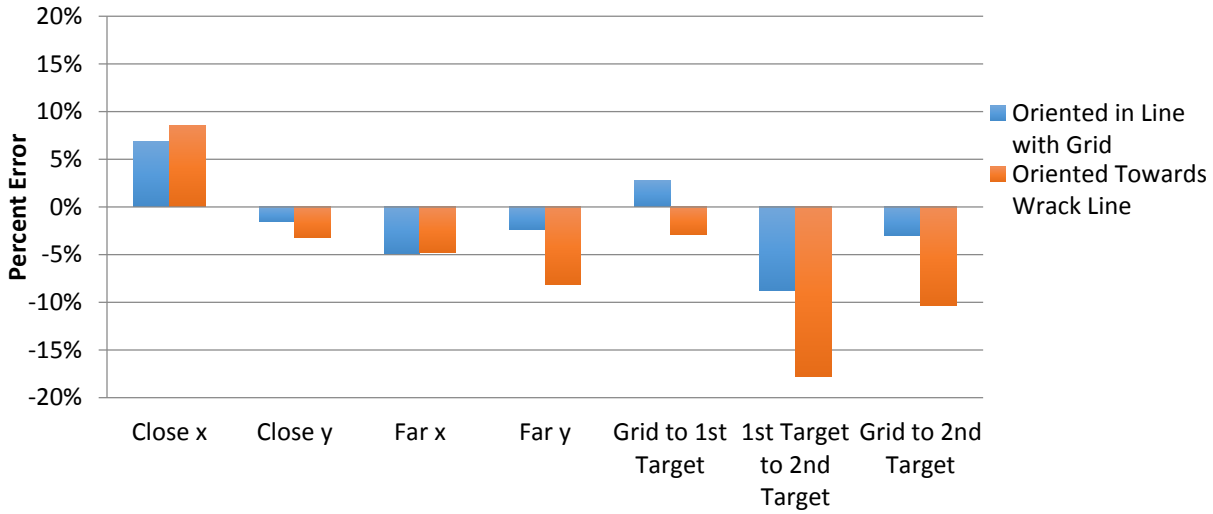


Figure 22. Percent errors associated with two one-foot by one-foot squares in gridded area at Stroubles Creek and grid edge to targets. All rectifications were performed using 6 points in the image. Negative percent error indicates that the distance in the rectified image was smaller than the real-world distance.

Usefulness of Method and Limitations

The pictures taken from the tower at The US Army Corps of Engineers FRF looking north down the shoreline produced rectified images, but they were very skewed. When the picture from the tower at FRF looking north down the shoreline (Figure 23 (a)) was rectified (Figure 23 (b)) little to nothing was even identifiable in the image. We believe this is because all of the points used to rectify the images are nearly inline with the camera view, so there is not enough data in the x direction for the DLT program to produce accurate coefficients. Similarly, pictures taken in the Drill Field used points that appeared in a relatively straight line perpendicular to the camera view, as shown in Figure 19, so a rectified image could not be obtained. We believe that this is because the DLT program did not have enough data in the y direction to produce accurate coefficients.

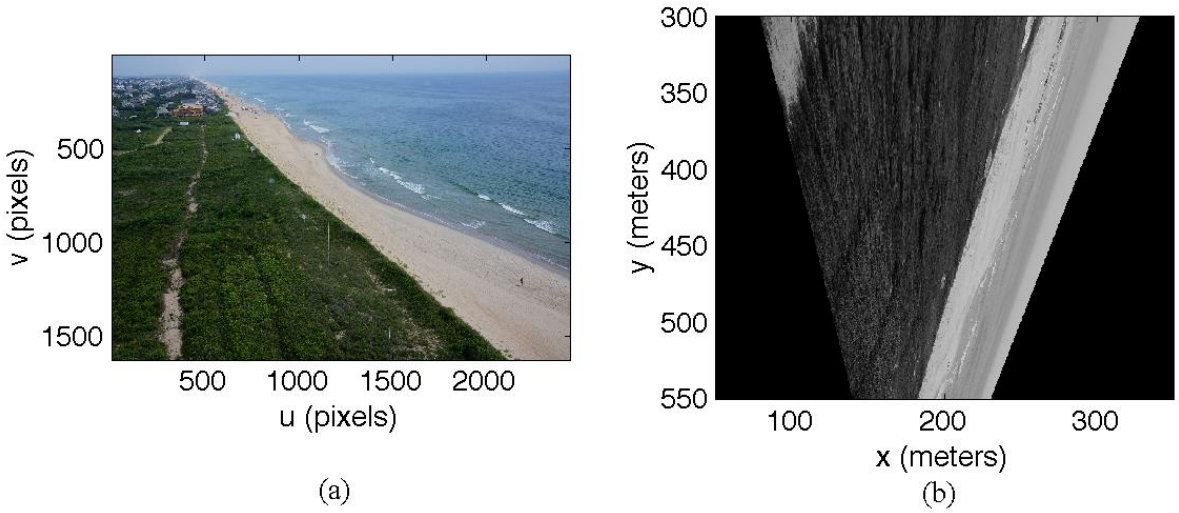


Figure 23. Original image taken from the tower at The Army Corps of Engineers FRF looking north down the shoreline (a) and the corresponding rectified image (b).

The pictures taken at the gazebo at The US Army Corps of Engineers FRF could not be rectified. This may be because of the large differences in elevation between the points, since the method requires the implementation of a spatial constraint so that the three-dimensional real-world objects can be related to the two dimensional picture. The algorithm used to find the DLT coefficients was written for the use of a single-value

constraint, meaning that the elevation of all the ground control points used should be the same. Therefore, this method is clearly not applicable when the relative elevations of the points vary greatly.

However, this method is useful when accurate, random, non-collinear data points can be surveyed within the camera view. For instance, Figure 24 (a) was rectified, as shown in Figure 24 (b) to obtain the length of the pier in the image. The actual length of the entire pier is 1840 feet. The estimated length of the pier within the image was 1760 feet. Based on the rectified image the length of the pier within the image was estimated to be about 2123 feet with accuracy of about 80%. Additional known locations within the rectified image were measured, such as the distance between the pier pillars, with errors generally less than 25% except at the edges of the image where errors increased to about 50%. Therefore, it can be estimated that the boat in the image is 1059 feet from the end of the pier with at least 75% accuracy.

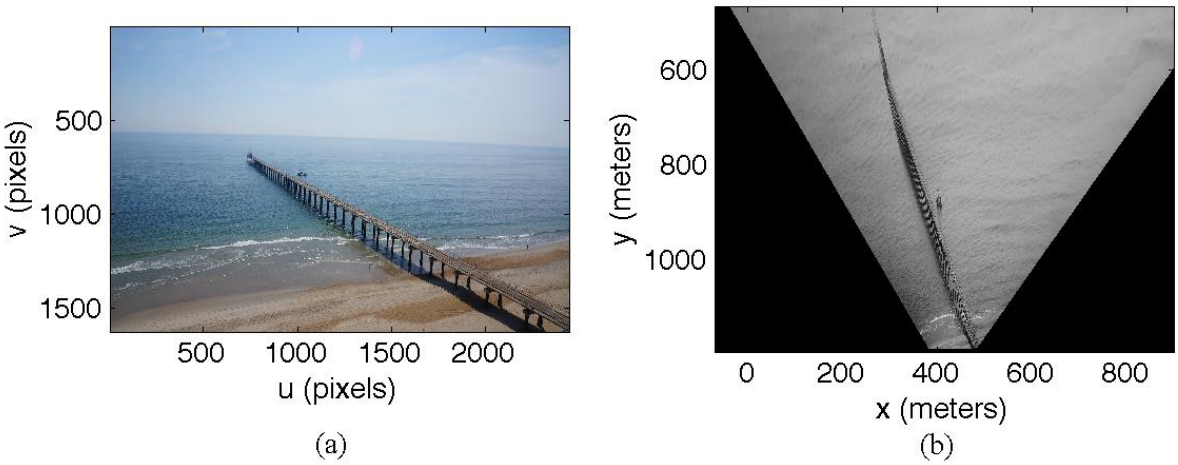


Figure 24. Original image of The Army Corps of Engineers FRF pier from the tower (a). Rectified image of The Army Corps of Engineers FRF pier (b).

Conclusion

A method has been developed and tested to use handheld digital cameras to estimate distances within an image. Center of image and optical distortion were tested to enhance the accuracy of our estimations based on rectified images. The effects of focal length, auto focus, camera orientation, and ground control point layout on the accuracy of our estimations were also analyzed.

It was determined that the center of image and auto focus had little effect on the accuracy of the rectified images. Optical distortion was found to have the most effect at no zoom, or 18 mm zoom, decreasing the error in our estimations by 2.8%. Also, the focal length causes a high variance in the accuracy of the rectified images ranging from less than 1% to about 7%. We found that the camera's orientation may have an effect on the accuracy of the rectified image, where an orientation in line with the survey area gives most accurate results. Therefore, we determined that the method is not effective if the ground control points are arranged in line with the camera's view or perpendicular to the camera's view.

We suggest further research on the center of image at different levels of zoom, correcting for other types of distortion, and looking closer into the effects of camera orientation. Also, the use of different numbers of ground control points to calculate DLT coefficients may be beneficial since we used six or more points for all of our testing. Lastly, this method should be tested with different types of cameras, and a similar method should be developed to use two different camera positions to obtain elevation as well as horizontal distances.

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Analysis of Benthic Macroinvertebrate Density and Distribution in Stroubles Creek

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ABSTRACT

Stroubles Creek, a third order stream in Blacksburg, Virginia is classified as impaired by the Virginia Department of Environmental Quality based on a benthic macroinvertebrate assessment. Because these organisms have diverse responses to environmental stressors and their communities reflect environmental conditions over time, benthic macroinvertebrate assessments provide information about stream quality. The Biological Systems Engineering Department at Virginia Tech recently instituted a stream restoration project on a 1.3-mile section of Stroubles Creek. One goal of the BSE project is to improve the aquatic habitat of the stream to support benthic macroinvertebrate diversity. My research evaluated the density and distribution of benthic macroinvertebrates in Stroubles Creek, used this data to assess the effectiveness of the stream restoration area, and compared benthic macroinvertebrate diversity in Stroubles Creek to that of Toms Creek. The macroinvertebrate data indicate continued impairment of Stroubles Creek. However, the extent of impairment found in Stroubles Creek was not uniform.

Keywords: water quality, benthic macroinvertebrate, biomonitoring, stream restoration

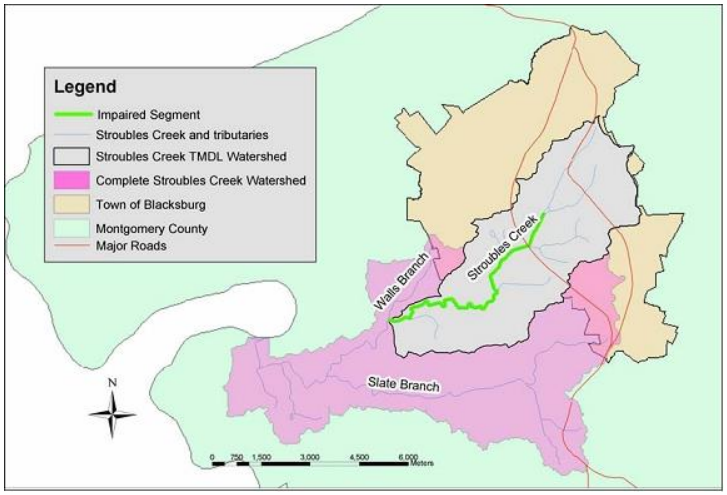
Introduction

In the State of Virginia over 2,000 miles of rivers and streams are classified as impaired based on benthic macroinvertebrate assessment (Virginia DEQ 2012a). According to the Virginia Department of Environmental Quality (VDEQ), a waterway is considered impaired if it contains one or more contaminants that exceed water quality standards and inhibit the body of water from serving in one or more of the six designated uses of waterways. These uses are aquatic life, fish consumption, public water supplies, recreation, shell fishing, and wildlife (VDEQ 2012a).

Stroubles Creek is one stream that is classified as impaired by the Virginia DEQ. Stroubles Creek originates from multiple springs within the town limits of Blacksburg, Virginia. This third order tributary of the New River flows through the town of Blacksburg, the Virginia Tech campus, as well as agricultural and wooded areas in Montgomery County (Parce et al. 2010).

Significant anthropogenic changes to Stroubles Creek have occurred since the area was first settled. In 1937, a portion of Stroubles Creek was directed underground to allow for the expansion of the Virginia Tech Drillfield (Parece et al. 2010). Additionally, a dam was constructed at the convergence of Webb Branch and main branch of Stroubles Creek to create the Duck Pond on the Virginia Tech campus. Population growth and urbanization in the Town of Blacksburg, specifically the increase in impervious surface and storm water run off, have contributed to the degradation of stream quality (Parece et al. 2010). The increase in impervious surface and storm water runoff has caused increase water flow in Stroubles Creek during wet weather periods, resulting in stream bank erosion and increased sedimentation (Paul & Meyer 2001).

In 1996, Stroubles Creek was first put on the Virginia Impaired Waterways List by the VDEQ (Parece et al. 2010). In 2003, sedimentation was identified as the primary stressor causing impairment in Stroubles Creek (VDEQ & DCR 2006). In 2012, the VDEQ studied two sections of Stroubles Creek. A 2.1-mile section of Stroubles Creek from the Slate Branch mouth to the Walls Branch mouth was classified as impaired under the recreation designation due to *E. coli* contamination (VDEQ 2012b). A 5-mile section of Stroubles Creek from the Walls Branch mouth to the Duck Pond (Figure 1) was classified as impaired under the recreation designation and



aquatic life designation do to *E. coli* contamination and poor benthic macroinvertebrate bioassessments (VDEQ 2012b).

Figure 1. Map of Stroubles Creek Watershed showing impaired region based on macroinvertebrate assessments (Center for TMDL and Watershed Studies).

Benthic macroinvertebrates are defined as bottom dwelling aquatic animals that can be seen with the naked eye (Hauer & Lamberti, 1996 339). This diverse group includes aquatic insects, crustaceans, worms, mollusks, and others. Benthic macroinvertebrates play an integral role in nutrient cycling, decomposition, and nutrient translocation in aquatic ecosystems (Wallace & Webster 1996). Macroinvertebrates have a diverse response to environmental stresses and communities of benthic macroinvertebrates serve as a reflection of environmental conditions overtime; therefore, the presence, absence, and relative abundance of benthic macroinvertebrates can be used to assess stream quality (Barbour et al. 1999).

In response to the impaired classification of Stroubles Creek, the department of Biological Systems Engineering (BSE) at Virginia Tech instituted a stream research area on a 1.3-mile section of Stroubles Creek (BSE VT 2013). The overall goal of the restoration project is to remove Stroubles Creek from the Clean Water Act List of Impaired Streams. Furthermore, objectives of the project include improving aquatic habitat, reducing sediment and bacteria loading, and assessing implemented methods of stream restoration (BSE VT 2013).

In light of Stroubles Creek impairment based on benthic macroinvertebrate assessment and the recent instillation of a stream restoration area on a portion of the stream, this research will focus on assessing stream quality through benthic macroinvertebrate characteristics in Stroubles Creek. The objectives of this research were to: (1) evaluate the density and diversity of macroinvertebrates in Stroubles Creek, (2) use these data to assess the effectiveness of the BSE stream restoration effort, (3) compare macroinvertebrate density and diversity in Stroubles Creek with that in Toms Creek, and (4) analyze the relationship between streambed substrate composition and stream quality. The objectives were achieved through the analysis of qualitative and quantitative macroinvertebrate samples, analysis of streambed substrate size, and qualitative assessment of aquatic and riparian habitat.

Research Methods

Field Procedure

Two streams in the Blacksburg area, Stroubles Creek and Toms Creek, were examined in this study as a means to assess the effectiveness of the BSE stream restoration area and compare the benthic macroinvertebrate health of the two streams. There were 13 total sample sites located in Blacksburg and Montgomery County (Table 1).

Table 1. Research sample site names and description.

	Site Name	Site Description
T1	Toms Creek Deerfield	Stream with tall grass, shrubs, and some overhanging trees.
T2	Toms Creek Lower	Wide stream in forested area with some overhanging vegetation.
W1	Webb Branch Lucas Drive	Narrow stream in forested area with overhanging vegetation.
S1	Stroubles Fire Station	Narrow stream with "stabilized banks" and some forest upstream.
W2	Webb Branch Surge Building	Narrow stream with grassy banks and spars birch and willow trees.
W3	Webb Branch Emerging from Derring Parking Lot	Stream with open ground cover and grassy banks.
S2	Stroubles Emerging from Drillfield	Stream with open ground cover and grassy banks.
S3	Stroubles Parking Lot Oak Lane	Stream in forested area with overhanging vegetation.
S4	Stroubles Restoration Area Bridge One	Stream with steep banks and tall grass and shrub ground cover.
S5	Stroubles Restoration Area Bridge Two	Stream with tall grass and shrub ground cover.
S6	Stroubles Restoration Area Bridge Three	Stream with steep banks and tall grass and shrub ground cover.
S7	Stroubles Merrimac	Stream with forested land cover, tall grass, and shrubs.
S8	Stroubles 705	Stream in forested area with some overhanging vegetation.

Benthic macroinvertebrate samples were collected from six sites upstream of the restoration area, three sites in the restoration area, two sites downstream of the restoration area, and two sites in Toms Creek. Qualitative sampling was used for samples sites W1, W2, and W3 and quantitative sampling was used at the remaining sample sites (Figure 2).

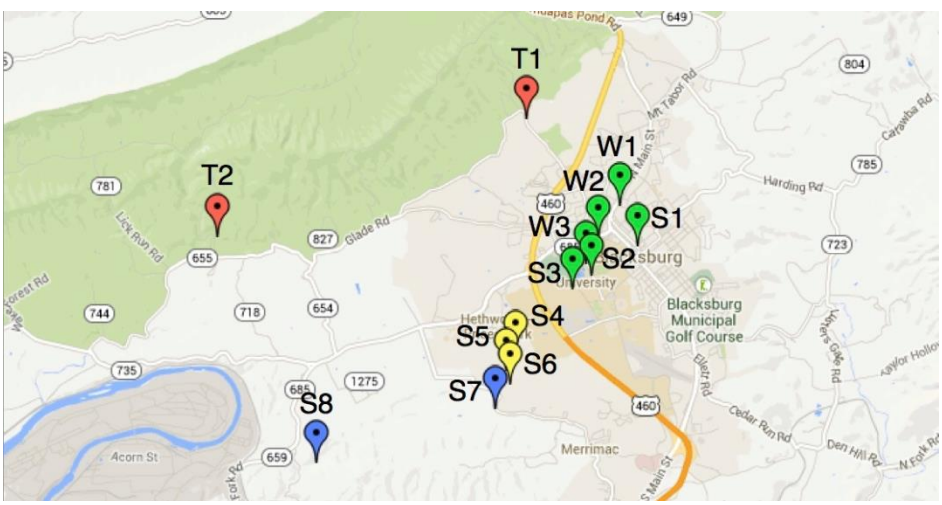


Figure 2. Map of 13 collection sites on Stroubles and Toms Creek. Red sites are located on Toms Creek, green sites are located upstream of the restoration area, yellow sites are located in the restoration area, and blue sites are located down stream of the restoration area.

For the qualitative sampling procedure, two samples were collected from each site using a dip net. The dip net was used to collect the sample while simultaneously kicking for 10 minutes to disturb the stream area. For the quantitative sampling procedure, three samples were collected from each site using a D-frame net. The three samples were taken from lotic-erosional macro-habitats, commonly known as riffles. The sampling began downstream and proceeded upstream. The samples were collected for two minutes by disturbing the area within a

0.9x0.45 meter metal frame attached to the D-frame net. A 250-µm mesh net was used to collect all samples. Following sample collection, the benthic macroinvertebrates and any other organic or inorganic material found in the sample were rinsed at the stream side into Whirl-Pak ® bags and 95 percent ethanol was added to achieve an approximately 80 percent ethanol solution to preserve the samples. In addition to benthic macroinvertebrate samples, specific conductance and temperature were measured using meter. Specific conductance is a proxy measurement for total dissolved solids and can be an indicator of stream impairment (U.S. EPA 1997). Qualitative assessments of the riparian and aquatic environments were also recorded.

On a separate day of fieldwork, a USGS standard substrate analysis procedure was performed at each of the 13 sample sites (West Virginia DEP). A 25-meter stream reach was measured at each sample site. Particle sizes were collected by walking in a zigzag pattern and randomly selecting a pebble every two steps. A gravelometer was used to measure the particles (Figure 3). For particles too large to be measured with the gravelometer, the shortest axis was measured. Approximately 100 particles were measured from each sample site. Furthermore, recent flow depth data of Stroubles Creek provided by the StREAM lab of Virginia Tech was assessed to choose a sampling day in which recent storms did not result in rising flows.

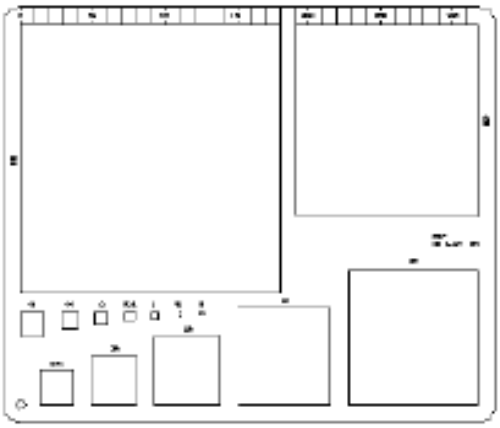


Figure 3. Gravelometer used for substrate analysis (Stream Systems Technology Center, 1996).

Laboratory Procedure

After the field sampling was complete, benthic macroinvertebrate samples were taken back to the laboratory for further processing. Each sample was placed into a rectangular white enamel pan. The sample was rinsed using tap water into a system of two stacked sieves. The purpose of this procedure was to separate the benthic macroinvertebrates from most of the other organic and inorganic material found in the sample. This created a raw sample. For 10 of 36 samples a sub sampling procedure was performed to process the raw samples and remove organic debris. This was necessary do to high amounts debris in the sample. In this procedure, the raw sample was placed on a gridded rectangular sieve. A random number generator was used to randomly select four out of twelve grids (Figure 4). The contents of the four selected grids were used for analysis and the remainder of the sample was saved.

After the raw sample processing was complete, a dissecting microscope set on 0.65x or 1.0x was used to remove benthic macroinvertebrates from the raw sample to create the picked sample. Next the dichotomous keys of Merritt, Cummins, and Berg (2008) and Bouchard (2004) were used to identify the benthic macroinvertebrates to Family. For some non-insects, identification was only possible to Order or higher.

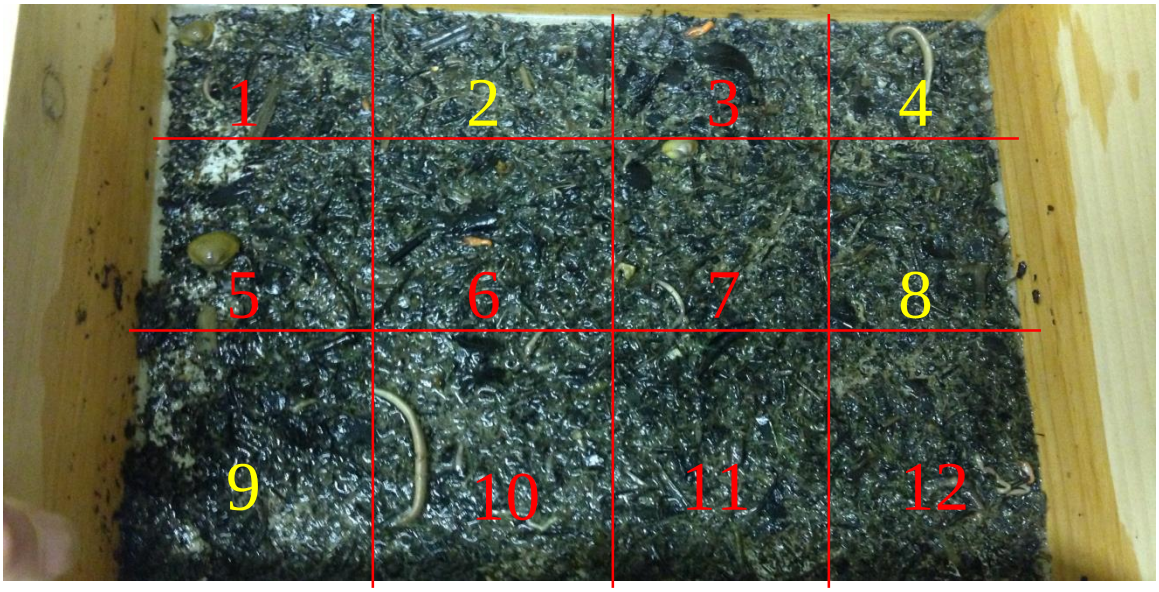


Figure 4. Example of sub sampling procedure. Yellow numbers were picked and identified and red numbers were saved.

Sample Analysis Procedure

After the benthic macroinvertebrates were identified and counted, the data were keyed into the VDEQ's family-level Ecological Data Assessment System (EDAS) and Virginia Stream Condition Index (VSCI) scores were calculated using the EDAS. The VSCI is a multi-metric index used to assess benthic macroinvertebrate density and diversity in non-coastal Virginia Streams. Metrics are measurable characteristics of a biological community that are expected to change in response to stream impairment (Burton & Gerristen 2003). The VSCI is calculated using a total of eight metrics that assess taxonomic richness, composition, trophic groups, diversity, and tolerance (Table 2). Each metric is given a score from 0-100 and scores from each metric are averaged to create the VSCI. Streams with a score of less than 60 are categorized as impaired (Burton & Gerristen 2003)

Table 2. Virginia Stream Condition Index metric descriptions (Burton & Gerristen 2003).

Metric	Definition	Pollution Response
	Taxonomic Richness	
Total Taxa (RTOTAL)	Number of distinct tax in sample	Decrease
EPT Taxa (REPT)	Number of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) taxa in sample	Decrease
	Composition	
%Ephemeroptera (ZEPHM)	Percent Ephemeroptera (mayflies) in sample	Decrease
%PT less Hydrospychidae (ZPTLH)	Percent PT not including the tolerant Trichoptera family Hydrospychidae in sample	Decrease
% Chironomidae (ZCHIR)	Percent Chironomidae (midge) larvae and pupa in sample	Increase
	Trophic Groups	
% Scrapers (ZSCRA)	Percent benthic macroinvertebrates in the sample that belong to the scrapers functional feeding group	Decrease
	Diversity	
% 2 Dominant Taxa (Z2DOM)	Percent two most abundant taxa found in sample	Increase
	Tolerance	
HBI	Abundance-weighted average tolerance of benthic macroinvertebrates in sample	Increase

Statistical Analysis

The mean and coefficient of variation (CV) for each metric and the final VSCI score was calculated for each sample site. A linear regression was used to analyze the association between substrate size and VSCI Score. A residual plot and a normal probability plot of the regression data was made and a P-value was determined to assess if the relationship was significant or not. It was predetermined that a P-value of less than 0.05 would indicate statistical significance.

Results and Discussion

Taxonomic Richness

The sample sites from Toms Creek had an average of 22 and 19 total taxa and 11 and 10 EPT taxa respectively. The sample sites from Stroubles Creek upstream of the restoration area had an average of 5-8 total taxa and 0-2 EPT taxa. The sample sites from the Stroubles Creek restoration area had an average of 10-11 total taxa and 2-4 EPT taxa (Table 3). The sample sites from Stroubles Creek downstream of the restoration area had an average of 14-16 total taxa and 4-8 EPT taxa (Table 3).

These data indicate that taxonomic richness in Toms Creek is high and environmental disturbances in the sampling area are minimal. The data also indicate that taxonomic richness in Stroubles Creek is low, suggesting increased environmental disturbances and poor stream quality.

Composition

The 13 sites from Toms Creek and Stroubles Creek showed noteworthy differences in composition. Sample sites T1 and T2 from Toms Creek and S7 and S8 from Stroubles Creek downstream of the restoration area showed the highest percent of Ephemeroptera (mayflies) and percent Plecoptera (stoneflies) plus Trichoptera (caddisflies) minus the pollutant tolerant Trichoptera family Hydrospychidae (Figure 5). High percentages of mayflies, stoneflies, and pollution intolerant members of the caddisfly family are indicators of good stream quality. While the aforementioned sample sites have the highest percentages of pollution intolerant benthic macroinvertebrates among the 13 sample sites, 3 of 4 sites would be considered impaired because the VASCI scores were <60 (Table 3).

Sample sites W1 and W2 and S1-S3 from upstream of Stroubles Creek contain a small to zero percentage of pollution intolerant benthic macroinvertebrates, indicating that this sample area had the lowest stream quality and highest environmental disturbance (Figure 5). Sample sites S4-S7 collected from the BSE Stroubles Creek stream restoration area also had very small percentages of pollution intolerant macroinvertebrates; however, the percentage of pollution intolerant macroinvertebrates increased from 0.46 percent at sample site 9 to 4.86 percent at sample site 11 (Table 3). This increase in benthic macroinvertebrates, which are intolerant to pollution points toward improved stream health.

Dissimilarly to the presence of mayflies, stoneflies, and pollution intolerant caddisflies, the high presence of Chironomidae indicates poor stream health. The high abundance of Chironomidaes found in sample sites W2, W3, S1-S2, and S4-S6 are indicative of stream impairment (Figure 5).

Table 3. Benthic macroinvertebrate analysis results.

	RTOTAL				REPT				ZEPHM			
	Metric		Score		Metric		Score		Metric		Score	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
T1	22.33	0.09	96.97	0.05	11.00	0.09	96.97	0.054	9.23	0.19	15.05	0.19
T2	18.67	0.03	84.85	0.03	9.67	0.06	87.88	0.060	10.36	0.26	16.90	0.26
W1	7.50	0.09	34.09	0.09	1.00	0.00	9.09	0.000	0.00	NA	0.00	NA
S1	7.00	0.20	31.82	0.20	2.00	0.00	18.18	0.000	0.00	NA	0.00	NA
W2	5.00	0.00	22.73	0.00	0.00	NA	0.00	NA	0.00	NA	0.00	NA
W3	6.67	0.09	30.30	0.09	0.00	NA	0.00	NA	0.00	NA	0.00	NA
S2	8.00	0.43	36.36	0.43	2.00	1.00	18.18	1.000	0.84	0.87	1.37	0.87
S3	7.33	0.08	33.33	0.08	1.00	0.00	9.09	0.000	0.00	NA	0.00	NA
S4	10.00	0.10	45.45	0.10	2.00	0.00	15.15	0.346	0.46	0.72	0.75	0.73
S5	11.33	0.44	51.51	0.44	2.33	0.66	21.21	0.655	3.25	0.12	5.31	0.12
S6	11.33	0.10	51.52	0.10	3.67	0.16	33.33	0.157	3.83	0.24	6.25	0.24
S7	14.33	0.18	65.15	0.18	4.33	0.27	39.39	0.266	6.51	0.47	10.63	0.47
S8	16.33	0.14	74.24	0.14	8.00	0.13	72.73	0.125	6.63	0.14	10.82	0.14

	ZPTLH				ZCHIR				ZSCRA			
	Metric		Score		Metric		Score		Metric		Score	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
T1	14.44	0.33	40.56	0.33	30.46	0.30	69.54	0.13	36.97	0.14	71.66	0.14
T2	10.43	0.65	29.31	0.65	39.71	0.32	60.29	0.21	25.17	0.15	48.79	0.15
W1	NA	NA	0.00	NA	25.82	0.78	74.19	0.27	5.50	0.68	10.66	0.68
S1	1.30	0.12	3.65	0.12	84.25	0.08	15.76	0.40	1.90	0.36	3.67	0.36
W2	NA	NA	0.00	NA	74.62	0.10	25.39	0.30	3.78	0.46	7.33	0.46
W3	NA	NA	0.00	NA	90.22	0.03	9.78	0.27	0.30	0.87	0.58	0.88
S2	NA	NA	0.00	NA	53.66	0.36	46.34	0.42	0.71	0.94	1.37	0.95
S3	NA	NA	0.00	NA	21.95	0.52	78.05	0.15	0.36	0.17	0.70	0.17
S4	NA	NA	0.00	NA	42.61	0.15	57.39	0.11	2.71	0.93	5.25	0.93
S5	1.01	0.00	0.95	1.73	47.29	0.10	52.71	0.09	13.61	0.55	26.39	0.55
S6	1.03	0.74	2.89	0.74	59.39	0.31	40.61	0.45	18.23	0.78	35.34	0.78
S7	2.29	0.27	4.31	0.91	19.77	0.42	80.23	0.10	35.51	0.14	68.82	0.14
S8	11.05	0.36	31.03	0.36	38.93	0.10	61.07	0.07	24.83	0.14	48.12	0.14

	Z2DOM				HBI				VSCI	
	Metric		Score		Metric		Score			
	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
T1	52.11	0.14	69.20	0.16	4.44	0.04	81.85	0.03	67.72	0.09
T2	54.68	0.19	65.49	0.23	4.76	0.09	77.10	0.08	58.82	0.12
W1	78.16	0.12	31.57	0.44	5.50	0.09	66.26	0.11	28.23	0.11
S1	89.93	0.05	14.55	0.42	5.95	0.00	61.31	0.02	18.40	0.14
W2	87.37	0.04	18.25	0.27	5.83	0.01	59.58	0.01	16.88	0.01
W3	97.05	0.02	4.26	0.77	5.99	0.00	59.04	0.00	13.00	0.07
S2	93.69	0.03	9.12	0.50	5.94	0.01	59.72	0.01	21.56	0.16
S3	77.33	0.14	32.75	0.49	6.02	0.01	58.55	0.02	26.56	0.06
S4	78.58	0.14	30.95	0.52	5.90	0.02	60.34	0.02	26.91	0.07
S5	73.39	0.11	38.03	0.31	5.49	0.04	66.26	0.05	32.79	0.21
S6	79.91	0.20	29.03	0.79	5.46	0.06	66.83	0.07	33.22	0.24
S7	50.45	0.23	71.61	0.23	4.55	0.01	80.13	0.01	52.53	0.11
S8	52.11	0.05	69.20	0.06	4.55	0.01	80.10	0.01	55.91	0.02

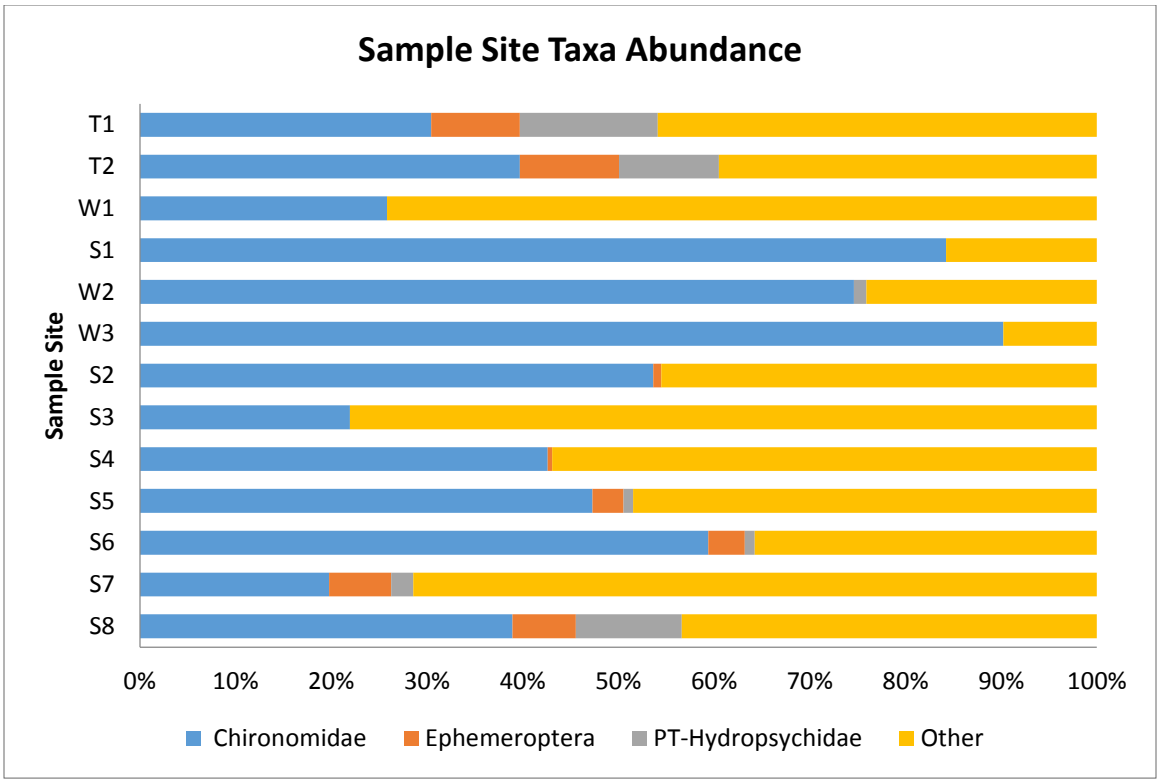


Figure 5. Percent abundance of Chironomidae, Ephemeroptera, and Plecoptera plus Trichoptera minus Hydrospychidae by sample site.

Trophic Groups

The percentage of scrapers, benthic macroinvertebrates that feed on substrate-attached algae or periphyton and associated materials, tend to decreases as pollution in streams increase (Burton & Gerristen 2003). Percent scrapers for the Toms Creek samples ranged from 25-37 percent. Percent scrapers for the Stroubles Creek samples upstream of the restoration area ranged from 0.30-5.50 percent. Percent scrapers for the Stroubles Creek samples in the restoration site ranged from 2-18 percent. Percent scrapers for the Stroubles Creek samples downstream of the restoration area ranged from 35-35 percent. Once again, these metrics indicate that stream quality is highest in Toms Creek and the downstream area of Stroubles Creek and lowest in the upstream area of Stroubles Creek and the restoration area.

Diversity

The percentage of benthic macroinvertebrates belonging to the top two dominant taxa can be a measure of diversity. Sample sites with more families are more diverse than sample sites with fewer families. If a community is dominated by one or two taxa, it has low diversity, which indicates poor water quality. The higher the percentage of macroinvertebrates belonging to the two dominate taxa, the lower the stream quality (Burton & Gerristen, 2003). The mean percent of the top two dominant taxa for each sample site was greater than 50 percent. This denotes low diversity and poor stream quality in Toms Creek and Stroubles Creek. The percentage of top two dominant taxa was highest for S1, W2, and S2 indicates that stream quality is lowest in these areas of Webb branch and Stroubles Creek.

Tolerance

The HBI index has possible values from 0.00 to 10.00 (Hilsenhoff, 1988). Two sites in Toms Creek and 2 sites in Stroubles Creek downstream of the restoration area scored in the good water quality range, 4.26-5.00 (Table 3). One site from upstream of the restoration area and two sites in the restoration area scored in the fair water quality range, 5.01-5.75 (Table 3). The remaining sites scored in the fairly poor water quality range, 5.76-6.50 (Table 3). Good water quality indicates “some organic pollution probable”, fair water quality indicates

"fairly substantial organic pollution likely", and fairly poor water quality indicates very substantial pollution likely (Hilsenhoff, 1988).

VSCI score

The VSCI score is determined from the metrics discussed above, as a result, the score demonstrates similar trends to those previously described. The VSCI scores were highest for sites in Toms Creek, sites T1 and T2, followed by the sites in Stroubles Creek downstream of the restoration area, sites S7 and S8 (Figure 6). Of these four sites, only site 1 had a VSCI above 60, which is the threshold for stream impairment (Table 3). This indicates that while the above sites had the highest VSCI scores, the data from this study still indicate stream impairment.

The lowest VSCI scores, ranging from 13.00 to 28.23, came from the samples sites upstream of the Stroubles Creek restoration area, sites W1-W3, S1-S3, and the sample taken from the first bridge in the restoration area, site S4. Sites S5 and S6, from the restoration area, had VSCI scores of 32.79 and 33.22, respectively (Table 3).

All the VSCI scores for Stroubles Creek fall below 60 and therefore indicate stream impairment (Figure 6). However, the severity of impairment is not constant throughout the stream. The highest VSCI scores found in Stroubles Creek were from sample sites downstream of the restoration area, S7-S8 and the lowest VSCI scores were from sample sites upstream of the restoration area, W1-W3 and S1-S3 (Table 3).

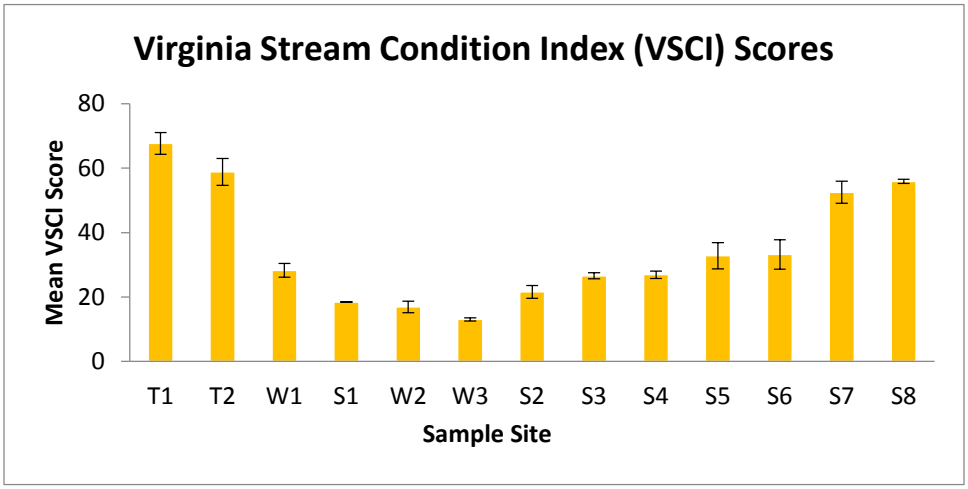


Figure 6. Mean VSCI scores by sample site with standard error

One reason for the variance in VSCI scores found in Stroubles Creek could be habitat availability. One indicator of habitat availability is substrate size. A review of studies examining the relationship between substrate and benthic macroinvertebrate diversity found that medium to large substrate size is positively correlated with taxa richness (Vinson & Hawkins 1998). However, a linear regression of substrate size verse VSCI score resulted in a coefficient of determination value (r^2) of 0.35, indicating a weak positive correlation between average substrate size and VSCI scores (Figure 7). The P-value for the regression was 0.033, indicating that there is a statistically significant correlation between substrate size and VSCI score. This suggests that substrate size may be related to VSCI scores; however, other variables of habitat availabiltiy also influence VSCI scores.

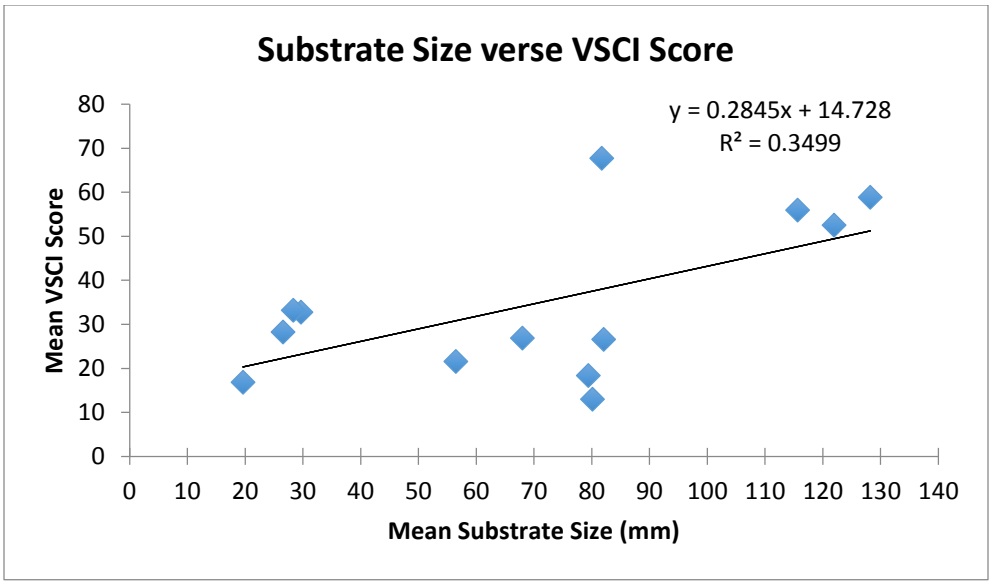


Figure 7. Plot of mean substrate size verse mean VSCI score for Toms Creek and Stroubles Creek sample sites.

Another factor influencing VSCI is substrate stability. Benthic macroinvertebrate diversity generally increases with substrate stability (Vinson & Hawkins 1998). Stroubles Creek is a flashy stream, as seen by the sharp rises and falls of flow depth, which correspond with storms (Figure 8). Furthermore, in non-forested areas, such as the agricultural and urban land surrounding much of Stroubles Creek, water infiltration decreases and surface runoff increases, resulting in floods that rise more rapidly and create more sediment disturbances (Paul & Meyer 2001). The lowest VSCI scores for Stroubles Creek were found in urban and agricultural areas, whereas the higher VSCI scores for Stroubles Creek were found in more forested areas. This is indicative that sediment disturbances are a factor in the poor benthic macroinvertebrate health of Stroubles Creek.

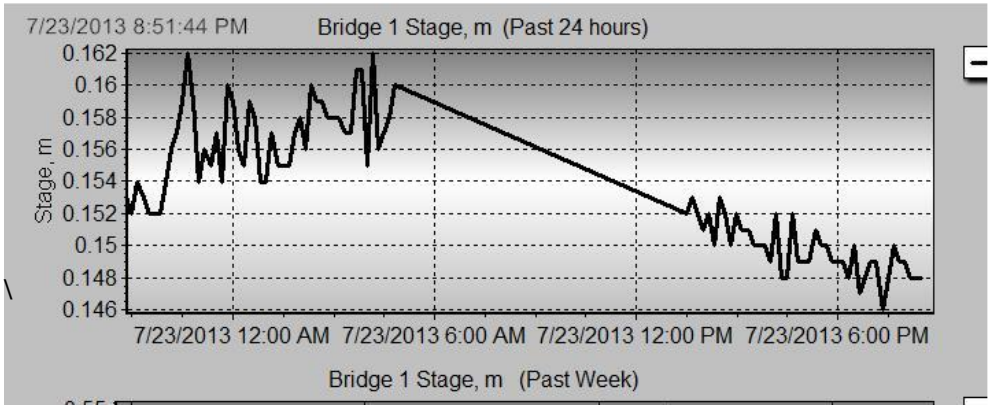


Figure 7. Stream flow height from Bridge 1 in the Stroubles Creek Restoration Area (BSE VT 2013).

Conclusions

The macroinvertebrate data from this research indicate Stroubles Creek continues to be impaired. However, the extent of impairment found in Stroubles Creek was not uniform. The highest levels of impairment were found upstream of the BSE restoration site and the lowest levels of impairment were found downstream of the BSE restoration site. The level of impairment in the Stroubles Creek Restoration was improved from the upstream impairment levels, but still significantly below the VSCI scores of Toms Creek and Stroubles Creek downstream of the restoration area. Thus, the restoration project has not yet restored aquatic habitat suitable of supporting benthic macroinvertebrate diversity.

The macroinvertebrate data from this research also indicated continued unimpaired stream quality of Toms Creek; nevertheless, the average VSCI for the sample sites in Toms Creek was only marginally above the threshold between impaired and unimpaired stream quality.

Analysis of stream substrate found that stream quality as indicated by VSCI scores was weakly related to stream substrate size. Nevertheless, this relationship was statistically significant. However, qualitative assessment of the riparian ecosystem of the sample sites showed that non-forested samples sites had lower stream quality values and were more susceptible to substrate disturbances, suggesting a relationship between substrate stability and benthic macroinvertebrate diversity. Further research regarding the relationship between substrate stability and benthic macroinvertebrate diversity in Stroubles Creek is needed.

Additional research is needed to determine the causes of impairment variation present in Stroubles Creek. Benthic macroinvertebrate monitoring of Stroubles Creek for effects of the BSE restoration effort on benthic macroinvertebrate density and diversity in the stream should be continued in order to evaluate effectiveness of the restoration.

While the objectives of this research project were achieved, improvements could be made to achieve more representative results of benthic macroinvertebrate makeup of Stroubles Creek. First, benthic macroinvertebrate populations differ by time of year. A better understanding of overall stream health could be obtained by taking benthic macroinvertebrate samples throughout the year. Furthermore, collecting more benthic macroinvertebrate samples from each site could add to more comprehensive results and a better understanding of stream health.

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Effect of Nutrient Enrichment and Copper Sulfate Application on Phytoplankton Growth in Falling Creek Reservoir

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ABSTRACT

The Western Virginia Water Authority supplies potable water to the City of Roanoke and Roanoke County from four local reservoirs, including Falling Creek Reservoir (FCR). FCR exhibits algal blooms, which can increase human health risks and treatment costs. The primary goals were to determine the limiting nutrient for phytoplankton growth and the effect of copper sulfate on phytoplankton. Results indicate that the primary limiting nutrient for phytoplankton in FCR is phosphorus, and the minimum concentration of copper sulfate needed to reduce phytoplankton concentrations 24h after application is between 0.125mg/L and 0.250mg/L, though its effectiveness diminishes by 72h after application.

Keywords: copper sulfate, Falling Creek Reservoir, nitrogen, phosphorus, phytoplankton, water quality

Introduction

Water quality is declining worldwide due to anthropogenic impacts (Jos et al. 2005). Climate change and unsustainable withdrawal has decreased water quality, yet the most prevalent water quality problem is nutrient enrichment, known as eutrophication (Jos et al. 2005). Eutrophication is due to high nutrient loads, namely phosphorus and nitrogen, and substantially impairs beneficial uses of water. Algal blooms can result from eutrophication, which can disrupt ecological food webs and increase health risks and treatment costs associated with drinking water (Callinan et al. 2013). Therefore, managing water quality is an essential component in sustaining environmental resources and public health.

Reservoirs commonly used for drinking water sources may be susceptible to eutrophication. The Western Virginia Water Authority (WVWA) supplies an average 87.1 million liters per day (MLD) of potable water to more than 155,000 people in the city of Roanoke and Roanoke County, in Virginia. The primary sources for the water supply are four local reservoirs, including the Falling Creek Reservoir (FCR) (WVWA 2013). The water column in FCR becomes thermally stratified in the warm summer months, which can cause algal blooms to occur. The eutrophication and, consequently, algal growth in FCR are dependent on concentrations of nitrogen and phosphorus in the reservoir. Specifically, phosphorus is often considered to be the limiting nutrient for algal growth (Schindler 1974).

The WVWA has attempted to control these blooms with various methods. In 2007, a surface circulator was installed to counter thermal stratification by mixing the reservoir's hypolimnion and epilminion layers. However, this attempt at controlling blooms was determined to be unsuccessful because algal counts remained high in the summer months. A hypolimnetic oxygenation system was installed in fall 2012, and the WVWA plans to couple the system with a destratification system in the upcoming year. The oxygenation system introduces oxygen into the hypolimnion, preventing the reservoir from becoming anoxic. The destratification system will aid in mixing the reservoir's stratified layers, reducing cyanobacterial scums dependent on a stable water column and further decreasing anoxia. When anoxia occurs, phosphorus bound to the reservoir's sediment is released. Increased concentrations of soluble phosphorus in a reservoir increase the rate of photosynthesis for phytoplankton. Therefore, the oxygenation and destratification system can work in conjunction to limit algal blooms by preventing the release of a potentially limiting nutrient for growth (Nurnberg 1984).

Algal blooms can be controlled with algaecides, though their effectiveness is variable (García-Villada et al. 2004). Currently, the WVWA relies on using a common algaecide, copper sulfate pentahydrate ($CuSO_4$-$5H_2O$), to control algal blooms (Rissky et al. 2012). The WVWA spreads ~45.4kg of the algaecide onto the surface of FCR when algae counts are high in the summer months, which usually occurs every three to four weeks from May to September. However, although the average concentration of copper sulfate applied to the reservoir is 0.285mg/L when the pond is at full volume, the method of spreading the algaecide undoubtedly causes the concentration of the copper sulfate to vary in the water column.

The effect of copper sulfate on phytoplankton species has been examined in controlled growth inhibition experiments. These experiments primarily examine the effect of a copper-based algaecide on a specific species of algae, because different types of algae have different sensitivities to the copper-based algaecide. Copper sulfate dosing also has a varying effect on algae species overtime. The half maximal inhibitory concentration (IC_{50}) and half maximal effective concentration (EC_{50}) have been identified for certain freshwater algae species over specific experimental durations (Table 8). Guidelines for performing freshwater algal and cyanobacteria growth inhibition experiments from the Organization for Economic Cooperation and Development (OECD) recommend testing algal samples at 24-hour intervals for at least 72 hours (OCED 2011). Furthermore, the LD_{50} of copper sulfate for rats and mice has been determined for copper sulfate pentahydrate (Table 9).

Table 8: Copper Sulfate IC_{50} and EC_{50} Values for Algal Species

Algal Species	Phylogenetic Group	IC_{50} (mg/L)	EC_{50}		Duration (hr)	Source
			(mg/L)	(g Cu^{2+}/m^2)		
Microcystis aeruginosa	Cyanobacteria	0.250	N/A	N/A	24	Chow et al. 1999
Desmodesmus subspicatus	Chlorophyta		2.45	N/A	96	Guclu, Z. 2012
Diatom bloom (consisting primarily of *Melosira* spp., *Stephanodiscus astraea* var. *minutula* and *Asterionella formosa)*	Chrysophyta		N/A	0.400	96	Button et al. 1976

Table 9: Copper Sulfate Pentahydrate LD_{50} Values for Specific Mammals

Animal	LD50 (mg/kg)	Source
Rat (Oral)	300	EPA 2001
Rat (Intraperitoneal)	19000	
Rat (Subcutaneous)	43	
Rat (Intravenous)	49	
Mouse (Oral)	370	
Mouse (Intraperitoneal)	33	
Mouse (Intrevenous)	23	

The primary goal of this research is to investigate the effect of nutrient enrichment and copper sulfate application on phytoplankton growth in FCR. Specific research objectives include the following: 1) determine the limiting nutrient for the growth of algal species in FCR in response to the oxygenation system and 2) to analyze the effect of varying copper sulfate dosages on phytoplankton species in FCR. The second objective can be used to determine an appropriate copper sulfate dosage for the WVWA to apply to FCR when algal blooms occur.

Research Methods

FCR is located in Bedford County, Virginia and covers approximately 21 acres. FCR stores approximately 322 million liters (ML) of water at full pond, and though the reservoir has the capacity to supply 3.79MLD, the reservoir more commonly supplies between 1.14 and 1.51MLD (WVWA 2013). The WVWA uses

an oxygenation system and sporadic copper sulfate dosing to control phytoplankton blooms in FCR. These phytoplankton control methods were manipulated and analyzed in two experimental procedures to meet the stated research objectives.

Experiment 1: Nutrient Enrichment Assay

I performed nutrient enrichment assays to accomplish the first research objective. In order to determine the response of algal concentrations to varying nutrients and the status of the oxygenation system, I performed these assays using samples taken from when the system was both on and off. I collected subsurface grab samples for both assays from the epilimnion, approximately 0.3m below the reservoir's surface, at a site where the reservoir reaches its maximum depth of approximately 9.5 m (Figure 1). I collected these samples on the same day that the assays were performed. Grab samples for the first nutrient enrichment assay were collected on 18 June 2013 while the oxygenation system was turned on and the dissolved oxygen (DO) approximately 1.5m above the sediment was measured to be 10.4mg/L using a Winkler test. Grab samples for the second assay were taken approximately three weeks after the oxygenation system was turned off, on 15 July 2013, and measured DO approximately 1.5m from the sediment was determined to be 6.4mg/L using a Winkler test.

Figure 25: Samples were taken at the deepest spot in FCR (shown in red) (Google Maps 2013).

The procedure had a fully factorial design, with three replicates of each of the following treatments: controls (no added N or P), nitrogen-dosed (N) samples, phosphorus-dosed (P) samples, and nitrogen- and phosphorus-dosed (NP) samples. The nitrogen- and phosphorus- dosed samples included 0.032M NH_4 and 0.0032M PO_4 stock solutions, respectively. In addition, we analyzed three replicate samples of the phytoplankton community at the beginning of the experiment, which was used to determine the species present in reservoir water. These samples were preserved with 1% Lugol's solution immediately after collection. The remaining samples- controls, N, P, and NP- were filtered with an 80µm zooplankton net to remove large zooplankton grazers and kept in separate flasks for four days. These 500mL flasks had 300mL of sample and were stored in a water bath that remained at 20-22C. I placed the water bath near a window for the incubation period, and the flasks were gently stirred and randomly reordered daily (Figure 2). After the 4-day period, 250mL of all samples were put into amber bottles and preserved with 1% Lugol's solution.

Figure 26: Flasks for assay were kept in water bath near a window for the 4-day incubation period.

I measured phytoplankton densities to determine the limiting nutrient for the growth of various phytoplankton species in response to the oxygenation system. 1mL of each sample was individually placed on a slide and examined under a Micromaster microscrope by Fisher Instruments using a PLAN 10/0.25 lens at a magnification of 100X. I identified and counted phytoplankton species in one row of a slide for each sample. Since 11 rows are visible per slide with this magnification, representation concentrations of species present in 1L of reservoir water were estimated by multiplying raw counts by 11,000.

I analyzed differences among the phytoplankton communities in the four treatments at the end of experiment using ANOVA in Excel with a 95% confidence interval. I also examined whether significant differences existed among treatments using Tukey's honest significance test (HST). The concentrations of phytoplankton species present in samples taken when the oxygenation system was on and approximately three weeks after being turned off were also compared.

Experiment 2: Copper Sulfate Dosing

I analyzed the response of FCR's phytoplankton community to varying concentrations of copper sulfate pentahydrate to accomplish the second objective. I collected grab samples from FCR's epilimnion, approximately 0.3m from the reservoir's surface, on 8 July 2013. I collected these samples at the same sampling site where the

reservoir is deepest, 9.5m, and DO at the surface was measured to be 8.4mg/L using a Winkler test (Figure 1). The samples were filtered with an 80µm zooplankon net. Three replicates of pre-incubation samples containing 250mL were immediately preserved with 1% Lugol's solution after collection.

The protocol for the growth inhibition experiment incorporated recommendations from the OECD (OECD 2011). That is, replicates of samples were dosed with at least 5 different concentrations of copper sulfate and were incubated for at least 72 hours. Specifically, the concentrations used in the experiment included 0, 0.0625, 0.125, 0.250, 0.500, and 1.000mg of copper sulfate/L of sample, which spans the dosages currently used by the WVWA to treat algae in FCR. I dosed samples with a 0.457mM copper sulfate stock solution, composed of 57mg of copper sulfate pentahydrate and 500mL of nanopure water (**Error! Reference source not found.**). I then stored samples in flasks kept in a water bath at 21-24C for 3 days and removed 25mL from each flask at two time intervals, 24h and 72h. The individual volumes removed from samples were stored in separate amber bottles and immediately preserved with 3mL of 1% Lugol's solution. I gently swirled and reordered flasks in the water bath daily using a random number generator.

Figure 27: : Soluble copper sulfate pentahydrate was mixed with nanopure water to compose a stock solution for the experiment.

As described above, I measured phytoplankton densities in all samples to determine the effect of varying copper sulfate dosages over time. Concentration response curves were fitted using the estimated phytoplankton concentrations from each flask. The concentration of copper sulfate used to dose each flask was plotted against respective algal concentrations determined overtime to produce response curves for two time intervals, 24h and 72h. I also calculated differences among the phytoplankton communities in the six treatments at the end of experiment using ANOVA in Excel and Tukey's HST.

Results and Discussion

Experiment 1

Nutrient Enrichment Assay 1

The nutrient enrichment assay performed using grab samples from FCR on 18 June 2013 indicated that phosphorus is the limiting nutrient for the growth of specific phytoplankton species when the oxygenation system was on. Concentrations of phytoplankton species were counted in pre-incubation, control, N, P, and NP samples (Figure 28). ANOVA results demonstrated that the means of the control, N, P, and NP concentrations were significantly different (one-way ANOVA; $F_{3,8} = 21.22$, $p = 0.0004$). Tukey's HST indicated that significant differences were present amongst the mean phytoplankton concentrations in the following samples: control and P, control and NP, N and P, N and NP. These results verified that phosphorus was the limiting nutrient when the oxygenation system was on at FCR. Phytoplankton concentrations in P and NP samples were not significantly different because phosphorus drove phytoplankton growth in NP samples.

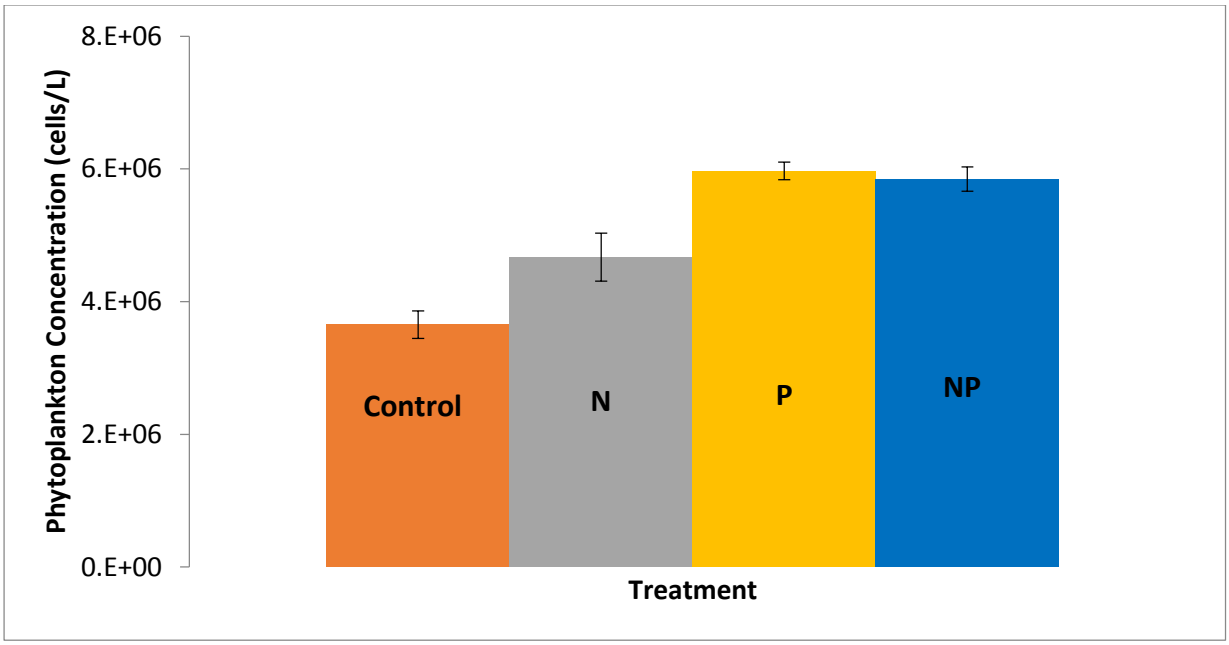

Figure 28: Phytoplankton concentrations (shown with +/- standard error) were significantly higher in replicates dosed with a phosphorus stock solution.

Samples from the first nutrient enrichment assay had significantly different concentrations of phytoplankton species. All samples contained the following taxa: *Mallomonas, Ankistrodesmus, Cocconeis, Ulothrix, Oocystis, Chlorella, Chrysococcus, Cyclotella, Anabaena,* and *Anacystis* (Figure 29). The pre-incubation and control samples also had a small concentration of *Euglena*. *Anacystis* and *Anabaena*, the most prevalent species in all samples, are cyanobacterial taxa, and are known to adversely affect the taste and odor of drinking water most substantially. Our results indicated that significant differences are present amongst the following species in the samples: *Anacystis* (one-way ANOVA; $F_{3,8} = 20.17$, $p = 0.0004$), *Cyclotella* (one-way ANOVA; $F_{3,8} = 8.79$, $p = 0.007$), and *Chlorella* (one-way ANOVA; $F_{3,8} = 5.86$, $p = 0.02$). Thus, phosphorus was the limiting nutrient for these phytoplankton species when the oxygenation system was on at FCR. However, the null hypothesis that means of phytoplankton densities were the same amongst varying groups could not be rejected for the remaining species.

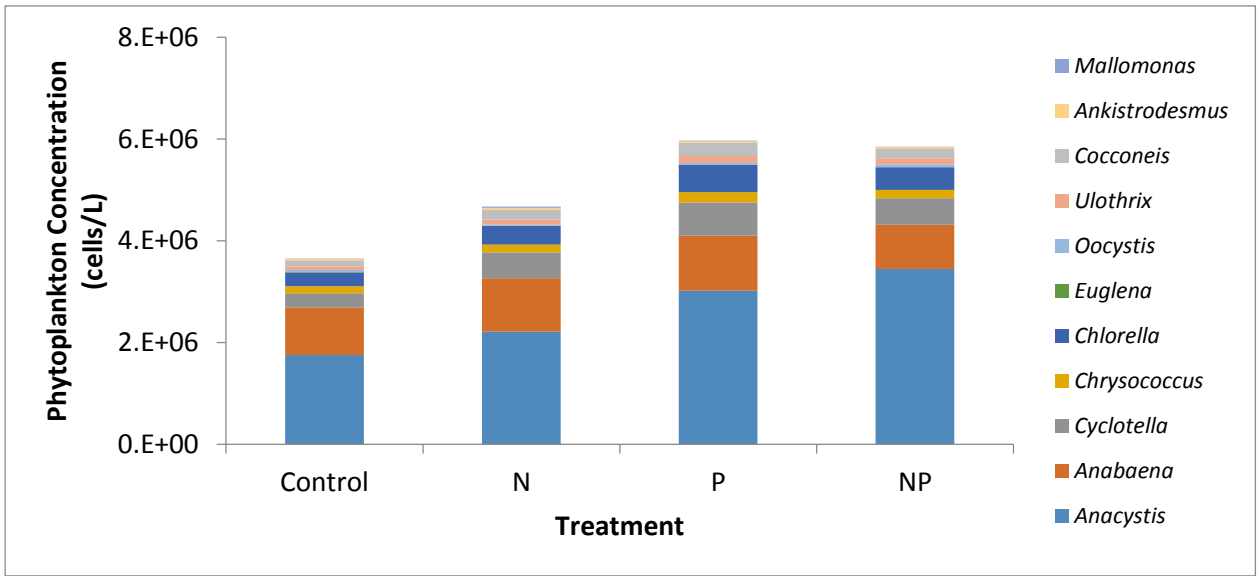

Figure 29: Phytoplankton concentrations (shown with +/- standard error) in replicates varied based various samples in the experiment.

Phytoplankton groups exhibited different sensitivity to the limiting nutrients. The phytoplankton species identified in the samples were grouped into the following phytoplankton phylogenetic groups: Cyanobacteria, Chrystophyta, Chlorophyta, and Euglenophyta (Figure 30). ANOVA results indicated that Cyanobacteria (one-way ANOVA; $F_{3,8}$ = 12.81, p = 0.002), Chrysophyta (one-way ANOVA; $F_{3,8}$ = 17.28, p = 0.0007), and Chlorophyta (one-way ANOVA; $F_{3,8}$ = 4.14, p = 0.05) groups had significantly different mean concentrations amongst the varying samples, and mean concentrations of Euglenophyta were not significantly different in various samples due to the small concentration of Euglena species present. Therefore, phosphorus was the limiting nutrient for Cyanobacteria, Chrystophyta, and Chlorophyta groups when the oxygenation system was on at FCR. Tukey's HST indicated that significant differences existed amongst the mean phytoplankton concentrations in control and NP samples and control and P samples, yet not in control and N samples, for these groups. It is important to note that the hypolimnetic oxygenation of FCR limits Cyanobacterial growth, and consequently, adverse changes to taste and odor in drinking water, most substantially by preventing the release of phosphorus from the reservoir sediments.

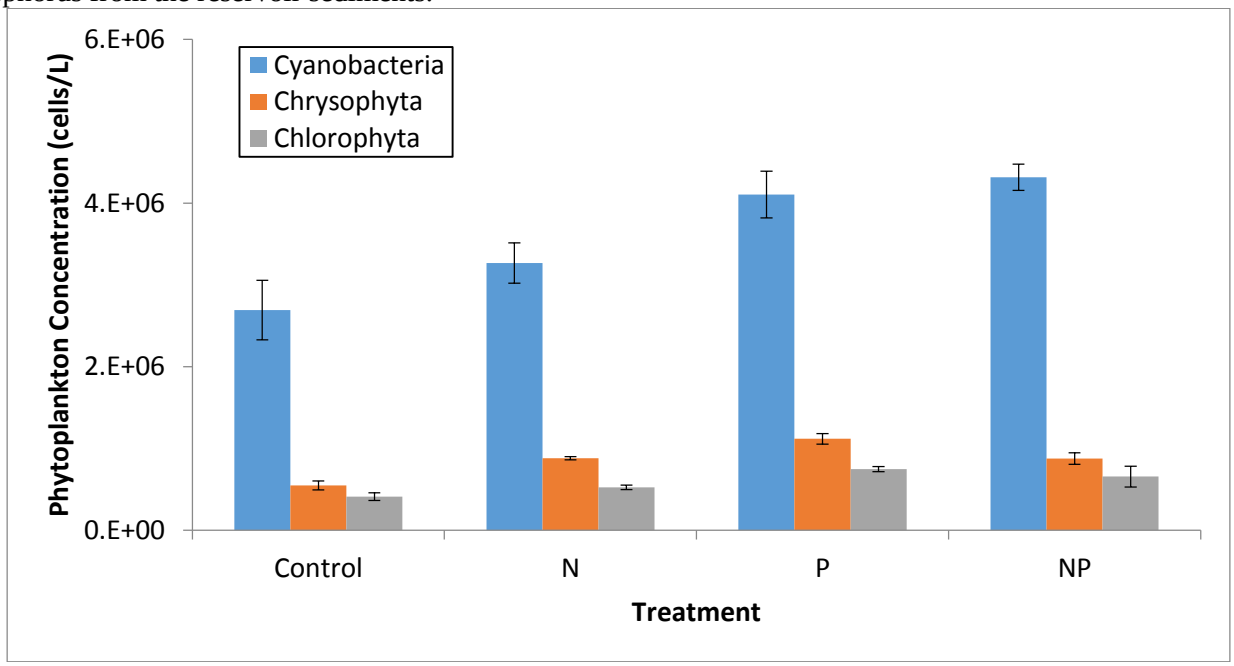

Figure 30: Significant differences in phytoplankton phylogenetic groups (shown with +/- standard error) were present amongst samples.

Experiment 1

Nutrient Enrichment Assay 2

The second nutrient enrichment assay performed using surface samples from FCR on 15 July 2013 indicated that phosphorus remained the limiting nutrient for the growth of specific phytoplankton species after the oxygenation system was turned off for approximately three weeks. I observed significant differences among the mean phytoplankton concentrations in the various samples (one-way ANOVA; $F_{3,8}$ = 9.58, p = 0.005). Increased phytoplankton concentrations in P samples indicated that phosphorus was the limiting nutrient for phytoplankton present in the samples (Figure 31). The Tukey's test determined that significant differences among the mean phytoplankton concentrations existed for the following samples: control and NP, control and P, N and NP, N and P. Results from Tukey's HST indicated that concentrations in P and NP samples were not significantly different, and increased phytoplankton concentrations in NP samples was primarily driven by the presence of phosphorus. Though the oxygenation system had been off at FCR for several weeks, the DO concentration 1.5m from the sediment had not fallen below 2 to 3mg/L- concentrations which would indicate an anoxic system. Phosphorus had not been released from sediment substantially, so the limiting nutrient for phytoplankton growth remained the same for both nutrient enrichment assays.

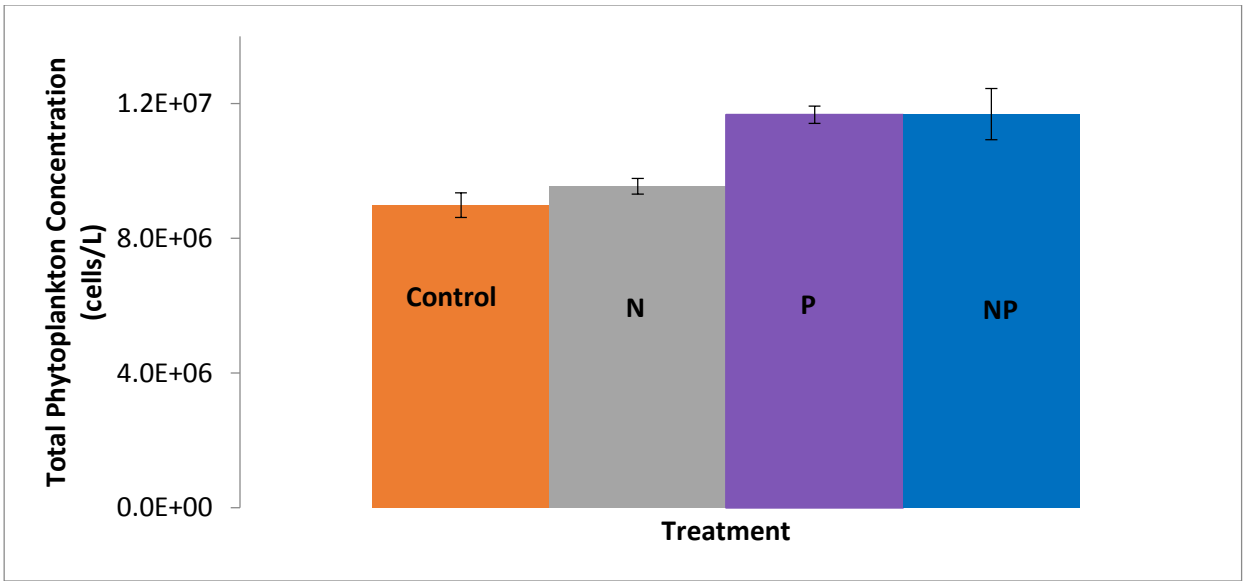

Figure 31: Increased phytoplankton concentrations in P and NP samples (shown with +/- standard error) indicated that phosphorus was the limiting nutrient for phytoplankton growth approximately three weeks after the oxygenation system in FCR had been turned off.

Concentrations of phytoplankton species varied amongst samples, though distributions of species remained fairly constant. Ten phytoplankton species were present in the assay samples, including *Anacystis*, *Anabaena*, *Ulothrix*, *Closterium*, *Cyclotella*, *Cocconeis*, *Ankistrodesmus*, *Chrysococcus*, *Cryptomonas*, and *Chlorella*. ANOVA results determined that significant differences among mean specie concentrations were present for one of the species- *Anacystis* (one-way ANOVA; $F_{3,8} = 17.26$, $p = 0.0004$). Therefore, *Anacystis* growth was limited by phosphorus concentrations after the oxygenation system at FCR had been turned off for approximately three weeks, and the DO concentration 1.5m from sediment was 6.4mg/L or above at the reservoir's deepest point. *Anacystis* remained the most prevalent species amongst the samples (Figure 32). *Cyclotella* and *Chlorella*, non-cyanobacterial species determined to be limited by phosphorus in the first assay, were no longer considered to be limited by the nutrient with decreased DO concentrations. Thus, *Anacystis* primarily drove the phosphorus limitation in FCR.

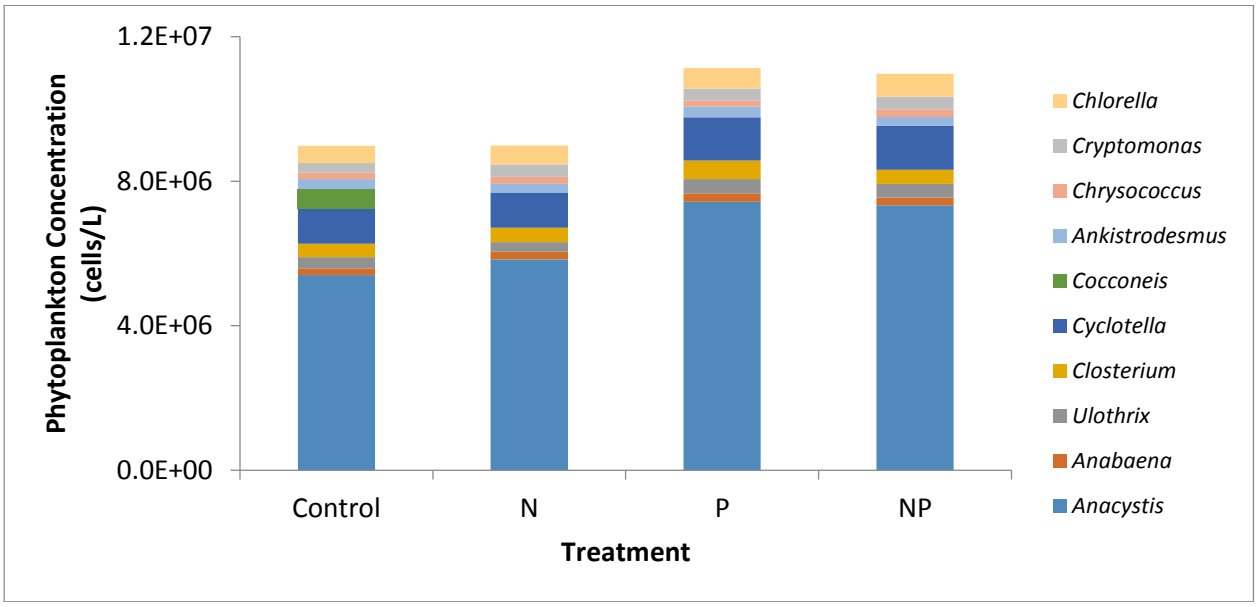

Figure 32: Anacystis remained the most prevalent phytoplankton species in the samples (shown with +/- standard error), indicating that its growth had an increased response to phosphorus concentrations.

Cyanobacterial taxa responded significantly to phosphorus, while the other three divisions present did not. ANOVA results indicated that mean concentrations of Cyanobacteria in the samples were significantly different among the treatments (one-way ANOVA; $F_{3,8}$ = 17.91, p = 0.0007). The remaining three divisions present, Chlorophyta, Chrysophyta, and Cryptophyta, were not significantly affected by the presence of the nutrient. Phosphorus was determined to limiting for Chlorophyta and Chrysophyta growth when the first assay was performed, yet decreased DO concentrations near the reservoir's sediment appear to have decreased phosphorus limitation for these taxa.

Experiment 2: Copper Sulfate Dosing

Phytoplankton concentrations were determined 24h and 72h after dosing samples with varying concentrations of a copper sulfate stock solution. The following phytoplankton species were determined to be present in all samples: *Anacystis*, *Anabaena*, *Ankistrodesmus*, *Cyclotella*, *Cocconeis*, *Chrysococcus*, *Chlorella*, *Ulothrix*, *Closterium*, and *Cryptomonas*. *Mallomonas* and *Euglena* were also present in a majority of samples counted after 24h and 72h, though concentrations of these algal species remained minimal. The phytoplankton species present in the experiment's samples can be classified into 5 divisions- Cyanobacteria, Chlorophyta, Chrysophyta, Cryptophyta and Euglenophyta.

Phytoplankton concentrations measured in samples after 24h of exposure to the copper sulfate solution generally decreased with increased concentrations of copper sulfate. ANOVA results indicated that significant differences were present amongst means of samples exposed to various concentrations within a 95% confidence interval (one-way ANOVA; $F_{3,8}$ = 10.35, p = 0.0005). Tukey's HST determined that phytoplankton concentrations did not significantly change in samples dosed with 0.000 to 0.125mg/L and 0.250 to 1.000mg/L of copper sulfate stock solution. However, total phytoplankton concentrations decreased by approximately 40% when copper stock solution concentrations increased from 0.125mg/L to 0.250mg/L (Figure 33). Because phytoplankton concentrations generally decreased in samples exposed to increased concentrations of copper sulfate, the algaecide was effective in decreasing the natural phytoplankton community in FCR.

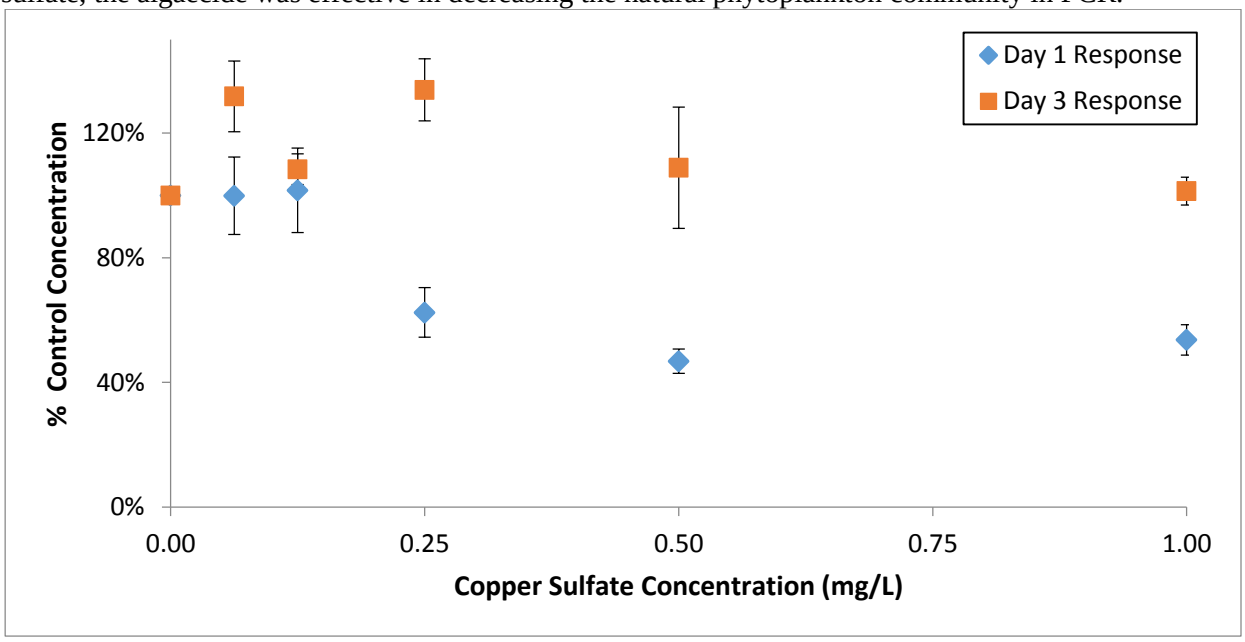

Figure 33: Reductions in samples dosed with varying concentrations of copper sulfate stock solution (shown with +/- standard error) compared to mean concentrations of 3 control replicates, represented as % of control, varied substantially from 24h to 72h after application of the algaecide.

Concentrations of phytoplankton species measured in samples exposed to copper sulfate stock solution for 24h responded nonlinearly to dosing concentrations. ANOVA results determined that significant differences were present among the means of samples exposed to varying dosages for three major divisions, Cyanobacteria (one-way ANOVA; $F_{3,8}$ = 18.72, p = 0.00003), Cryptophyta (one-way ANOVA; $F_{3,8}$ = 5.61, p = 0.007), and Chrysophyta (one-way ANOVA; $F_{3,8}$ = 9.90, p = 0.0006). Cyanobacterial concentrations decreased most

substantially in samples dosed with 0.125mg/L of copper sulfate stock solution to those dosed with 0.250mg/L of copper sulfate stock solution (Figure 34). Specifically, Cyanobacteria concentrations decreased from 90% to 66% of concentrations present in control samples. Copper sulfate effectively decreased the concentration of Cyanobacteria, the most problematic species present in FCR, after 24h of exposure to the algaecide. Therefore, an application of copper sulfate between 0.125 to 0.250mg/L should be applied to FCR to reduce phytoplankton concentrations. However, its effectiveness was reduced prior to 3 days after application.

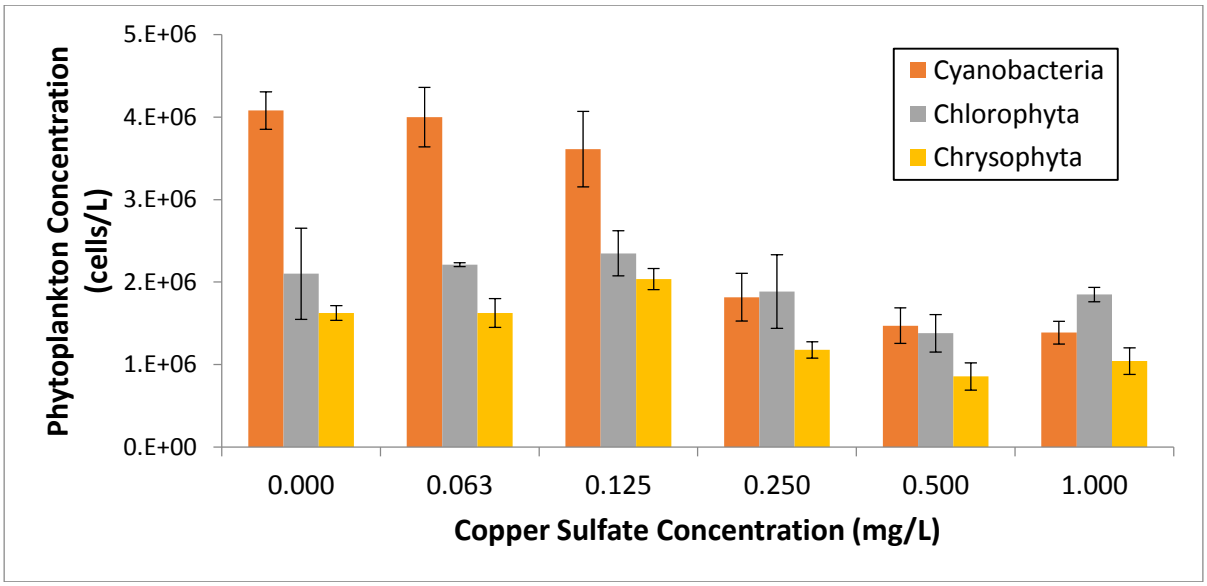

Figure 34: Concentrations of phytoplankton species grouped by division (shown with +/- standard error) decreased variably as concentrations of copper sulfate stock solution in samples increased.

Species present in FCR's natural phytoplankton community responded to copper sulfate exposure variably after 24h. Significant differences were present amongst the concentration means of the following phytoplankton species in samples dosed with 0.000 to 1.000mg/L of copper sulfate stock solution as compared to that of the control: *Anacystis* (one-way ANOVA; $F_{3,8} = 17.85$, $p = 0.00003$), *Anabaena* (one-way ANOVA; $F_{3,8} = 14.68$, $p = 0.00009$), *Mallomonas* (one-way ANOVA; $F_{3,8} = 16.92$, $p = 0.00005$), *Cyclotella* (one-way ANOVA; $F_{3,8} = 7.90$, $p = 0.002$), *Cocconeis* (one-way ANOVA; $F_{3,8} = 6.80$, $p = 0.003$), and *Cryptomonas* (one-way ANOVA; $F_{3,8} = 5.61$, $p = 0.007$). However, significant differences were not present among mean concentrations for *Ankistrodesmus*, *Chrysococcus*, *Chlorella*, *Ulothrix*, *Euglena*, and *Closterium*. Thus, copper sulfate dosing has a species-dependent effect on FCR's natural phytoplankton community.

Overall phytoplankton responses to copper sulfate dosing were not significant 72h after exposure. ANOVA results indicated that no significant differences were present amongst the total phytoplankton concentrations in samples exposed to varying doses of copper sulfate stock solution. Total phytoplankton concentrations in dosed samples were higher than those measured in 72h control samples (Figure 33). Therefore, effects of copper sulfate on FCR's phytoplankton community diminished prior to 72h after exposure. One possibility for these results is that copper resistant mutant species that survived the first day of exposure began to dominate the community thereafter (García-Villada et al. 2004). Continuous applications of copper sulfate on FCR can increase the concentration of copper resistant mutant species in the reservoir, decreasing the effectiveness of the algaecide over time.

Conclusions

Results from the study were used to determine the limiting nutrient for phytoplankton growth in response to the oxygenation system and the effect of varying copper sulfate dosages on the natural phytoplankton community in FCR. We determined that FCR's oxygenation system limits phytoplankton growth by preventing the release of phosphorus from sediment. The threshold to reduce phytoplankton concentrations 24h after copper sulfate application falls between 0.125mg/L and 0.250mg/L. However, the effectiveness of copper sulfate diminished by 72h after application. Recommendations for further study include performing a nutrient enrichment

assay when FCR is anoxic and dosing samples with a narrower range of copper sulfate concentrations (0.125mg/L to 0.250mg/L) to determine an optimal application to reduce phytoplankton concentrations 24h after application.

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NSF REU Interdisciplinary Watershed Sciences and Engineering Assessment Report

Virginia Tech, Summer, 2013

John Muffo

The following is an independent assessment of the level of success of the program conducted during the summer of 2013. As in 2007 through 2009 and in 2011-2012, 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. Most came to the program with some commitment to attend graduate school, though this group seemed more interested in part-time and master's level study but wanting to use the experience as an opportunity to explore the matter further. The levels of commitment stayed the same or weakened slightly over the summer; most do not expect to go on for a Ph.D. immediately after their undergraduate programs.

The students in the program reported the greatest gains in appreciation of the role of graduate students in research and understanding the processes used to monitor water quantity and water quality. The third largest gain was in those planning to go to work soon after graduation. Other areas of reported growth include being aware of the many ways in which scientists from different fields interact with each other in conducting research in water sciences and gaining an appreciation for the role of faculty in research. In addition, they seemed to genuinely enjoy each other's company; the esprit des corps of the undergraduate students was quite obvious and came through in their comments. The suggestions for improving the program were modest and generally reflected logistical matters. On the positive side, they found a number of the seminar presentations interesting and were complimentary regarding the field trips as well, though they did offer suggestions for improving both.

Entering Survey

There were ten students who completed the pre-test during the summer of 2013. Their responses are below, in order of the highest to lowest responses. (The questions were developed in cooperation with the faculty who are the Principle Investigators for the project. They were revised in 2011-2012 based on the questions asked in 2007-2009.)

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 have an appreciation for the role of faculty in advising students. – 4.40
- Water research can be challenging. – 4.40

- I have a good understanding of the role of ethics in scientific investigations. – 4.20
- I have an appreciation for the role of faculty in research. – 4.10
- I am aware of many ways in which scientists serve their communities. – 4.00
- I have an appreciation for the role of graduate students in research. – 3.80
- I can communicate scientific concepts effectively to a scientific audience. – 3.80
- I am aware of many opportunities for employment in the water field. – 3.70
- I plan on attending graduate school soon after I graduate. – 3.60
- I am confident that I understand how to conduct scientific research. – 3.50
- I know everything that I need to know to conduct scientific research in the library. – 3.50
- I understand the processes used to monitor water quantity and water quality. – 3.00
- I am aware of the many ways in which scientists from different fields interact with each other in conducting research in water sciences. – 3.00
- Social network sites (e.g., Facebook, YouTube, etc.) are a good way to share scientific research results. – 3.00
- I plan on going to work soon after I graduate. – 2.90
- There are winners and losers in environmental conflicts; it's as simple as that. – 2.30
- The use of statistics is not important in water research. – 1.40

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 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 during this 10-week long NSF/REU program.

Exiting Survey

At the completion of the program the same ten students completed the same survey with the same questions. Their responses are below, again in order from the highest to lowest.

- I have an appreciation for the role of graduate students in research. – 4.80
- I have an appreciation for the role of faculty in research. – 4.70
- I have an appreciation for the role of faculty in advising students. – 4.60
- Water research can be challenging. – 4.50
- I have a good understanding of the role of ethics in scientific investigations. – 4.40
- I am aware of many ways in which scientists serve their communities. – 4.15
- I can communicate scientific concepts effectively to a scientific audience. – 4.00
- I understand the processes used to monitor water quantity and water quality. –3.90
- I am aware of many opportunities for employment in the water field. – 3.80
- I am aware of the many ways in which scientists from different fields interact with each other in conducting research in water sciences. – 3.75
- I am confident that I understand how to conduct scientific research. – 3.70
- I plan on going to work soon after I graduate. – 3.70
- I know everything that I need to know to conduct scientific research in the library. – 3.45
- I plan on attending graduate school soon after I graduate. – 3.40
- Social network sites (e.g., Facebook, YouTube, etc.) are a good way to share scientific research results. – 3.05
- There are winners and losers in environmental conflicts; it's as simple as that. – 2.40
- The use of statistics is not important in water research. – 1.30

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 field trips that you participated in during the past 10 weeks. Feel free to list the trips 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.

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. 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 have an appreciation for the role of graduate students in research. – 1.00

- I understand the processes used to monitor water quantity and water quality. – 0.90

- I plan on going to work soon after I graduate. – 0.80

- I am aware of the many ways in which scientists from different fields interact with each other in conducting research in watershed sciences. – 0.75

- I have an appreciation for the role of faculty in research. – 0.60

- I am confident that I understand how to conduct scientific research. – 0.20

- 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. – 0.20

- I have an appreciation for the role of faculty in advising students. – 0.20

- I am aware of many ways in which scientists serve their communities. – 0.15

- There are winners and losers in environmental conflicts; it's as simple as that. – 0.10

- I am aware of many opportunities for employment in the water field. – 0.10

- Water research can be challenging. – 0.10

- Social network sites (e.g., Facebook, YouTube, etc.) are a good way to share scientific research results. – 0.05

- I know everything that I need to know to conduct scientific research in the library. – (0.05)

- The use of statistics is not important in water research. – (0.10)

- I plan on attending graduate school soon after I graduate. – (0.20)

To summarize, the greatest reported gains over the summer were in the areas of appreciation of the roles of graduate students and faculty in research and observing the ways in which scientists from different fields interact with each other in conducting research in water sciences. Other than increasing the likelihood of going to work soon after graduation, most of the other changes over the summer were somewhat minimal.

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 participated in it. 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?

- It was not centrally controlled. The students were able to work on individual projects with graduate students and professors.
- There was freedom as part of research projects. The students were treated as professionals, having to figure out things for themselves, be accountable. In this sense it simulated graduate school, was equivalent to being a graduate student.
- Liked the diversity of the field trips.
- Liked the majority of the seminars, especially the ones about library resources and the graduate seminar.
- Deadlines were flexible, research-oriented. Would recommend the summer program. They can continue working on the research after leaving VT.
- Professors value quality more than quantity or a deadline.
- Liked the concept of the build-up of timing of presentations, building up the amount of time; many have not done scientific presentations before.
- Enjoyed the symposia, presenting to other researchers.
- Liked getting paid for their work.
- Liked the freedom to research a topic, the flexibility to explore within a topic area.
- 5-minute presentations were good in the beginning.
- They liked the group. They learn from each other. There is mutual support and a building of friendships, coming back to people you know at night. They will continue to stay in touch with each other. They also like the diversity within the group, the different academic and college backgrounds.

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

- Some projects still being brainstormed upon arrival; student(s) did not know what to present initially.
- Desire for more consistency among projects; there is too much variation among mentors, which is seen as not being fair to all students.
- One student complained of being shoved into a room all day with nothing to do and minimal supervision, minimal mentor supervision.
- A lack of central control leads to more variability in mentorship; sometimes the students are not provided a lot of guidance but are allowed the freedom to do their research.
- The interview beforehand with the professor and the graduate student helped match the student with the project and the expectations.
- Sometimes they spent too much time in meetings discussing the logistics in the labs, doing trivial things.
- The reflection papers were not as useful as they could have been because the students were afraid of insulting the faculty and graduate students. They were not totally honest, since they were afraid of upsetting somebody.
- The responses to their questions were not always done in a timely manner, e.g., regarding expectations regarding the final presentations.
- Overall communication was weak, e.g., regarding status reports with professors.
- Earlier feedback on final presentations would have been helpful.
- Some seminars were not helpful. For example, the way that ethics was discussed. There could have been a discussion of environmental issues rather than a presentation. The format should be more discussion than lecture. The last one was good, could be a model for others.
- There was variability in student experiences regarding relationships with graduate students. Some had poor relationships.

3. List the top three things that you learned (within and outside of your discipline) during this program.

- Counting bacteria
- Using machines in labs
- Using machines to measure carbon

- Endochronic disruptor
- Nanotechnology and medical research
- Formal lab experience

- Stormwater network system
- Clean sensor in stream
- Communicating with other departments on campus

- Hierarchy in a department and how to act
- Working with lab equipment
- Setting up a lab experiment

- Use of MATLAB
- Lot about coastal engineering
- How research works and what it takes to get useful results

- Water quality and quantity, which is outside of my field

- Data processing
- Use of LABVIEW in programming

- Putting data together to tell a story

- That I don't want to go to graduate school for engineering. I find qualitative analysis more interesting than data and experiments. I will go in a different field.

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

- Seven (out of ten)
- Basically the summer experience let them try out graduate school but did not change their minds very much. Two students did say that they are going to professional school but not graduate school for a Ph.D. but rather an M.D. in one case and a Masters in Public Health in the other. Both of them raised their hands when asked if they are going to graduate school, since both of these are graduate school beyond a bachelor's degree. As in other years, others also suggest that they might go to graduate school part-time while working.

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

- Seven (out of ten)

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

- They could see improvement in everybody's public speaking.
- They learned to communicate more tactfully with supervisors.
- Regarding written communication, a lot was done through e-mail. There were daily reports in the labs also for some while others did not write as much. Blog reporting for some was also helpful.
- Only two students reported using Facebook very much.

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

- The wastewater treatment plant was a useful one.
- The fire station visit was a good one, but not for the intended reason.
- ICTAS presentation was dynamic.

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

- Social/cultural part was great.
- Good night life.
- Enjoyed the outings.
- Small things like sinks, elevators, etc. in dorms need work.
- Needed gluten-free options in dining hall from the beginning.
- The meals were repetitious.

8. Other comments?

- None.

Concluding Comments

The group in 2013 differs from that in the five prior years in that that they did not seem to have a high commitment to Ph.D. research when they began the program and did not share a strong knowledge of how graduate students and faculty conduct research. At the end of the summer they report having learned a lot about how research is conducted by graduate students and faculty and how faculty from different disciplines interact with each other in conducting water research, yet their commitment to doctoral research, if anything, weakened a bit. The rankings of most of the survey answers generally followed the patterns of prior years, however, and their opinions about the program itself were generally positive.

Appendix A

Open Ended Questions – Beginning of the Summer

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

- I appreciated my graduate mentor contacting me via e-mail to introduce himself + the lab. That was very useful and inviting. Overall, very impressed with logistical details + communications.
- I liked the application process.
- Better communication of requirements/materials on professor's end.
- I found the application process to be comprehensive & appropriate for the program for the most part. However, I would have liked longer descriptions of each project before I was encouraged to rank my selection. I also liked the emphasis on the interview – I liked that I was able to speak to both the faculty advisor & graduate student.
- The application process was pretty streamlined, I thought, though the Lewas website was a little un-navigable, as I remember it.
- No suggestion.
- I believe a phone interview would be beneficial for the candidates to discuss the research further before coming to Virginia Tech.
- I thought that it was a good process. There was a quick response, but very little time for me to decide.
- Possibly add another short essay question for students on the application.
- I would suggest incorporating a longer personal essay to be included in the application or additional short-answer questions.

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

- I am nervous that I will be asked/responsible for a very difficult/complicated/ advanced project that I won't do perfectly.
- I am concerned about being unprepared.
- None that come to mind.
- I am concerned that my capabilities will not be as extensive as Dr. xxx might expect & that I will be expected to have knowledge of procedures or data that I have not yet encountered.

- My concerns are that I don't have the biggest background in what I am studying; more of a groundwater type. I'm also still a little unsure about what I'll be doing/ studying as I haven't been in major communication with anyone from the program.
- The only concern – building a database will be challenging to me since I do not have any experience in doing it.
- That I do not have the necessary skills to be an adequate resource for my Professor.
- My only concern is that this research will not be of assistance to me in the long run.
- I am concerned about how little I know about the specifics of my research program + about the REU in general.
- I do not have concerns but am excited to get started & learn more about the program along the way.

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

#1

- Field research (collecting samples) and testing them in the lab, articulating results; full "cycle" to project.
- ??? over fields/labs; interdisc./??? cooperation.
- Become familiar with Virginia Tech campus + its departments and what it has to offer. I'd like to develop the confidence and experience to establish a rapport with faculty + staff to possibly consider applying for graduate school. (I'm entering my fifth year of my Bachelor's program.)

#2

- Work in a chem lab.
- The outdoor Virginia experience.
- An intro into groundwater research.

#3

- Learn how to properly operate lab equipments like GCMS, AA etc.
- Learn how to write a publishable scientific report.
- Learn how to work alongside graduate students in a lab.

#4

- How to interact appropriately in a laboratory setting with other scientists & researchers.
- The role of water quality in human health & the impacts of various filters on drinking water.
- Compiling & presenting research in appropriate ways to exhibit my work to others of professional backgrounds.

#5

- Expanded horizons w/ regards to knowledge & understanding of hydrosphere.
- An appreciation of how real life research is undertaken, and perhaps a glimpse into what that would look like in grad school.
- A little insight into whether or not I can do this w/ my life.

#6

- Build Labview based water monitoring system

- Build a database using my SQL command.
- Website interface.

#7

- Improved knowledge about Research and Graduate Study.
- Current Water Issues and possible ways to resolve them.
- A publication of my worth here.

#8

- How better to do research.
- Ways that digital cameras can be used in the field.
- How better to communicate results of research.

#9

- Gain hands-on research experience in the field of water quality.
- Interact with fellow students, graduate students, + faculty to conduct research.
- Learn more about presenting + communicating scientific findings.

#10

- To learn more about the effect of ayanotoxins on aquatic health & necessary treatment for drinking water.
- To obtain samples and perform water quality analyses.
- To have a great internship experience at Virginia Tech.

Appendix B

Open Ended Questions – End of the Summer

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

- I enjoyed the recreational/social activities Blacksburg had to offer, and was happy that the program has a couple BBQs and fancy lunches/dinners, breakfasts, etc. I did have time/energy to travel with my friends. However, I was way too tired, stressed, or weighed down with work to want to DO anything. Perhaps alleviating some of the pressures next year so students can explore other opportunities.
- I had a wonderful group of my fellows during the 10-week program. We did many outdoor activities. However, because of my task responsibilities, I could not be involved in all. A suggestion to any one coming next year is to be prepared for all kinds of challenges and enjoy the job/responsibilities.
- Planned but optional social excursions to areas across Virginia. Maybe something to the ocean.
- Better planning of program. I got the feeling that my research topic was still in a brainstorming phase upon my arrival. Also in the case of multiple advisors, better communication between both parties.
- Social activities such as hiking, traveling to historic sites, and activities in Roanoke were successful. Travel to other nearby states is encouraged.

- I enjoyed the hands-on approach taken with students concerning social activities this summer. It gave us a chance to work together and form natural, smaller groups for weekend and evening excursions. The "what to do in Blacksburg" pamphlets given to us at the beginning were very helpful!
- Socially I think the group was pretty self-contained: beach trips, volleyball games, hikes were all part of the scene. As the program wore on, schisms sort of developed in the group, leading to social clumping, and then, towards the end, papers came before parties. It was good that Blacksburg folk were here to direct the group to local highlights, but the group would have figured it out without them.
- I really enjoyed the two grill outs that we did as a full group and wish there could have been more of them.
- The social activities that we did on our own were very fun and we did a variety of things (hiking, road trips, triathlon, etc.). However, the events organized by the REU could be more interesting. Maybe participate in more of the programs put on by the office of undergraduate research.
- Claytor Lake is a bit far to travel for a BBQ. BBQ's were a nice way to get together. I enjoyed going out to lunch prior to the LEED field trip. Social activities outside of those that were mandatory were more enjoyable.

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.

- I was very impressed with the organization of these seminars and felt that they've contributed a lot to the program. I feel that meeting the experts in these fields have made me more aware of career and research options. However, I got the feeling that not everyone agreed with being forced to go. Perhaps cramming less into each Friday and making it optional.
- Most of the seminars were not related to my field of study (computer engineering), but I enjoyed being part of the team. It was exciting and a new experience to know about the aspects of civil and environmental engineering/water science.
- I liked the water and wastewater treatment plants the most. More infrastructure trips. The LEED buildings were cool but not as cool.
- The weekly seminars, well I understood their purpose and see sense to it. I enjoyed Dr. Edward's speech as well as Dr. Lohani's Indian colleagues. An inclusion of round table discussion of water related issues should be taken into consideration.
- Most presentations were done well. I found the nano-tech and Mr. Edwards most interesting. I feel like the graduate student panel was excellent.
- I enjoyed the weekly seminars for the most part. I think that it would have been beneficial to either keep them in a more consistent time slot or make sure schedule we got at the beginning had all the correct times so that we could plan weekend activities accordingly.
- For some, I wish the seminars had been specifically related to professor's research. Liked: Edwards, Microfinance/water in India, Nanotech. The rest I don't remember.
- I felt the seminars were very useful. I wish the library seminar had been in the first two weeks. I also wish that the impromptu seminar on the tablets used here had been more interactive than just hearing about it.
- I liked the design and nanotechnology seminars best. I enjoyed the field trips. I did not enjoy the seminars focused on VT (especially the ethics seminar because it wasn't about ethics, it was just about how ethics is integrated into the classroom at VT). The graduate student panel was excellent.

- Seminars were not always useful (including library research, ethics video, 10-minute presentation, using tablets, and Dr. Vess' talk. However, doing two presentations throughout (5 and 15 minute) was a good way to practice speaking in front of peers. I enjoyed Dr. Edwards' talk, as well as learning about water treatment technologies in India. The library seminar should happen in week one or two, not later.

Please comment on the field trips you participated [in] during the past 10 weeks. Feel free to list the trips you liked and didn't like. Suggestions for next year are most welcome.

- Excellent. Water treatment, Nanotech were very good. I'd recommend maybe going on a group hike to App Trail to explore Riparian ecosystems there with a prof to guide tour of the nature.
- The field trips were great opportunities for knowing about the real world situations of our studies in school.
- Have research tips before ethics. Have a graduate panel with a diverse range of student backgrounds.
- I enjoyed most of the field trips especially the one to the nanotechnology labs. Wasn't too thrilled by the trip to the wastewater facility, however the trip is very relevant to the program.
- I felt like all trips were interesting except for the LEED program. I would suggest a trip to VTTI.
- While I enjoyed the weekly seminars, I found the field trips to be more engaging, interesting, and overall a better use of time. After working in the labor field on research, it was exciting to see the real-world applications and job opportunities in water-related fields. Dislike/neutral/least liked=LEED gold buildings. Most liked=Nanotech.
- I liked most all the field trips except NOAA. Don't waste our time, is my only precaution.
- I enjoyed all of the field trips. I was a bit disappointed in the LEED building field trip though, because the fire station had not kept up with most of what made them LEED certified, and the elementary school was not open, so we could not see a whole lot.
- Maybe include a trip to the smart road. One thing is that the schedule should try and stay the same without deviations from the original as much as possible.
- Most liked: nanotechnology. Least liked: LEED (but was okay nonetheless). In between: water treatment and wastewater treatment. These were enjoyable components of the Friday seminars overall; continue doing them.

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

- I personally presented 7 times, which I thought was a bit much. I was happy to have had to present at conferences/symposiums with the LEWAS lab and it was very encouraging to be treated as a valued member of the team. But, I think the presentations every couple of weeks as part of the program were a little stressful. I do, however, feel like I've become a better public speaker.
- I attended all seminars/conferences during the 10-week program (except our meeting in the first week). I had a total of 5 presentations including the one I am doing throughout the program. Having the opportunity to do presentations was one of the most essential benefits I have received during the program. It has prepare me as a more confident presenter in front of the public.
- I did not like the fact that we had 3 presentations in a week.
- I think the presentations were planned out pretty well. The 5-minute one was a bit of a challenge due to too limited dissemination of my project by advisors, which can be improved by better communication. It helped me overall prepare for the symposium, and I am in agreement with the current standards.
- The number of presentations was sufficient for the REU.

- As much as I disliked having to listen to everyone give seminar presentations 5 times over the 10 weeks, I grudgingly admit that it was extremely helpful in elevating my preparedness level, confidence, and speaking ability. I do not think (and I am fairly sure of this) that I would have practiced this extensively otherwise, and I am glad that my presentations encouraged a more well developed and comprehensive PowerPoint and presentation.
- I'm a little non-committal about this, but I think 5 was the right amount. Mine really didn't come together until the final. However, there really shouldn't be 3 presentations in the last week. That's a time sink and redundant when you're trying to finish a paper, and since many had results coming in that final week, it meant 3 new presentations and a lot of work.
- I liked getting to practice our presentations three times before our two final ones. And I liked the timing of them. The first one being two weeks in I think is good to better give our fellow researchers an idea of what we're doing.
- The frequency and timing of presentations was good. It was nice building from a five minute presentation to a 15 minute presentation. The peer feedback was excellent and the standardized procedure for feedback was very helpful. I felt that the practice presentations helped me to enhance my communication skills and helped me learn how to relate scientific ideas to the community.
- At this point I'm extremely bored hearing everyone's presentation – and bored of my own! (extreme repetitiveness – 5 x presentations)! I think the five minute presentation was a good way to get ideas/goals solidified early on in the program and the minute one was good practice prior to the final presentation. However, the ten minute presentations was not needed – namely because results were not clear for a majority of the fellows half way into the program. Also, during the 15 minute presentation, Dr. Lohani should ask questions of the presenter.

NSF/REU Site Announcements

Short Announcement

Summer 2013 (May 26 – August 3, 2013) - Undergraduate Research Fellowships Announcement

National Science Foundation Research Experiences for Undergraduates (REU) Site
INTERDISCIPLINARY WATER SCIENCES AND ENGINEERING
Virginia Tech, Blacksburg, Virginia
Application Deadline February 18, 2013 (Monday)

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 26-August 3, 2013) summer research in interdisciplinary water sciences 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 26, 2013 (arrival day) at Virginia Tech and end on August 3, 2013 (departure day: Aug. 3 or 4). The research internship includes a stipend of $450/week, subsistence costs (dormitory and most of the meals) and round trip travel expenses, limited to a maximum of $500 per person, to Virginia Tech. We have already graduated **45** excellent undergraduate researchers from our program during 2007, 2008, 2009, 2011, and 2012. Application materials, details of the ten Research Mentors along with possible research projects and other program activities are posted on following website:

http://www.lewas.centers.vt.edu/

Example Projects:

- Natural Attenuation of Contaminants in Groundwater
- Hydrology and Hydraulics Impacts on Ecological Health of Surface Waters
- Bacterial Contamination of Water Distribution and Plumbing Pipelines
- Water Quality for Human Health and Aesthetics
- Investigation of Occurrence and Fate of Organic Contaminants in a Watershed Impacted by Urban Development
- Hypolimnetic Oxygenation: Coupling Bubble-Plume and Reservoir Models
- Design and Application of a Real-Time Water Monitoring System
- Development of a Still-Camera Remote Sensing Tool for Measuring Nearshore and Onshore Coastal Features
- Bioremediation of Oil Spills
- Analysis of Patterns of Macroinvertebrate Density and Distribution in Strouble's Creek
- Biogeochemical Controls on Trace Element Transport and Transformation

Deadline for application submission is **February 18, 2013**. Successful applicants will be informed by March 12, 2012. Please contact **Dr. Vinod K Lohani** (Phone: (540) 231-9545; Fax: (540) 231-6903; E-mail: vlohani@vt.edu) for questions.

Long Announcement

Summer 2013 - Undergraduate Research Fellowships Announcement
National Science Foundation Research Experiences for Undergraduates (REU) Site
INTERDISCIPLINARY WATER SCIENCES AND ENGINEERING
Virginia Tech – Application Deadline February 18, 2013 (Monday)

Program Description: Applications are invited from qualified and motivated undergraduate students (rising sophomores, juniors and seniors – engineering and science majors) from all U.S. colleges/universities to participate in a 10-week summer research in interdisciplinary water sciences 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. This NSF REU site has successfully hosted a total of 45 undergraduate researchers in summers of 2007, 2008, 2009, 2011, and 2012.

Successful applicants (hereafter referred to as REU fellows) will join one of the ongoing research projects in water sciences and engineering and conduct research under the supervision of Virginia Tech faculty and graduate students. Research projects address issues related to sustainable management of water resources and water infrastructure, and facilitate opportunities of field research, laboratory work and testing of theoretical concepts. See Appendices 1 and 2 for list of faculty advisors and typical 2013 summer research projects, respectively. The summer research program is complemented by other professional activities. For example, REU fellows will attend weekly forums and participate in a few field trips. Speakers at these forums will include VT faculty members, graduate students and experts from water industry and government. These weekly forums provide an excellent opportunity to REU fellows to learn about commonalities between their various research projects, interact with each other and with other research mentors. REU fellows will make frequent presentations to their peers about their research progress and ultimately prepare a research report in collaboration with their research mentors suitable for conference presentation and/or publishing in a refereed journal or other appropriate publications.

Social interaction and networking is a major goal of the program. Several social activities are organized to encourage informal personal interaction between REU Fellows and the research team and the larger university community. See Appendix 3 for possible recreational activities.

Financial Support: The 10-week internship will begin on May 26, 2013 (arrival day) at Virginia Tech and end on August 3, 2013 (departure day: Aug. 3 or 4). The research internship includes a stipend of $450/week, subsistence costs (dormitory and most of the meals) and round trip travel expenses, limited to a maximum of $500 per person, to Virginia Tech.

Application: The deadline to receive all application materials is **February 18, 2013**. Applications should be submitted online via the website: **http://www.lewas.centers.vt.edu/.** The application should include:

1. A 300-word essay about your interest in water/environment research and professional goals, and indicate top two choices of summer research project and include a brief justification (see Appendix 2). The justification should be part of your essay. This should be uploaded as a PDF document in the online application form.
2. Unofficial College transcripts, to be uploaded as a PDF document in the online application form.
3. Two letters of reference to be sent by your referees to Dr. Lohani (See email address below). *Letters should address candidate's motivation, enthusiasm, reliability, team-work and personality.*

Successful applicants will be announced by March 11, 2013. For questions, please contact: **Dr. Vinod K Lohani**, NSF REU Program Director, e-mail: vlohani@vt.edu; Phone: (540) 231-9545; FAX: (540) 231-6903

Appendix 1. Program Management Team and Research Mentors

Name	Organization	Responsibility	Academic Discipline and Field of Study
Dr. Widdowson	Virginia Tech	Research Mentor; Participant Selection	Civil & Environ Eng.; Groundwater Hydrology
Dr. Hester	Virginia Tech	Research Mentor; Participant Selection	Civil & Environ Eng.; Ecohydraulics
Dr. Edwards	Virginia Tech	Research Mentor; Participant Selection	Civil & Environ Eng.; Water Infrastructure
Dr. Dietrich	Virginia Tech	Research Mentor; Participant Selection	Civil & Environ Eng.; Analytical Chemistry
Dr. Xia	Virginia Tech	Research Mentor; Participant Selection	Crop & Soil Environ Sciences – Soil Chemistry
Dr. Little	Virginia Tech	Research Mentor; Participant Selection	Civil & Environ Eng. ; Lakes & Reservoirs
Dr. Lohani*	Virginia Tech	Project Director (PI); Program Coordinator; Recruitment & Selection; Assessment; Cohort Experiences/ Professional Development; Dissemination; Research Mentor	Civil and Agricultural Engineering; Watershed Instrumentation, Hydrology, and Engineering Education
Dr. Irish	Virginia Tech	Research Mentor; Participant Selection	Civil & Environ Eng.; Coastal Engineering
Dr. Pruden	Virginia Tech	Research Mentor; Participant Selection	Civil & Environ Eng.; Environmental Contaminants
Dr. Benfield	Virginia Tech	Research Mentor; Participant Selection	Biology; Ecology; Macroinvertebrates
Dr. Schreiber	Virginia Tech	Research Mentor; Participant Selection	Hydrogeosciences; Chemical Hydrogeology
Dr. Muffo	Independent Assessment Consultant	Evaluation/Assessment	Academic Assessment

* Project Management

Appendix 2. 2013 NSF REU Potential Research Projects

2013 NSF REU Potential Research Projects

Please select your top two choices and enter the corresponding project IDs in the application form.

Project ID: 001 Title: Natural Attenuation of Contaminants in Groundwater
Mentor: Dr. Widdowson
Complex contaminants are known to biodegrade at slow rates in groundwater systems under the right environmental conditions. This process, known as natural attenuation, is linked to the flux and bioavailablity of electron donors and electron acceptors. Bioavailable ferric iron in aquifer sediments is thought to be a critical component in the long-term sustainability of natural attenuation. This research will employ the use of computational tools developed at Virginia Tech to determine the limits of sustainable natural attenuation of contaminants in several model aquifers. In addition, the REU participant will develop laboratory skills related to the measurement of ferric iron concentration using samples collected at several field sites. He/She will work with Dr. Widdowson to publish the results of this research in the form of a conference proceedings paper.

Project ID: 002 Title: Hydrology and Hydraulics Impacts on Ecological Health of Surface Waters
Mentor: Dr. Hester
This research aims to understand the mechanisms connecting human activities in stream corridors and watersheds with degradation of aquatic ecosystems and water quality, to allow better informed ecological stream and river restoration design, pollutant attenuation by natural processes, and watershed planning. Current projects include field experiments and associated data analysis to evaluate the effect of human activities such as urbanization on surface water-groundwater exchange, floodplain hydraulics, and temperature dynamics in streams and rivers, all of which can strongly impact aquatic organisms and water quality. The REU participant's role will vary but typically entail installing piezometers or using of geophysical techniques to monitor surface water-groundwater exchange; installing, monitoring, or downloading hydraulic and water quality sensors; surveying streambed and floodplain topography; collecting water quality samples; analyzing sensor or survey data; and presenting results in a written report or oral presentation.

Project ID: 003 Title: Bacterial Contamination of Water Distribution and Plumbing Pipelines
Mentor: Dr. Edwards
The growth of pathogens in home plumbing poses a significant human health threat and is currently a primary source of waterborne disease in the US. Two pathogens, Legionella pneumophila and Acanthamoeba, are of particular concern when present together, because Acanthamoeba can induce rampant growth of L. pneumophila by serving as a host organism. This research will develop techniques to identify and enumerate L. pneumophila and Acanthamoeba using PCR and Q-PCR methodology. When applied to practical experiments in simulated potable water heaters effects of nutrients such as organic carbon on L. pneumophila and Acanthamoeba growth can be established. The goal of this work is to establish organic carbon thresholds in which Acanthamoeba (and by extension L. pneumophila) can proliferate. The REU participant would first develop, and then execute, a sub-set of experiments to address this issue, in collaboration with a graduate student, under the direction of Dr. Edwards. He/She would also write up the results and hopefully, present the work at a major research conference.

Project ID:004 Title: Water Quality for Human Health and Aesthetics
Mentor: Dr. Dietrich
The increasing demands on the world's water supplies had led to the need for using lower quality water sources for drinking water supplies. Unresolved issues related to use of these lower quality sources include increased treatment, concern about nutritional and aesthetic content of drinking water. Projects for the REU fellows could include: 1) evaluating human exposure to dissolved aqueous metals and aqueous particulates through inhalation from vaporizers and showers; 2) performing sensory evaluations with human subject to determine suitable mineral content of drinking waters and to determine the concentration range at which humans can detect and describe tastes and odors; 3) isolating and identifying the odorous chemicals in natural and engineered drinking water; 4) research related to communicating the value of drinking water for healthy living.

Project ID: 005 Title: Investigation of occurrence and fate of organic contaminants in a watershed impacted by urban development

Mentor: Dr. Xia

Strouble's Creek is a major stream draining about 80% of the town of Blacksburg and most of the VT campus. Many large apartment complexes, residential neighborhoods, and VT campus are located along the Strouble's Creek Watershed. Past effort of the long-term stream quality monitoring program at the Strouble Creek Watershed has focused on nutrient loading and biological indicators. No information is available on the impact of organic contaminants at the Watershed. A REU project is proposed to assess urban impact on the Strouble's Creek Watershed by monitoring the levels of 4-nonylphenol, an anthropogenic organic compound, often used as an indicator for urban impact. This project combines field sampling and laboratory mesocosm experiments to evaluate the occurrence and fate of 4-nonylphenol in the Strouble's Creek Watershed. Participating student will learn latest techniques for analysis of organic contaminants in environmental samples and gain hands on experience with the state-of-the art analytical instrument such as gas chromatography-tandem mass spectrometry (GC/MS/MS). Under the direction of Dr. Xia, the participating student is expected to write up the results and present the work at a research conference.

Project ID: 006 Title: Hypolimnetic Oxygenation: Coupling Bubble-Plume and Reservoir Models

Mentor: Dr. Little

The REU participant will be engaged in a recently funded NSF project entitled "Hypolimnetic Oxygenation: Coupling Bubble-Plume and Reservoir Models." Bubble-plumes are increasingly used to replenish oxygen in the hypolimnion of stratified reservoirs. These devices are required to sustain cold-water fisheries, improve raw water quality in water-supply reservoirs, and mitigate the environmental consequences of hydropower generation. However, bubble-plumes may induce significant mixing that in turn can change stratification, enhance sediment oxygen demand, and cause hypolimnetic warming. The plume action also alters the prevailing vertical density gradient in the water column, which affects the performance of the bubble-plume in a feedback loop.

Project ID: 007 Title: Design and Application of a Real-Time Water Monitoring System

Mentor: Dr. Lohani

A LabVIEW Enabled Watershed Assessment System (LEWAS) has been successfully tested on VT campus for remotely assessing real-time water quality and quantity data from a creek that flows through Virginia Tech campus. LEWAS integrates a water quality sonde and a flow meter with LabVIEW and provides the capability to sense temperature, conductivity, dissolved oxygen, turbidity, and pH of water. In addition, a weather station has also been integrated into LEWAS to allow real-time monitoring of weather parameters like precipitation, temperature, humidity, etc. The data is shared with remote clients via Wireless LAN. The field implementation of LEWAS employs an industrial computer (compactRIO from the National Instruments) as server which can run remotely and continuously without user intervention. The REU participant will: (i) participate in integrating software and hardware components of the LEWAS, (ii) conduct data collection work at LEWAS outdoor site and carry out analysis of real-time water data and associate these with ongoing activities on Stroubles Creek watershed, and (iii) document research results and experiences in a paper for a conference / journal publication.

Project ID: 008: Development of a Still-Camera Remote Sensing Tool for Measuring Nearshore and Onshore Coastal Features

Mentor: Dr. Irish

Historically, high-resolution video cameras have been fixed-mounted at beach locations to study nearshore processes, or features of the area near the coastline. In this study, a less expensive approach for observing beach features will be investigated. The purpose of this investigation is to develop a method for using photographs taken from a low-cost, hand-held digital camera and coordinates from a handheld GPS to determine the location of objects within the image. For example, an investigator on a site visit to a post disaster beach may bring a digital camera and take pictures to quantify coastal impacts of a hurricane or tsunami. Using surveyed positions of fixed objects within the photos, the investigator can then determine locations of subaerial features, such as shoreline position, inundations, or dune scarp, and submerged features, such as locations of sand bars. If a series of pictures is taken over time, an estimate of the erosion or accretion rate can be estimated. The student will be responsible

for evaluating and testing a method for using digital cameras and GPS for these applications. A portion of testing will be conducted at the beach, where the student will plan and conduct a small field experiment to take photographs and locate beach features in the images. The student will be expected to write a document and present results of the development of the method, its application, and field results.

Project ID: 009 Title: Bioremediation of Oil Spills
Mentor: Dr. Pruden
Dr. Pruden's research focuses on the application of molecular biological tools in order to harness microbes to clean-up contaminants in the environment. In this project, the REU student will learn basic molecular biological tools, such as DNA extraction, polymerase chain reaction (PCR), and denaturing gradient gel electrophoresis (DGGE) in order to characterize bacterial communities involved in oil spill clean-up. A field excursion to an oil spill site in Minnesota is anticipated in order to sample and analyze contaminated groundwater and aquifer sediment. Laboratory experiments will be performed in order to determine if the native bacterial populations can be stimulated to biodegrade the oil at optimal rates. Molecular tools will be applied to identify key characteristics of the microbial communities that best degrade the oil.

Project ID: 0010 Title: Analysis of Patterns of Macroinvertebrate Density and Distribution in Strouble's Creek
Mentor: Dr. Benfield
Strouble's Creek is a 3rd-4th order stream draining about 80% of the town of Blacksburg and most of the VT campus. Downstream of campus and the Duck Pond, Strouble's Creek passes through several miles of farmland before entering a woodland area and travelling on to the New River. Members of the Biological Systems Engineering Department recently initiated a stream restoration project in Strouble's Creek in an open pastureland reach downstream from campus. I propose an REU project evaluating the effectiveness of the restoration on the macroinvertebrate assemblage in the creek. This would involve but not be limited to quantitatively sampling macroinvertebrates upstream in an open reach and in a wooded reach, at one or more sites within the restoration reach, and at downstream sites. The macroinvertebrates would be identified to the lowest practical taxonomic level and the data would be subjected to a suite of standard multimetric analysis. There would also be a number of geomorphic analyses of the stream bed, banks and associated variables leading to an attempt to explain patterns of macroinvertebrate density and distribution.

Project ID: 0011. Biogeochemical Controls on Trace Element Transport and Transformation
Mentor: Dr. Schreiber
Our research goal is to examine the biogeochemical controls on contaminant transport and transformation in natural waters. To do this, we utilize hydrologic, geochemical, and biological techniques in the field and laboratory to determine rates of reaction and properties of the medium/contaminant, in order to construct quantitative models that can be used to simulate transport and transformation. The REU participant would conduct field monitoring and/or lab experiments for a project on manganese mobility in natural waters. Experience (and interest) in field sampling, analytical chemistry, and environmental chemistry would be highly beneficial.

Appendix 3

Recreational Activities around Blacksburg, Virginia

Virginia Tech is located in Blacksburg, Virginia and surrounded by the Blue Ridge Mountains. The Appalachian Trail runs through the area and affords many hiking trails. Other hiking trails off the Appalachian Trail include a 2-mile hike to the Cascades Waterfall and Wind Rock, which affords panoramic views of nearby mountain ridges. The New River is located nearby providing kayaking, canoeing, inner tube floating, and fishing during the summer. Other outdoor activities include mountain biking at Pandapas Pond, road biking the Blue Ridge Parkway, and walking, running or biking the Huckleberry trail. The Salem Avalanche, a Class A Affiliate of the Houston Astros, play in nearby Salem, VA.

Live music in both indoor and outdoor venues is available. Friday Night Jamboree in Floyd, VA has been listed as one of the two best places to hear bluegrass music in the United States. Friday nights on Henderson Lawn (located on campus and next to downtown) is an opportunity to hear live music free during the summer. Several restaurants provide live music throughout the week such as Jazz and Bluegrass. Unique eating experiences include local eateries such as Mike's Grill (burgers and fries), More than Coffee (Mediterranean cuisine), Cabo Fish Taco, Boudreaux's (Cajun style food), The Cellar (Greek cuisine), Gillie's (vegetarian fare), Excellent Table (Ethiopian fare) as well as numerous coffee shops located next to campus. Next to campus is The Lyric, a non-profit venue that shows weekly movies and with occasional live performances and a large stadium style movie theatre is located 5 miles away in Christiansburg adjacent to the New River Mall. This is just a sample of the wide varieties of things to do and see in and around Blacksburg.

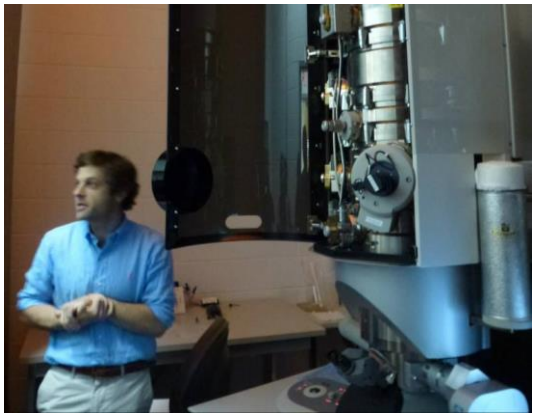
Cascade Falls, Jefferson National Forest, near Blacksburg, Virginia

2011 Site REU Fellows Visiting a Water Treatment Facility near Virginia Tech

Orientation and Concluding Ceremonies

Pictures from Summer 2013 Site

FIELD TRIPS



Environmental Nanotech Lab at Virginia Tech

Field trip to LEED Sites in Roanoke, Virginia

NOAA Weather Service Forecast Station
Blacksburg, Virginia

Wastewater Treatment Plant - Fairlawn, Virginia

SOCIAL EVENTS