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# Interdisciplinary Water Sciences and Engineering

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1- Engineering Education, 2- Civil and Environmental Engineering, 3- Crop and Soil Environmental Sciences, 4- Biological Sciences 5- Geosciences, 6- Forest Resources and Environmental Conservation

Host Lab: <http://www.lewas.ictas.vt.edu/>



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## Acknowledgements

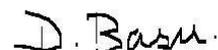
*We would like to express our sincere thanks to REU/NSF Site Research Mentors for their kind cooperation and excellent mentoring during summer 2015. Our thanks are also to our graduate student mentors, REU Fellows for their dedication and excellent performance, laboratory staff, seminar speakers, professionals 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. 1359051).*

Editorial Staff



**Dr. Vinod K Lohani**

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# Summary

This Research Proceedings includes papers of undergraduate research that was conducted at Virginia Tech during summer 2016 as part of an NSF/REU Site on Interdisciplinary Water Sciences and Engineering. This was the third year of the 3<sup>rd</sup> cycle of the NSF/REU Site that was implemented during 2014, 2015, and 2016. This 3<sup>rd</sup> cycle of this Site follows two very successful REU Site cycles that were implemented 2007-09 and 2011-13. Research Proceedings of all these years are available at: [www.lewas.ictas.vt.edu](http://www.lewas.ictas.vt.edu). At the end of summer 2016, 85 REU Fellows (55 women and 30 men) have graduated from REU Sites. Our Site continues to expose qualified undergraduates to interdisciplinary research issues in water sciences and engineering. Faculty members from five departments (Engineering Education, Civil and Environmental Engineering, Biological Sciences, Geo-sciences, Biological Systems Engineering, and Crop and Soil Environmental Sciences) at Virginia Tech mentored 9 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.

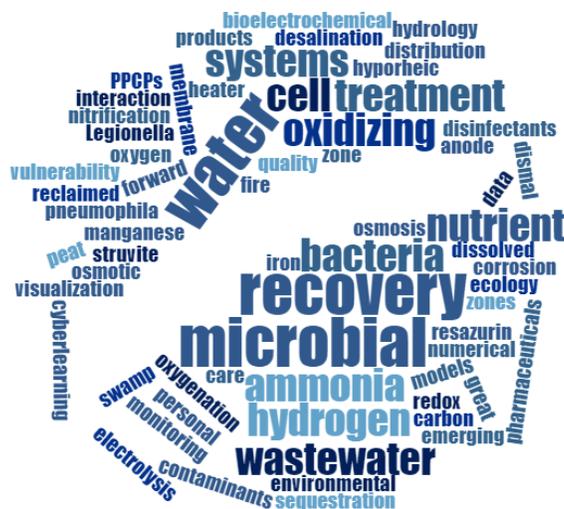


Figure 1: Word Cloud of Keywords – 2016 Research Work

Mr. Verghese, and REU scholar from Virginia Tech, and his co-authors worked in the Learning Enhanced Watershed Assessment System (LEWAS) and contributed in advancing the data visualization and interactive features of the Online Watershed Learning System (OWLS) which is the public interface of the LEWAS. A JavaScript library called Data-Driven Documents (D3) was used to update the existing live data graph (scatter plot) within the OWLS. Further, this library was used to create a grouped and stacked bar graph representation of collected water quantity and rain data over a user-specified number of days. Ms. Chapman (an REU scholar from NS State) and her co-authors examined the microbial community and redox zones in simulated reclaimed water distribution systems (SDSs). The SDSs were constructed out of polyvinyl chloride pipes consisting of three disinfectant types (free chlorine, chloramine, and no

disinfectant residual) and two nutrient levels (high and low assimilable organic carbon (AOC)). Results showed that chloramination did in fact caused nitrification, particularly in the low AOC SDS which resulted in rapid chloramine decay suggesting chlorination is a better secondary disinfectant method to maintain reclaimed water quality. Ms. FitzGerald (an REU scholar from SUNY College at Geneseo) and her co-authors studies the presence of forty Pharmaceuticals and Personal Care Products (PPCP) compounds in various water sources including an urban-impacted stream, wastewater treatment influent and effluent, and private well water. In total, seventeen PPCPs were detected in the bioretention pond, twenty-one in wastewater, and eleven in private well water at estimated concentrations from low ppt to low ppb. Ms. Gile (an REU scholar from Villanova University) and her co-authors examined the influence of wetland hydrology on physical and chemical properties of surface peat that indicate its degree of decomposition and affect its vulnerability to fire. Excluding an outlier transect, peat bulk density and organic bulk density were found to decrease significantly with increased wetness. Nitrogen concentration was positively correlated with mean water level, while organic matter fraction and carbon content appeared to be influenced significantly by factors other than hydrologic regime. Ms. Hynes (an REU scholar from UT, Austin) and her co-authors investigated the recovery of ammonia from wastewater using an Osmotic Microbial Electrolysis Cell. Excessive content of ammonia in the water supply is toxic to aquatic life. The authors used a simulated wastewater solution as anode influent and a NaCl draw solution a cathode influent and reported removal of  $41.54 \pm 6.73\%$  of ammonia from the stimulated wastewater. Ms. Krueger (an REU scholar from Virginia Tech, the home institution) and her co-authors studied iron and manganese oxidation cycles in an oxygenated reservoir in Roanoke, Virginia. The authors discussed the use of an in situ method - a side stream supersaturation (SSS) system to oxygenate the reservoir - to prevent low DO in the reservoir which typically happens in summer months due to thermal stratification. The study results showed that the SSS significantly lowers the concentrations of soluble Mn and Fe in the hypolimnion of the oxygenated reservoir in comparison to a reference reservoir in the area. Ms. Lopez (an REU scholar from the Univ. of Southern California) and her co-authors used bioelectrochemical systems to remove and recover from wastewater in the form of struvite. A four-chamber microbial desalination cells (MDCs) was constructed to test the feasibility of struvite precipitation from synthetic wastewater. The MDC - after one month - produced a stable current of 14.0 mA for a hydraulic retention time (HRT) of 16 hours and a stable average current of 14.6 mA for a 24-hour HRT. The study has potential within the MDC and wastewater treatment nexus for the large scale applications. Ms. Martinez (an REU scholar from Arizona State University) and her co-authors evaluated the growth potential of Legionella in simulated glass water heaters. The study involved use of the Bench-scale reactors to evaluate growth potential of Legionella growth in two different hot water system designs (namely, standard water heater tanks and tanks with recirculation). Preliminary data showed that agitation resulted in increased microbial growth. Ms. McCutchan (an REU scholar from Oklahoma State University) and her co-authors conducted a study to investigate natural attenuation of groundwater contaminants plumes in the hyporheic zone. For this purpose, the investigators studied the reaction of resazurin to resorufin for lab simulation of the hyporheic zone, a zone where groundwater and surface water mix.

**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**

# Implementation of Interactive Graphs to Facilitate Environmental Monitoring with a Cyberlearning System

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## Abstract

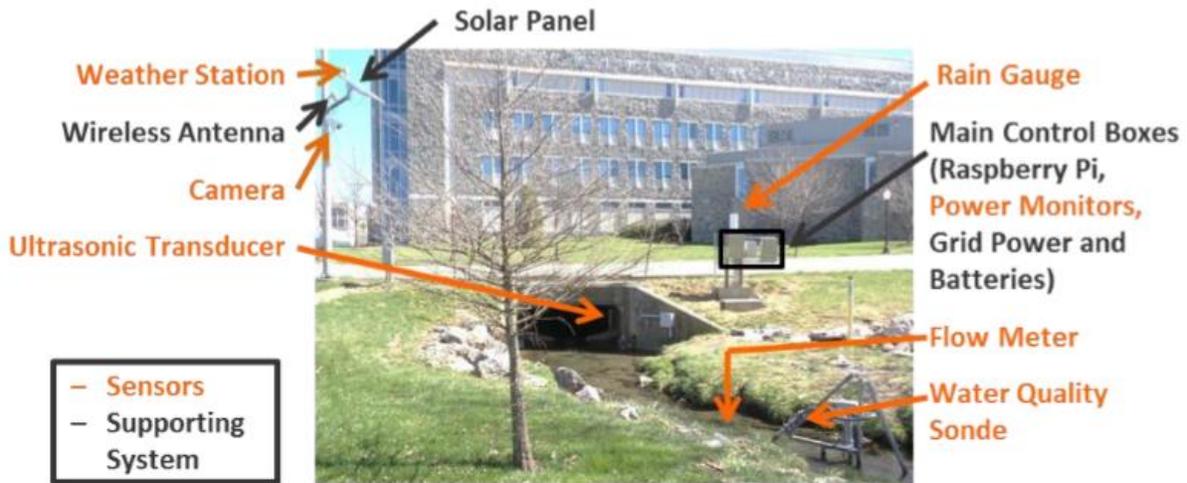
The Learning Enhanced Watershed Assessment System (LEWAS) is a unique, high-frequency, real-time environmental monitoring lab on the campus of Virginia Tech. The LEWAS has the following four stages: 1) data inputs which consist of environmental instruments including an acoustic Doppler current profiler, a water quality Sonde and a weather station each taking measurements every 1-3 min., 2) data processing occurring locally on a Raspberry Pi, 3) data storage on a remote server and 4) data visualization through an Online Watershed Learning System (OWLS) ([www.lewas.centers.vt.edu/dataviewer/](http://www.lewas.centers.vt.edu/dataviewer/)) through which end users access the LEWAS data for research and education. This paper presents the advancement of the OWLS by the addition of new data visualization and interactive features in order to facilitate cyberlearning. Data-Driven Documents (D3), a JavaScript library, was used to update the existing live data graph (scatter plot) within the OWLS with more data visualization and interactive features. Moreover, D3 was also utilized to create a grouped and stacked bar graph representation of collected water quantity and rain data over a user-specified number of days. A survey was implemented to assess the effectiveness of the new features added to the existing live data graph (scatter plot) and of the grouped and stacked bar graph. The results indicate that the new features facilitate the learning process for the environmental data. Future work will include the improvement of the OWLS and its advancement with more data visualization and interactive features based on the users' feedback through the survey.

**Keywords:** cyberlearning, interaction, data visualization, D3, environmental monitoring

## 1. Introduction

The Learning Enhanced Watershed Assessment System (LEWAS) is a unique real-time high-frequency environmental monitoring lab established to promote environmental monitoring education and research. The LEWAS field site (Figure 1) is located at the outlet of a small creek (Webb Branch) that flows through the Virginia Tech campus (W. McDonald, Dymond, Lohani, Brogan, & Clark, 2014). The LEWAS site drains a watershed which is approximately 2.78 km (Delgoshaei, 2012), and approximately 95% urban. The field site has instruments to measure water quality and quantity parameters including velocity, depth, pH, dissolved oxygen, turbidity, oxidation reduction potential, specific conductance, and temperature. In addition, weather parameters (temperature, barometric pressure, relative humidity, precipitation and wind) are also measured at the site. The LEWAS draws power from both the grid and a photovoltaic panel located at the site. The system is connected to the campus wireless network through a high-gain antenna to transmit sampled data to the LEWAS database every 1-3 minutes. The public can view the LEWAS data through the Online Watershed Learning System (OWLS). The LEWAS-based modules, including OWLS, have been implemented in 21 courses across 6 institutions in 3 countries (Debarati Basu, 2016; Delgoshaei, 2012; Delgoshaei, Lohani, & Green, 2010; Delgoshaei & Lohani, 2012, 2014; Lohani, Delgoshaei, & Green, 2009; W. McDonald et al., 2014; W. M. McDonald, Brogan, Lohani, Dymond, & Clark, 2015; Randel Dymond, 2013; D. S. B. Walter M. McDonald, Vinod K.

Lohani, Randel L. Dymond, 2015). Currently, LEWAS is expanding towards international use of its system with partners in KLE Tech of India and University of Queensland in Australia (R. L. D. Walter M. McDonald, Vinod K. Lohani, Daniel S. Brogan, Debarati Basu, 2014).



**Figure 1. LEWAS Field Site**

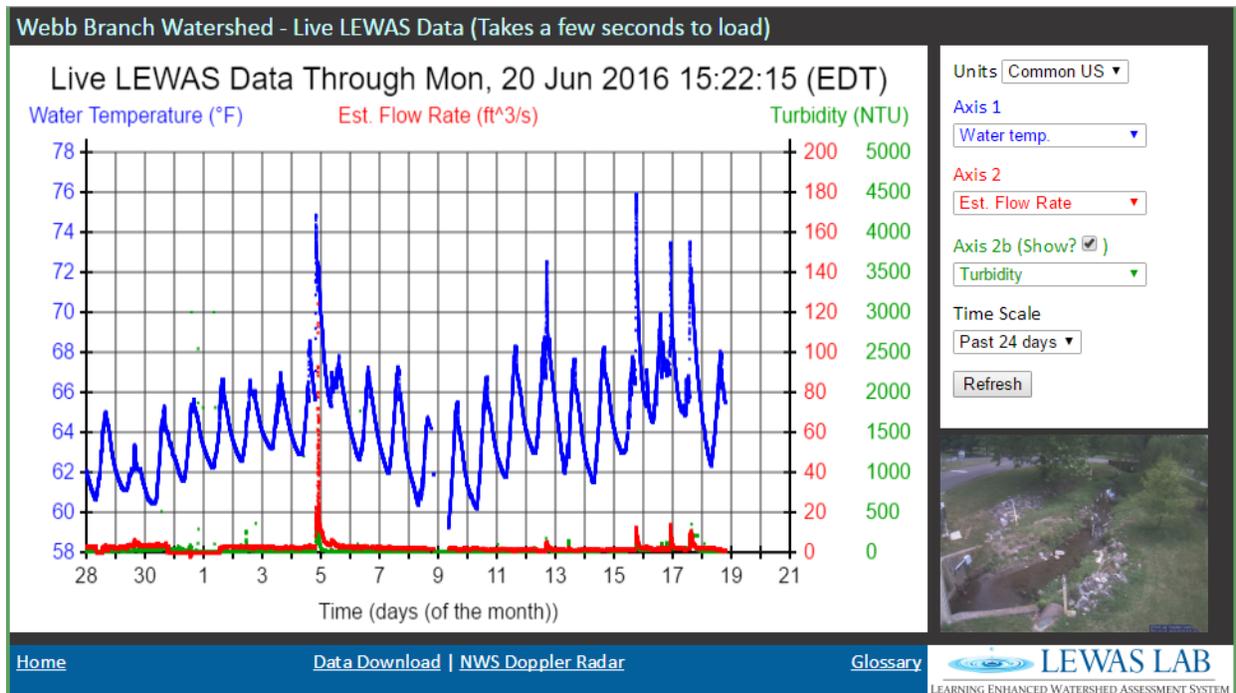
The Online Watershed Learning System (OWLS) was developed to allow the public to monitor the local Webb Branch watershed and to learn about the watershed and its environmental problems (Daniel S. Brogan, 2016). Table 1 describes various components of the OWLS and lists the intended purpose for each component (Brogan et al., 2014; Brogan et al., 2016).

Table 1.

Various Components of the OWLS with its Intended Purposes

OWLS Components	Purpose
Real Time data (of the current month), Anywhere/Anytime Access	Data availability
Interactive Graphs, Live Camera, and Weather Radar	Data visualization
Overhead View/Map, Background Information, Case Studies	Supporting information
How-to-use Guide	Operating instructions
Unique user id (UUID), time of visit, URL visited, city of the user, operating system, browser and version, referring source, service provider, and display size in pixels of the user's device, etc.	User Tracking

The OWLS live data graph provides data of a variety of parameters. These data values provide important insights into hydrologic processes, the overall health of the watershed, and the impacts of environmental issues. Furthermore, the OWLS is a hybrid online lab because it utilizes the benefits of a virtual lab and a remote lab. There is a high potential for student learning through this cyberlearning system that provides real-time data. (Daniel S. Brogan, 2016). Since the OWLS is constantly being used by more students, the features need to be updated to help them learn better.



**Figure 2. OWLS Live Data Graph (Daniel S. Brogan, 2016)**

The contents of this paper describe the advancements made to the OWLS with more data visualization and interactive features in order to facilitate the learning process through it. Section 2 is the literature review highlighting the importance of interaction and data visualization in the learning process as well as indicating the purpose for each of the added features. Section 3 of the paper is used to describe Data-Driven Documents (D3), a library of JavaScript, and the ways it was utilized to add more data visualization and interactive features to the OWLS. Section 4 provides the results from the pilot survey used to assess the effectiveness of the new features. Section 5 describes the conclusions made based on the collected data. Section 6 briefly discusses future work for further improvements of the OWLS.

## 2. Literature Review

Interaction, data visualization methods, transitions, and a clutter-free interface are all important in helping users understand data. Interaction refers to the user having the ability to make changes. Data visualization methods are ways to present data in a way that is easy to understand. Transitions are gradual changes in animated form towards a new object. Finally, clutter in an interface refers to having so many objects on a webpage that it causes confusion to the user. The goal is to keep the user focused with active (interaction), easy to understand (data visualization), easy to follow (transitions), traversable (clutter-free) data, which is outputted in the OWLS live data graph (scatter plot).

Interaction is an important part of the learning process. According to Wolfgram (1994), people remember 60% of what they interact with, which is much higher than the content they remember by hearing or seeing. If interaction is an effective tool for remembering content, then it has high potential for learning that content. Interactive multimedia is more effective than linear multimedia because it provides the user with ability to make choices. For example, according to Mohler (2001), linear multimedia such as linearly playing videos or slide-based self-learning tutorials are a possible explanation for students' short attention spans. The user must actively think about the situation presented with interactive multimedia,

providing more engagement and understanding (Mohler, 2001). The constant thinking involved with interactive material helps keep students focused. According to Moreno and Mayer (2007), the five types of interactivity are dialoguing, controlling, manipulating, searching and navigation. Advanced controlling, manipulating, and searching features help to provide a more user-focused interface by allowing the user to feel ownership for the material.

Simple representations of data, for example graphs, make it easy to understand complexities in data (Chen, Härdle, & Unwin, 2007). Although, if a graph is too complex, then the user can feel distressed and lose interest. Good data visualization methods help to ease the process of understanding data. Scatter plot often present time series data because the x axis works well for time and the y axis can hold the actual data values. More y axes can be added to output more plots based on different scales, in an organized way. Bar graphs typically present quantity data well and grouped and stacked bar graphs can present aggregated quantity data. This data visualization method (grouped and stacked bar graph) was used to present water quantity and rain data on a daily basis for the OWLS. The quantity per day of rain accumulation, rain duration, and volumetric flow of water can be put into each bar or stack, respectively, to present a day's worth of data. The data visualization method helps to present the data in a more meaningful way.

Changing a graph to a new focus can be highly disorienting to the user. In OWLS, changing a plot parameter to another plot parameter can also be highly disorienting since the chart is redrawn from scratch and the changes are not made gradually. Transitions in an animated form enforces constraints on the new chart to keep it similar to the original chart, allowing the user to follow the changes (Yee, Fisher, Dhamija, & Hearst, 2001). For example, if a plot transitions with a change in parameter from a drop down bar, then the plot change can be followed. A gradual change to the new chart provides less confusion. Rather than seeming brand new, the new chart only has the appearance of a slightly altered version of the original chart.

Clutter in an interface can move focus away from the intended object (Wei, Ward, & Rundensteiner, 2004). Therefore, it is important to allow the user to keep the interface clean to focus on what is important. The user should have the option to remove specific parts of the page that are not important to him or her.

### **3. Research Methods**

#### *3.1. Data-Driven Documents*

Data-Driven Documents (D3) is a library of JavaScript which aids in adding data visualization and interactive features to graphical displays. D3 works with HTML5 (Hyper Text Markup Language 5), CSS (Cascading Style Sheets), and SVG (Scalable Vector Graphics) to create complex graphs (Bostock, 2015). The addition of SVG provides more complex visualization methods which are aesthetically pleasing. Additionally, because it works with HTML5 and CSS, developers are able to include it in current code without too much of a transition. All D3 requires to run is a quick download and a line of code within the head of an HTML script (Bostock, 2015).

D3 focuses on selections within the canvas (section of webpage). One of the most important features of D3 is its ability to select all elements of a certain tag or class because it's such a simple, yet powerful tool. Instead of changing all instances of a tag or class manually or using an if statement or for

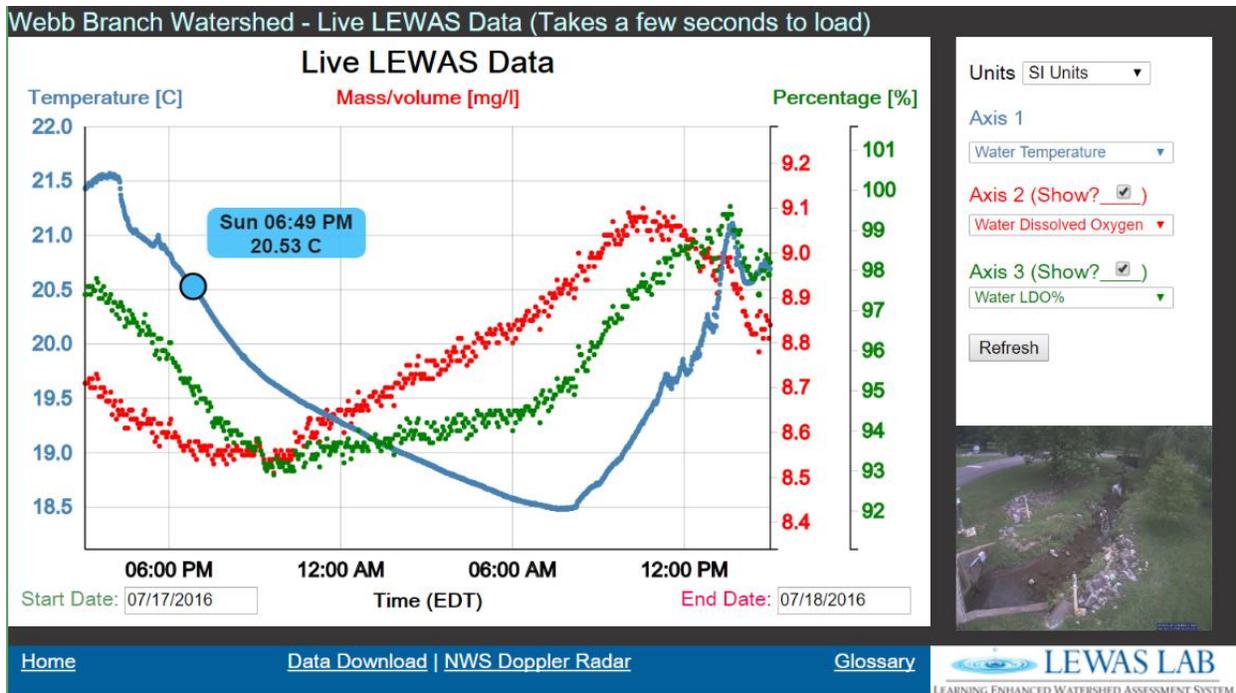
loop, a single command can be used to select all of a specified object. Additional objects can be appended by entering objects and unwanted objects can be removed by exiting at any time. Entering and exiting are part of the enter, update, exit process when working with dynamic applications that are constantly changing. D3 utilizes these *data-joins* to allow the manipulation of a large portion of data and also provide an easy way to dynamically update graphs (Bostock, 2012a). Additionally, there is a transition method which uses animations between updates to allow the user to follow changes in the data (Bostock, 2012b). Scales are another powerful tool in D3. They act as objects and functions in order to keep data within axes, provide color changes, and do many other important tasks.

### 3.2 OWLS with D3

According to Moreno and Mayer (2007), the five types of interactivity are dialoguing, controlling, manipulating, searching and navigation. In order to improve control, searching, and manipulation features in the graph, specific time intervals, hover and on click points, improved dates, a one plot output option, and transitions were added, as shown in Figure 3.

The date picker buttons allow the user to easily choose desired data. If the user wants to look at data from a specific day from a long time ago, then the specific interval can be chosen. The original OWLS live data graph (scatter plot) did not have the option of outputting data from over a month in the past or outputting only a day's worth of data from the past. Additionally, a feature was added to increase the size of data points if the user hovers over a point. When the user clicks a point, the point will get even bigger and also change color. The text within the tool tip (parameter value and date pair pop out) will be bolded to emphasize the value. These interactive features help with user-control by allowing the user to feel as though he or she has access to every specific point. The precise value shown makes the data easier to grasp. As a result, it becomes less abstract and more concrete. Date and time presentation were also improved in the new system. If the time interval is within one day of data, then the x axis tick labels will present the time of day and the tool tip will display the abbreviated day of the week and the specific time of day. The user is most likely trying to understand data by the hour if one day of data is requested, so the time by the hour is presented to improve readability of the data. If the time interval is greater than a day, but shorter than a week, then the x axis tick labels will be the days of the week. It is assumed that the user is searching for a recent day if a time interval shorter than one week is requested, therefore the weekday name is outputted to improve data acquisition. Any time interval that starts at a date longer than a week away, will present the abbreviated month name and the day of the month on the x axis. The same information will be shown in the tool tip, but the time will be included as well. The weekday name is not provided in these cases since the weekday name is less beneficial than the specific date for long time intervals.

Additionally, the graph was changed to allow the user to remove two plots rather than just one. This way, if the user wants to focus on one parameter, then there will be the option of only presenting one plot, allowing for less clutter on the graph. The new feature provides the ability to manipulate data to a desired state. Therefore, interaction within the graph view increases. Another implementation is the addition of transitions between plot changes. The built in transitions option provided by Mike Bostock, creator of D3, is used to update graphs on a change in parameter. The transition between different plots is more gradual, so the user can follow the changes rather than see a sudden, static re-plot (Bostock, 2012b). The transitions were added to provide better manipulation of the data. If the change is static, there does not always appear to be a difference in the data values, but when a dynamic transition is applied, the differences are easily followed. The new developments to the live data graph were added to improve two out of the five types of interactivity, controlling and searching, in order to facilitate the understanding of the LEWAS data.



**Figure 3. Existing Live LEWAS Data Graph Update**

### 3.3 Addition of Grouped and Stacked Bar Graph with D3 within the OWLS

D3 is a powerful tool for many different data visualization chart options, including grouped and stacked bar charts. The grouped and stacked bar chart were developed to display rain and water quantity data. As displayed in Figure 4, each bar represents a day's data value from the specified parameter signified by the x axis labels. Each day's data value is presented as a percentage based on the total data from the week as shown on the y axis. Each parameter has a specific number of bars based on the time interval, each representing the percentage of a specific day's worth of data compared to the data from the whole time interval. For example, during the week long time interval from July 14 to July 20 presented in Figure 4, 31.5% of the rain duration was on July 16, 2016. The legend indicates the date of the bar of data and the tool tip (pop out bubble with information about stack) additionally outputs the parameter value and the percentage of that value compared to the total value for the whole time interval. Similar to the Live LEWAS Data scatter plot, there is an option to choose specific time intervals for the grouped and stacked bar graph. The maximum interval is set to two weeks because when there are more than 14 stacks on each bar, the data becomes difficult to interpret. Transitions between grouped and stacked bar graph views were implemented to allow the user to follow the data. The stacked bar graph with the same tool tip displayed can be viewed in Appendix A. Each bar for a specific parameter is stacked on top of its preceding bar to add up to 100% of the data for that week, so it's important to be able to follow that change.

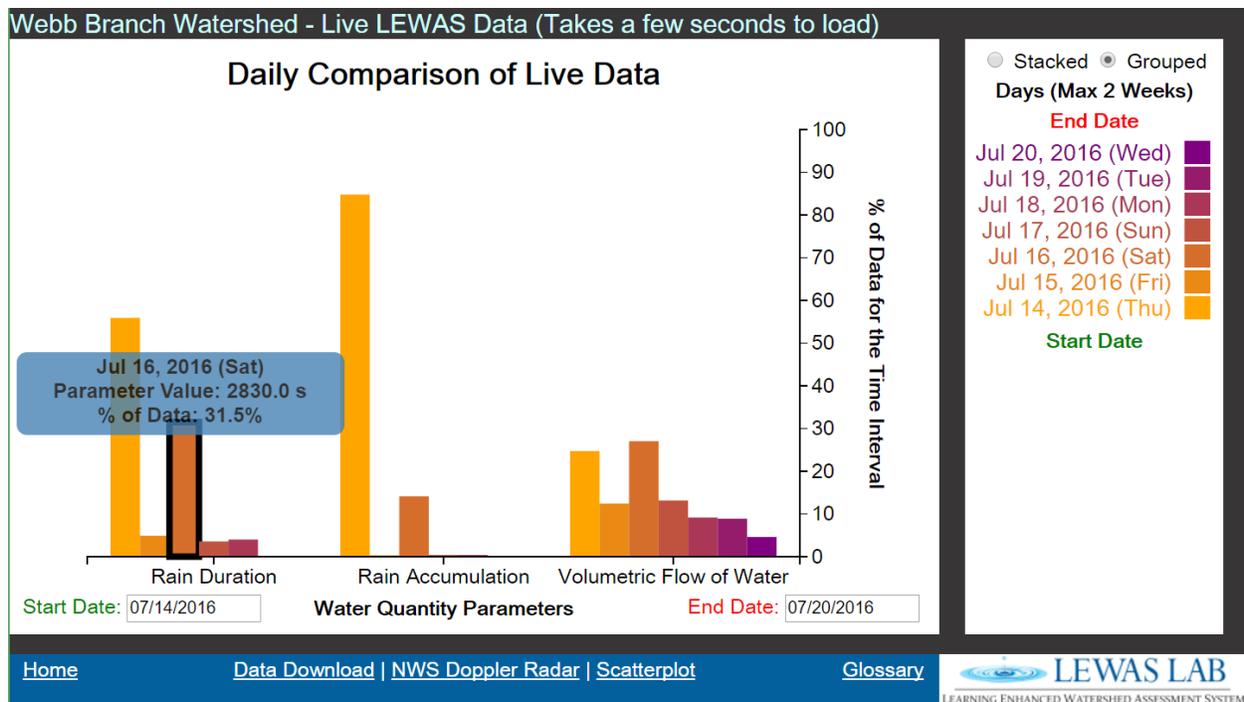


Figure 4. Daily Comparison of Live Data (Grouped Bar Chart)

#### 4. Results and Discussion

A pilot study was conducted to assess the effectiveness of the new data visualization and interactive features, to collect feedback for improving these features and to get ideas about new features that can be added to the OWLS in the future. An online survey was created in Qualtrics with some tasks on the new version of the OWLS followed by qualitative and quantitative questions specific to each task (Appendix B). Students in the Research Experience for Undergraduates (REU) program on - Water Sciences and Engineering program (n = 8) were requested to participate in this anonymous survey. The first task was to open the updated live data graph and choose a specific time interval (June 2 to June 6) when it heavily rained (The students were not told it rained during that interval). After they had accomplished this task, they were to answer questions that included Likert scaled multiple choice questions (7 options from extremely useless to extremely useful), yes or no questions, and specific fill in the blank questions about the new features. The second task was to open the grouped and stacked bar graph and answer Likert scaled questions (7 options from strongly disagree to strongly agree) and fill in the blanks about the features provided to the new stacked and grouped bar chart. At the time of the survey, the specific time interval was not yet developed for the stacked bar graph, so the students were told to just answer questions based on the effectiveness of the potential design. They also had the option to provide suggestions for what would help them learn better.

Since there were only eight responses to the survey, a statistically significant conclusion about the updated system cannot be made. The group consisted of students in a variety of engineering and science majors, but they all had a focus on water, so the results cannot be generalized to all engineering and science majors. Additionally, the students were all acquainted with the student conducting the survey, so there may have been inaccurate responses due to biased opinions.

The first question in task 1 was to find the rain intensity at a specified date and time (9:11 PM on June 4). 100% of the students used the hover point tool tips (parameter value and date pair pop out) to find the specific point. Also, 75% used the both check boxes to remove two of the plots in order to focus

in on one plot. An unexpected issue did show up though, only 50% of the students were able to find the actual rain intensity value at the specified date and time. The point was supposed to be difficult to search for, but it was still expected to be found with the hover and on click tool tips. The specified point was adjacent and slightly covering another point, so the students may have had issues hovering over the correct data value. The points get much larger when hovering, so in the future, the points will be developed so that they do not get too large on hover. Also, one of the students stated that “*selecting one day as the time interval*” would have helped in the understanding of the data. After the survey, the one-day feature was implemented so that a specific range of values could be viewed. Also, the specific point at 9:11 PM on June 4 should be easier to locate with the improvement. Also, another student said that having the ability to search for a specific time and date with an input box would be helpful, so an implementation of this improvement will be conducted in the future. It will make it much easier to find specified points that are desired by the user. A different student stated that a table of the data values and their respective time and dates would be helpful. Currently, OWLS has a link to a new page where data can be downloaded as a text file or csv file if the same date range is inputted again. In the future, a button will be created to highlight on a change in time interval so that the user can download data straight from the graph view. As a result, students would then be able to download data and then search for specific points within that text or csv file. After the students had finished locating the rain duration value at the specified date and time, they were asked to also include the pH plot to their graph and explain the environmental situation that was occurring at the time. The graph displayed a pH plot that was decreasing rapidly as rain duration spiked up. 87.5% of the students accurately answered that the pH was dropping because of the recent heavy rain that occurred during the time interval.

The features seemed to have positive impacts on the user’s experience with the OWLS. The majority of the student answered that the specific time interval, tool tips on hover, date on x axis, specific day and time on tool tip, and two check boxes to remove plots were extremely useful. Transitions were primarily rated as moderately useful (50% of the group). The on click points tool tips were primarily rated a slightly useful and moderately useful (37.5% of the group for both). Since the on click points were not as popular, additional improvements will be made to the on click points tool tips to make them more useful.

Task 2 was more general because the grouped and stacked bar graph had not been completely finished at the time. The majority of the students agreed that the layout was aesthetically pleasing and the tool tips contained useful water quantity information. Also, most students strongly agreed that the different data visualization chart makes learning about environmental data more interesting (37.5% of the group). An unexpected result from task 2 was that 25% of the students somewhat disagreed that the stacked and grouped bar graph would help in understanding water quantity and rain data from the past few days. After the survey, a specific time interval was implemented for the stacked and grouped bar graph. Therefore, in a future survey, this same questions will be asked with the ability to search for specific dates. In the free response section, someone asked “*will it be possible to switch between the [scatter plot] graph and bar graphs for the same dates*”. It would be helpful to be able to see data displayed in different ways for a specific time interval. Additionally, a student said to “replace parameter value with rain duration, rain accumulation, or volumetric flow” because the tool tip label of “parameter value” can be confusing. Both of these suggested changes will be implemented in future developments to the OWLS.

## **5. Conclusion and Future Work**

The results from the survey reveal that the updates to the scatter plot were effective since they helped the users analyze the given data. There were only two features that were deemed slightly useless (transitions and the two check boxes), so those will be improved in future developments. It was expected that the transitions would be useful since they allow the user to follow changes in the data, but they weren unnecessary to the users. This could be because the LEWAS plot all have labels that signify the parameter

used, and each parameter is completely different. Since the parameters were completely different, the gradual change during a change in parameter was most likely unnecessary. Gradual changes are only effective for similar data, that way the user can follow slight alterations. The check boxes were also expected to be useful since they provide more control to the user, one of the five types of interaction. Evidently, the control added to the user was insignificant. This could be because having two or three plots does not produce too much clutter. Implementing effective user-control features will be looked into in the future.

The results for the grouped and stacked bar graph revealed that there were a lot improvements required for the grouped and stacked bar graph. Even though most of the users agreed that the grouped and stacked bar graph would be helpful in understanding environmental problems and that they made the data more interesting, there was still a significant portion who slightly disagreed (25%). Additional meaningful information will be included in the grouped and stacked bar graph in order to make the data more useful. Also, the grouped and stacked bar graph was completed later after the survey was conducted, so another survey will be distributed in the future to retest the results.

Based on the results retrieved from the survey, the developed features will be further improved. They will be focused more on the user's desires when learning about environmental data. Also, additional data visualization and interactive features will be included based on survey comments describing features the users would have liked to be included. There will be a new data visualization chart made specifically for daily water quality data, since daily water quantity data was covered by the grouped and stacked bar graphs. After these improvements and new developments are implemented, the OWLS interface will be tested with a survey by a larger sample size. This way the conclusion will be generalized to a larger population.

## **6. Acknowledgements**

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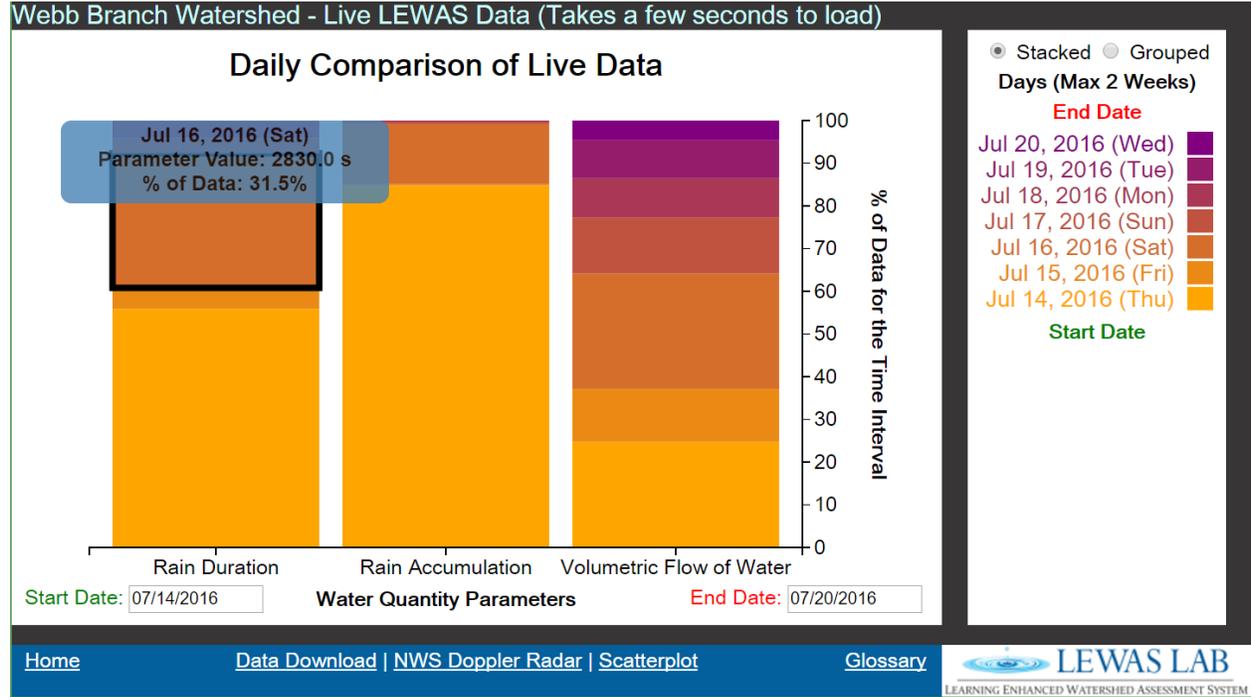
“We acknowledge the support of the National Science Foundation through NSF/REU Site GrantEEC-1359051. Any opinions, findings, and conclusions or recommendations expressed in this paper are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.”

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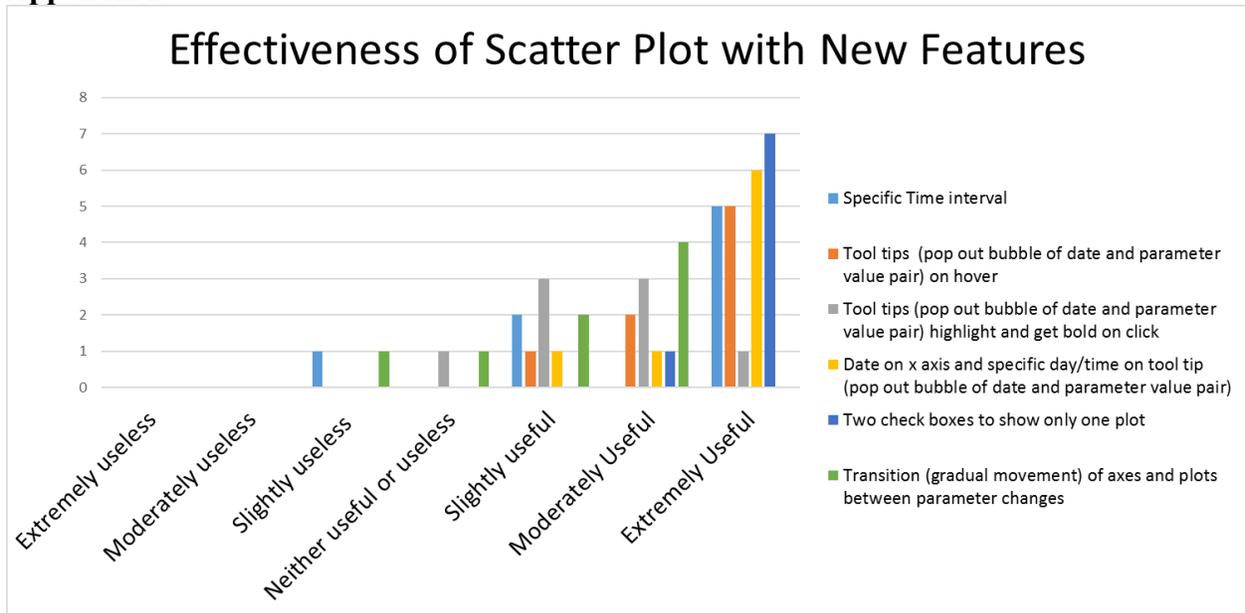
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# Appendix A



Daily Comparison of Live Data (Grouped Bar Chart)

## Appendix B



# Examination of the Microbiological Community and Redox Zones in Simulated Reclaimed Water Distribution Systems

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## Abstract

As water scarcity and demand for water sustainability become increasingly critical across the world, reclaimed water has gained a lot of interests as an alternative water source. While there are clear opportunities associated with reusing reclaimed water, there are also significant risks and uncertainties about the water chemistry and microbiological characteristics of reclaimed water, especially regarding the wide spectrum of water reuse applications. This research project seeks to relate water chemistry profiles in simulated reclaimed water distribution systems (SDSs) to microbiological communities. Previous research has demonstrated that drinking water distribution systems exhibit distinct redox zones that dominate key microbiological activities, including nitrifiers, denitrifiers and sulphate-reducing bacteria (SRB) (Masters *et al.*, 2014). In this study, the SDSs were constructed out of polyvinyl chloride pipes consisting of three disinfectant types (free chlorine, chloramine, and no disinfectant residual) and two nutrient levels (high and low assimilable organic carbon (AOC)). Four sample ports are located along each pipe to allow investigation of the effects of water age up to 5 days. It was hypothesized that high AOC distribution system treated with chloramine would exhibit the greatest growth potential of microbiological communities due to contribution of nitrification from chloramine, and rapid depletion of secondary disinfectant. Data show that chloramination did in fact caused nitrification, particularly in the low AOC SDS which resulted in rapid chloramine decay suggesting chlorination is a better secondary disinfectant method to maintain reclaimed water quality.

**Keywords:** reclaimed water distribution systems, redox zones, microbial ecology, nitrification, disinfectants

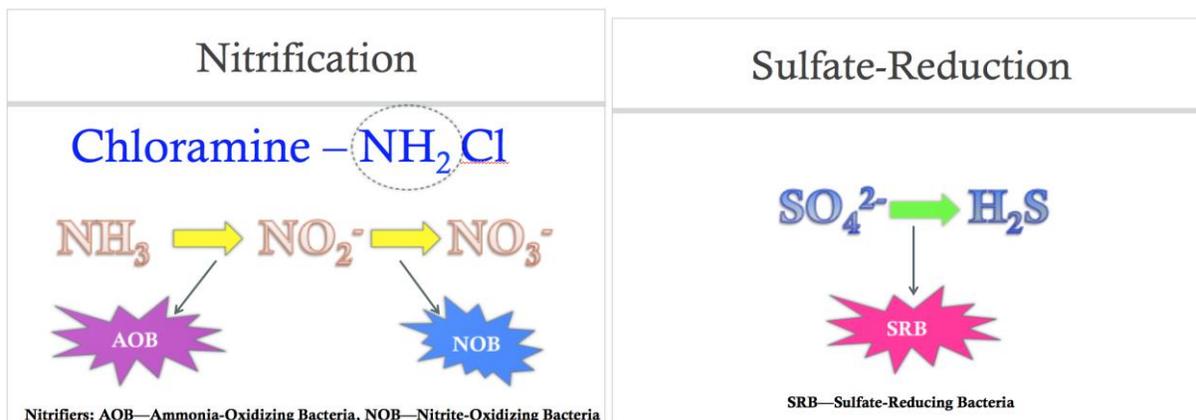
## 1. Introduction

Recently research on reclaimed water has grown rapidly to provide solutions to water demand and water scarcity issues. Reclaimed water is used for a wide range of applications, including non-potable agricultural and industrial use, unplanned use such as that which occurs when a drinking water plant is located downstream from wastewater effluent, and most stringent direct potable reuse. As an emerging source of water supply, there is currently a lack of well-established understanding and federal regulations to guide safe use of reclaimed water making the development of regulations and guidelines the responsibility of state and local governments. The most common reclaimed water quality parameter restrictions that are imposed are limited to biochemical oxygen demand, turbidity, total or fecal coliform bacteria, and chlorine contact time and residual (AWWA, 2009). Compared to potable water, reclaimed water has significantly more uncertainties in its composition and water qualities. Of particular concern is

a higher availability of nutrients and other favorable water chemistry conditions for microbial regrowth (Garner *et al.*, 2016).

Disinfectants have been used for a long time in water distribution systems to prevent bacterial regrowth with increased water age. In recent years, preference has shifted from chlorine to chloramine because it has been observed to be more persistent in the distribution systems and to have less potential to form harmful disinfectant byproducts. However, chloramine is only more persistent in the absence of nitrification (Zhang & Edwards, 2009). Thus, there is a need to evaluate the efficacy of disinfectant levels in reclaimed water distribution systems (RWDSs), given the suspected higher nitrification levels in RWDSs.

As chloramine decays, it releases ammonia in the water, which is the first step to initiate nitrification. Nitrification converts ammonia into nitrite and further into nitrate in a two-step process performed by nitrifiers (Figure 1a). Nitrite can be oxidized by chloramine, after the first step, consequently accelerating disinfectant decay (Wang *et al.*, 2014). It has been a consistent problem in drinking water distribution systems as it can produce microbial metabolites, potentially supporting growth of heterotrophic bacteria with an abundant source of nutrients. Nitrification is anticipated to have a greater prevalence in RWDSs because reclaimed water has a higher concentration of ammonia due to inconsistent nitrification at the wastewater treatment plant (Wang *et al.*, 2016). Adverse effects of nitrification, in addition to faster chloramine decay, include rapid microbial growth and lowered levels of dissolved oxygen (DO) and pH (Wang *et al.*, 2012). Denitrification in drinking water has not been of significant priority in research as there have not been negative consequences reported with the process. Changes and conversion between nitrogen species involved in nitrification and denitrification provide a better understanding of redox zones and associated physiochemical processes in RWDSs (Masters *et al.*, 2014).



**Figure 1. Redox reactions: a) Nitrification and b) Sulfate-Reduction**

Sulfate is reduced to hydrogen sulfide (Figure 1b), under anaerobic conditions, which produces a strong "rotten egg" odor in distribution systems and causes adverse aesthetics concerns and customer complains. Sulfate-reduction occurs where there is a high availability of organic compounds, however the process utilizes hydrogen rather than oxygen as an electron donor thus (Madigan *et al.*, 2009). Sulfate-reducing bacteria (SRB) are often found in biofilms, which provide an anaerobic environment even in the presence of aerobic bulk water (Gomez-Smith *et al.*, 2015). Although chlorination has limited effectiveness in destroying biofilm niches where sulfide has accumulated, maintaining chlorine residual to destroy SRBs prior to their growth of biofilms is an effective approach (Lewis, 1965).

Previous research has demonstrated that distribution systems exhibit distinct redox zones that dominate key microbiological activities, including nitrifiers, denitrifiers and sulphate-reducing bacteria (Masters *et al.*, 2014). The aim of this research project is to relate water chemistry profiles to microbiological communities in SDSs that compared disinfectant type (chlorine, chloramine, no disinfectant residual), nutrient level (high or low assimilable organic carbon), and water age (days 0, 1,

2.5, and 5). This research aims to identify specific, relevant risks associated with the use and transportation of reclaimed water.

## 2. Research Methods and Experiment Setup

### 2.1 Simulated Reclaimed Water Distribution Systems Set Up and Operation

Each simulated distribution system (SDS) is constructed out of polyvinyl chloride (PVC) pipes comparing high or low assimilable organic carbon (AOC) water treated with chlorine, chloramine, or breakpoint chlorination. The low AOC parallel SDSs are shown in Photo 1. Feed water flows through the SDS at a rate of 0.001 L/min, which allows the water to remain in the system for up to 5 days. 8-L containers with different feed waters are prepared based on the previously outlined six conditions and replenished every 30 hours. The water ages from the reservoirs to the sampling ports were approximately 0, 1, 2.5, and 5 days. Four sample ports are located along each pipe to allow examination at each water age. High AOC (HAOC) water is the final effluent water received from the a local wastewater treatment plant, while low AOC (LAOC) water undergoes granular activated carbon (GAC) filtration in a recirculating system for approximately 60 hours prior to the appropriate disinfectant adjustments. In the control conditions feed water is breakpoint chlorinated to satisfy initial chlorine demand and to remove any ammonia present in the raw waters. Chlorinated (CL) and chloraminated (CH) waters are dosed to a concentration of 4 mg/L of free chlorine and total chlorine, respectively. The SDSs are maintained in a constant temperature room maintained at each target temperature (14° C, 22° C, 30° C, 22° C, 14° C) for an ideal period of 2 months if sampling is successful. This study is conducted exclusively at the 30° C phase of the temperature cycle, potentially the most reactive phase for water chemistry and microbiological communities.

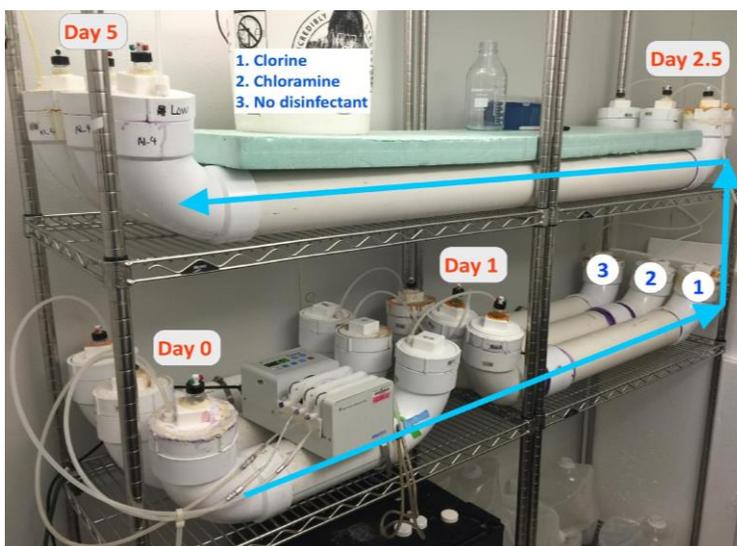


Photo 1. The “rigs”: simulated reclaimed water distribution systems.  
(Haniyyah Chapman, 07/25/2016)

### 2.2 Water Quality Analysis

To maintain an effectively pressurized system, water quality parameters were measured in each SDS starting from day 5 to day 0. DO was measured using an Orion Star DO probe (Thermo Scientific, Beverly, MA). Free chlorine, total chlorine, ammonia, nitrite, and sulfide were measured using a DR500 spectrophotometer (HACH, Loveland, CO). pH was measured using a pH 110 meter (Oakton Instruments, Vernon Hills, IL). Total Organic Carbon was measured using a Sievers 5310 C autosampler

(GE, Fairfield, CT). Total Cell Count (was measured using an Accuri C6 flow cytometer (BD Bioscience, San Jose, CA). Nitrate and sulfate were measured using an ion chromatograph (Dionex, Sunnyvale, CA). Nitrifying, Denitrifying, and Sulfate-Reducing Bacteria Biological Activity Reaction Tests (N-BARTs, DN-BARTs, SRB-BARTs) were used to examine the presence of active nitrifying, denitrifying, and sulfate-reducing bacteria.

### 3. Results and Discussion

#### 3.1 Disinfectant Decay

Chloramine is a more persistent secondary disinfectant than chlorine in the absence of nitrification (Zhang & Edwards, 2009). Our sampling results, in Figure 2, showed greater than 97% disinfectant decay between the reservoirs and day 0 in both chloramine SDSs, compared to 66% and 70% in the LAOC chlorine SDS and HAOC chlorine SDS, respectively. It was previously assumed that the time between the reservoirs and day 0 is negligible, however there was a noticeable amount of biofilm in the thin, pump tubing between the reservoirs and day 0. Thin tubing provided a high surface contact ratio allowing bacteria to further accelerate disinfectant decay, suggesting this assumption is not applicable without proper maintenance and renewal of the pump tubing. By day 5, each SDS reached a minimal disinfectant residual level of 0.7 mg/L or less. In contrast to earlier scenarios when the SDSs were newly operated and maintained at lower temperatures, it was observed that chloramine was more persistent than chlorine.

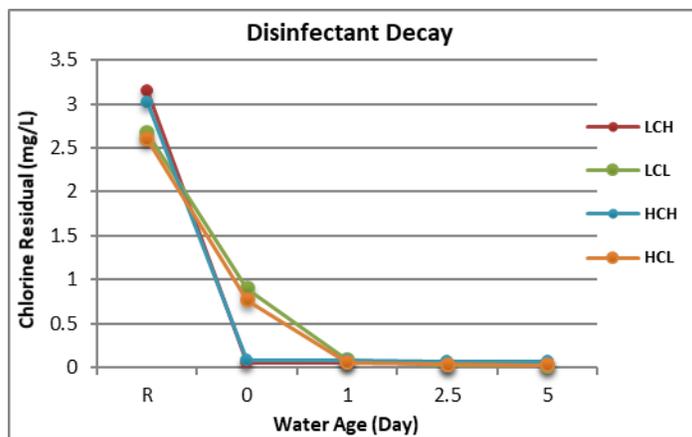
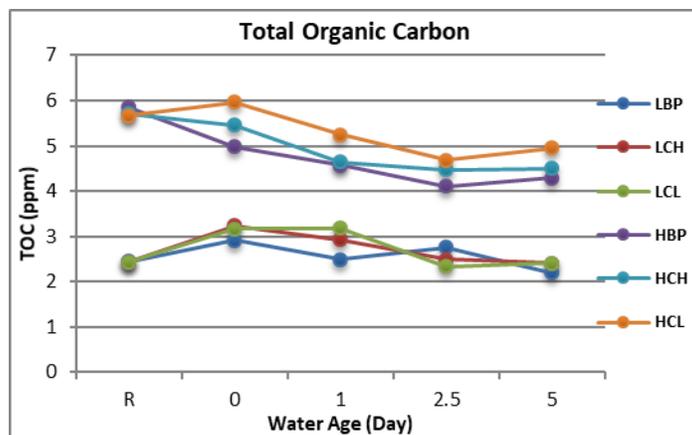


Figure 2. Disinfectant decays the fastest in the chloramine SDSs.

#### 3.2 Nutrient Level

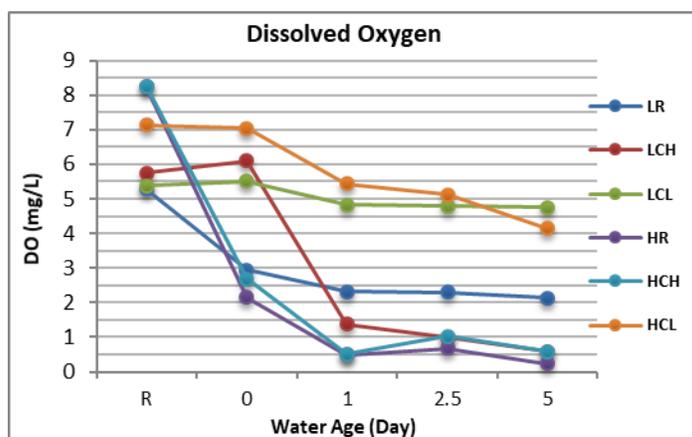
Total organic carbon (TOC) concentrations in HAOC bulk water are approximately two times higher than that of LAOC bulk water. There was an overall decrease in TOC levels in each SDS, suggesting a consumption of organic carbons by microorganisms. TOC concentrations in the HAOC SDSs decreased more than LAOC SDSs. This is believed to be due to an abundance of heterotrophic bacteria present to metabolize organic matter due to lack of disinfection. The HAOC breakpoint chlorinated SDS had a total change of 1.57 ppm TOC, the greatest decrease of all the SDSs (Figure 3).



**Figure 3. Total organic carbon concentration of each system.**

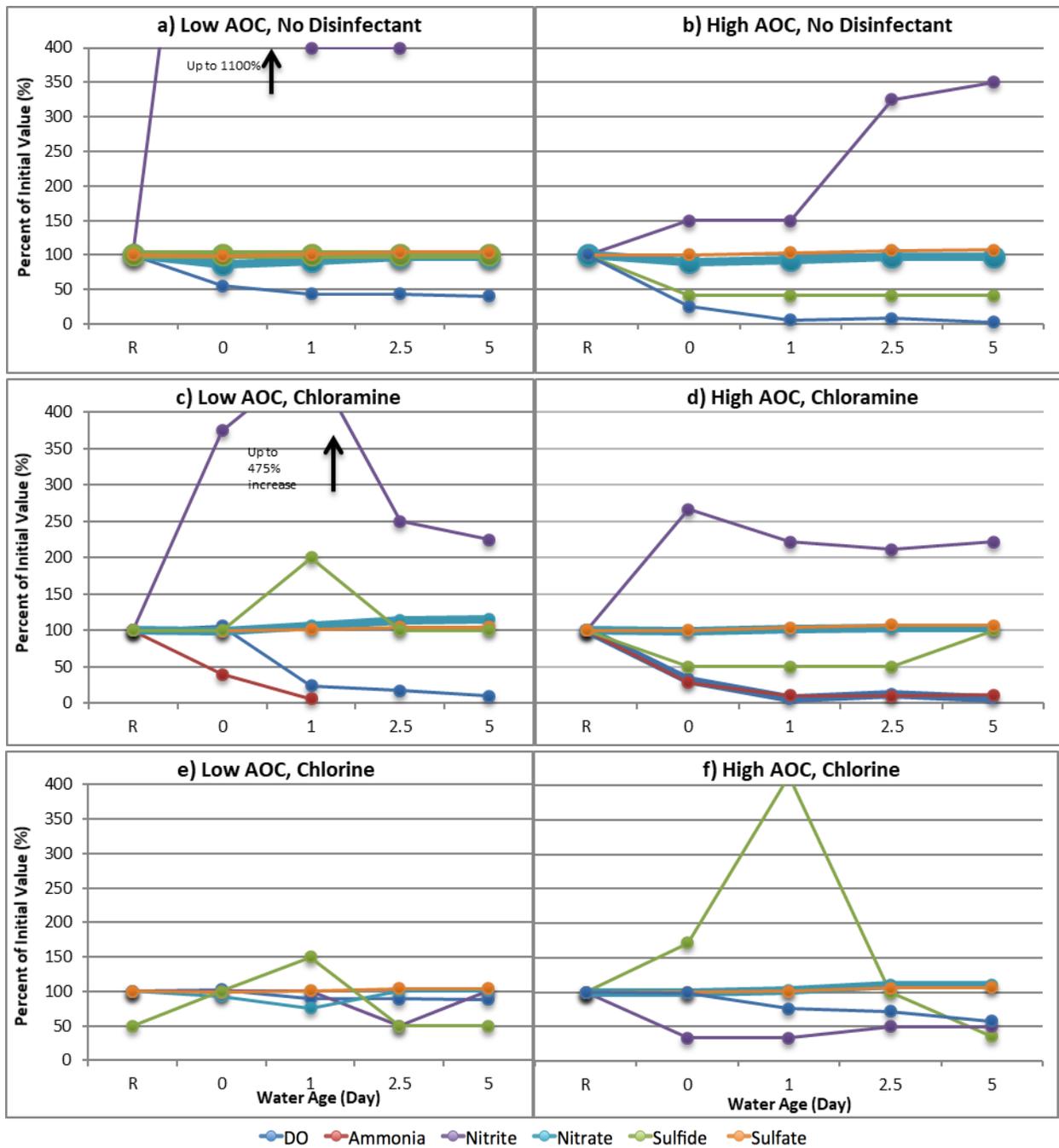
### 3.3 Chemistry and Redox Zones

Dissolved oxygen decreased with increasing water age in each SDS (Figure 4). The breakpoint chlorinated HAOC (HBP) system was the only system to reach anoxic conditions with a measured DO value of 0.28 mg/L at 2.5 days. There appears to be more active microbial communities, particularly aerobic bacteria, in the HBP SDS. The greatest change in DO occurred in the chloraminated HAOC system (HCH), decreasing a total of 9.85 mg/L from day 0 to day 5. The highest levels of DO at day 5, 4.75 mg/L and 4.14 mg/L were found in LAOC chlorine and HAOC chlorine SDS, respectively. This suggests there is minimal microbial activity depleting dissolved oxygen in the chlorinated systems.



**Figure 4. Dissolved oxygen concentration of each system.**

There were no detectable levels of ammonia in the chlorinated and breakpoint chlorinated systems (Figure 5a, 5b, 5e, 5f). Ammonia was completely oxidized by day 2 in the LAOC chloraminated SDS, however in the HAOC chloraminated system ammonia concentration was 0.13 mg/L by day 5 (Figure 5c, 5d). This corresponds to a greater increase in nitrite and nitrate concentration in the HAOC chloraminated SDS than the LAOC chloraminated SDS. Nitrification causes an increase in nitrite followed by a decrease due to nitrite-oxidation and denitrification. Thus, a decrease in nitrite not succeeded by an increase in nitrate, such as shown in the HAOC chloramine SDS between days 0 and 5, suggests there is potential denitrifier activity (Figure 5d).

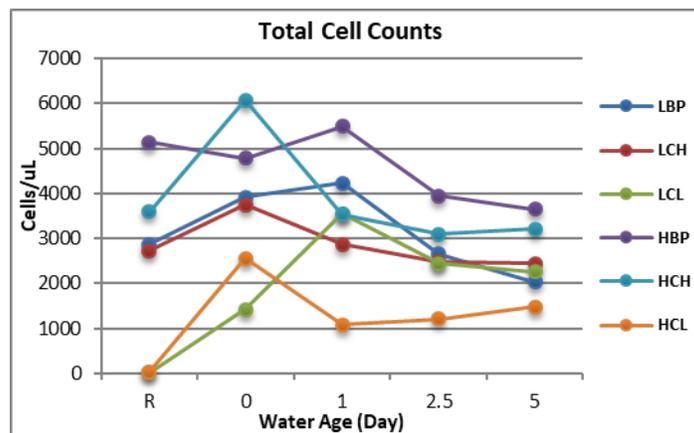


**Figure 5. Redox species: percent of initial values of in each system.**

### 3.4 Microbial Indicators

Proliferation in total cell counts (TCC) was observed in the chlorinated and chloraminated SDSs systems were observed as disinfectants decayed between the reservoir and day 1 (Figure 6). The chlorinated SDSs had the lowest TCC values in the reservoirs having contained 26 cells/ $\mu\text{L}$  and 42 cells/ $\mu\text{L}$  in the LAOC and HAOC systems, respectively. Compared to 2733 cells/ $\mu\text{L}$  and 3589 cells/ $\mu\text{L}$  in the LAOC and HAOC chloraminated systems, this suggests that chlorine is more effective at killing individual cells than chloramine. The greatest change in TCC occurred in the HAOC chloramine SDS with a decrease of 2547 cells/ $\mu\text{L}$  between day 0 and day 1. This happened after an increase of 2487

cells/ $\mu\text{L}$  between the reservoir and day 0. Once bacteria reach a high capacity, competition for nutrients and dissolved oxygen can lead to a decrease in population in attempts to regain stability with the supporting environment. Upon disinfectant depletion, SDSs with the same AOC condition tend to converge to a narrow total cell count range. The large amount of bacteria predicted to be in the breakpoint chlorinated SDS, as a result of high consumption of total organic carbon, is consistent with total cell count results.



**Figure 6. Total cell counts at each sampling port.**

Biological Activity Reaction Tests for nitrifying, denitrifying, and SRB were used to analyze the occurrence of these bacteria in each water age in all SDSs (Table 1). Presence of active nitrifying and denitrifying bacteria were detected by positive N-BARTs and DN-BARTs in the chloramine and breakpoint chlorinated systems. An aggressive presence of nitrifiers was found in the LAOC chloraminated SDS from day 1 and to day 5, which confirmed nitrification is responsible for the rapid decay of chloramine in this system. Despite anticipation that HAOC chloramine SDSs would be a more ideal environment for nitrification due to a higher available nutrient level, the N-BART results were consistent with previous research reporting organic matter supports the growth of heterotrophic bacteria. Autotrophic nitrifiers are out-competed by heterotrophic bacteria for oxygen and nutrients in environments with higher concentrations of total organic carbon [Zhu, 2001]. Denitrifiers thrive in anoxic conditions, reached by both chloramine SDSs and the HAOC breakpoint chlorinated SDS. It was anticipated that there would be higher activity of denitrifiers at the water ages resulting in anoxic conditions, particularly at day 5. There were moderately aggressive DN populations found at day 5 in the HAOC breakpoint chlorinated and chloramine SDSs. There was also a moderately aggressive DN population at day 2.5 in the HAOC breakpoint chlorinated SDS. This is likely due to anoxic conditions at day 1 before increasing to a DO concentration of 1.03 mg/L at day 2.5, and resuming anoxic level at day 5 (Figure 4). Overall, there appears to be no aggressive denitrification occurring in any of the distribution systems. Moderate to non-aggressive occurrences of nitrifiers and denitrifiers were present in the HAOC and LOAC chloraminated and breakpoint chlorinated SDSs. SRB-BARTs showed an aggressive population of SRB only in day 5 of the HAOC chloraminated SDS confirming the increase in sulfide between days 2.5 and 5, once the concentration of DO decreased to an anoxic level. If the system allowed sampling at a higher water age, it is predicted that greater sulfate-reduction would ensue. In a real distribution system, water age at the point of use would likely be higher than 5 days, thus it is possible for users to experience odor and aesthetic issues not apparent in the SDSs. Chlorinated SDSs all have negative N-BARTs, DN-BARTs, and SRB-BARTs at each water age. The absence of nitrifiers and denitrifiers is consistent with non-detectable ammonia concentrations. It appears that chlorine is better able to limit microbial activity, according to BARTs as well as higher DO concentrations, slower disinfectant decay rates and lower total cell counts.

**Table 1. N-BART, DN-BART, and SRB-BART Results (Haniyyah Chapman, 07/27/2016)**

<b>Sampling Port</b>	<b>N</b>	<b>DN</b>	<b>SRB</b>
<b>H0</b>	++	++	BB, ++
<b>H1</b>	+	+	BB, ++
<b>H2.5</b>	++	+	CL, ++
<b>H5</b>	++	++	BB, ++
<b>HCH0</b>	+	+	BB, ++
<b>HCH1</b>	+	+	-
<b>HCH2.5</b>	+	++	-
<b>HCH5</b>	+	++	BB, +++
<b>HCL0</b>	-	-	-
<b>HCL1</b>	-	-	-
<b>HCL2.5</b>	-	-	-
<b>HCL5</b>	-	-	-
<b>L0</b>	++	++	-
<b>L1</b>	+	+	-
<b>L2.5</b>	+	+	-
<b>L5</b>	+	+	-
<b>LCH0</b>	++	++	-
<b>LCH1</b>	+++	+	-
<b>LCH2.5</b>	+++	+	-
<b>LCH5</b>	+++	+	-
<b>LCL0</b>	-	-	-
<b>LCL1</b>	-	-	-
<b>LCL2.5</b>	-	-	-

<b>LCL5</b>	-	-	-
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+++ : Aggressive, ++ : Moderate, + : Not aggressive, - : Not present  
**BB-Dense anaerobic SRB consortium, CL-anaerobic bacteria present.**

#### 4. Conclusion

Chlorination appears to be a more effective secondary disinfectant method to minimize the negative effects of microbial activity, primarily at higher temperatures. Chloramination caused nitrification, which resulted in rapid chloramine decay. No significant occurrence of denitrification or sulfate-reduction occurs likely because the systems did not remain anaerobic for enough time to allow denitrifying and sulfate-reducing bacteria populations to stabilize. The interaction of disinfectant, nutrient level, and water age influence microbiological communities, redox zones and water chemistry in a unique way that affects overall water quality in reclaimed water distribution systems.

#### 5. Acknowledgements

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# Occurrence of Pharmaceuticals and Personal Care Products (PPCPs) in Various Water Sources

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## Abstract

Pharmaceuticals and personal care products (PPCPs) are a class of emerging contaminants that includes prescription, over-the-counter, veterinary, and illicit drugs in addition to products intended to have primary effects on the human body, such as sunscreens and insect repellants. PPCPs have been studied in the U.S. since 1999, when a USGS study found such compounds in 80% of streams analyzed. Recently, a 2016 study by the USGS identified pharmaceuticals in all 59 streams sampled in the southeastern U.S., with only 17 streams having a point source of pollution such as wastewater discharge.

The objective of this study was to screen for the presence of forty PPCP compounds in various water sources including an urban-impacted stream, wastewater treatment influent and effluent, and private well water. Water samples were collected from the two inlets and one outlet of Duck Pond, a bioretention pond intended to manage stormwater runoff from the Town of Blacksburg, VA. Private well water samples were collected from two counties in Virginia based on their reported proximity to various potential contamination sources. Influent and effluent samples were collected from wastewater treatment plants across the U.S. The samples were extracted and cleaned up using solid phase extraction and screened for target PPCPs on an ultra performance liquid chromatography-tandem mass spectrometry (UPLC/MS/MS). In total, seventeen PPCPs were detected in the bioretention pond, twenty-one in wastewater, and eleven in private well water at estimated concentrations from low ppt to low ppb.

**Keywords:** Pharmaceuticals, personal care products, PPCPs, emerging contaminants

## 1. Introduction

Due to exponential worldwide population growth and an increased demand for freshwater, preservation of freshwater resources is a major goal of the 21<sup>st</sup> century. Freshwater comprises only 2.5% of the total amount of water on Earth; of that percentage, 31.3% is accessible to humans in the form of groundwater and surface water (Shiklomanov, 1993). Because of limited availability and increased demand from a rising human population, preventing pollution of these freshwater resources has become increasingly important. One of the guiding objectives behind the Environmental Protection Agency's Safe and Sustainable Water Resources 2016-2019 research plan is to address water resource issues related to complex chemical pollutants, including developing new methods for the detection, quantification, and treatment of emerging contaminants (U.S. EPA, 2015, p. 1). One such class of emerging contaminants is pharmaceuticals and personal care products (PPCPs).

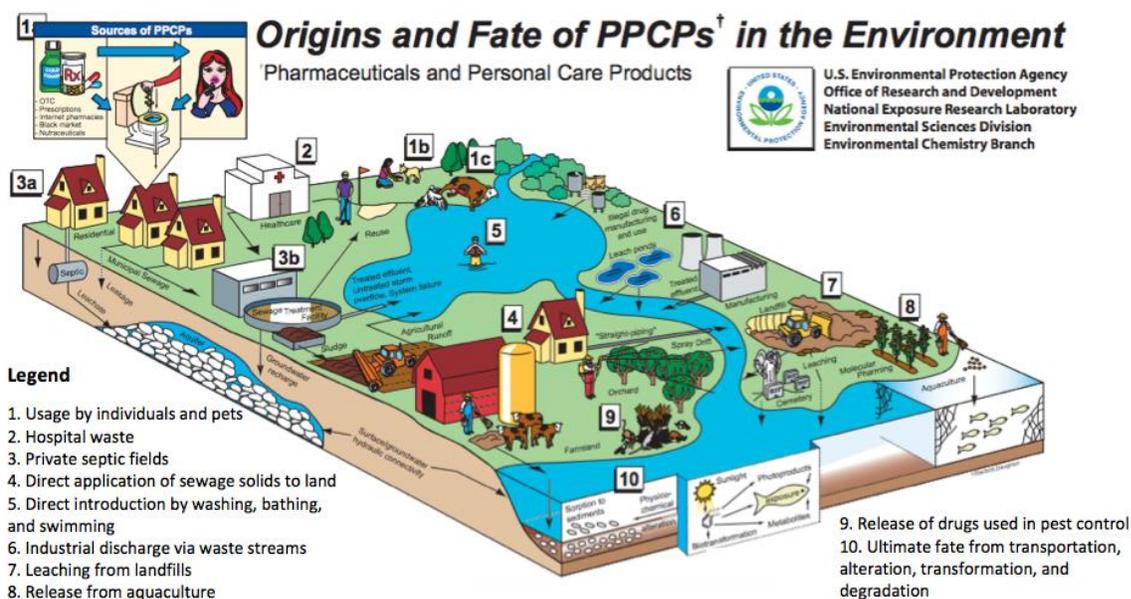
### 1.1 Pharmaceuticals and personal care products

The term pharmaceuticals refers to prescription and over-the-counter medications, along with veterinary and illicit drugs. Personal care products are those that are marketed for the direct use of the consumer and intended to have effects primarily on the human body (i.e. not through digestion)

(Daughton and Ternes, 2009, p. 930). This includes the active ingredients and preservatives used in fragrances, cosmetics, and sunscreen agents. While there has been widespread interest in the fate and consequences of pharmaceuticals in the environment, little attention has been given to those of personal care products and there are fewer regulations regarding the concentrations of personal care products in water (Daughton and Ternes, 2009, p. 930). In addition, personal care products differ from pharmaceuticals in that they are manufactured and used in larger quantities and can be introduced directly into the environment, such as into recreational waters and volatilized into the air.

### 1.2 How PPCPs enter the environment

In their Catchment Quality Control study, the Thames Water Authority outlined two main routes through which PPCPs are introduced into the water cycle (Fig. 1) (Richardson and Bowren, 1985). In the industrial sector, waste of 1-5% of product at a pharmaceutical manufacturing plant is deemed consented discharge, which can end up going down the drain and to a wastewater treatment plant (WWTP). This percentage is generally lower than in the manufacture of other products, due to the high cost of pharmaceuticals and stringent guidelines such as Good Manufacturing Practice (Richardson and Bowren, 1985). PPCPs are also introduced to the environment through domestic use. Pharmaceuticals are introduced to WWTPs through human excretion, and other personal care products can similarly be washed down the drain. In addition, a large quantity of prescription and over-the-counter drugs are never consumed, many of which are flushed down toilets for disposal (Daughton and Ternes, 2009). Through both the industrial and domestic route, PPCPs are primarily introduced into waterways by WWTPs, which are designed to handle naturally occurring human waste but not necessarily the anthropogenic chemicals that are introduced along with it (Rogers, 1996). This effluent is then discharged into surface waters or, in the case of residual solids, to landfills where it can be introduced to groundwater or surface waters through run-off (Rogers, 1996). However, recent research by the USGS suggests that WWTP effluent is not sole contributor to PPCPs in the environment. In a study of 59 small streams spanning Alabama, Georgia, North Carolina, South Carolina, and Virginia, one or more pharmaceuticals were detected in all 59 streams (Bradley, 2016). Of these streams, only 17 have reported wastewater discharges, indicating that non-point sources such as an aging sewer infrastructure, septic tank leakage, and stormwater runoff most likely contribute to the presence of PPCPs in the environment, an issue which must be further investigated (Bradley, 2016).



**Figure 1. Origins and Fate of Pharmaceuticals and Personal Care Products (PPCPs) in the Environment (adapted from EPA 2006)**

### *1.3 Presence of PPCPs in various water sources*

The presence of PPCPs in watersheds impacted by urbanization has been widely recorded, as they have been identified in WWTP effluent, surface water, and ground water across the world. The occurrence of prescription drugs in the environment was first reported in the late seventies. In a study published in 1976, Garrison et al. detected the presence of clofibrac acid at concentrations of 0.8-2.0 µg/L in effluent from WWTPs across the United States. Around the same time, a study conducted by Hignite and Azarnoff (1977) detected salicylic acid and clofibrac acid in the influent and effluent of a WWTP in Kansas City, Missouri. For antibiotics in particular, Hirsch et al. (1999) analyzed WWTP effluent in Germany for 18 antibiotic compounds, all of which were found in the µg/L range except for tetracycline and penicillin.

In the United States, the first national reconnaissance of the occurrence of organic wastewater contaminants (OWCs), including pharmaceuticals and hormones, was conducted by the USGS in 1999 (Koplin et al., 2002). Of the 139 streams surveyed across 30 states, OWCs were detected in 80% of those streams (Koplin et al., 2002). Similarly, a study conducted in South Korea detected 11 out of 13 PPCP compounds in surface water from the Mankyung River, the most prevalent compounds across the five sampling sites being ibuprofen (not detected (ND) to 414 ng/l), carbamazepine (ND-595 ng/l), and atenolol (ND-690 ng/l) (Kim et al., 2009). Boyd et al. (2003) sampled surface water from the Mississippi River and Lake Pontchartrain in Louisiana, as well as the Detroit River in Ontario, Canada, and detected naproxen (22-107 ng/l) at all sites and clofibrac acid (103 ng/l) in the Detroit River. They further tested the water during multiple phases of drinking water treatment at plants in Louisiana and Ontario, and found that conventional drinking water treatment processes did not remove naproxen from Mississippi River water (Boyd et al., 2003). However, additional methods including chlorination, ozonation, and dual method filtration reduced naproxen below detection limits in Mississippi and Detroit River waters, in addition to lowering the concentration of clofibrac acid in Detroit River water (Boyd et al. 2003).

PPCPs can be introduced into the environment through non-point sources as well. A study by Dougherty et al. (2009) analyzed the occurrence of PPCPs, herbicides, and a flame retardant in groundwater and surface water around Liberty Bay, Puget Sound, Washington. The community does not have point sources such as wastewater treatment plant effluent, and instead 70% of the population relies on septic systems. Twelve out of the twenty-five compounds analyzed for were detected at least once, suggesting that the community is in fact contaminating local water through non-point sources (Dougherty et al., 2009).

### *1.4 PPCPs in drinking water wells*

Despite these studies, little research has been performed into the presence of PPCPs in drinking water wells. One such study was conducted by the Silent Spring Institute in Cape Cod, Massachusetts in order to screen for 92 emerging contaminants, including pharmaceuticals, hormones, personal care products, herbicides, alkylphenols, flame retardants and perfluorinated chemicals, in 20 drinking water wells (Schaidler, 2010). At least one emerging contaminant was detected at parts per trillion levels in 75% of the wells tested, the most popular being the antibiotic compound sulfamethoxazole (Schaidler, 2010). The authors contribute the presence of these emerging contaminants in private water wells to the seepage of chemicals from household and commercial wastewater into groundwater from septic systems (Schaidler, 2010). However, there remains a need to screen for emerging contaminants in a larger number of privately owned wells in different areas around the country, in order to add to our understanding of their abundance in the environment. In the United States, over 15 million households rely on private wells for their drinking water (U.S. Census Bureau, 2008). In Virginia in particular, 1 in 5 residents use private wells as their primary source of drinking water, for a total of 1.7 million residents (USGS, 2010). Thus, screening for PPCPs in well water is an important step in understanding the prevalence of such emerging contaminants in drinking water, in the hope that future steps can be taken to reduce their abundance.

### *1.5 Effects of PPCPs on the environment*

The effects of PPCPs in the environment must be studied with regard to both aquatic organisms and ecosystems in addition to as human health. Determining the ecotoxicity of compounds such as PPCPs is difficult because they were not created with the purpose of affecting wildlife, so it is hard to predict what effects to look for. In addition, the effect of PPCPs on a complex ecosystem is difficult to discern from the impact they have on a select few organisms. Thus, investigations into the ecotoxicology of PPCPs have been limited. One of the major concerns with PPCPs is the unintended and unpredicted effect that they may have on both humans and non-target organisms. Kurelec (1993) has outlined this idea with a concept called genotoxic disease syndrome (GDS), in which pharmaceuticals have subtle and unperceivable effects on a single organism's behavior, but when accumulated over generations the effect becomes profound. One such example of behavioral alteration, as reported by Kurelec (1992), is the fact that fish exhibit dramatic avoidance patterns when exposed to the drug verapamil. In addition to their effect on the environment, the rampant use and release of antibiotics into the environment is the leading proposed cause of antibacterial resistance. Stream surveys conducted by Ash et al. (1999) demonstrate the prevalence of native bacteria that are resistance to a multitude of antibiotics including vancomycin, often considered a "drug of last resort" due to its efficiency in treating drug-resistant infections.

### *1.6 Research objectives*

This is a preliminary study that aims to screen for PPCPs in multiple water sources impacted by anthropogenic activities including 1) surface water that is heavily impacted by urbanization and stormwater runoff, 2) influent and effluent from multiple WWTPs across the United States and 3) well water from several locations within Virginia.

## **2. Materials and Methods**

### *2.1 Sample collection*

#### *2.1.1 Duck Pond/Stroubles Creek samples*

The Stroubles Creek watershed encompasses most of the town of Blacksburg, Virginia. Stroubles Creek runs into the New River, which is the source of drinking water for Montgomery County. The creek has been on Virginia's list of impaired water bodies since 2002, when biological monitoring of the stream done by Virginia Tech's Department of Biological Systems Engineering indicated that the stream did not meet the "general standard" of water quality in Virginia due to benthic impairment, which constitutes an alteration of the aquatic biological community (Department of Biological Systems Engineering, 2003). Since this designation, the Stream Restoration, Education, and Management (StREAM) Lab has been working with the community of Blacksburg in order to restore the stream in the hopes of removing it from this list.

Samples were collected on 6/2/16 and on the afternoon of 6/23/16 following an hour-long rain event with a total precipitation of 0.06 inches. Water samples were collected from inlets 1 and 2 of Duck Pond, a bioretention pond located on the Virginia Tech campus, and at the pond's one outlet (Fig. 2). Inlet 2 runs directly under the Drillfield on Virginia Tech's campus, and the outlet is located right after a dam. Duck Pond acts as the boundary between the upper and lower Stroubles Creek watershed, and is intended to act as a stormwater management facility for urban runoff from the Virginia Tech campus and portions of Blacksburg in the upper watershed (Parece, 2010). Additionally, on 7/18/16 samples were collected from Stroubles Creek 3 km and 8 km downstream of the pond's outlet. The latter site is located near where Stroubles Creek flows into the New River, a source of drinking water for surrounding counties.

Samples from these sites were collected in triplicate in 1L mason jars. Jars were rinsed with water from the creek three times before water was collected from the center of the channel. The samples were kept in a cooler filled with ice then extracted upon arrival back at the lab.



**Figure 2. Sampling site map of Duck Pond**

### *2.1.2 WWTP samples*

Influent and effluent samples were collected from three WWTP in various locations around the U.S. Treated wastewater from these plants becomes reclaimed water that is used for non-potable applications, such as irrigation.

Secondary wastewater effluent was collected before UV disinfection from the secondary clarifier outlet of the Blacksburg-VPI sanitation authority treatment plant. Samples were collected in sterile polypropylene containers and transported to the lab in a cooler filled with ice, and SPE was conducted immediately upon arrival.

### *2.1.3 Private well water samples*

Samples from private water wells were collected from residents of Virginia through the Virginia Household Water Quality Program, an effort of the Virginia Cooperative Extension. Participants received a questionnaire regarding their well and a sampling kit with four plastic labeled sample bottles. They were instructed to collect samples from a cold-water faucet in the kitchen or bathroom first thing in the morning. The residents turned their faucet on and let it run for one minute, then collected the samples. They then stored the bottles in the refrigerator or on ice in a cooler until they could drop them off at the designated location.

## *2.2 Sample preparation*

### *2.2.1 Duck Pond/Stroubles Creek samples*

Following collection, 500mL of each sample were measured out and transferred into clean 1L mason jars. In addition to the samples, 500mL of ultrapure MilliQ water were also measured out as a method blank and transferred to a mason jar. These ten samples were extracted using solid-phase extraction (SPE) with a vacuum pump. Oasis HLB cartridges (60mg, 3cc) were preconditioned using 3mL of methanol followed by 3mL of ultrapure water. The pre-measured 500mL samples were passed through the HLB cartridges at a flow rate of 5mL/min. Samples were then eluted with 1mL of 100% methanol, filtered with a 1mL syringe and 0.2  $\mu$ m filter membrane, and transferred to clean 1mL amber bottles for analysis.

### 2.2.2 WWTP samples

500mL of each sample were measured out and filtered using Whatman 42 ashless quantitative filter paper (2.5  $\mu$ m) due to the anticipated high organics content of these samples. The samples were then transferred into clean 250mL Erlenmeyer flasks and extracted using SPE with a vacuum pump.

Oasis HLB cartridges (60mg, 3cc) were preconditioned using 3mL of methanol followed by 3mL of ultrapure water. The samples were passed through the HLB cartridges at a flow rate of 5mL/min. Samples were then eluted with 1mL of 100% methanol, filtered with a 1mL syringe and 0.2  $\mu$ m filter membrane, and transferred to clean 1mL amber bottles for analysis.

A recovery test was performed by spiking six flasks of 50mL MilliQ water with a 1ppm mixture of antibiotics including sulfamethazine, chlortetracycline, and tylosin in order to achieve spiked concentrations of 0.01ppb, 0.04ppb, and 0.1ppb. Three samples were spiked before SPE, and three after SPE. The recoveries of sulfamethazine, tylosin, and chlortetracycline were 62.2%-73.8%, 32.5%-86.1%, and 10.0%-54.0%, respectively.

### 2.2.3 Private well water samples

50 mL of each well-water sample were measured and transferred into 125 mL Erlenmeyer flasks. 50 mL of ultrapure MilliQ water were also measured and transferred to an Erlenmeyer flask as a method blank. A recovery test was performed by spiking six flasks of 50mL MilliQ water with a 1ppm mixture of antibiotics including sulfamethazine, chlortetracycline, and tylosin in order to achieve spiked concentrations of 0.1ppb, 0.4ppb, and 1ppb. Three samples were spiked before SPE, and three after SPE. The recoveries of sulfamethazine, tylosin, and chlortetracycline were 73.6-102.1%, 69.1%-184.1%, and 15.8%-30.4%, respectively.

Oasis HLB cartridges (60mg, 3cc) were preconditioned using 3mL of methanol followed by 3mL of ultrapure water. Samples were passed through the HLB cartridges at a flow rate of 5mL/min. Samples were then eluted with 0.3mL of 100% methanol and transferred to 1mL amber bottles with insert for analysis.

### 2.3 LC/MS/MS analytical procedure

Analysis was performed using an Agilent 6400 Series Triple Quadrupole LC/MS (6490, Agilent, USA). LC/MS/MS analytical conditions are listed in Table 1. Target compounds that were screened for in this study can be found in Table 2.

**Table 1. LC/MS/MS analytical conditions**

Column	Agilent ZORBAX Extend-C18 Columns, 4.6x50mm, 5 $\mu$ m	
Column temp.	40 °C	
Sample solvent	Methanol:water = 1:1	
Injection volume	20 $\mu$ l	
Autosampler temp.	8°C	
Mobile phase	A) Water + 0.1% formic acid B) 95% acetonitrile + 0.1% formic acid	
Flow rate	0.3 mL/min	
Gradient program	Time (min)	B%
	0	5
	10	100
	13	100
	13.5	5

**Table 2. Target PPCP compounds**

Compound	Retention time (min)	Molecular ion	Quantifier ion	Qualifier ion
Metformin	1.6	130.0	71.0	60.0
Triclocarban	2.1	313.0	35.0	160.0
MDMA	3.5	194.0	77.0	163.0
Ibuprofen	3.9	205.0	161.0	131.0
Thiabendazole	4.7	202.0	131.0	175.0
Triamterene	4.9	254.0	237.0	104.0
Lidocaine	5.2	235.0	86.0	58.0
Mefenamic acid	5.6	242.0	208.0	220.0
EDDP	5.7	278.0	234.0	249.0
Amitriptyline	5.7	278.0	91.0	202.0
Sulfamethazine	5.7	279.0	92.0	186.0
Venlafaxine	6.1	278.0	260.0	58.0
Dextromethorphan	6.5	272.0	128.0	171.0
Sertraline	6.6	306.0	159.0	275.0
Carbamazepine	6.6	237.0	194.0	193.0
m-Hydroxybenzoylecgonine	6.6	306.0	65.0	168.0
Cocaine	6.8	304.0	77.0	182.0
Escitalopram	8.2	325.0	80.0	98.0
Continine	8.5	177.0	275.0	98.0
Clenbuterol	8.6	277.0	203.0	132.0
Lorazepam	8.7	321.0	275.0	229.0
Diltiazem	8.7	415.0	109.0	178.0
Atrazine	9.1	216.0	174.0	68.0
DEET	9.2	192.0	119.0	91.0
Nifedipine oxidized	9.4	345.0	284.0	268.0
Buprenorphine	9.4	468.0	55.0	84.0
Primidone	9.9	219.0	91.0	162.0
Vancomycin	10.25	724.80	144.0	100.1
Propranolol	11.2	260.0	56.0	116.0
Trimethoprim	11.37	291.14	230.1	123.0
Gabapentin	11.4	172.0	55.0	154.0
Ormetoprim	11.88	275.15	259.1	123.0
Cefotaxime	12.27	456.06	125.0	126.0
Tetracycline	12.44	445.16	410.1	154.0
Ornidazole	13.01	220.04	128.0	82.1
Chlortetracycline	14.31	479.12	444.1	462.1
Sulfamethoxazole	15.11	254.06	92.1	108.0
Oxolinic acid	16.08	262.07	244.0	216.0
Erythromycin	16.79	734.50	158.1	576.0
Tylosin	17.55	916.52	174.1	772.4
Nalidixic acid	18.19	233.09	215.1	187.0
Flumequine	18.66	262.08	244.0	202.0

### 3. Results and Discussion

#### 3.1 Duck Pond/Stroubles Creek

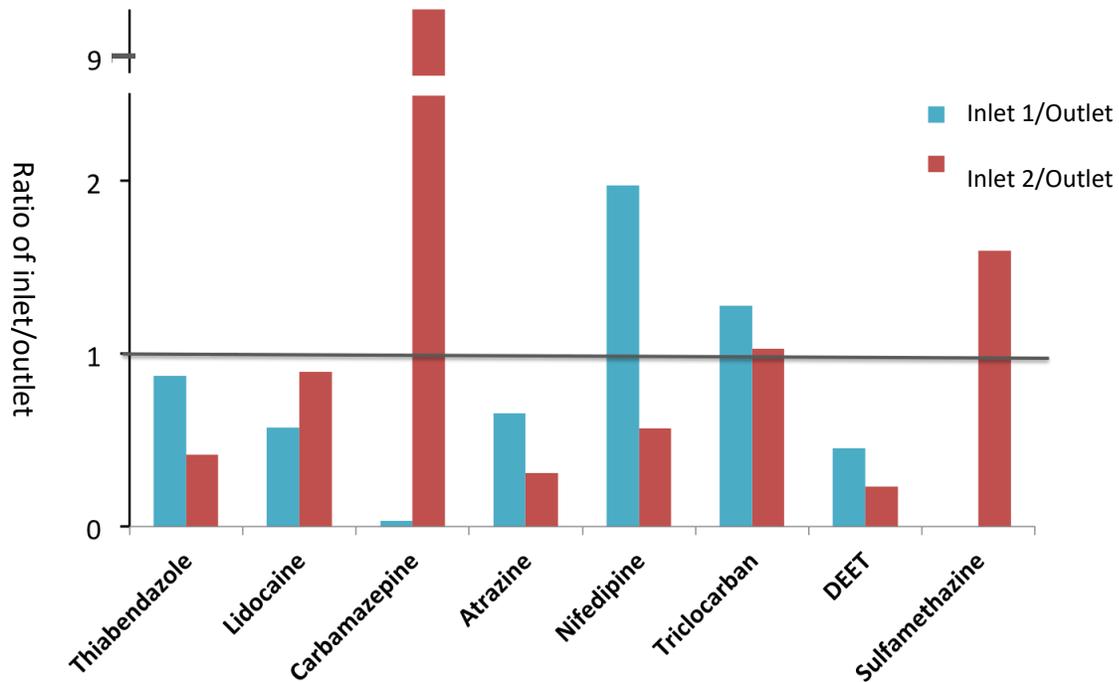
Of the target compounds analyzed, eleven compounds were detected in Duck Pond (Table 3). At inlet 1, eight compounds were detected. At inlet 2, nine compounds were detected. At the outlet of the bioretention pond, ten compounds were detected. Downstream of the pond, at both 3 km and 8 km, six compounds were detected (Table 3).

The peak areas of the compounds detected at both the inlet and outlet were compared in order to determine the effect of the bioretention pond on the compound's abundance (Fig. 3). The concentrations of 5 out of 7 compounds increased from inlet 1 to the outlet, while the concentrations of 5 out of 8 compounds increased from inlet 2 (Fig. 3). This indicates that the bioretention pond is not fully serving its purpose as a stormwater management facility intended to reduce the amount of pollution that flows downstream from the pond.

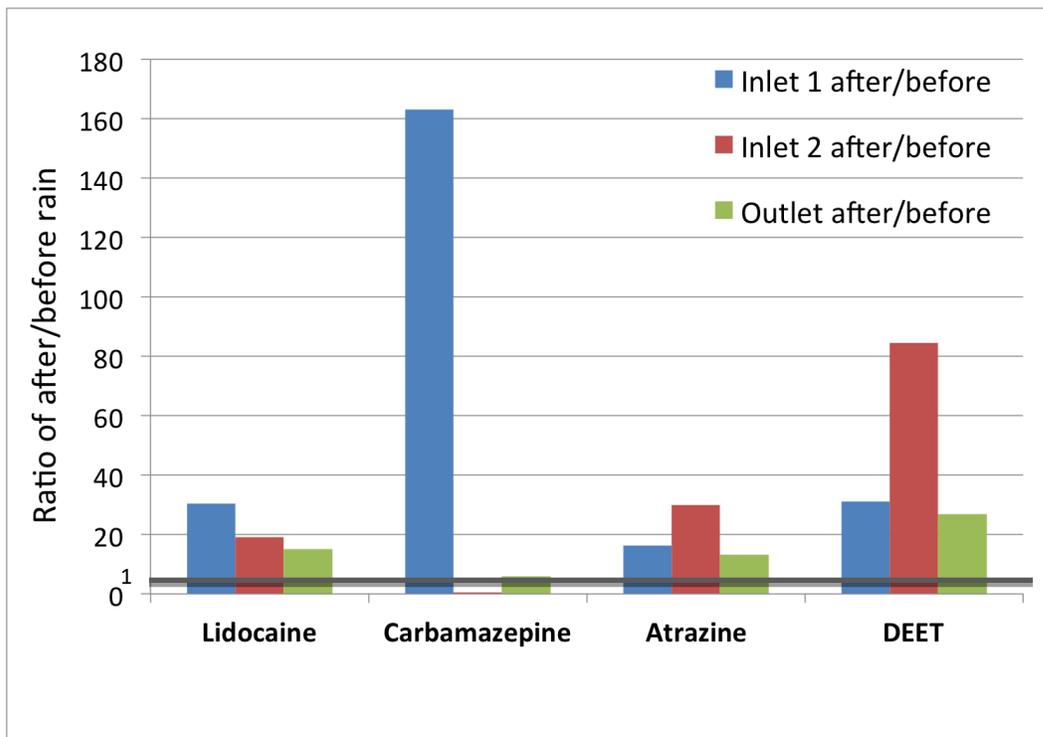
**Table 3. Presence of PPCPs in Duck Pond and Stroubles Creek**

Compound	Duck Pond before rain			Duck Pond after rain			Downstream	
	Inlet 1	Inlet 2	Outlet	Inlet 1	Inlet 2	Outlet	3 km	8 km
Metformin								
Triclocarban				N/A	N/A	N/A	N/A	N/A
MDMA								
Ibuprofen				N/A	N/A	N/A	N/A	N/A
Thiabendazole								
Triamterene								
Lidocaine				↑30	↑18	↑15		
Carbamazepine				↑163	↓0.5	↑6		
Cocaine								
Atrazine				↑16	↑30	↑13		
DEET				↑31	↑85	↑27		
Nifedipine oxidized								
Primidone								
Gabapentin								
Sulfamethazine								
Sulfamethoxazole								
Tylosin								
↑ indicates increase, ↓ indicates decrease; numeric value indicates x times of difference								

After the rain event, seven compounds were detected at inlet 1, eight at inlet 2, and seven at the outlet (Table 3). The peak areas for compounds detected at a location both before and after the rain event were similarly compared in order to determine the effect of a rain event on the compound's abundance (Fig. 4). The concentration of each compound increased after the rainfall, ranging from approximately 15 to 160 times greater. The only exception was the compound carbamazepine at inlet 2, which decreased by half after the rain event. Possible factors that may influence the presence of a certain compound after a rain event is the compound's solubility and its ability to be retained in the sediment of the pond.



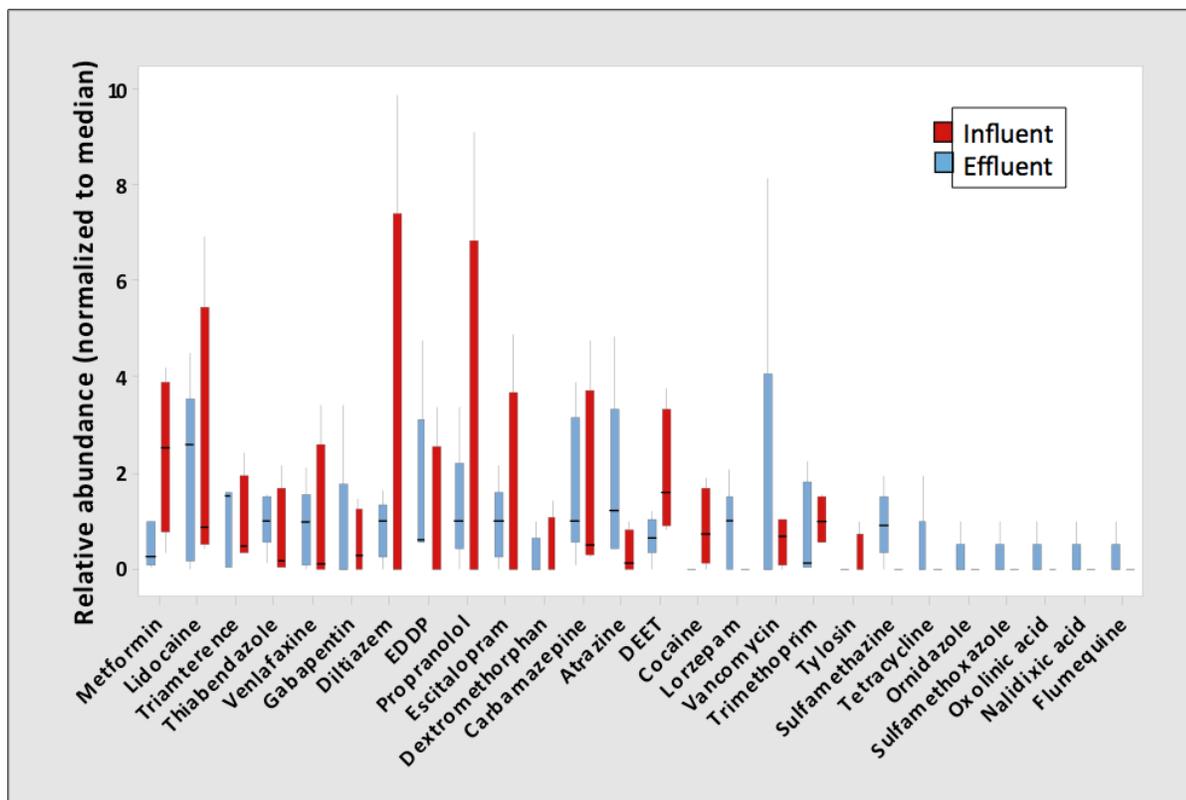
**Figure 3. Effect of a bioretention pond on PPCP abundance. Values <1 indicate an increase in a compound's concentration from inlet to outlet.**



**Figure 4. Effect of a rain event on PPCP abundance. Values >1 indicate an increase in a compound's concentration after a rain event.**

### 3.2 WWTP

Eighteen compounds were detected in the influent from wastewater treatment plants, and nineteen were detected in the effluent. For a majority of the compounds detected at both the influent and effluent points, their concentrations decreased as a result of wastewater treatment (Fig. 5).



**Figure 5. Effect of a wastewater treatment plant on PPCP abundance**

### 3.3 Private well water

The private well water samples were categorized based on possible contributions of pollution to the well. Residents reported if their well was located within 100 feet of a septic tank drain field or pond/freshwater stream, or 0.5 miles within a field of crops, nursery, or farm animal operation. Two samples were analyzed from wells that were near a septic drain field, two from field crops or nursery, two from a farm animal operation, and three from a pond or freshwater stream.

Of these samples, 8 compounds were detected in wells near a septic drain field, 3 near field crops or nursery, 5 near farm animal operations, and 8 near a pond or freshwater stream (Table 4). The presence of PPCPs in wells located near septic drain fields highlights the importance of non-point sources of pollution such as leaky septic systems in introducing PPCPs to the environment. The fact that antibiotics such as sulfamethazine, chlortetracycline, and sulfamethoxazole were detected in wells near field crops or a nursery may be due to the use of biosolids and reclaimed wastewater as fertilizer for such crops. Antibiotics were also prevalent in wells near a farm animal operation, as well as in those nearby a pond or freshwater stream.

Compound	Septic drain field		Field crops/nursery		Farm animal operation		Pond/freshwater stream	
Sulfamethazine								
Carbamazepine								
Clebuterol								
Atrazine								
Propranolol								
Meprobanate								
Tetracycline								
Chlortetracycline								
Sulfamethoxazole								
Tylosin								
Nalidixic acid								

**Table 4. Presence of PPCPs in private well water**

## 5. Conclusion

This study provides insight into the occurrence of PPCPs in multiple water sources, including surface water impacted by urbanization, wastewater treatment influent and effluent, and private well water. The prevalence of PPCPs in such water supplies highlights the importance of nonpoint sources of pollution, such as stormwater runoff and leaky septic systems, in introducing such pollutants into our water. This study will enable future research into the exact concentrations of the compounds detected at each source.

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# Hydrologic Influences on Surface Peat Characteristics at the Great Dismal Swamp: Implications for Carbon Storage and Fire Vulnerability

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## Abstract

Peatland ecosystems are a critical environmental resource for biodiversity and global carbon sequestration; drainage of peatlands for agricultural, residential, and commercial development can severely impair these functions. The purpose of this research was to examine the influence of wetland hydrology on physical and chemical properties of surface peat that indicate its degree of decomposition and affect its vulnerability to fire. Peat cores were collected from the Great Dismal Swamp at 20 locations along a hydrologic gradient and analyzed for bulk density, organic matter content, and carbon/nitrogen concentrations. These soil properties were evaluated against mean water levels at each sampling location to assess hydrologic controls on peat characteristics. Excluding an outlier transect, peat bulk density and organic bulk density were found to decrease significantly with increased wetness. Nitrogen concentration was positively correlated with mean water level, while organic matter fraction and carbon content appeared to be influenced significantly by factors other than hydrologic regime. Evaluated soil parameters serve as important indicators of peat decomposition, as well as influence the vulnerability to peat-consuming fires, both of which have implications for carbon storage. Understanding the hydrologic controls on these parameters at the Great Dismal Swamp will not only aid in the assessment of current conditions, but also will serve to guide ongoing restoration efforts focused on rewetting the Swamp.

**Keywords:** hydrology, peat, Great Dismal Swamp, carbon sequestration, fire vulnerability

## 1. Introduction

Wetland structure and function are largely driven by hydrology. Saturation or flooding during all or part of the year creates anaerobic conditions that control biogeochemical processes involved in soil formation and that select plants adapted to tolerate such conditions (Mitsch & Gosselink, 2007). As such, wetlands are typically identified by their soil and vegetation, as these factors are indicative of long-term hydrologic conditions. Wetland hydrology and the unique biogeochemical processes that result support a suite of important functions, including water quality improvements, hydrologic buffering, carbon sequestration, and unique habitat for flora and fauna (Mitsch & Gosselink, 2007). Understanding the key drivers of these processes is critical to wetland management and restoration.

Peatlands, one specific class of wetlands most common to boreal regions, have unique soil development processes that enable important functions. Peatlands are characterized by their peat soil, which is primarily composed of partially decayed organic matter. Peat forms when the rate of organic matter production exceeds that of decomposition. Decomposition of organic matter progresses slowly in peatlands for a number of reasons. First, the acidic pH and low nutrient availability of peatland soil reduce growth of microorganisms that facilitate the decay process (Lichtler & Walker, 1974). Additionally, peat decomposition is slowed dramatically by the anaerobic soil conditions that exist below the water table (Reddy & Patrick, 1974; Hartford, 1993; Klavins *et al.*, 2008). Among other factors, the

formation of peat depends on limited drainage at and beneath the soil surface, which helps create these saturated, anaerobic conditions (Lichtler & Walker, 1974). As peat accumulates, it further restricts drainage of surface water and decreases pH and nutrient availability, thereby encouraging continued peat formation (Lichtler & Walker, 1974). Peatlands have global significance to the carbon cycle, as they play an important role in carbon sequestration (Yu *et al.*, 2010; Chambers *et al.*, 2011; Davies *et al.*, 2013). Peatland carbon storage worldwide is estimated to exceed 600 GtC, with northern peatlands accounting for approximately 90% of this pool (Yu *et al.*, 2010).

Peat soil development and characteristics are largely controlled by hydrologic regime. When the water table is lowered in a peatland, aerobic conditions return to the soil and facilitate an increased rate of decomposition (Reddy & Patrick, 1974; Blodau *et al.*, 2004). A fluctuating water table can also have influences, as oscillations between aerobic and anaerobic conditions can promote carbon and nitrogen losses equal to or greater than those under continuous aerobic conditions (Reddy & Patrick, 1974). Peat collected from drier sites would be expected to have a lower organic content because of the mineralization that occurs during decomposition (Drzymulska, 2010). Both Drexler *et al.* (2009) and Minkinen and Laine (1998) studied peat *in situ* and found that peat bulk density was much greater at drained sites than corresponding undrained sites. Minkinen and Laine explain that this observed compression was due to subsidence of the peat soil. Soon after a region is drained, the peat compacts as it loses the support of saturating water. Decomposition progresses more rapidly in the dry environment, which further breaks down the plant material that provides structure for the peat soil. Organic bulk density increases with decomposition by the same processes (Chambers *et al.*, 2011). Carbon/nitrogen content, bulk density, organic bulk density, and organic/mineral composition are important soil properties because they function as diagnostic parameters for decomposition and fire vulnerability. Advanced decomposition in peat is indicated by increased bulk density and organic bulk density, decreased organic content, and a relatively low ratio of carbon to nitrogen (Kuhry & Vitt, 1996; Minkinen & Laine, 1998; Blodau *et al.*, 2004; Drzymulska, 2010; Chambers *et al.*, 2011). Elevated vulnerability of peat to ignition and sustained smoldering is signified by low moisture content, high organic content, and low bulk density (Frandsen, 1987; Hartford, 1993; Davies *et al.*, 2013).

The Great Dismal Swamp is a temperate peatland located in the southeastern United States on the border between Virginia and North Carolina. The wetland is protected as a national wildlife refuge that encompasses 109,000 acres of forested terrain managed by the United States Fish and Wildlife Service (USFWS) [GDSNWR, 2001; Bradley, 2013]. Near the center of the Swamp is a shallow body of water, the 3,100-acre Lake Drummond (GDSNWR, 2001). The origin of Lake Drummond is unknown, although Whitehead (1972) concludes that the lake might have formed from a deep peat burn between 3,500 and 6,000 years ago during a period of low water table elevation. Surface water flows into the Swamp via streams from the west, and primary outflow is through a network of manmade drainage ditches (Lichtler & Walker, 1974). Beginning in colonial times, these ditches were constructed to facilitate transportation through the Swamp and to make the land more suitable for agricultural, commercial, and residential development (Lichtler & Walker, 1974; GDSNWR, 2001). The ditch system functioned to drain standing water from inundated regions of the Swamp and to lower the water table throughout. Soil material removed from the ditches during excavation was used to form raised beds for the approximately 140 miles of roads that were built within the Swamp to facilitate access to logging sites (GDSNWR, 2001). These developments impacted the hydrology of the Swamp ecosystem: the roads blocked the established flow of surface water, and the ditches drained water from the Swamp, creating drier conditions (GDSNWR, 2001). Corporate ownership and development of Swamp land continued until 1973, when a large plot was donated to the federal government (Bradley, 2013). This paved the way for the establishment of the Great Dismal Swamp National Wildlife Refuge the following year. Today, the USFWS oversees management programs intended to restore the Swamp's hydrology and biodiversity (GDSNWR, 2001).

In addition to those trends observed more generally across peatland ecosystems, several consequences of impaired hydrology have been detected specifically within the Great Dismal Swamp. One observed consequence of drier wetland conditions is the loss of habitats and their native

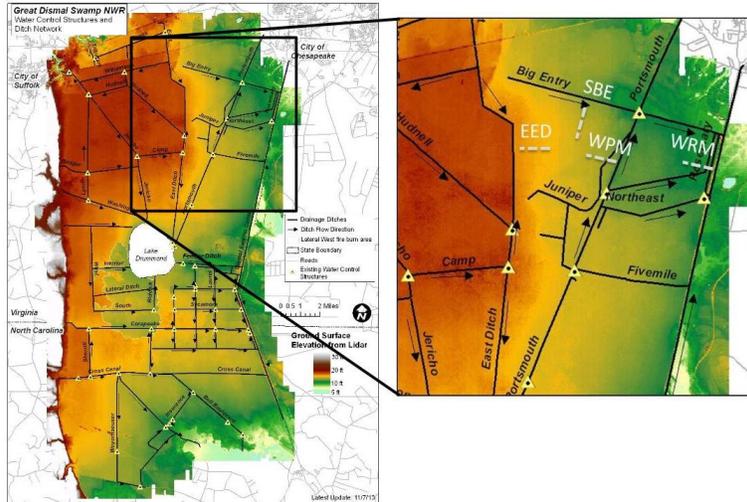
biodiversity. Upland trees, most notably red maple (*Acer rubrum*), have become prevalent in areas previously dominated by wetland species (Whitehead, 1972; Lichtler & Walker, 1974; Mitsch & Gosselink, 2007). A large portion of the wetland habitat has been destroyed through drainage and subsequent land development, such that the Swamp as it stands today probably encompasses less than half of its original area (GDSNWR, 2001). Visual subsidence is evident in many locations throughout the refuge. Hydrologic impairments within the Swamp may contribute to an increased vulnerability to peat fires; recent examples include the South One fire in 2008 and the Lateral West fire in 2011, which spanned an area of 25 km<sup>2</sup> (Reddy *et al.*, 2015). Estimates by Reddy *et al.* (2015) indicate that the Lateral West fire released 1.10 Tg of carbon from the soil.

In this work, we explored the influence of wetland hydrology on physical and chemical properties of surface peat that indicate its degree of decomposition and affect its vulnerability to smoldering. Whereas previous studies have tested the effects of impaired hydrology by comparing peat properties between sites categorized as “drained” or “undrained,” (Minkinnen & Laine, 1998; Drexler *et al.*, 2009), this study explores the relationship between hydrology and peat characteristics along a hydrologic gradient within a single peatland system. This study examined connections between various water level metrics and soil properties including bulk density, organic content, organic bulk density, and carbon/nitrogen composition. Understanding the impact of peatland drainage at the Great Dismal Swamp will not only aid in the assessment of current conditions, but also will serve to guide ongoing efforts to restore and protect this important ecological resource.

## **2. Research Methods**

### *2.1 Site Description*

Peat samples were collected from the northeastern corner of the Great Dismal Swamp, located on Virginia’s Coastal Plain. The Swamp has a temperate climate with humid summers and mild winters, and on average, the region receives 128 cm of rainfall each year (Lichtler & Walker, 1974). Topographic relief is low, but there is a gradual slope downward from the Suffolk Scarp, which forms the western boundary of the Swamp, to the Deep Creek Swale along its eastern edge (Lichtler & Walker, 1974). The Swamp contains a network of drainage canals depicted in Figure 1. Transects used in this study were established near the drainage ditches. These transects, 300 m in length, originate at the midpoint of the drainage ditch and run perpendicular to the ditch. Wells located at both ends of each transect record water levels every 15 minutes. Plot centers have been established at 20-m intervals along each transect, for a total of 15 plots per transect. Sampling for this study was performed along four transects, located east of East Ditch (EED), west of Portsmouth Ditch (WPM), south of Big Entry Ditch (SBE), and west of Rosemary Ditch (WRM). Along each of the four transects, samples were taken from the five plots closest to the drainage ditch, yielding a total of 20 sampling locations.



**Figure 1. Map of sampling locations at the Great Dismal Swamp. Transects displayed are located east of East Ditch–EED, west of Portsmouth–WPM, south of Big Entry–SBE, and west of Rosemary–WRM (USFWS).**

## 2.2 Field Methods

Cylindrical peat cores measuring 15 cm in diameter and 5 cm in height were taken from the surface of the peat layer. This large sample size contributes to the quality of laboratory analyses because the sample is more likely to be representative of peat characteristics at that location (Givelet *et al.*, 2004). Care was taken throughout the sampling process to avoid disturbing or compressing the soil. At each sampling location, the litter and root mat were cleared to expose the peat soil beneath. A corer measuring 15 cm in diameter was inserted into the ground to cut the lateral wall of a peat cylinder away from the surrounding soil (De Vleeschouwer *et al.*, 2010). The corer was then removed from the ground, and a PVC ring with a diameter of 15 cm and a height of 5 cm was inserted into the slot cut by the corer (Photo 1). A wide metal spatula was positioned underneath the peat cylinder, and the soil inside the ring was carefully lifted out of the ground. Excess roots and soil material that protruded from the top and bottom of the ring were removed so that the volume of the sample matched that of the PVC ring. The sample was then deposited into a sealed plastic bag and stored at a cool temperature (~4°C) to prevent decomposition (Drexler *et al.*, 2009). Three replicates were obtained in this manner from each of the twenty sites. In addition to each main sample, a small volume of surface peat was collected for elemental (carbon and nitrogen) analysis. These smaller samples were collected separately so that they could be dried at a reduced temperature to minimize bias due to nutrient volatilization.



**Photo 1. Collection of peat cores (6/30/2016).**

### 2.3 Hydrologic Data

This study utilized hydrologic data assembled by Morgan Schulte in connection with her graduate work at Virginia Tech.

Wells located at both ends of each 300-m transect recorded water table depth every 15 minutes between March 2015 and May 2016. The transect was surveyed to determine the elevation of each plot center. Using known ground elevations and interpolating water table depths between the two wells, it was possible to estimate the water table depth at each plot center. In this study, hydrologic behavior was represented by water levels measured in this manner.

Because our well data captures only several months of hydrologic behavior, we cannot assume that recorded water table heights are typical for that region. Factors such as rainfall vary throughout the course of a single year, and without long-term data, we cannot speak to whether a particular year is representative of usual conditions (Mitsch & Gosselink, 2007). However, since our data was obtained from set locations over the same period of time, the sites' hydrology relative to one another should be consistent. Any bias resulting from the available span of hydrologic data will be observed across all sites. In other words, the driest site should always be the driest of the sites, and so on, regardless of whether its recorded mean water table height is numerically representative of long-term conditions.

### 2.4 Bulk Density

Samples were placed in an oven and dried to constant weight at 105°C (Heiri *et al.*, 2001; Wang *et al.*, 2001; Givelet *et al.*, 2004). Upon removal from the oven, samples were weighed to the nearest 0.01 g. Any large root material (diameter > 0.5 cm) was removed from the sample and set aside. The mass of the root material was recorded, and the volume was determined through water displacement in a graduated cylinder. The corrected dry mass of the sample was calculated by subtracting the root mass from the dry mass of the sample. The corrected volume of the sample was calculated by subtracting the root volume from the total volume of the sample. Bulk density was then determined by dividing the corrected dry mass of the sample by its corrected volume.

### 2.5 Loss on Ignition and Organic Bulk Density

After the bulk density of the sample had been determined, a subsample was taken for loss-on-ignition (LOI) analysis. Because the sample had been uniformly spread across a tray for drying, a representative subsample could be obtained by visually dividing the tray in two and using the peat spread across one half of the surface (De Vleeschouwer *et al.*, 2010). The subsamples were ground and passed through a 2-mm sieve to promote complete combustion (Hoogsteen *et al.*, 2015). The large (~100 g) subsamples of dried, ground peat were then placed in a muffle furnace at 500°C for 24 hours (Kristensen & Anderson, 1987; Heiri *et al.*, 2001; Wang *et al.*, 2011). Organic content was calculated as the quotient of the mass lost during ignition divided by the original mass of the subsample. Organic bulk density was then determined by multiplying the sample's bulk density by its organic matter fraction.

### 2.6 Carbon/Nitrogen Analysis

Samples were placed in an oven and dried to constant weight at 65°C. Each sample was then ground and passed through a 2-mm sieve in order to improve homogeneity (Schumacher *et al.*, 1990; Gandois *et al.*, 2012; Novak *et al.*, 2014). A subsample of mass 0.25 g was obtained from each sample and analyzed for carbon and nitrogen in an Elementar Analysensysteme GmbH vario Max CN Elemental Analyzer. The molar ratio of C to N was calculated using the molar masses of the elements and their relative gravimetric concentrations.

### 2.7 Statistical Methods

Regressions were used to explore hydrologic controls on each of the soil parameters evaluated. Hydrology was represented by the mean, standard deviation, 10<sup>th</sup> percentile, and 90<sup>th</sup> percentile water levels corresponding to each sampling location.

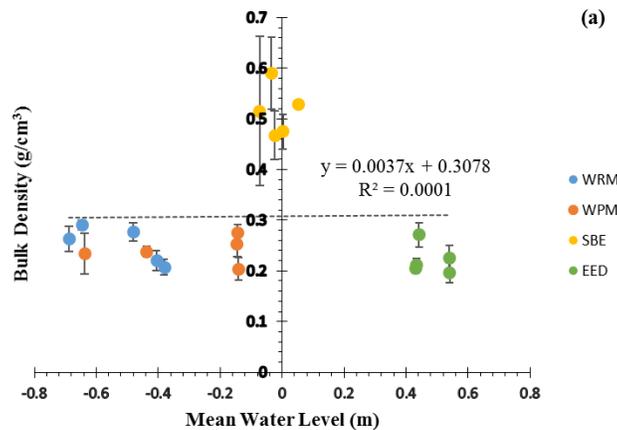
### 3. Results

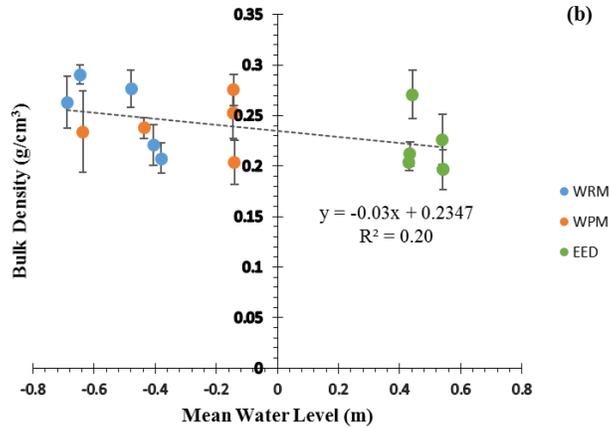
#### 3.1 Bulk Density

As evident from Figure 2, peat samples collected from the transect south of Big Entry Ditch (SBE) proved to be significant outliers in terms of bulk density and composition, so data analyses were performed with and without these outlier points for bulk density and other impacted variables.

Bulk density values of surface peat collected from non-outlier transects ranged from 0.18-0.30 g/cm<sup>3</sup>. The average standard deviation among the three replicate samples taken from each of the fifteen sampling locations was 0.02 g/cm<sup>3</sup>. By contrast, surface peat from SBE had bulk density values between 0.36 g/cm<sup>3</sup> and 0.67 g/cm<sup>3</sup>. Variation among replicates was also greater along this transect, with an average standard deviation of 0.06 g/cm<sup>3</sup> at the five sampling locations.

Excluding SBE, a significant ( $p < 0.01$ ) negative correlation was found between mean water level and surface peat bulk density. Relationships of similar strength were found between bulk density and other hydrologic representations, specifically 10<sup>th</sup> and 90<sup>th</sup> percentile water levels; meanwhile, bulk density was found to be positively correlated to water table standard deviation (Table 1).

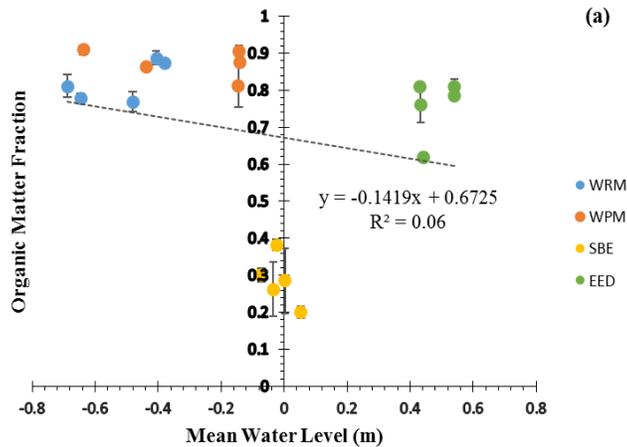


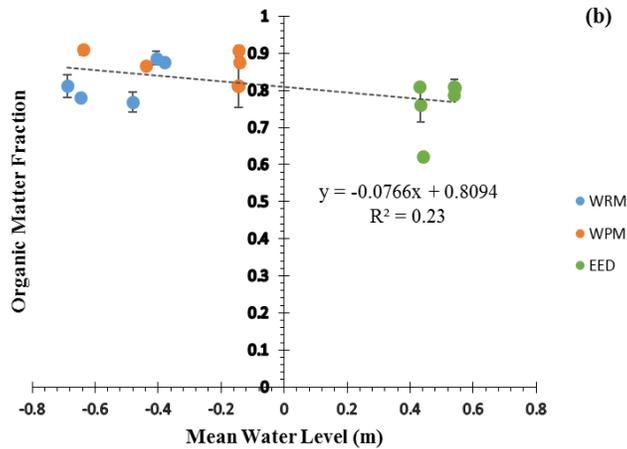


**Figure 2. Variation in bulk density of surface peat across a hydrologic gradient within the Great Dismal Swamp, both (a) including SBE and (b) excluding SBE.**

### 3.2 Organic Matter Content

The organic matter fraction of surface peat collected from non-outlier transects ranged from 0.61 to 0.92, with a median value of 0.82 (Figure 3b). By contrast, surface peat from SBE had organic matter fractions between 0.18 and 0.40, with a median value of 0.30 (Figure 3a). The average standard deviation among the three replicate samples taken from each of the twenty sampling locations was 0.03. Excluding SBE, a significant ( $p < 0.01$ ) negative correlation was found between mean water level and surface peat organic matter content. Similar correlations related organic matter to 10<sup>th</sup> and 90<sup>th</sup> percentile water level metrics, while a positive correlation existed between organic matter and water table standard deviation (Table 1).

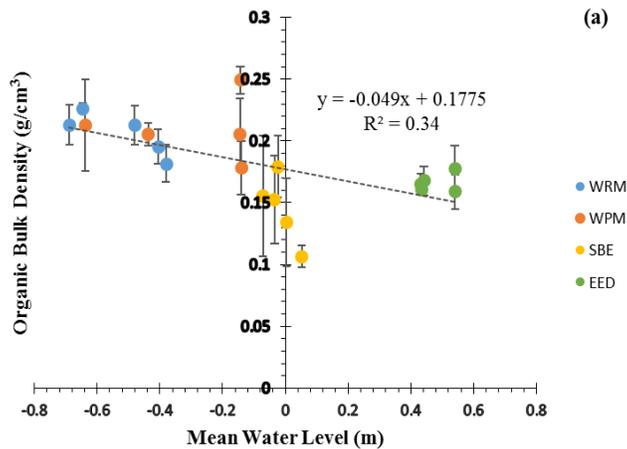


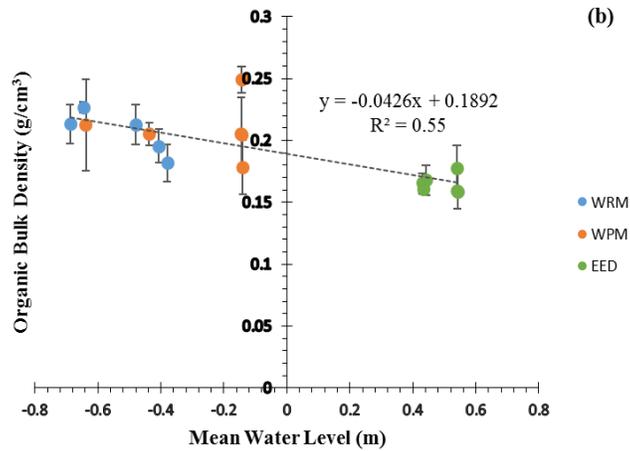


**Figure 3. Variation in organic matter content of surface peat across a hydrologic gradient within the Great Dismal Swamp, both (a) including SBE and (b) excluding SBE.**

### 3.3 Organic Bulk Density

The organic bulk density of surface peat collected from all four transects ranged from 0.09 g/cm<sup>3</sup> to 0.26 g/cm<sup>3</sup> (Figure 4). Samples from SBE tended to occupy the lower end of this range, but values from this transect for organic bulk density were less extreme than those for other parameters measured. A significant ( $p < 0.01$ ) negative correlation was found between mean water level and organic bulk density, both including and excluding SBE. Relationships of similar strength were found using 10<sup>th</sup> and 90<sup>th</sup> percentile water metrics to represent site hydrology, whereas a trend in the opposite direction was detected between organic bulk density and water level standard deviation (Table 1).

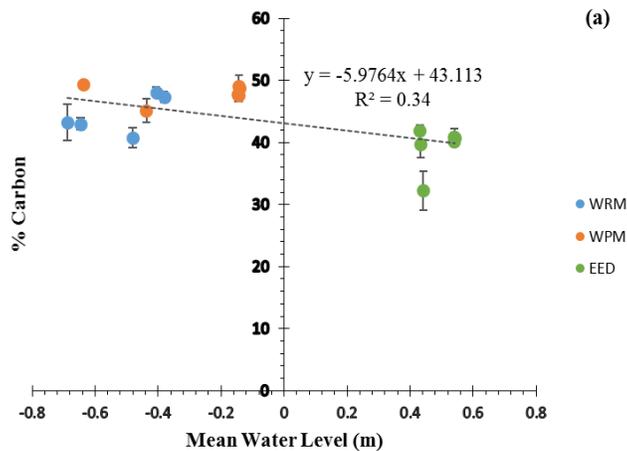


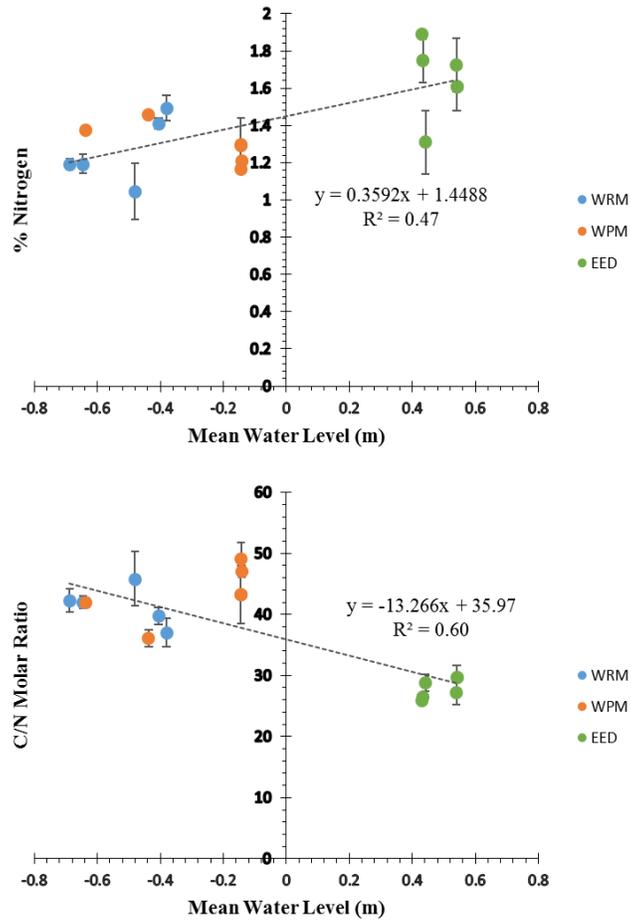


**Figure 4. Variation in organic bulk density of surface peat across a hydrologic gradient within the Great Dismal Swamp, both (a) including SBE and (b) excluding SBE.**

### 3.4 Carbon and Nitrogen Concentrations

Excluding SBE, a significant ( $p < 0.01$ ) negative correlation was found between mean water level and the carbon content of surface peat (Figure 5a). Relationships of similar strength were found using 10<sup>th</sup> and 90<sup>th</sup> percentile water level metrics (Table 1). Among the same three transects, nitrogen concentration was found to increase significantly ( $p < 0.01$ ) with wetness as expressed by mean, 10<sup>th</sup> percentile, and 90<sup>th</sup> percentile water levels (Figure 5b, Table 1). Molar ratios of carbon to nitrogen varied considerably, ranging from 19 to 52 across all twenty sampling locations. A significant ( $p < 0.01$ ) negative correlation existed between C/N ratio and site wetness as expressed by mean, 10<sup>th</sup> percentile, and 90<sup>th</sup> percentile water levels (Figure 5c, Table 1). For all three of these parameters, the direction of correlation with standard deviation was the opposite of that observed with mean, 10<sup>th</sup> percentile, and 90<sup>th</sup> percentile water levels (Table 1).





**Figure 5. Variation in (a) carbon concentration, (b) nitrogen concentration, and (c) C/N molar ratio of surface peat across a hydrologic gradient within the Great Dismal Swamp, excluding SBE.**

**Table 1. Regression statistics for soil parameters vs. hydrologic metrics, excluding SBE.**

	P-VALUE (DIRECTION OF CORRELATION)			
	Mean	10 <sup>th</sup> Percentile	90 <sup>th</sup> Percentile	Std. Dev.
<b>BULK DENSITY</b>	0.008 (-)	0.009 (-)	0.006 (-)	0.02 (+)
<b>ORGANIC MATTER</b>	0.001 (-)	0.0004 (-)	0.008 (-)	3 E-5 (+)
<b>ORGANIC BULK DENSITY</b>	1 E-6 (-)	3 E-7 (-)	6 E-6 (-)	1 E-7 (+)
<b>CARBON</b>	6 E-5 (-)	6 E-6 (-)	0.0009 (-)	1 E-8 (+)
<b>NITROGEN</b>	1 E-6 (+)	3 E-7 (+)	5 E-6 (+)	3 E-8 (-)
<b>C/N</b>	3 E-9 (-)	3 E-11 (-)	1 E-7 (-)	4 E-17 (+)

#### 4. Discussion

To explore hydrologic controls on peat characteristics and attendant functions, here we collected and analyzed surface peat from the Great Dismal Swamp along a hydrologic gradient and in conjunction with hydrologic data. The physical and chemical properties tested are important because they function as diagnostic parameters for peat decomposition and vulnerability to fire. In examining the effects of peatland drainage within the Great Dismal Swamp, this study aims to guide ongoing efforts to restore and protect this important ecological resource.

##### 4.1 South of Big Entry as an Outlier

Peat collected from the transect south of Big Entry (SBE) proved to be a significant outlier for many of the parameters measured, including bulk density, organic matter content, and carbon/nitrogen concentrations. A map of peat depth throughout the Great Dismal Swamp indicates that SBE is located near the edge of the Swamp's peat deposits (Oaks & Coch, 1973). Because of this, the shallow peat layer there is likely mixed with sand and clay sediments reported by Oaks and Coch (1973) to lie beneath the peat deposit. USFWS hydrologist Fred Wurster suggests that in addition to this mineral influence, peat near SBE is well oxidized due to century-long drainage and severe fires reported to have occurred in that region during the 1920's (personal communication, July 15, 2016). The high mineral content of the peat is likely responsible for its high bulk density, as the particle density of mineral soil is much greater than that of organic matter (Minkinen & Laine, 1998). Similarly, a higher mineral concentration would naturally result in lower concentrations of organic compounds. SBE was therefore excluded from certain analyses because variation in measured parameters is thought to be primarily due to mineral input, not hydrologic controls.

##### 4.2 Bulk Density

Bulk density serves as an important diagnostic of peat decomposition and fire vulnerability, both of which critically impact the peatland's ability to store carbon. Decomposition is essentially a collection of physical, chemical, and biological processes which break down organic matter into simpler organic and inorganic units (Klavins *et al.*, 2008). Higher bulk density indicates greater levels of decomposition because plant fibers provide structure for the soil, so when this material breaks down, peat loses its

support and becomes more compact (Minkkinen & Laine, 1998). Hartford (1993) found that greater bulk density served to impede sustained smoldering due to a higher mineral density and reduced airflow from the diminished porosity. She also noted that peat with greater bulk density has a greater heat capacity and therefore requires a greater quantity of energy to preheat for smoldering. Despite the counter effect of bulk density, peatland regions with impaired hydrology due to drainage are still particularly vulnerable to ignition (Davies *et al.*, 2013).

In this study, peat bulk density was found to decrease with increasing wetness. This trend agrees with previous studies that have found bulk density to be greater at drained peatland sites than corresponding undrained sites (Minkkinen & Laine, 1998; Drexler *et al.*, 2009). Non-outlier values of peat bulk density ranged from 0.18–0.30 g/cm<sup>3</sup>, which was somewhat higher than expected. For instance, Chambers *et al.* (2011) found the bulk density of temperate peat to generally fall between 0.05 g/cm<sup>3</sup> and 0.2 g/cm<sup>3</sup>, and a survey of Great Dismal Swamp peat performed in August 2013 by the Natural Resources Conservation Service (NRCS) reported a range of 0.09–0.22 g/cm<sup>3</sup>. One possible explanation for this difference is that samples analyzed in this study consisted exclusively of surface peat, which is particularly vulnerable to compaction from drainage (Minkkinen & Laine, 1998). Conditions near the surface are the first to become aerobic as water levels fall or fluctuate, so it makes sense for consequences of drainage to be experienced most acutely in the topmost layers of peat. The negative correlation between bulk density and wetness indicates that peat in drier regions of the Swamp has undergone more extensive decomposition than peat in wetter regions, but is less vulnerable to fire than peat at wetter sites with equivalent moisture and mineral contents.

#### 4.3 Organic Matter Content

As with bulk density, organic matter content can indicate peat's degree of decomposition and likelihood of ignition. With all other variables constant, organic content will be lower in well-decomposed peat because of the mineralization that occurs as part of the decay process (Klavins *et al.*, 2008; Drzymulska, 2010). Hartford (1993) found that as the mineral content of peat increased, its probability of sustained smoldering decreased. Mineral content inhibits smoldering because the inorganic matter absorbs heat from the smoldering mass but does not itself smolder and produce heat (Frandsen, 1987).

In this study, organic matter content was found to be negatively correlated with the wetness of the sampling location. This observed trend does not match our prediction that drier conditions permit faster decomposition and therefore increase mineralization, meaning peat collected from wetter sites should have a higher fraction of organic matter. One possible reason for these unexpected results is given by Chambers *et al.* (2011), who note that the organic matter fraction of peat varies considerably according to mineral inputs from external processes. This explanation is supported by certain instances in our data where large differences in organic matter occurred at sites with similar hydrologic characteristics. Delesantro (2013) reported a negative correlation between percent organic matter and water level standard deviation, whereas our data displayed the opposite trend. The results from our study suggest that mineralization during decomposition has an insignificant effect on overall soil composition compared to other processes that introduce mineral material into the soil. Because of this, organic matter fraction alone may not be a reliable indicator of *in situ* peat decomposition. Sustained smoldering in peat is limited by both moisture content and mineral content (Frandsen, 1987; Hartford, 1993). Surface peat at a drier site typically has a lower moisture content, which makes it more vulnerable to ignition. If mineral content could be expected to increase with dryness, then the mineral influence would partially counteract that of the dryness, mitigating the risk of fire. However, such a relationship with mineral content and wetness was not found here, suggesting increased vulnerability to peat fire at drier sites.

#### 4.4 Organic Bulk Density

Organic bulk density is a useful, albeit less common, indicator of decomposition and fire vulnerability. This parameter expresses the mass of organic matter per unit volume of undisturbed soil (Frandsen, 1987). Low organic bulk density is characteristic of peat that has undergone low levels of

decomposition (Chambers *et al.*, 2011). Chambers *et al.* (2011) reason that since regular bulk density is influenced by mineral inputs that typically result from external mechanisms, organic bulk density serves as a more effective indicator to target organic matter decay. Additionally, organic bulk density is advantageous because it allows for comparison among peat with varying fractions of organic matter. The influence of organic bulk density on fire vulnerability is less straightforward because the factors that determine organic bulk density (organic content and bulk density) have opposite effects on sustained smoldering. On one hand, peat with a higher concentration of organic matter has a greater amount of fuel per unit volume; however, this greater density can also restrict the supply of oxygen to the combustion reaction (Frandsen, 1987). All things considered, Frandsen (1987) suggests that the overall effect of increased organic bulk density might be a moderate increase in flammability.

This study found the organic bulk density of peat to decrease with increasing wetness, matching the trend observed with regular bulk density. Organic bulk density was more strongly correlated with hydrology, which is reasonable if variation in regular bulk density is also influenced by other factors such as mineral input. These findings concerning organic bulk density affirm the implication of regular bulk density that peat at drier sites is measurably more decomposed than peat at wetter sites. Peat at drier locations within the Swamp is also indicated to be more vulnerable to fire because of its increased organic bulk density.

#### 4.5 Carbon and Nitrogen Concentrations

Peat deposits represent an important store of carbon, which is sequestered indefinitely in the soil unless oxidized through smoldering combustion or through the rapid decomposition that results from drainage (Mitsch & Gosselink, 2007). Accordingly, carbon content decreases as carbon is released from the soil through these processes. This study detected a decrease in carbon content with wetness, which contradicts indications of decomposition presented by trends in bulk density and organic bulk density. However, carbon concentration is also affected by mineral inputs (similar to organic matter content) and the quality of organic matter; the latter means that variation can be attributed in part to the composition of plant material from which the peat formed (Chambers *et al.*, 2011). A significant ( $p < 0.01$ ) positive correlation was found between carbon concentration and red maple dominance (as measured by relative basal area; data from M. Schulte not shown), suggesting an important influence of vegetative controls on this parameter. A possible explanation for these findings is that the baseline carbon concentration of surface peat is influenced primarily by vegetative factors, with subsequent changes controlled by hydrology. Therefore, since the negative correlation between carbon content and wetness does not account for the original carbon content of the soil, the trend might exist simply because drier sites support different vegetation. Further study is recommended to assess the relative roles of hydrology and vegetation in determining the carbon content of peat.

Nitrogen, another component of the elemental analysis performed in this study, is an essential nutrient for biomass production and often limits plant growth in peatland bogs. Novak *et al.* (2014) note that peatlands can store greater amounts of nitrogen in a given area of land than most other types of terrestrial ecosystems. In this study, non-outlier samples had a median nitrogen content of 1.39% by weight, which closely matched the 1.44% reported by the NRCS survey, but was somewhat higher than the 1% approximation given by Novak *et al.* (2014) for surface layers of bog peat. The nitrogen content of our surface peat was found to increase with wetness, suggesting that a greater capacity for plant productivity exists at these wetter sites. Hydrologic controls on nitrogen concentration may therefore affect the continued formation of peat, as an increase in biomass production contributes to a more rapid accumulation of organic matter.

Together, carbon and nitrogen concentrations can be evaluated using the molar ratio between them. Kuhry and Vitt (1996) reason that since nitrogen is often a limiting factor to plant growth in peat bogs, an increase in nitrogen availability usually results in an increase in biomass production, with little effect on the overall C/N ratio of the soil. Instead, they propose that changes in this ratio typically correspond to changes in carbon content, and a decrease in C/N can be interpreted to represent carbon loss through decomposition. However, given the relationship between vegetative quality and carbon

concentration, our results seem to support the explanation presented by Drzymulska (2010), who cautions that trends in C/N ratio can be muddled by the changes in vegetation that accompany hydrologic differences. As with carbon, the unexpected trend in C/N ratio with wetness suggests a need for future work exploring the role of vegetative controls in shaping peat characteristics, as well as the influence of hydrology on these vegetative factors.

## **5. Conclusion**

Hydrology plays a critical role in processes that govern the rate of decomposition and vulnerability to fire of surface peat at the Great Dismal Swamp. Surface peat collected from sites most impacted by drainage was measurably more decomposed, as indicated by its greater bulk density and organic bulk density. Further study is needed to understand the vegetative and hydrologic controls on carbon content and to measure carbon loss due to decomposition. Increased bulk density is known to limit the sustained smoldering of peat at drier sites, but ignition is encouraged by the dry conditions and high organic bulk density at these locations. Furthermore, organic content varied by mineral input more so than degree of decay, so a greater mineral concentration cannot be counted upon to mitigate fire risk at drier, more decomposed sites. Carbon released into the atmosphere through the decay and combustion of peat impacts the carbon cycle on a global scale and could potentially contribute to global warming. To preserve the Great Dismal Swamp as an ecological and environmental resource, it is recommended that flow control structures in ditches be used to minimize drainage and stabilize water levels, thus emulating hydrologic conditions conducive to effective peatland function.

## **6. Acknowledgements**

The authors would like to thank the USFWS staff at the Great Dismal Swamp National Wildlife Refuge for their support. Thanks to Dave Mitchem for his guidance in the laboratory and to Ray Ludwig for his help collecting samples. We acknowledge the support of the National Science Foundation through NSF/REU Site Grant EEC-1359051. 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.

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# Ammonia Recovery using an Osmotic Microbial Electrolysis Cell

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## Abstract

Removing Ammonia from wastewater is important because excessive ammonia in the water supply is toxic to aquatic life. Recovering Ammonia is important because Ammonia is an important chemical for agricultural practice. Microbial electrochemical technologies (METs) are an emerging wastewater treatment technology that have several advantages over the common Activated Sludge process. An OsMEC is a type of MET. In this study, we tried to see if an OsMEC could recover more ammonia from wastewater than a Microbial Electrolysis Cell (MEC). An OsMEC is different from an MEC in that it has a Forward Osmosis Membrane (FOM) between its anode and cathode instead of a Cation Exchange Membrane (CEM). Forward Osmosis Membranes are cheaper than CEMs, and can produce higher current and clean water at the cathode. We used a two-chamber OsMEC, with both anode and cathode in continuous mode. We used a simulated wastewater solution as anode influent and a NaCl draw solution and cathode influent. We were able to remove  $41.54 \pm 6.73\%$  of ammonia from the stimulated wastewater. We were also able to recover 50 mg hydrogen when using a 10 ohm resistance.

**Keywords:** Osmotic Microbial Electrolysis Cell, Forward Osmosis Membrane, Wastewater Treatment, Nutrient Recovery, Ammonia Recovery, Hydrogen Recovery

## 1. Introduction

*1.1 What issues are we facing about wastewater treatment?*

*1.1.1 Wastewater treatment consumes a lot of energy.*

Disposing of wastewater safely is vital to civilization. We have made many advances in disposing of waste hygienically and in massive quantities. The conventional treatment method is currently the activated sludge process, which uses bacterial sludge, as well as disinfectants like chlorine, to remove contaminants. Now there may be need of wastewater processing methods that are more energy efficient and sustainable. So what's the problem with conventional wastewater treatment?

First of all, wastewater treatment consumes a lot of energy. Wastewater treatment currently uses about 3-5% of electricity in the United States—around 15 GW—but using organic matter in waste to produce energy could curb that significantly (B. E. Logan & Rabaey, 2012; B. Logan, 2014). All wastewater produced in the USA stores about 17 GW of power—a number which could be increased dramatically to more than 600 GW if industry practices were optimized to make even more wastewater (B. E. Logan & Rabaey, 2012). These figures show that wastewater contains more energy than is used to process it. It would be in our best interest to try and recover that stored energy, even partially, to mitigate the cost of processing wastewater.

### *1.1.2 Nutrients and contaminants need to be removed from wastewater.*

Nitrogen and Phosphorous are both nutrients that must be removed from wastewater (Kelly & He, 2014). According to a report published by the USGS in 2013, “The major sources of nutrients to streams and ground water are precipitation, dissolution of natural minerals from soil or geologic formations, fertilizer application, and effluent from sewage-treatment plants.” High phosphorous causes eutrophication and algal blooms. High levels of nitrate (NO<sub>3</sub>) can cause blue baby syndrome, a dangerous condition effecting the oxygen in the blood of young infants. High levels of ammonia (NH<sub>4</sub><sup>+</sup>) can be toxic to fish and other animals living in the water (Mueller & Helsel, n.d.).

Ammonia is the form of Nitrogen that is usually found in wastewater. Nitrogen can be removed from wastewater by the processes of nitrification and denitrification (Kelly & He, 2014). In nitrification-denitrification, ammonia is turned into nitrate, then nitrite, then nitrogen gas, which can be released safely into the atmosphere. These processes require both anaerobic and aerobic bacteria. The aerobic bacteria require a stage of aeration which is very energy intensive—in fact it accounts for most of the energy spent in conventional wastewater treatment (B. E. Logan & Rabaey, 2012).

Our experiment focused on recovering ammonia from the water.

### *1.2 How can microbial electrochemical technologies (METs) recover nutrients and energy from wastewater?*

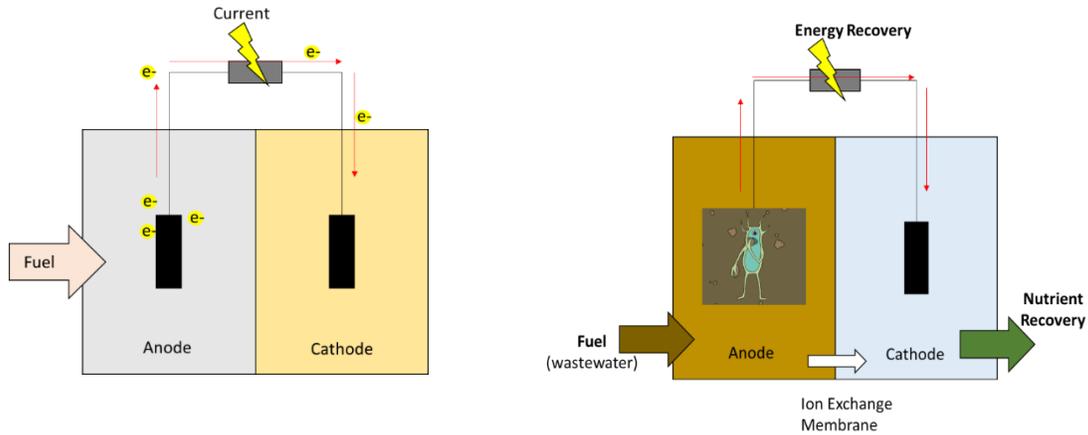
Recovering energy and nutrients from the biomass in wastewater may be possible through microbial electrochemical technologies (METs). Research on microbial electrochemical technologies (METs), such as the microbial fuel cell (MFC), has exploded in the last few years as researchers try to answer the problems of wastewater treatment from the perspective of microbial electrochemistry—“the study and application of interactions between microorganisms and electron conductors” (Schröder, Harnisch, & Angenent, 2015).

METs use microbes as catalysts for electrochemical reactions and use organic matter from wastewater as fuel. The particular bacteria of use in METs are called exoelectrogens. Exoelectrogens will live on organic matter like “simple molecules such as acetate, ethanol, glucose, and hydrogen gas; polymers such as polysaccharides, proteins, and cellulose; and many types of wastewaters from domestic, food processing, and animal sources” (B. E. Logan & Rabaey, 2012). In the anode, they oxidize the organic matter, like acetate, in wastewater and expel electrons (Kelly & He, 2014; B. E. Logan & Rabaey, 2012).

METs have a major advantage in that they can treat wastewater while producing much less sludge than the current treatment methods (B. E. Logan & Rabaey, 2012).

#### *1.2.1 How do METs work?*

The prototypical MET is the microbial fuel cell (MFC). A microbial fuel cell is much like a conventional fuel cell. A fuel cell is a device with two compartments—the anode and the cathode—which are connected by an electrode. Fuel is added to the anode. In the anode, the fuel goes through chemical reactions to generate electrons. Those electrons go from the anode to the cathode through an electrode, which creates a current. In the cathode, electrons are accepted by an electron acceptor, such as oxygen (O<sub>2</sub>).

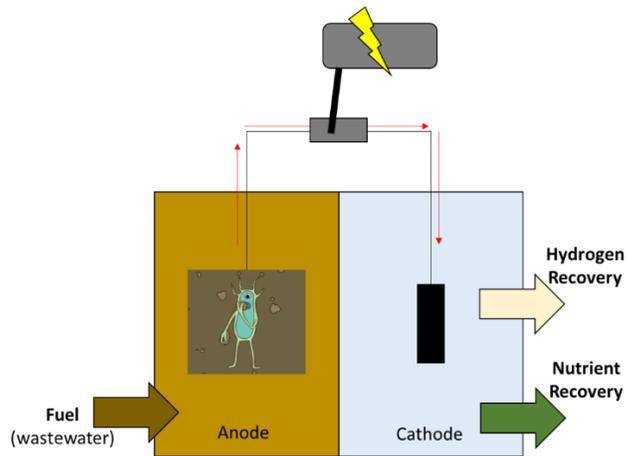


**Figure 1. (Left) Diagram of a basic fuel cell. (Right) Diagram of a microbial fuel cell.**

In the microbial fuel cell (MFC) bacteria are placed in the anode, where they act as a catalyst for the production of electrons. Wastewater is used as the fuel in the anode. An MFC is one of many METs. An MFC can be used to generate electricity from wastewater or to recover nutrients in the cathode by allowing ions to cross from anode to cathode through the ion exchange membrane.

MFCs cannot recover as much energy as a hydrogen fuel cell. MFCs have higher internal resistances; they cannot be connected in series to increase voltage; the microbes that catalyze them are picky about the temperature, pH, and conductivity of their environment; biofilm builds up in MFCs, causing membrane fouling; and “substrate degradability”. Hydrogen fuel cells “produce on the order of  $1 \text{ W cm}^{-2}$ ,” while MFCs often get around  $2\text{-}3 \text{ W m}^{-2}$  of cathode. MFCs used to treat wastewater have not been successful at efficiently recovering energy from the waste;  $.07 \text{ kWh m}^{-3}$  has been recovered, but wastewater contains  $2 \text{ kWh m}^{-3}$ . For this reason, it is appealing to focus on nutrient recovery, or generating chemicals like  $\text{H}_2$ , rather than energy recovery from wastewater (B. E. Logan & Rabaey, 2012). METs are able to remove nutrients effectively (Kelly & He, 2014).

In a hydrogen fuel cell, the negative charge of electrons crossing over from the anode to the cathode side is balanced by protons crossing the ion exchange membrane from anode to cathode. In MFCs, the negative charge of the electrons going over to the cathode side is balanced by cations, like ammonia ( $\text{NH}_4^+$ ), crossing the ion exchange membrane to the anode side. Since cations cross from anode to cathode, it is possible to collect cations, like ammonia, from the cathode. Adding additional energy to the reaction can make thermodynamically unfavorable reactions happen, expanding the products one can possibly recover from the MET (B. E. Logan & Rabaey, 2012). An MFC with an external power source added in this way is called a microbial electrolysis cell (MEC).



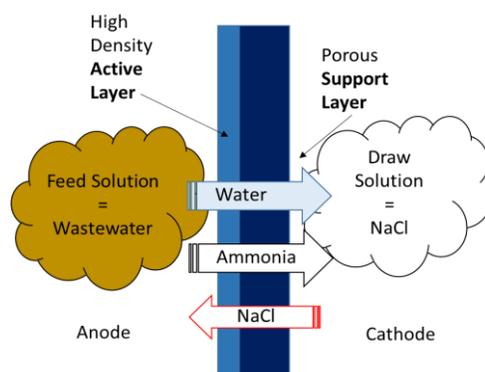
**Figure 2. Diagram of a microbial electrolysis cell.**

A microbial electrolysis cell (MEC) is like an MFC but with an external power source added to encourage thermodynamically unfavorable reactions, like hydrogen generation (Lee, Kim, Yang, & Kim, 2015).

### 1.2.2 How can METs recover nutrients?

In a METs, nitrogen can be removed by moving ammonia across a separator (Kelly & He, 2014). Wastewater, containing ammonia, is fed into the anode of an MET. The ammonia crosses from the anode to the cathode through a cation exchange membrane, allowing it to be collected from the cathode. Ammonia recovery from anode to cathode with METs has been shown to be feasible. One experiment showed that an MFC could recover 100% of ammonia (Desloover, Woldeyohannis, Verstraete, Boon, & Rabaey, 2012; Kelly & He, 2014). An analysis showed that ammonia recovery with METs saved energy when compared to conventional ammonia removal methods, and that high current density improved ammonia recovery (Haddadi, Elbeshbishy, & Lee, 2013; Kelly & He, 2014).

### 1.2.3 How are forward osmosis membranes relevant?



**Figure 3. Diagram of a forward osmosis membrane, as it would be applied in an MET, active layer facing the anode and supporting layer facing the cathode.**

### 1.3 Our project, the OsMEC

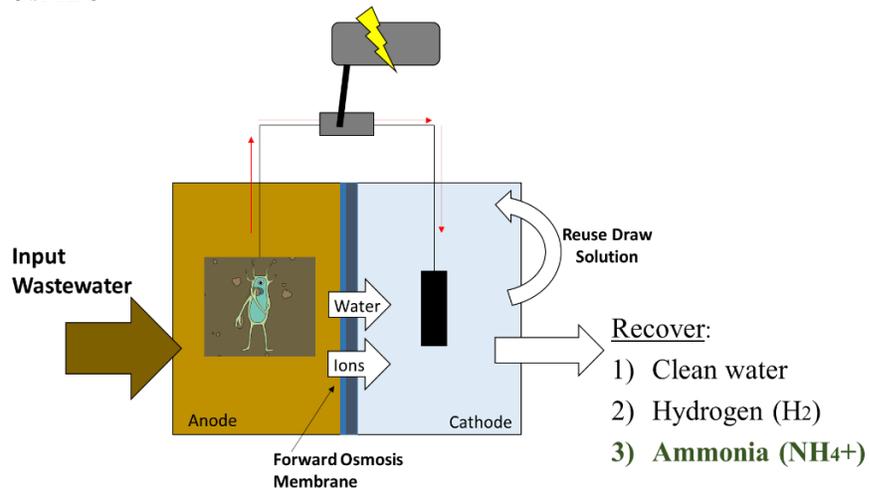


Figure 4. Diagram of an osmotic microbial fuel cell (OsMEC).

We want to combine FOM and MEC technology to recover ammonia because OsMECs have been shown to generate higher current, which correlates with higher ammonia recovery, and the water flux with FOM may encourage ammonia crossing over to the cathode (Kelly & He, 2014; Lee et al., 2015).

## 2. Research Methods and Experimental Setup

### 2.1 Experimental set up

#### 2.1.1 OsMEC set up

Our OsMEC reactor has two compartments, an anode and a cathode, each with a volume of 280 mL. The anode and cathode are connected to each other, a resistor, and an external power source. The external power source supplies a voltage of .5 V. The resistor was changed throughout the experiment to test the effect of different levels of current on the ammonia recovery. At different times we used a 1 ohm resistor, a 10 ohm resistor, and a 100 ohm resistor.

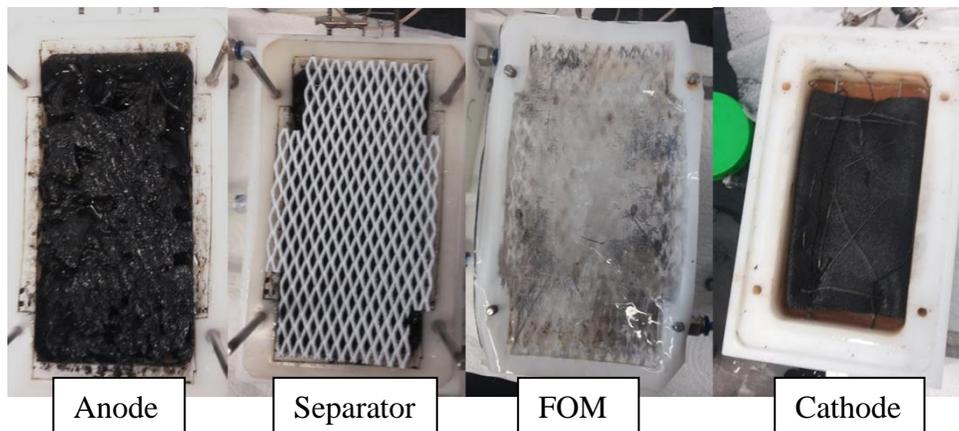
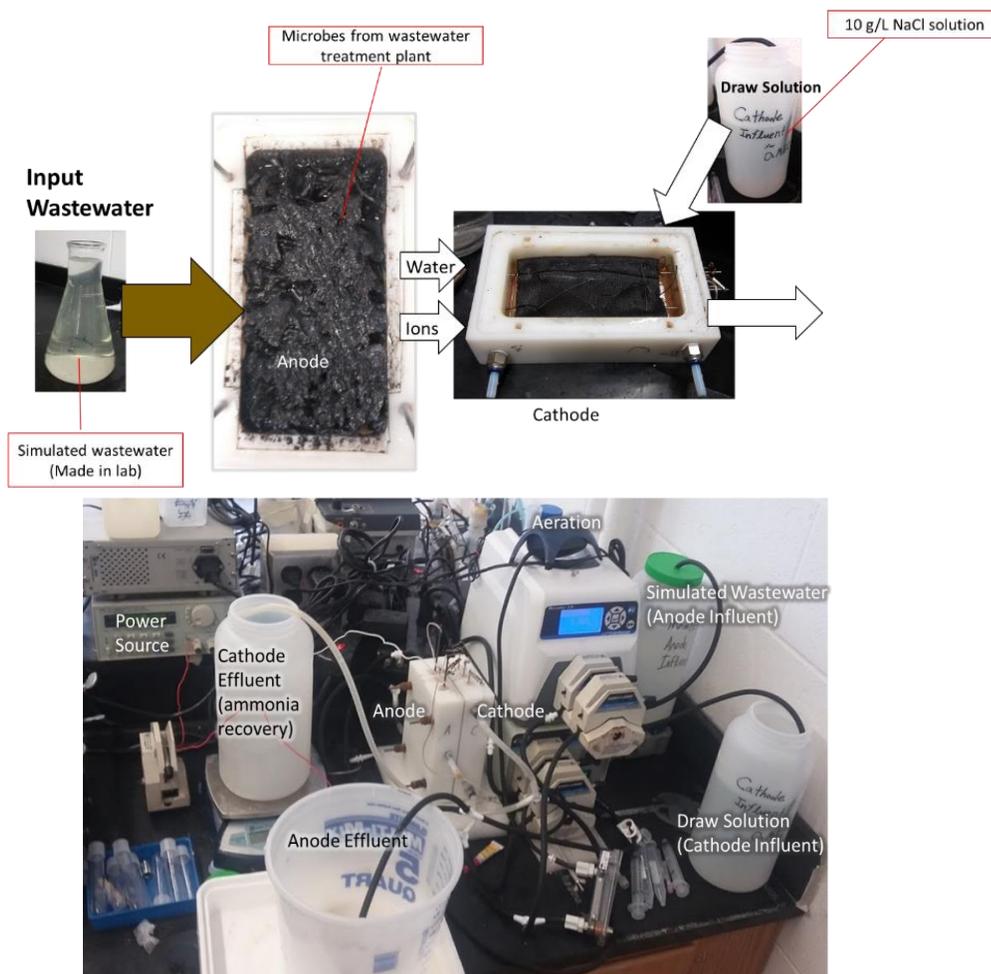


Figure 5. This diagram shows each layer of the fuel cell when it is taken apart. Starting from the left, the anode holds the microbial sludge, growing thickly on a carbon brush. The simple plastic

separator provides the FOM with a layer of protection from the microbes. The FOM shown here has a few days of biofouling darkening its surface. On the far right, the cathode contains activated carbon cloth, which acts as an electron acceptor.

The anode contains two carbon brushes (Gordon Brush Mfg. Co. Inc., Commerce, CA, USA) which provide a high surface area for microbes to grow. Wastewater flows into the anode. A porous plastic separator between the anode and the forward osmosis membrane (FOM) protects the membrane from the microbes in the anode, which could foul the membrane and decrease its effectiveness. The forward osmosis membrane (FOM) is placed with its active layer facing the cathode. The cathode contains a carbon cloth coated in platinum (0.3 mg Pt cm<sup>-2</sup>). Draw solution is added at the cathode.

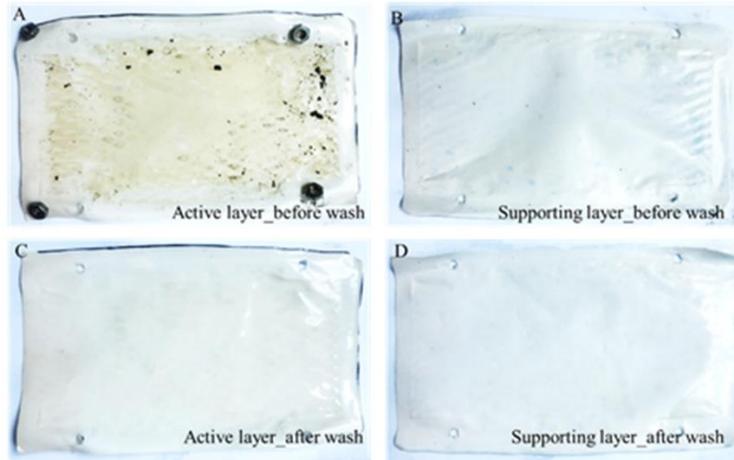


**Figure 6. (Above) Simple flow chart of my osmotic microbial electrolysis cell and its parts. (Below) The entire set up of the OsMEC, with parts labeled.**

Our simulated wastewater (also referred to as anode influent or anolyte) was made of 1.5 g/L sodium acetate, .2 g/L sodium carbonate, and 3 g/L ammonium chloride. Our draw solution (also referred to as cathode influent or catholyte) was a 10 g/L sodium chloride. Both anode and cathode were set up in continuous mode, meaning anolyte and catholyte continuously flowed in and effluent continuously flowed out of each compartment. We would add around 2 L of new catholyte and anolyte every 24 hours or as needed.

An aeration pump was connected to the cathode. Aerating the cathode provides oxygen to the cathode. Oxygen acts as an electron acceptor, which encourages high current generation. The first three experiments were completed with aeration at the cathode, and the last two without.

### 2.2.2 Obstacles to efficiency



**Figure 7. FOM with biofouling and after cleaning.**

Biofouling is when microbes grow on and in the forward osmosis membrane. We took steps to mitigate biofouling by cleaning the forward osmosis membrane every few days. One of the drawbacks of frequent FOM cleansing

Reverse salt flux occurs when the sodium chloride (NaCl) in the cathode flows “the wrong way”—into the anode. Reverse salt flux decreases the difference in conductivity between the anode and the cathode, thereby reducing the effectiveness of the forward osmosis membrane. Reverse salt flux was minimized by maintaining high current, and mixing the contents of the cathode and anode thoroughly—with a mixer in the anode influent container and recirculation tubes for both the anode and cathode. A high current prevents reverse salt flux by keeping negatively charged electrons headed to the cathode, thereby encouraging positively cations, like sodium, to remain in the cathode. Mixing prevents salt from building near the membrane by simply keeping it distributed throughout the fluid.

### 2.2 Data collection

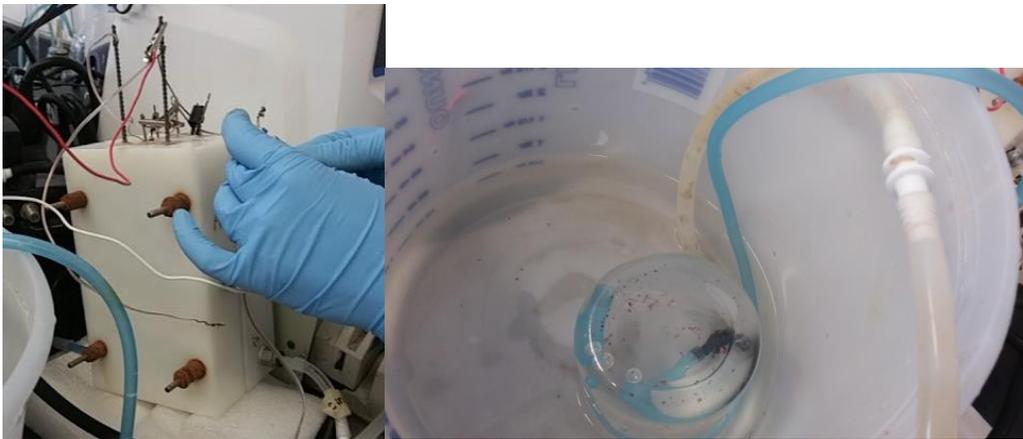
#### 2.2.1 Ammonia Concentration



**Figure 8. (Left) HACH Test'n'Tube vials to find the concentration of ammonia in the cathode effluent, anode effluent, and the simulated wastewater. (Right) Fully reacted test vials. The darker green that the vial is, the higher ammonia concentration. Accordingly, the simulated wastewater vial is darkest and the water vial is the lightest.**

We recorded ammonia concentration from the simulated wastewater, the anode effluent, and the cathode effluent. Ammonia found in the cathode effluent is successfully recovered ammonia. The samples reacted in the vials shown in Figure 8, taking on a shade of green related to their ammonia concentrations, they then were placed in a DR/890 colorimeter (HACH Co., Ltd., USA) which determined their ammonia concentrations based on their color.

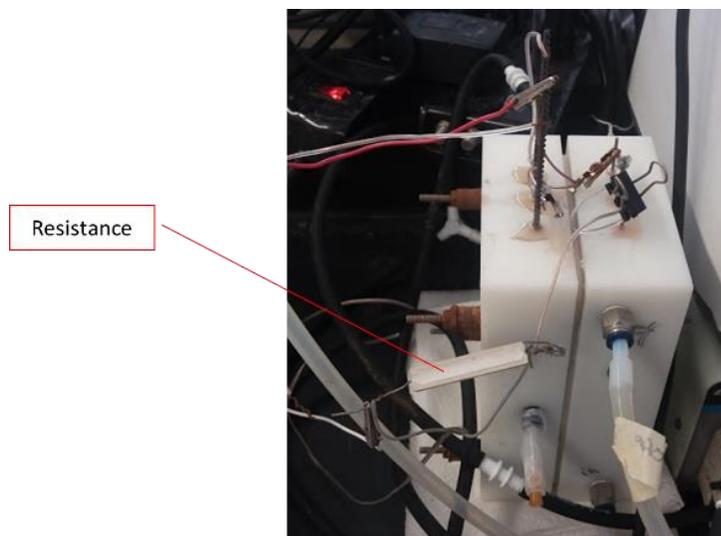
### *2.2.2 Hydrogen generation*



**Figure 9. We adjusted the OsMEC to collect hydrogen. (Left) The beaker where hydrogen collected.**

During our last two tests, the reactor (cathode and anode compartments together) was tilted up to make sure the hydrogen would bubble out of the cathode. (Right) The hydrogen was collected simply by placing an overturned beaker in the cathode effluent container. The gas that collected in the beaker could be suctioned out with a syringe and measured for hydrogen content.

### *2.2.3 Current generation and water flux*



**Figure 10.** Above you can see the wires connecting the cathode and anode. A resistor is connected between anode and cathode. To manipulate the current in the cell, we simply change the resistance. The resistor in the picture above is the 1 ohm.

The reactor was wired to a multimeter that recorded the voltage between the anode and cathode every 2 minutes (2700, Keithley Instruments Inc., Cleveland, OH, USA). We calculated the current generation using Ohm's law:

$$\text{Current} = \text{Voltage} / \text{Resistance}$$

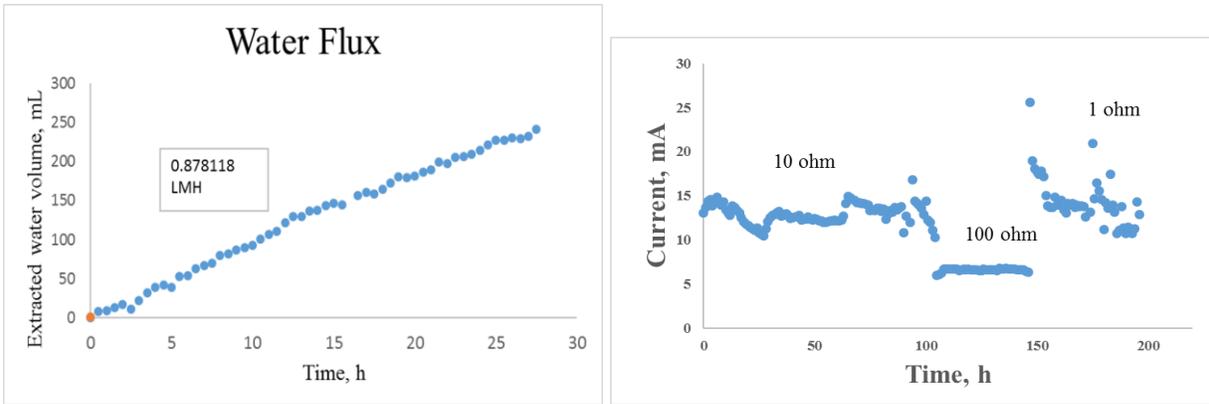
Water flux is the rate at which water was transferred from the anode to the cathode chamber. The water flux was calculated based on the mass of the cathode effluent, which was collected every minute on a scale (Scort Pro, Ohous, Columbia, MD, USA), and the flow rate at which the influent was pumped into the anode.

#### 2.2.4 Conductivity and pH

The conductivity and pH of anode effluent and cathode effluent were collected twice every 24 hours that each experiment ran, with a conductivity meter (Mettler-Toledo, Columbus, OH, USA) and a pH meter (Oakton Instruments, Vernon Hills, IL, USA) respectively. Conductivity reflects the concentration of ions in a substance. The difference in conductivity between anode and cathode affects the water flux. It is important to collect pH because if the conditions in the anode are too acidic, the microbes could die off.

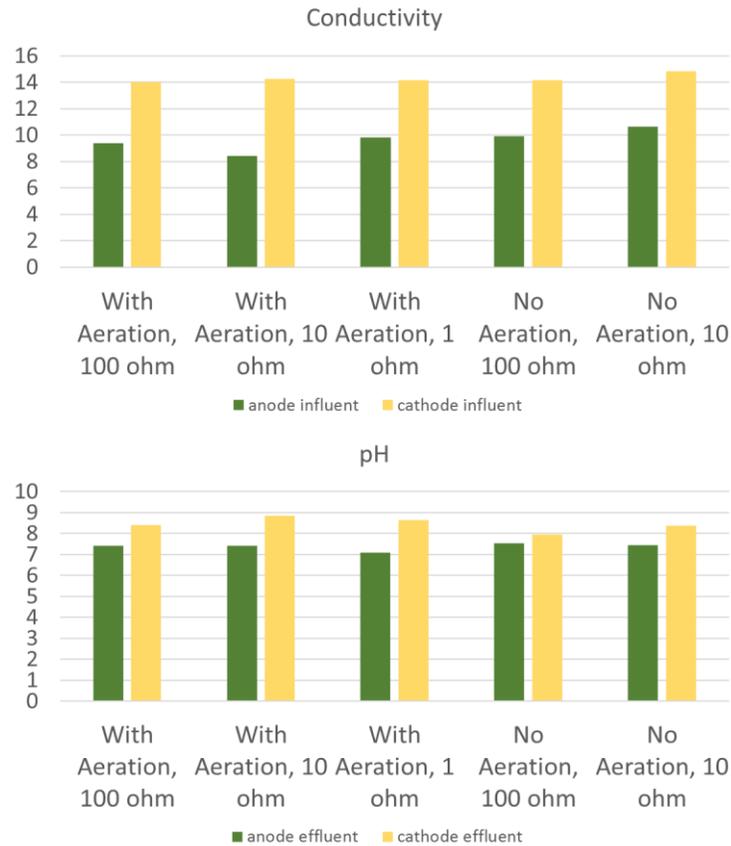
### 3. Results and Discussion

Five experiments were run. The first three were run with aeration, the last two without aeration. We tested with aeration with a 1 ohm, 10 ohm, and 100 ohm resistor. We tested no aeration with only 10 ohm and 100 ohm due to time restrictions.



**Figure 11. Water flux (left) and current generation (right).**

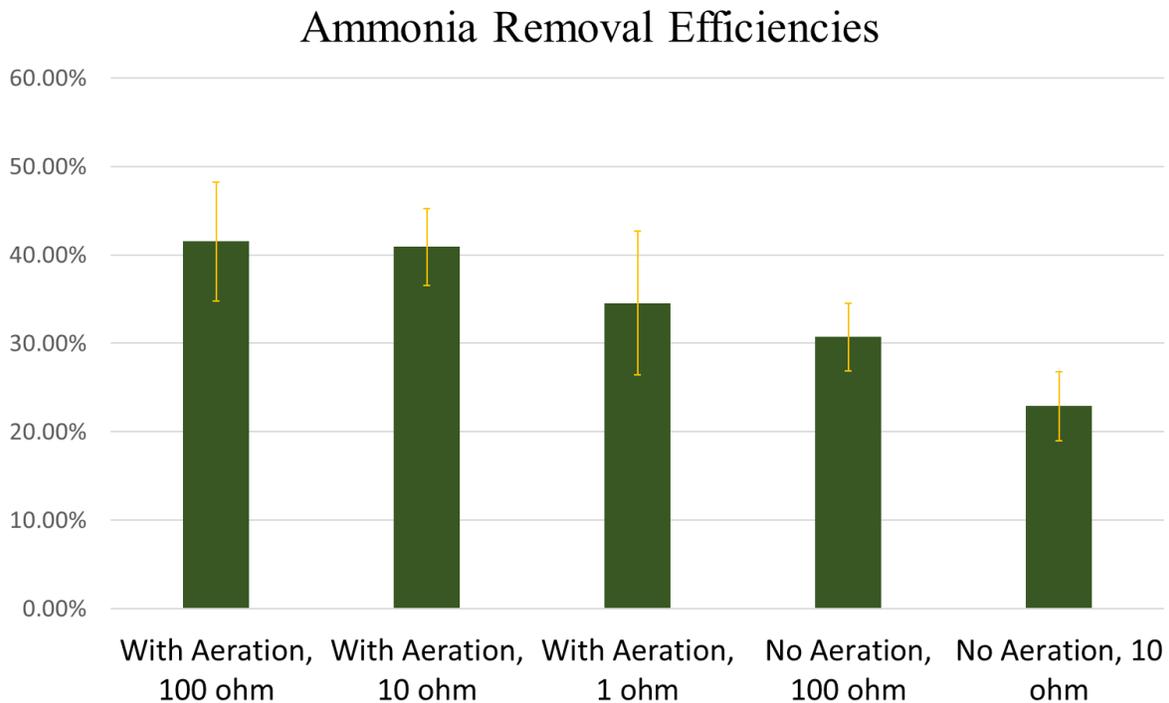
Figure 11 shows that the water flux in these experiments was quite low, at only around .878 LMH. This is probably due to our low concentration draw solution (10 mg/L). A higher concentration draw solution, say 35 mg/L, would encourage a higher difference in conductivity between the anode and cathode, thereby more effectively drawing water from anode to cathode. Below is a graph comparing the conductivity of the fluid in the anode (green) with the conductivity of the fluid in the cathode (yellow). It can be seen that the cathode is always higher in conductivity than the anode, which is caused by the salty cathode influent (draw solution). Without this difference, there would be little water flux between anode and cathode.



**Figure 12. (Above) Conductivity. (Below) pH.**

The graph of current shows how higher resistance results in lower current generation and vis versa. You can see from the 1 ohm section that the reactor was not very stable during the 1 ohm test, which could have affected the accuracy of our results.

The ammonia removal efficiency is the percentage of ammonia that was moved from the simulated wastewater (anode influent) to the cathode effluent. The ammonia removal efficiencies are shown in Figure 13. We were able to get ammonia removal efficiency as high as  $41.54 \pm 6.73\%$ . However, the results show higher ammonia removal for lower current, which was not an expected result. The reactor achieved higher ammonia removal with aeration at the cathode.



**Figure 13. This bar graph shows the percentage of ammonia removed from the simulated wastewater to the cathode.**

#### 4. Conclusion

These experiments need to be repeated to see if they are replicable. If they are correct than we would need to find out why lower current would result in higher ammonia removal for OsMECs.

We plan to test the OsMEC with more concentrated draw solution in order to increase flow rate to see how a higher water flux would affect ammonia removal. Then we will test different hydraulic retention times, because longer times will likely lead to more ammonia recovered. We also plan to use mathematical models to predict the ammonia recovery performance of a normal MEC so we can compare it to the OsMEC.

## **5. Acknowledgements**

I would like to acknowledge Mohan Qin who served as my graduate student mentor during my time at Virginia Tech, Dr. Richard He was my faculty mentor, and Dr. Vinod K. Lohani who was the director of the REU program. I would also like to thank Virginia Tech for hosting the program.

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*Energy Environ. Sci.*, 2015, 8, 513-519. doi:10.1039/c4ee03359k

## Iron and manganese oxidation cycles in an oxygenated reservoir

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### Abstract

Iron (Fe) and manganese (Mn) cause water quality issues in drinking water reservoirs, especially during summer months when thermal stratification can lead to low dissolved oxygen (DO) conditions. Under these conditions, Fe and Mn can be released from the sediment into the water column. These metals are costly to treat; thus, efforts have been made to address the issue *in situ* before the water reaches the treatment plant.

One *in situ* method, installed at a local drinking water reservoir in 2013, includes use of a side stream supersaturation (SSS) system to oxygenate the reservoir to prevent low DO. In past years (2013-2015), the SSS has been activated for different periods of time during the summer to evaluate the impact of oxygenation on metals and other water quality parameters. Results have shown that metals are released from sediment, regardless of the extent of oxygenation. However, increased oxygenation promoted Fe oxidation, with lesser impact on Mn oxidation.

In 2016, the SSS has been activated continuously since the start of thermal stratification. For this study, we are evaluating the impact of continuous oxygenation on metal release and cycling within the reservoir. Water samples were collected with depth over a ten week period from the oxygenated reservoir and also from a nearby reference reservoir that is not oxygenated. Results to date show that the SSS significantly lowers the concentrations of soluble Mn and Fe in the hypolimnion of the oxygenated reservoir (maximum soluble Fe = 0.1 mg/L, maximum soluble Mn = 0.9 mg/L) in comparison to the reference reservoir, which has elevated concentrations of Mn and Fe in the hypolimnion (maximum soluble Fe=12.7 mg/L, maximum soluble Mn = 2.4 mg/L). Comparison of these data with previous years shows that continuous oxygenation results in lower soluble metal concentrations in the hypolimnion in the oxygenated reservoir. However, even during continuous oxygenation, concentrations of soluble Mn in the lowest depths of the hypolimnion still exceed the secondary drinking water standard of 50 ppb.

**Keywords:** iron, manganese, water quality, oxygenation

### 1. Introduction

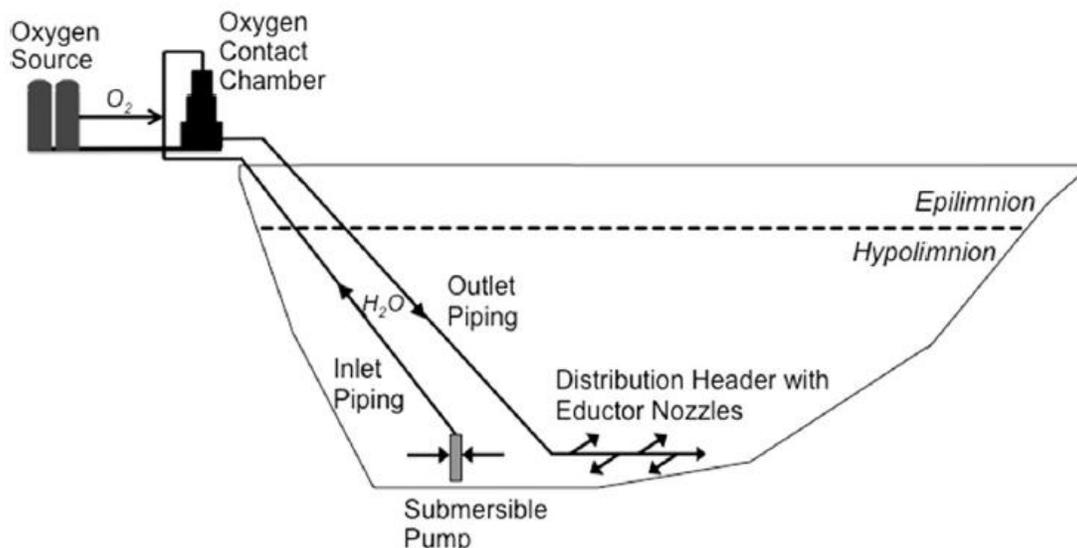
Iron (Fe) and manganese (Mn) are among the most abundant metals on Earth's surface (Hem, 1972). Both are commonly found in surface waters and are nuisance contaminants for water treatment industries (Stauffer, 1986). In their oxidized state, Fe and Mn have low aqueous solubility, which occurs in surface water conditions with dissolved oxygen (DO) conditions that are in equilibrium with the atmosphere (Hem, 1972). However, in hypoxic conditions (<2mg/L) (Dauer, Rodi, & Ranasinghe, 1992), Fe and Mn can be microbially reduced. In their reduced

form, Fe and Mn are soluble and can be released from the sediments into the water column (Davison, 1993).

The presence of Fe and Mn, cause issues in water, including taste, odor, and color (Kohl & Medlar, 2006). It has also been identified that high levels Mn can cause neurological issues in children (Maryse et al., 2007). In response to the water quality effects of Fe and Mn, the United States Environmental Protection Agency (EPA) has established secondary water quality limits for Fe and Mn concentrations in drinking water at 0.3 and 0.05 mg/L respectively (*Secondary Drinking Water Standards: Guidance for Nuisance Chemicals*, 2016).

Once in the water, Fe and Mn can be removed, mainly through oxidation and filtration techniques; however this process can be costly (Kohl & Medlar, 2006). Recent efforts have been made to treat the problem at the water's source using hypolimnetic oxygenation systems (Bryant, Hsu-Kim, Gantzer, & Little, 2011; Gantzer, Bryant, & Little, 2009; Gerling et al., 2014). The goals of hypolimnetic oxygenation systems are to add dissolved oxygen or other soluble gases to the hypolimnion (bottom waters) while preserving the stratification of the lake (Singleton & Little, 2006).

One method of hypolimnetic oxygenation uses a side stream supersaturation (SSS) system (Figure 1) to take water from the hypolimnion, inject it with oxygen and return the oxygenated water back into the hypolimnion. This method is effective for shallow reservoirs because the water is returned at a low pressure, which helps preserve lake stratification (Gerling et al., 2014). This type of system was installed at Falling Creek Reservoir in 2013, a shallow eutrophic reservoir in southwest Virginia.



**Figure 1. Schematic of the SSS system installed in Falling Creek Reservoir (Gerling et al., 2014)**

In past years (2013-2015), the SSS has been activated and de-activated for different periods of time during the summer to evaluate the impact of oxygenation on metals and other water quality parameters. Results have shown that metals are released from sediment, regardless of the extent of oxygenation. However, increased oxygenation promoted Fe oxidation (and subsequent settling within the water column), with lesser impact on Mn oxidation (Gerling et al., 2014; Munger et al., 2016 in review).

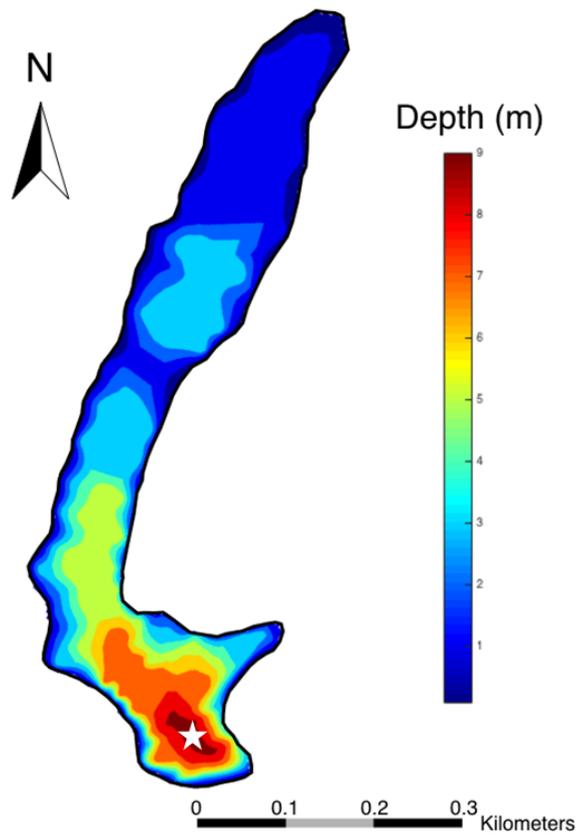
In addition to the SSS, a bubble plume vigorous epilimnetic mixing (VEM) system was installed at the thermocline in order to artificially mix the reservoir. The VEM simulates a storm (Dauer et al., 1992) in which the hypolimnion and epilimnion mix while preserving the stratification of the reservoir. One effect of this procedure is that oxygen levels increase at the thermocline where dissolved oxygen can become depleted.

This summer, the SSS has been used since the start of thermal stratification in early May. In addition the VEM was activated to artificially mix the lake twice during the study period. For this study we are evaluating the impact of continuous oxygenation on metal release and cycling within the reservoir in comparison to a reference reservoir that is not oxygenated.

## 2. Methods

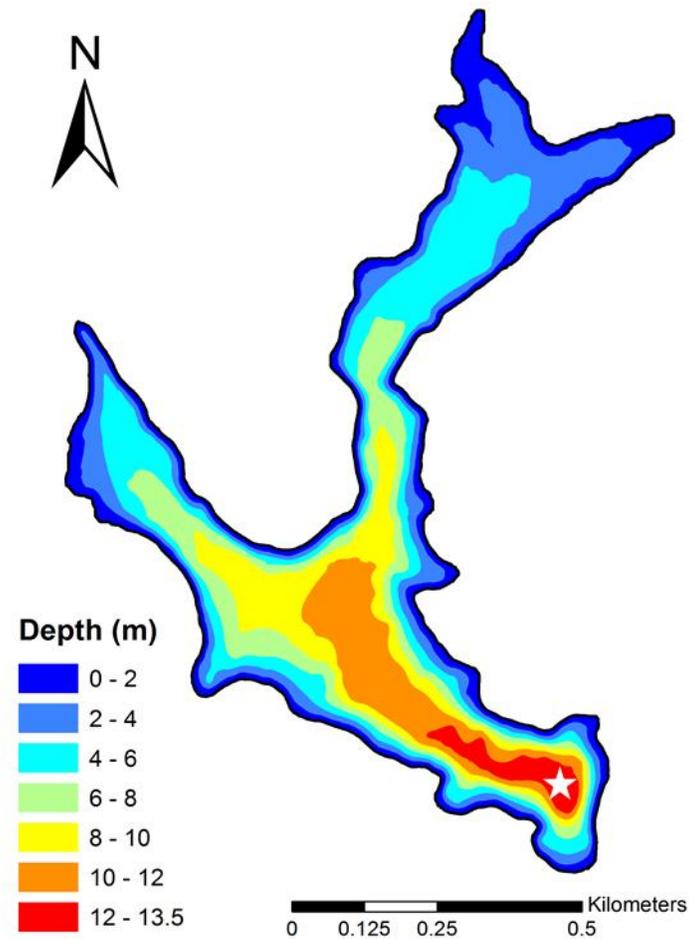
### 2.1 Site descriptions

Figure 2 shows Falling Creek Reservoir (FCR), a small eutrophic reservoir near Vinton, Virginia, USA (37° 18' 12"N, 79° 50' 14" W). The reservoir provides drinking water for residents of Roanoke, VA and is owned and operated by the Western Virginia Water Authority. FCR has a surface area of  $1.19 \times 10^{-1} \text{ km}^2$ , maximum depth of 9.3 m, mean depth of 4 m, and volume  $2.1 \times 10^5 \text{ m}^3$  (Gerling et al., 2014). The sampling site is located at the maximum depth of FCR, which is marked by a white star in Figure 2. FCR has historically exhibited thermal stratification from May to early October. To deal with issues of hypolimnetic hypoxia, an SSS was installed in 2013 (Gerling et al., 2014).

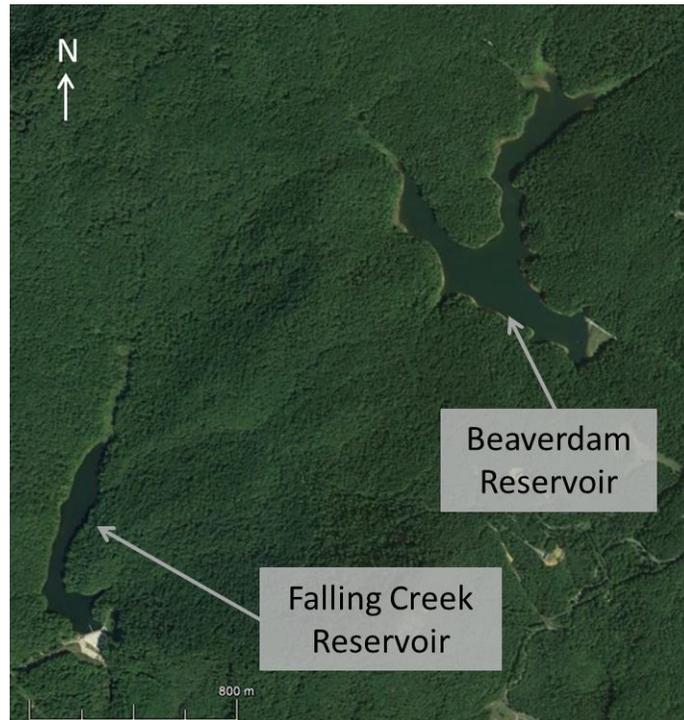


**Figure 2. Falling Creek Reservoir Bathymetry Map**

Beaverdam Reservoir (BVR) is another small eutrophic reservoir near Vinton, Virginia, USA (Figure 3). The reservoir acts as the primary inflow for FCR (Figure 4) and is owned and operated by the Western Virginia Water Authority. BVR has a surface area of  $3.9 \times 10^{-1} \text{ km}^2$ , volume of  $1.4 \times 10^6 \text{ m}^3$ , maximum depth of 13.5 m, and mean depth of 3 m. The depth at the sampling site is 12 m at full pond. The sampling site is marked by a white star in Figure 3. BVR has no oxygenation system and is used as a reference reservoir in this study. BVR continues to have seasonal hypoxia in the hypolimnion from May to early October.



**Figure 3. Beaverdam Reservoir Bathymetry Map**



**Figure 4. Google Earth image showing Falling Creek Reservoir and Beaverdam Reservoir**

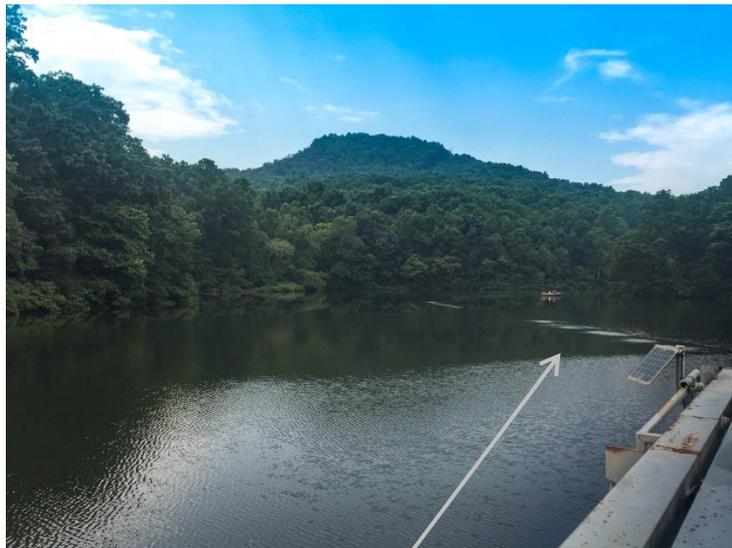
### *2.2 Metal sample collection and analysis*

We collected water samples for metals analysis weekly from each reservoir from late May – late July 2016. Water samples were collected along a depth profile (FCR: 0.1, 1.6, 3.8, 5.0, 8.0, 9.0 m and at BVR: 0.1, 3.0, 6.0, 9.0, 11.0 m). Samples were poured directly from a 4 L Van Dorn sampler (Wildlife Supply Company, Yulee, FL) into 15 mL centrifuge tubes. Soluble samples were filtered using .45 $\mu$ m nylon filters. Both total and soluble samples were preserved using trace metal grade nitric acid. Samples were analyzed for Fe and Mn concentrations using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Thermo Electron X-Series) following APHA Standard Method 3125-B (*Standard methods for the examination of water and wastewater, 20th ed.*, 1998). The results were processed using MATLAB to create color contour maps (“heat” maps) of the metal concentrations in the reservoir over time.

### *2.3 Vigorous Epilimnetic Mixing (VEM)*

Twice during the study period, FCR was artificially mixed using a bubble plume vigorous epilimnetic mixing (VEM) system installed at the thermocline (Figure 5). The VEM simulates a storm by releasing air bubbles from a diffuser using 95% pure oxygen scrubbed from the atmosphere. Like a storm, the VEM causes waters from the epilimnion and hypolimnion to mix while keeping the reservoir stratified. Near the beginning of the sampling period (May 30), the researchers ran the VEM for 5 hours (9:56 to 15:56) at 15 standard cubic feet per minute (SCFM). The VEM was run a second time (June 27 and 28) for a total of 24 hours (from 10:00 to 10:00).

The first six hours, the VEM ran at 15 SCFM and then for the remaining eighteen hours, the VEM was turned down to 10 SCFM. In contrast, BVR was not mixed.



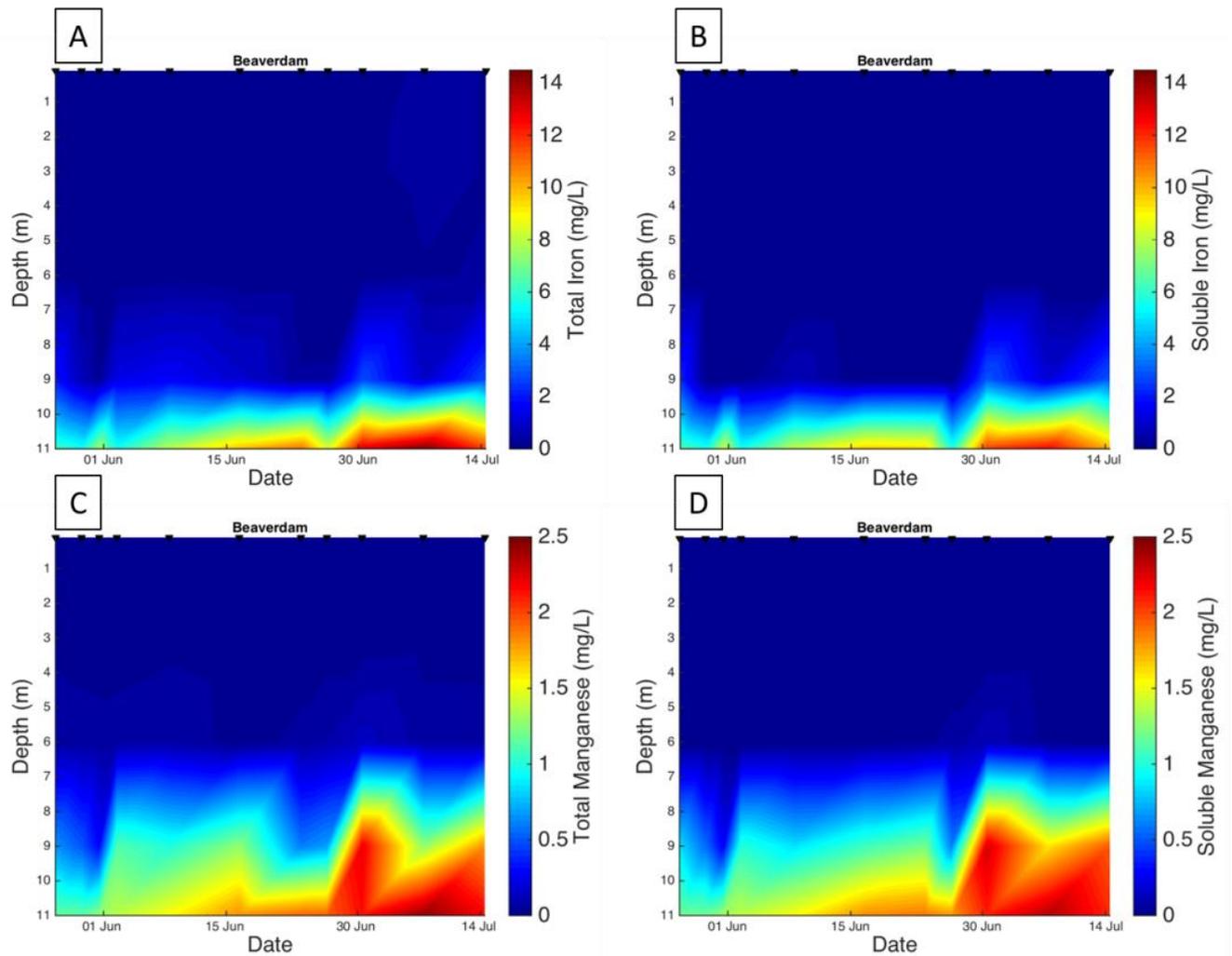
**Figure 5. Bubbles from VEM seen in FCR**

### **3. Results and Discussion**

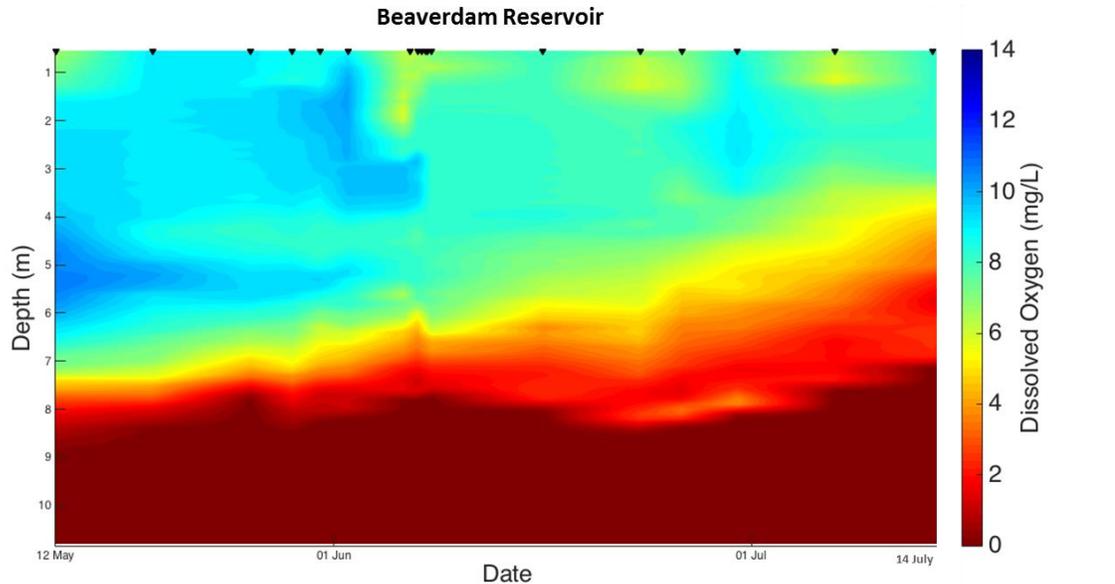
#### *3.1 Beaverdam Reservoir*

Figure 6 shows the change in Fe and Mn concentrations throughout the study period at Beaverdam Reservoir (BVR). The figure represents one sampling site at the deepest part of the reservoir. Through the sampling period, Fe and Mn concentrations were highest near the sediments, where oxygen is the lowest (Figure 7), and decreased near the water surface. Both Fe and Mn concentrations increased throughout the summer, with total Fe reaching levels as high as 14.5 mg/L in the hypolimnion in early July. Soluble Fe was also found in high concentrations near the sediments with concentrations reaching 12.7 mg/L in early July. The soluble Fe concentrations follow a similar pattern to the total Fe concentrations, indicating that the majority of Fe in the water is in its soluble, or reduced, form.

Mn concentrations were also highest near the sediments, with very little, if any, Mn found at the water surface. Like Fe, Mn concentrations increased beginning in late June. Total Mn concentrations were measured as high as 2.5 mg/L while soluble Mn concentrations were seen as high as 2.4 mg/L during the testing period. The similar patterns in total and soluble Mn concentrations indicate that the majority of Mn in the reservoir is present in its soluble form.



**Figure 6. The total and soluble concentrations of iron (A and B) and manganese (C and D) for Beaverdam Reservoir are shown above. Color scale on right shows concentrations in mg/L. Dates of sampling are noted as black triangles on top of figure. Between sampling dates, concentrations were interpolated using contouring methods in MATLAB.**

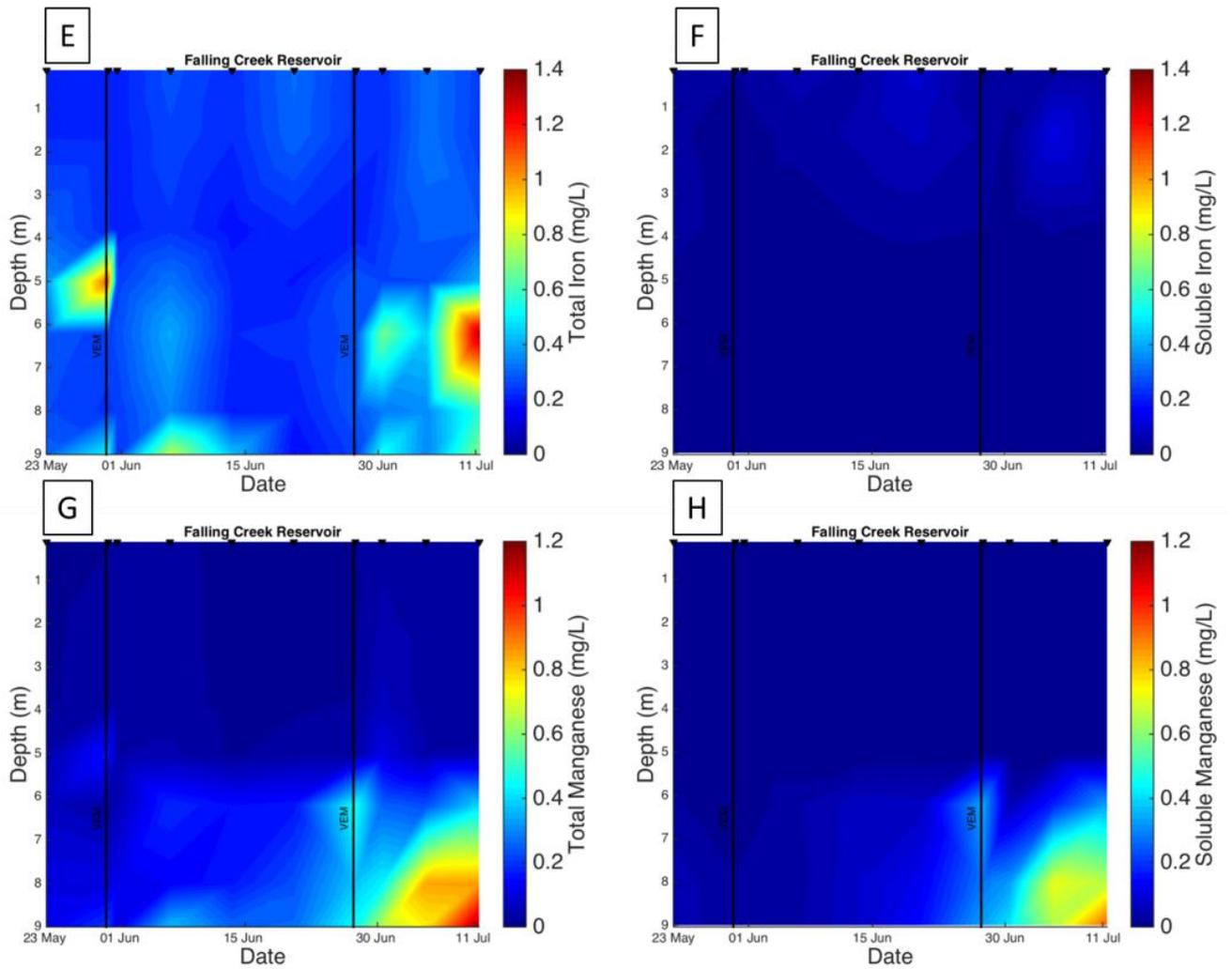


**Figure 7. Dissolved Oxygen (DO) concentrations in Beaverdam Reservoir from R.P McClure**

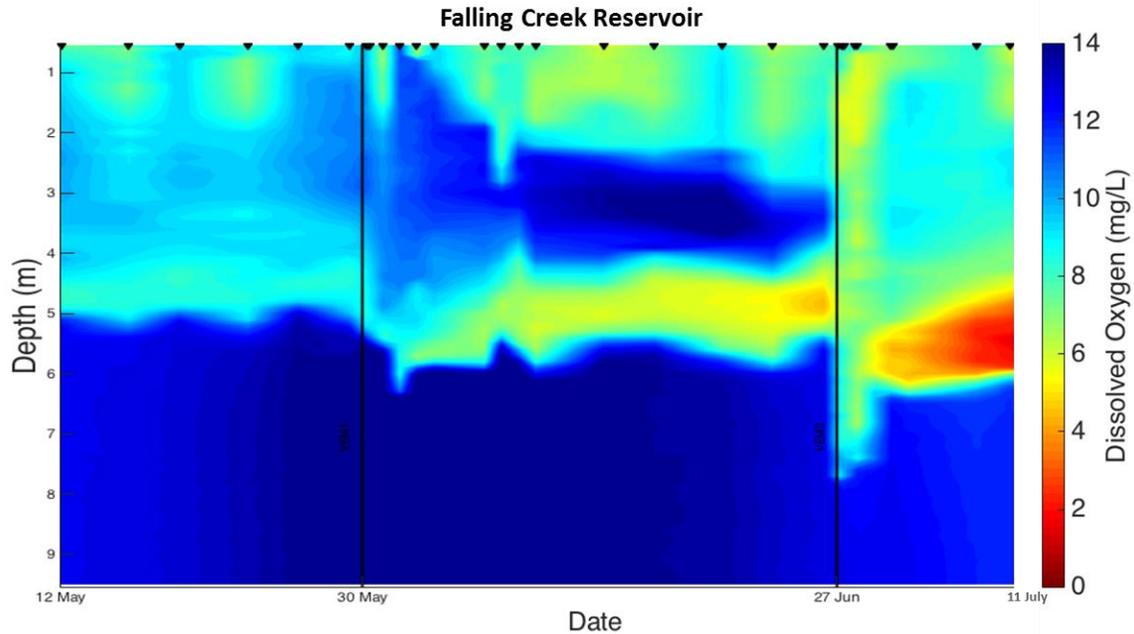
### *3.2 Falling Creek Reservoir*

Figure 8 shows the concentrations of Fe and Mn in FCR during the study period at Falling Creek Reservoir (FCR). Throughout the study, total Fe ranges from 0.17 mg/L to 1.32 mg/L, with an increase in the concentration in late June near the sediments. Soluble Fe was found in low concentrations for the duration of the study period (max = 0.12 mg/L) even when there was an increase in total Fe concentration. This indicates that the oxygenation system, which successfully oxygenated the hypolimnion (Figure 9), oxidized the Fe quickly and it settled out of the water column back into the sediments.

Concentrations of total Mn begin to increase in late June near the sediment, reaching a maximum of 0.93 mg/L by mid-July. Mn are highest in the deepest section of the hypolimnion, near the sediments. Soluble Mn also increased starting in late June. The pattern for soluble Mn was similar to total Mn, indicating that a significant amount of the Mn in the water is in reduced form. Soluble Mn concentrations in the deep hypolimnion exceed the EPA limit of 0.05 mg/L starting in June.



**Figure 8. The total and soluble concentrations of iron (E and F) and manganese (G and H) for Falling Creek Reservoir are shown above. Color scale on right shows concentrations in mg/L. Dates of sampling are noted as black triangles on top of figure. Between sampling dates, concentrations were interpolated using MATLAB. Vertical lines show dates of mixing (VEM) events.**

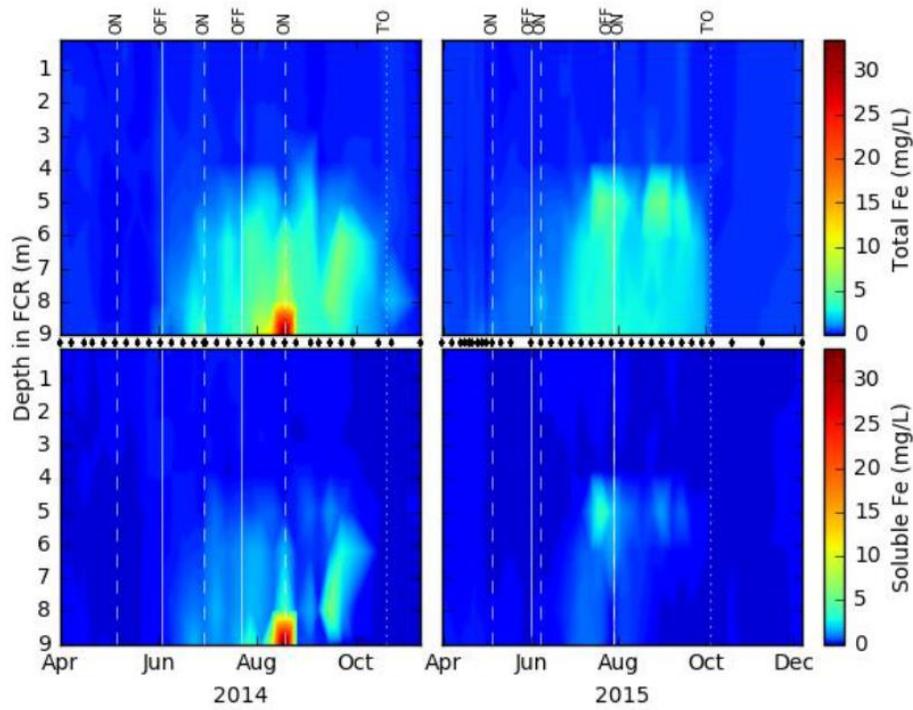


**Figure 9. Dissolved Oxygen (DO) concentrations in Falling Creek Reservoir from R.P McClure**

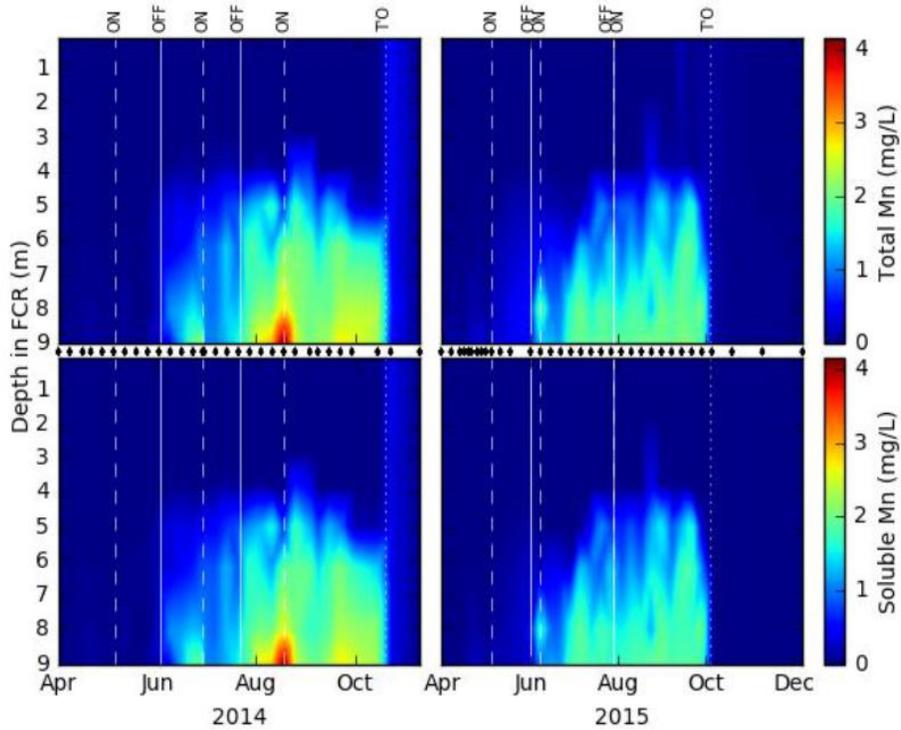
Both FCR and BVR show increases in metal concentrations beginning in late June. In FCR, the soluble Fe decreases greatly compared to BVR. Mn has slightly lower concentrations in FCR compared to BVR, however the concentrations of soluble Mn are significantly higher than the EPA limit of 0.05 mg/L.

Figure 10 and 11 show Fe and Mn concentration in FCR in both 2014 and 2015 (Munger et al. 2016 in review). These figures demonstrate that both total and soluble metal concentrations rapidly increase when the SSS is turned off. However, soluble Fe was generally lower while the SSS is activated and significantly increased when the SSS was turned off in both 2014 and 2015 (Munger et al, 2016 in review). These previous results are similar to the data collected in 2016 where soluble concentrations remain low throughout the study period due to the oxygenation of the hypolimnion.

In both 2014 and 2015 Mn accumulated in the hypolimnion during thermal stratification. The soluble Mn concentrations decreased when the SSS was turned on. However, similar to 2016, soluble Mn concentrations continued to be above the EPA limit of 0.05 ppb (Munger et al, 2016 in review).



**Figure 10. Total and soluble Fe concentrations in Falling Creek Reservoir (Munger et al, 2016 in review)**



**Figure 10. Total and soluble Mn concentrations in Falling Creek Reservoir (Munger et al, 2016 in review)**

During the study period, the VEM was only run twice in FCR. Given the limited samples that were collected during the two mixing periods, there is not enough data to accurately evaluate the impact of VEM events on metals.

Metal analysis should be continued this year at FCR until thermal stratification ends in October. This will allow the researchers to compare this year when the SSS has run continuously to years past where it has only been run periodically. Research also needs to be continued with VEM events to evaluate its effects on metal concentrations.

#### **4. Conclusion**

Comparison of the Fe and Mn concentrations in the hypolimnion of an oxygenated reservoir (FCR) and a non-oxygenated reference (BVR) demonstrates that oxygenation results in lower metals concentrations in the water column, thus improving water quality. Beaverdam Reservoir shows conditions common in seasonally stratified lakes. In comparison, continuous oxygenation of Falling Creek Reservoir during periods of stratification significantly lowers both Fe and Mn concentrations. However, there are still significantly high levels of soluble Mn (greater than the EPA limit) in the water column, especially near the sediment. This is likely due to the slower oxidation kinetics of Mn (Munger et al., in review). The effects of epilimnetic mixing on metal concentrations are not understood at this time; more research is needed to evaluate this process.

#### **Acknowledgements**

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# Nutrient Recovery of Nitrogen and Phosphorous as Struvite Using Microbial Desalination Cells

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## Abstract

Nutrient pollution is a widespread environmental problem caused by the discharge of excess nutrients, i.e., nitrogen and phosphorus. In this study, we use bioelectrochemical systems to remove and recover nutrients from wastewater in the form of struvite and achieve multiple benefits. First, the recovery of nutrients will limit the adverse effects of eutrophication while producing a beneficial fertilizer product for the agricultural industry. Second, this approach enables the integration of bioelectrochemical technologies like microbial desalination cells (MDCs) with conventional wastewater treatment processes to improve its sustainability. A four-chamber MDC was constructed to test the feasibility of struvite precipitation from synthetic wastewater. Ammonium and phosphate ions would in theory migrate across the ion exchange membranes and be concentrated in the second chamber. After one month of start-up, the MDC produced a stable current of 14.0 mA for a hydraulic retention time (HRT) of 16 hours and a stable average current of 14.6 mA for a 24-hour HRT. Influent and effluent carbon oxygen demand (COD), pH, ammonium and phosphate concentrations were monitored for both 16-hour and 24-hour hydraulic retention periods. Based on the successful concentration of ammonium and phosphate ions, the concentrated solution containing ammonium and phosphate could be transferred to an external bottle and precipitated by adjusting the pH and adding magnesium. The influent COD concentrations and the hydraulic retention times are expected to exert strong influence to MDC performance and would be examined in the following studies. Based on high electrical current generation and nutrient recovery concentrations, theoretically, it is expected to have struvite precipitation, which would encourage further development to improve the potential within the MDC and wastewater treatment nexus for larger scale applications.

**Keywords:** nutrient recovery, struvite, bioelectrochemical systems, microbial desalination cell, wastewater treatment

## 1. Introduction

Nutrient pollution is of growing concern in the United States, as it is one of the most challenging environmental issues faced across various sectors; it takes an extensive toll on the environment, health, and water quality. While nutrients, specifically, nitrogen and phosphorous are necessary for productive ecosystems, excess of nutrients from natural or anthropogenic sources can result in harmful algal blooms that may release toxins into aquatic environments (EPA, 2015). The subsequent decay of the plant matter can significantly decrease dissolved oxygen concentrations by the oxygen consumption of aerobic microorganisms in the breakdown of plant matter and produce aquatic dead zones (EPA, 2015). Among a multitude of additional sources, nutrient discharge to receiving waters can be attributed to wastewater treatment plant effluent, as these facilities may not remove nitrogen and phosphorus prior to discharge (EPA, 2016; Rao, 2013; USEPA, 2009). According to the Environmental Protection Agency, only 4% and 9.9% of wastewater treatment plants have nitrogen and phosphorus effluent limits, respectively (USEPA, 2009). Previous studies suggest that the widespread problems associated with nutrient pollution

are expected to be exacerbated due to warmer climates and continued population growth (Paerl and Huisman, 2009; O'Neil et al., 2012; Paerl and Paul, 2012; EPA, 2015). These challenges prompt the need for improved wastewater treatment that can efficiently remove and recover these nutrients.

The majority of current wastewater treatment technologies rely on the biological activated sludge process in which natural biological processes function to breakdown and remove organic matter and produce effluent that is safe for discharge (Rao, 2013). Constant aeration is necessary to sustain the microorganisms' metabolic reactions for oxidizing organic matter into carbon dioxide and water (Rao, 2013). Consequently, activated sludge treatment is significantly energy intensive, consuming an estimated amount of 0.349 kWh of electrical energy per cubic meter of wastewater which correlates to at least 21 billion kWh of energy per year (Goldstein & Smith, 2002). The aeration basins account for approximately half of the total energy for treatment, resulting in roughly 0.6 kWh/m<sup>3</sup> of wastewater treated (McCarty, Bae, & Kim, 2011). The substantial use of energy directly correlates to global environmental issues such as an increase in green house gas emissions (Emmerson et al., 1995). For a low capacity treatment plant of less than 1 MGD, the activated sludge process can contribute up to 1400 tons of carbon dioxide for operation with an additional 50 tons of carbon dioxide for maintenance during 15 years (Emmerson et al., 1995). These factors, along with the lack of recovery of valuable resources, further limits the treatment's sustainability (Li, Yu, & He, 2014). Additional studies analyzing the sustainability of wastewater treatment suggest that nutrient recovery and reuse for agricultural applications will be beneficial in limiting high concentration nutrient fluxes into receiving waters (Muga & Mihelcic, 2008).

The integration of bioelectrochemical systems and conventional wastewater treatment has the potential to result in a more sustainable approach to wastewater treatment due to the multitude of additional functions that these systems are capable of. Microbial fuel cells (MFCs) are bioelectrochemical systems that utilize microorganisms to oxidize organic matter and generate current (Logan et al., 2006). However, upscaling problems, low power density, and high cost are some of the current limitations of MFCs which can potentially be overcome with the development of integrated technologies that allow for resource recovery (Li et al., 2014). Microbial desalination cells (MDCs) are a variation of the traditional MFCs that are capable of simultaneous wastewater treatment, desalination, energy production and nutrient recovery (Li et al., 2014; Logan et al., 2006; Yuan & He, 2015). The multi-functionality of this system allows for the process to be more sustainable by increasing the resultant benefits; studies have found that in comparison to the activated sludge process, bioelectrochemical systems have the capacity to become energy sufficient and produce less sludge waste with significant COD removal, generally over 70% (Kim & Logan, 2013; Yuan, Hou, Abu-Reesh, Chen, & He, 2016). This approach is able to harness some of the energy potential of wastewater, through the oxidation of organic matter by electrochemically active microorganisms (Kim & Logan, 2013; Li et al., 2014). This interaction releases electrons that flow from anode electrode to cathode electrode where oxygen is the electron acceptor, resulting in the generation of an electrical current (Kim & Logan, 2013; Li et al., 2014). The use of alternating cation exchange membranes (CEM) and anion exchange membranes (AEM) results in a concentrating chamber and desalination chamber between the anode and cathode chambers (Kim & Logan, 2013). The electric potential gradient drives ions across the ion exchange membranes accumulating them in the concentrating chamber; this allows for nutrient ions, i.e., ammonium and phosphate to be separated from the influent synthetic wastewater for nutrient recovery. Nutrient recovery serves as a more sustainable approach than removal due to the depletion of natural nitrogen and phosphorus sources. The recovered product can be used as agricultural fertilizer.

The accumulation of ammonium and phosphate ions, with the addition of magnesium ions, provide suitable conditions for the precipitation of struvite ( $\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$ ), a phosphate mineral. Previously, struvite was a naturally precipitating nuisance in wastewater treatment plants that resulted in scaling problems and decreasing treatment efficiency (Kelly & He, 2014; Marti, Bouzas, Seco, & Ferrer, 2008). However, various studies have shown success in nutrient recovery using struvite due to its

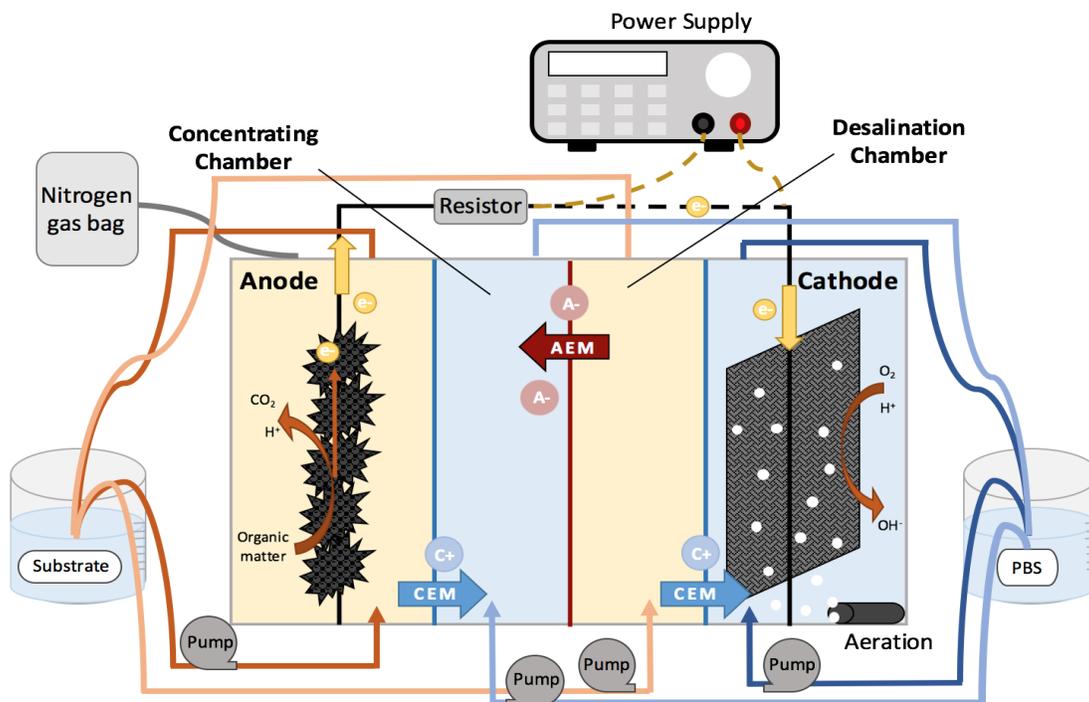
formation with naturally occurring ions in wastewater (Acelas, Flórez, & López, 2014; Booker, Priestley, & Fraser, 1999; Doyle & Parsons, 2002; Fattah, 2012; Ichihashi & Hirooka, 2012). Struvite precipitation occurs when its component ions, magnesium, ammonium, and phosphate are present at concentrations that exceed the struvite solubility limit (Ichihashi & Hirooka, 2012). Its precipitation is mainly affected by the concentration of magnesium, ammonium, and phosphate and the solution pH (Doyle & Parsons, 2002). Struvite is a slow-release fertilizer, its properties allow for nutrients to be released at a controlled rate over a longer time frame than conventional fertilizers (Booker et al., 1999; Kumar & Pal, 2015). Struvite as a fertilizer product is of high demand due to its additional properties that provide more benefits when compared to conventional fertilizers such as its lack of leaching and low heavy metals content (Doyle & Parsons, 2002; Fattah, 2012). The precipitation of nutrient ions in the form of struvite allows for the removal of phosphate and ammonium from wastewater and the precipitate can be recovered for use in the agricultural industry as fertilizer making the process more economical (Acelas et al., 2014; Booker et al., 1999).

The objective of this study is to test the feasibility of struvite precipitation from synthetic wastewater using a four-chamber MDC system. The MDC will be used to concentrate ions, particularly, phosphate and ammonium. Separately, magnesium may be added to the highly concentrated ionic solution that can be collected from the MDC. The MDC and wastewater nexus can be further advanced by continuing improvement experiments to increase the amount of nutrients recovered and bridge the gap between removal and recovery. Future advancements in the integration of sustainable technologies can help overcome the exacerbating water, energy, and nutrient challenges faced globally.

## **2. Research Materials and Methods**

### *2.1 Constructing the MDC*

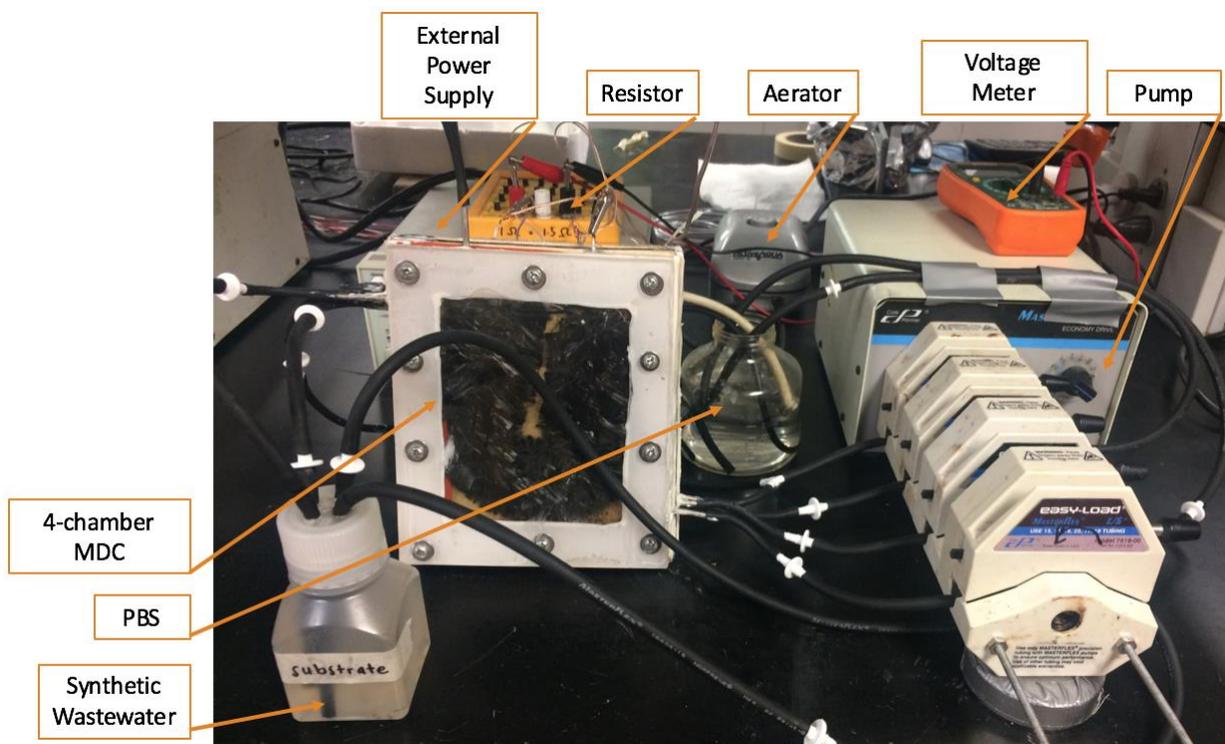
An MDC with four chambers consisting of an anode chamber, a concentrating chamber, a desalination chamber, and a cathode chamber was constructed according to the schematic shown in Figure 1. The MDC's external dimensions are of  $6.0 \times 1.6 \times 7.0$  in. The volumes of the anode, concentrating, desalination, and cathode chambers are 110 mL, 100 mL, 60 mL and 110 mL respectively. Volume measurements are approximate as membrane deformation can cause volume change. The external chambers were made of acrylic plastic plates and the internal chambers were made of rubber plates. Ion exchange membranes were placed between the chambers, with cation exchange membranes placed adjacent to the anode and cathode chambers and an anion exchange membrane placed between the concentrating chamber and desalination chamber. The anode electrode was composed of carbon brush and the cathode electrode was made of carbon cloth ( $500 \text{ cm}^2$ ) coated in activated carbon catalyst 5 mg/cm<sup>2</sup> (2.5 g).



**Figure 1. Diagram of the Functions within the Four-Chamber MDC Configuration.**

## 2.2 Experimental Setup

The system utilizes a plate-type four-chamber MDC for the oxidation of organic matter and concentration of nutrient ions for the production of struvite precipitate as seen in Photo 1. The cathode chamber was connected to an aerator to provide oxygen as the electron acceptor and the anode chamber was connected to a nitrogen gas bag to keep conditions anaerobic, suitable for microbial communities present in digested activated sludge. A pump with a pump head for each chamber was used for recirculation at a rate of  $40 \text{ mL min}^{-1}$ . External bottles were used for recirculation between pairs of chambers, the anode chamber with the desalination chamber and the cathode chamber with the concentrating chamber. The anode and cathode chambers were connected with electrical wire to allow for the flow of electrons. A  $1\text{-}\Omega$  resistor was connected to the anode and cathode and an external power supply of  $0.8 \text{ V}$  was connected to the system. The voltage generated by the system was recorded using an automatic digital multimeter (2700, Keithley Instruments Inc., Cleveland, OH, USA) every 5 minutes.



**Photo 1. Experimental Setup of Four-Chamber MDC (Elena Lopez, 6/29/16).**

### 2.3 Feasibility Study

The feasibility study observes the function of the bioelectrochemical system and its ability to recovery nutrients in the form of struvite. Theoretically, the MDC system should be capable of concentrating nitrogen and phosphorus in the form of ammonium and phosphate ions as the ions migrate across the ion exchange membranes into the concentrating chamber and cathode chamber. The concentrated fluid may be collected and in a separate container magnesium chloride can be added to precipitate struvite ( $\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$ ) from nutrients in the synthetic wastewater.

#### 2.3.1 Startup Stage

Synthetic wastewater (COD  $\sim 7.8$  g/L) was initially used as the anolyte solution for the MDC. The synthetic wastewater solution was composed of  $10 \text{ g L}^{-1}$  NaAc,  $10 \text{ mL L}^{-1}$  of a stock solution containing  $55 \text{ g L}^{-1}$   $\text{NH}_4\text{Cl}$ ,  $50 \text{ g L}^{-1}$  NaCl,  $1.5 \text{ g L}^{-1}$   $\text{MgSO}_4$ ,  $2 \text{ g L}^{-1}$   $\text{CaCl}_2$ , and  $10 \text{ g L}^{-1}$   $\text{NaHCO}_3$ ,  $1 \text{ mL L}^{-1}$  of a buffer solution containing  $107 \text{ g L}^{-1}$   $\text{K}_2\text{HPO}_4$  and  $53 \text{ g L}^{-1}$   $\text{KH}_2\text{PO}_4$ , and  $1 \text{ mL L}^{-1}$  of a trace element solution containing  $10000 \text{ g L}^{-1}$   $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ ,  $2000 \text{ g L}^{-1}$   $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $1000 \text{ g L}^{-1}$  EDTA,  $500 \text{ g L}^{-1}$   $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ,  $142 \text{ g L}^{-1}$   $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $123 \text{ g L}^{-1}$   $\text{Na}_2\text{SeO}_3$ ,  $50 \text{ g L}^{-1}$   $\text{ZnCl}_2$ ,  $50 \text{ g L}^{-1}$   $\text{H}_3\text{BO}_3$ ,  $38 \text{ g L}^{-1}$   $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $1 \text{ mL L}^{-1}$  HCl (37.7%),  $69 \text{ g L}^{-1}$   $\text{Na}_2\text{MoO}_4 \cdot 6\text{H}_2\text{O}$ , and  $90 \text{ g L}^{-1}$   $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ . The MDC was inoculated with  $10 \text{ mL}$  of digested activated sludge from a local domestic wastewater treatment plant. The anode chamber and desalination chamber were connected to an external  $100 \text{ mL}$  bottle and the cathode and concentrating chamber were connected to an external  $200 \text{ mL}$  bottle. The anolyte and catholyte were replaced approximately every 24 hours. All chambers were recirculated at a rate of  $40 \text{ mL min}^{-1}$ . By gradually decreasing the external resistance from  $10,000\text{-}\Omega$  to  $1\text{-}\Omega$  the electrical current generation was increased until a relatively stable system was obtained at  $1\text{-}\Omega$  resistance.

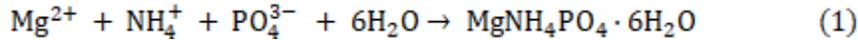
### 2.3.2 Operation Stage

After one month of start-up, the MDC produced a stable current of approximately 14 mA. A new stock solution (50 g L<sup>-1</sup> NaCl, 1.5 g L<sup>-1</sup> MgSO<sub>4</sub>, 2 g L<sup>-1</sup> CaCl<sub>2</sub>, and 10 g L<sup>-1</sup> NaHCO<sub>3</sub>) was used. All chambers of the MDC were drained and new solutions were pumped in. The influent synthetic wastewater composition was changed to 2 g/L Ac, 10 mL of the new stock solution, 1 mL of the aforementioned phosphate buffer solution, 1 mL of the trace element solution, 4.2 g/L NaHCO<sub>3</sub>, and 1.5 g/L NH<sub>4</sub>Cl. Additionally, the concentrating and cathode chambers were washed with tap water and the phosphate buffer solution in their external bottle was replaced by tap water. Three data sets were run using the 2 g/L Ac and another data set was run using a 1 g/L Ac concentration to observe how a lower organic matter concentration affects the performance of the system.

Samples were taken at this initial point and the initial COD and nitrogen and phosphorus concentrations in both the anolyte and catholyte were measured. After a hydraulic retention time of 24 hours for the 2 g/L Ac samples and a hydraulic retention time of 16 hours for the 1 g/L Ac samples, samples were taken and these parameters were measured again. The pH of the catholyte was also monitored throughout the hydraulic retention time.

#### 2.3.2.1 Struvite Formation

The concentrated ion solution from the MDC could be collected for struvite precipitation in an external container where the magnesium, ammonium, and phosphate ions would react in equimolar ratios to form struvite by the following reaction.



Based on the measured amounts of ammonium and phosphate in the catholyte, the theoretical amount of struvite precipitate was calculated.

### 2.4 Data Analysis and Calculations

During the operation stage, 10 mL samples of catholyte and anolyte were taken every 24 hours. Influent catholyte and anolyte samples were taken after 10 minutes of recirculation once new solutions were added to the MDC. After the 24-hour time period effluent catholyte and anolyte samples were taken then the reactor was drained, the cathode chamber was rinsed with tap water and new anolyte and catholyte was added.

All samples were filtered using a 0.22 μm membrane. Then the appropriate dilutions were made to test for the concentrations of COD, ammonium nitrogen, and phosphate (anolyte dilutions: 1:5, 1:10, 1:20; phosphate dilutions: 1:10 and 1:20) Samples were tested using a DR/890 Colorimeter (HATCH Co., Ltd., USA) according to the manufacture's instructions. The test read the nitrogen and phosphorus content in the sample; therefore, these concentrations were converted to ammonium and phosphate concentrations, respectively. The pH of the influent and effluent catholyte solutions was measured using a bench-top pH meter (Oakton Instruments, USA).

The voltage (V) of the MDC on a 1-Ω resistor was recorded with automatic digital multimeter and the current was calculated with the following equation:

$$I = \frac{V}{R} \quad (2)$$

The MDC performance is highly affected by the electrical current generation. Therefore, the Coulombic efficiency ( $\epsilon_{cb}$ ) of the system was calculated for every sample using the following equation:

$$\epsilon_{cb} = \frac{M \int_0^{t_b} I dt}{F b v_{An} \Delta COD} \quad (3)$$

where  $M$  is the molecular weight of oxygen ( $M = 32 \text{ g/mol}$ ),  $I$  is the average current throughout the 24-hour period,  $F$  is Faraday's constant ( $F = 9.6487 \times 10^4 \text{ C/mol}$ ),  $b$  equals the number of electrons exchanged for ever mole of oxygen ( $b = 4$ ),  $v_{An}$  is the volume of the anode chamber ( $v_{An} = 200 \text{ mL}$ ), and  $\Delta COD$  is the change in COD over the the time  $t_b$ .

The theoretical amount of struvite produced was calculated based on the measured ammonium concentrations, due to it being the limiting constituent for struvite formation. The daily production rate of struvite was also calculated based on the ammonium concentrations. Additionally, the energy consumption and efficiency of the system were also calculated based on the measured parameters and the following equations:

$$Energy = V \times I \times t \quad (4)$$

$$Efficiency = \frac{\text{amount of struvite produced}}{\text{energy consumption}} \quad (5)$$

### 3. Results and Discussion

#### 3.1 MDC Performance

The performance of the MDC reactor is influenced by multiple parameters, including reactor size and configuration, microbial activity, organic matter concentration, current generation, pH fluctuations, and the hydraulic retention time. The current generated by the MDC is based on the the flow of electrons from the anode to the cathode and thus the rate of degradation of organic matter. Figure 2 below, shows the current generation of the MDC throughout a 16-hour hydraulic retention time using the synthetic wastewater composed of 1 g/L Ac.

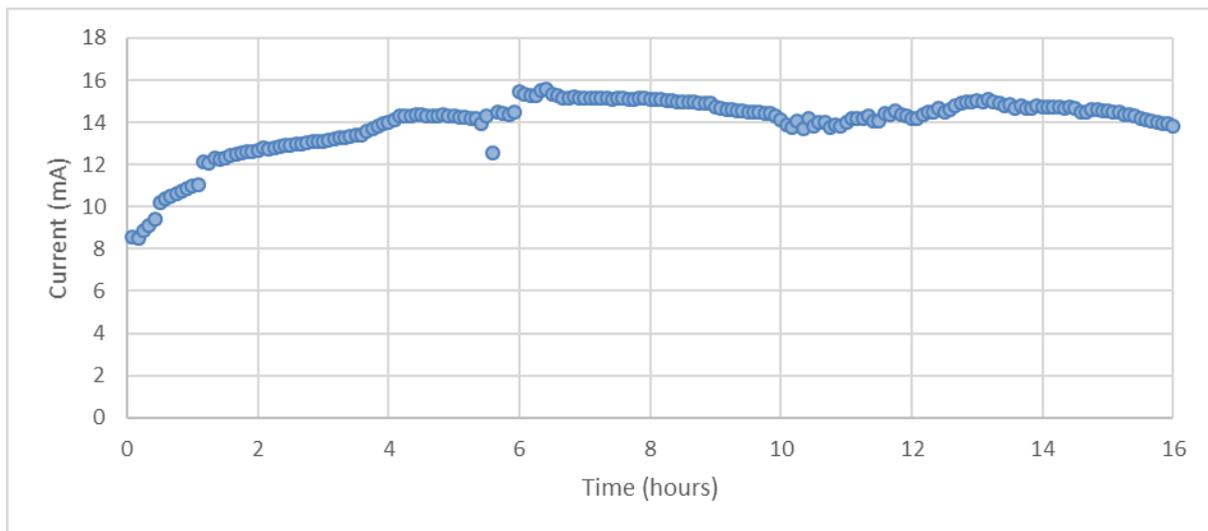
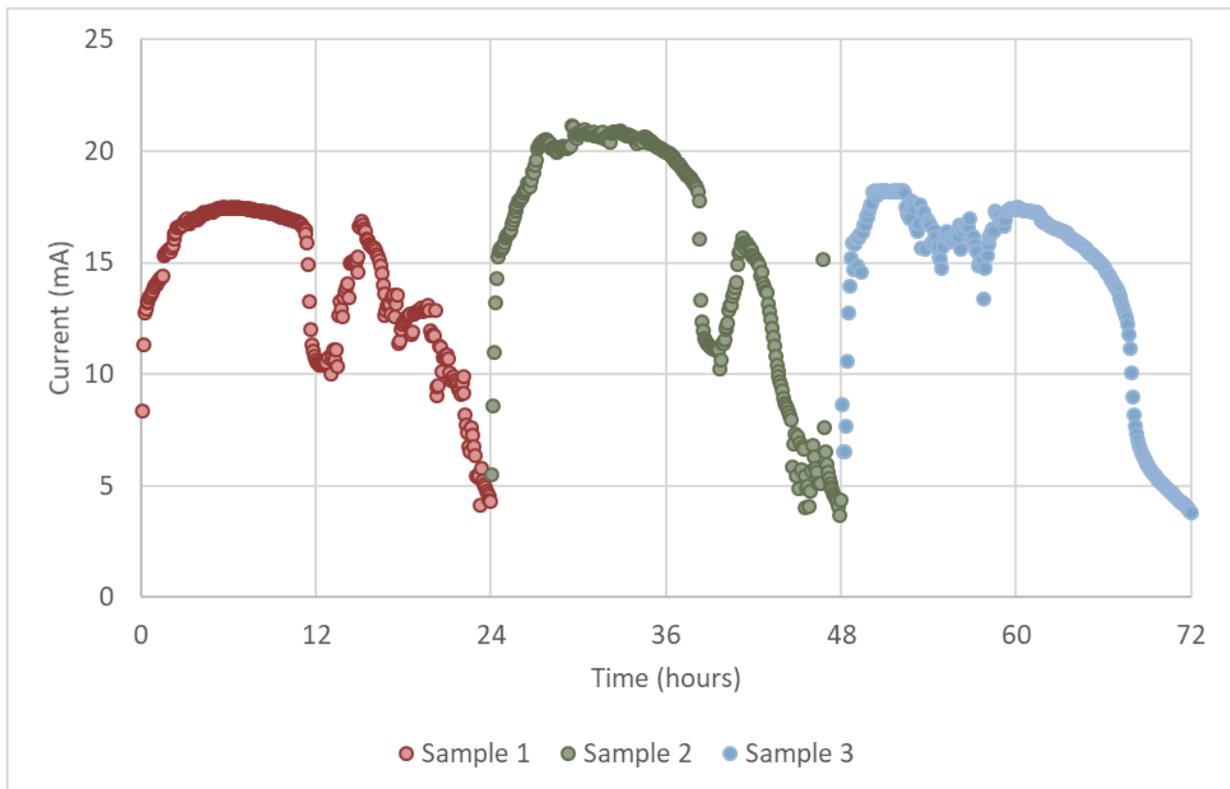


Figure 2. Current for for 16-hour Hydraulic Retention Time.

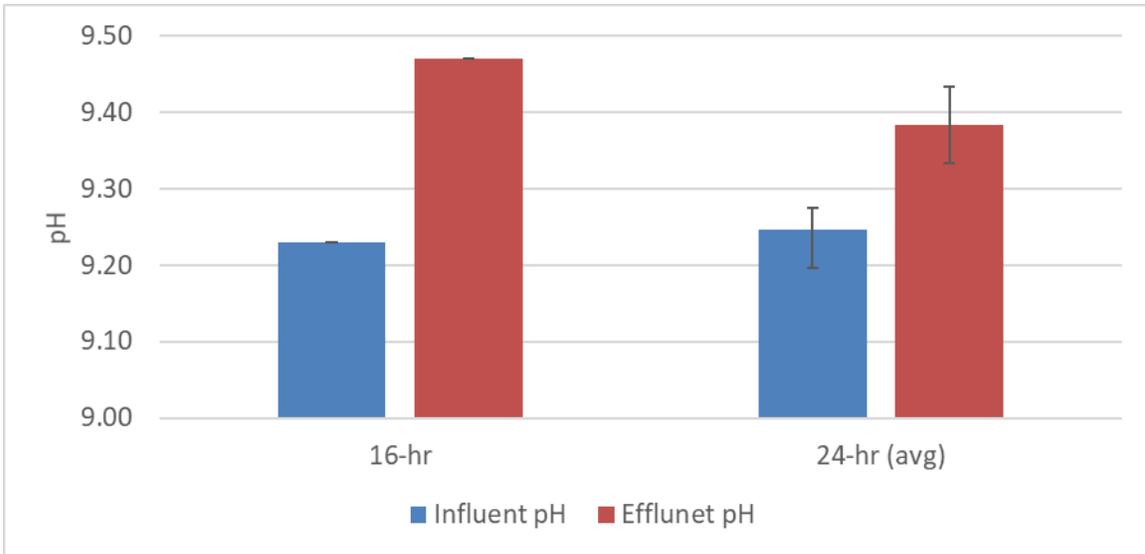
It was observed that the current was relatively stable throughout the 16-hour time period, averaging at a current of 13.97 mA with a maximum of 15.55 mA. However, since only one sample was run at this hydraulic retention time, no concrete conclusions can be made without testing more samples under the same conditions.

Three additional samples were run using the 2 g/L Ac synthetic wastewater solution under a 24-hour hydraulic retention period as can be seen in Figure 3. The average current for samples 1, 2, and 3 were 13.92 mA, 15.63 mA, and 14.26, respectively, with an overall average current of 14.60 mA for all three 24-hour data sets. It can be noted that similar trends are followed for all three data sets. There tends to be a more stable current during approximately the first 12 hours. The subsequent decrease in current can be an indication of COD depletion, as there is less organic matter present in the anode for microorganisms to oxidize. Consequently, there is a decrease in electron flow which can be seen by the decrease in current toward the end of the time period.



**Figure 3. Current for 3 Samples of 24-hour Hydraulic Retention Times.**

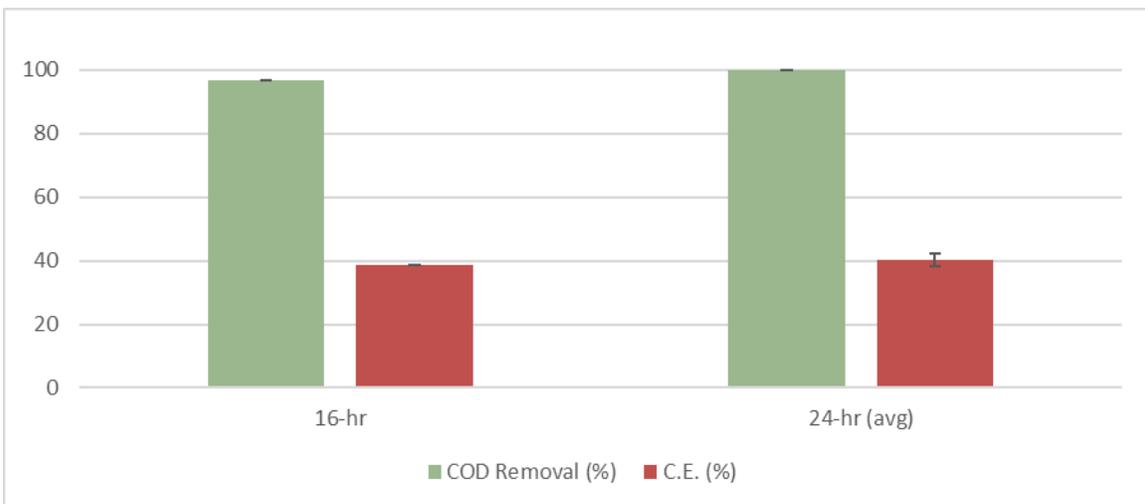
The formation of protons at the anode chamber due to the oxidation of organic matter and the formation of hydroxyl ions at the cathode chamber can lead to pH imbalances. These reactions may create significant pH imbalances within the MDC which can substantially lower current generation (Kim & Logan, 2013). Considerable decreases in pH in the anolyte can impair microbial communities and be detrimental to the productivity of MDC (Kim & Logan, 2013). The system's recirculation allowed for reduce pH imbalances. As can be seen in Figure 4, catholyte pH was measured at the start and end of the 24-hour hydraulic retention time. The 16-hour sample had a larger pH difference than the 24-hour data sets; however, since the shorter time frame data was not replicated so the results cannot be conclusive. The 24-hour data sets had an average pH change 0.14 with a standard deviation of 0.04.



**Figure 4. Changes in Catholyte pH over Hydraulic Retention Time.**

### 3.2 COD Removal and Coulombic Efficiency

The microbial oxidation of organic matter in the synthetic wastewater was measured by COD, the removal of COD indicates if the system can effectively treat the synthetic wastewater. All data sets showed significant COD removal above 95% removal; however, all three data sets within the 24-hour showed 100% COD removal, indicating that the MDC competently treated wastewater as can be seen in Figure 5. Coulombic efficiency was calculated based on equation 3 using average current and change in COD for each individual data set and an average as taken for all three 24-hour hydraulic retention time samples as seen in Figure 5. All data sets showed relatively constant Coulombic efficiency with an average Coulombic efficiency of 40.0% with a standard deviation of 1.9. However, it is expected that as the system becomes more stable the Coulombic efficiency will be improved.

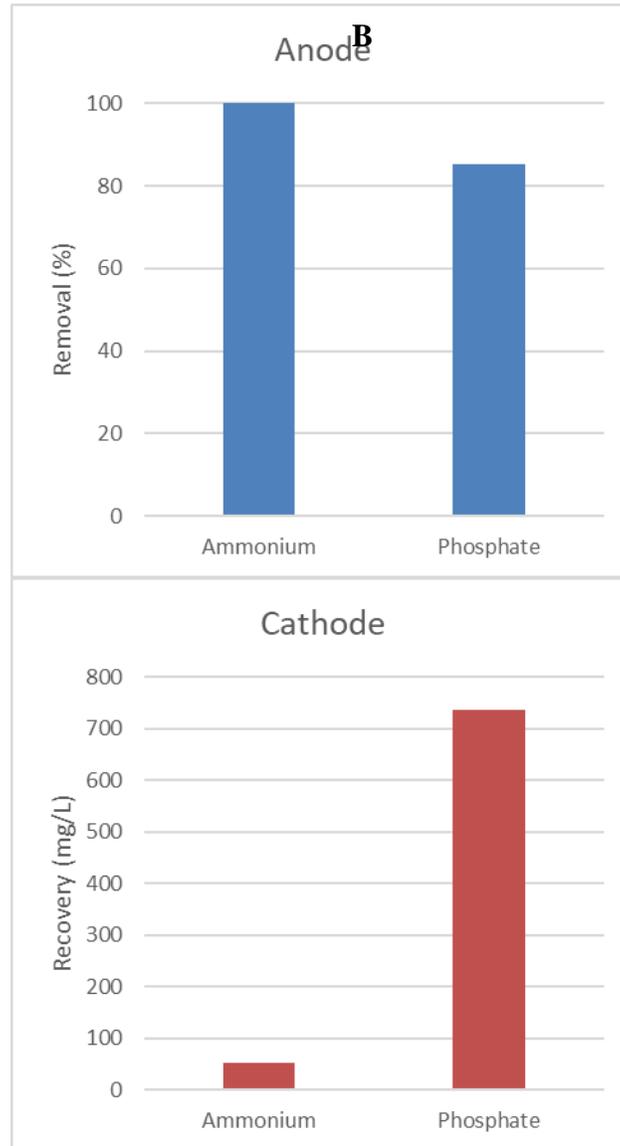


**Figure 5. COD Removal and Coulombic Efficiency.**

### 3.3 Nutrient Removal and Recovery

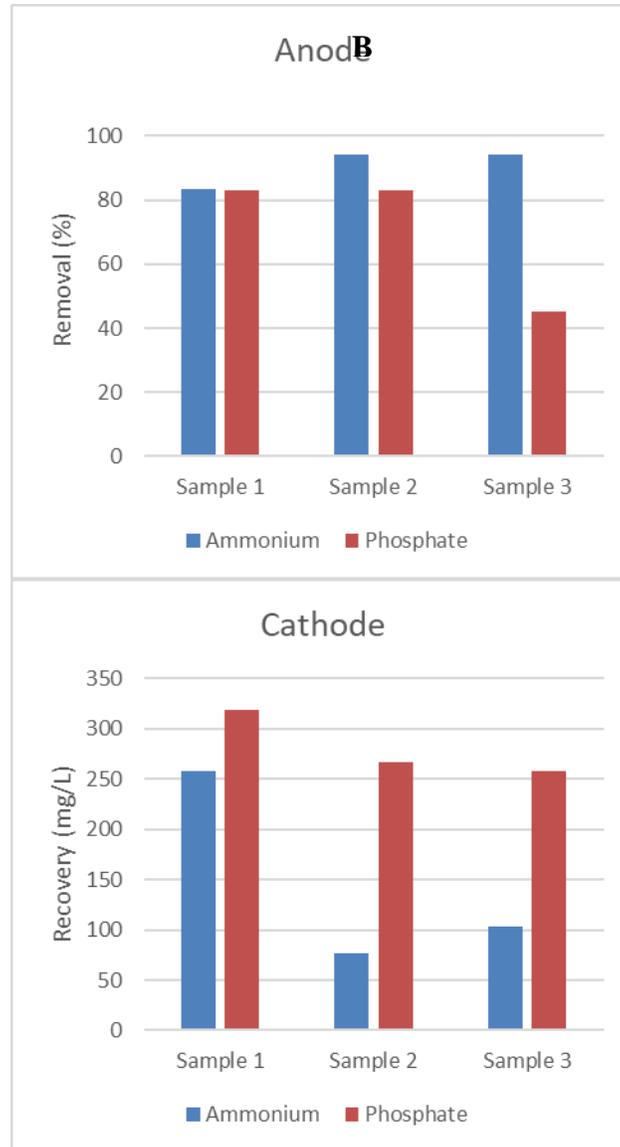
Based on colorimetry analysis of influent and effluent samples, the amount of nutrients in the form of ammonium and phosphate removed from the synthetic wastewater in the anode chamber and recovered in the catholyte were measured as seen in Figures 6 and 7 for the 16 and 24-hour retention periods, respectively.

**A**



**Figure 6. (A) Removal of  $\text{NH}_4^+$  and  $\text{PO}_4^-$  from anode chamber and (B) recovery of  $\text{NH}_4^+$  and  $\text{PO}_4^-$  in cathode chamber for a 16-hour HRT.**

A



**Figure 7. (A) Removal of  $\text{NH}_4^+$  and  $\text{PO}_4^-$  from anode chamber and (B) accumulation of  $\text{NH}_4^+$  and  $\text{PO}_4^-$  in cathode chamber for 24-hour HRTs.**

Overall, the removal of ammonium and phosphate from the anode chamber were high, but not all of the nutrients removed were recovered. This can be attributed to separate reactions that occur within the reactor. For instance, since ammonium recovery is notably lower than phosphate recovery, it is likely that due to the cathode chamber's aeration, ammonium reacts with oxygen to form nitrate and nitrite species. Similar trends can be observed between the two retention time sets. The average removal rate of ammonium and phosphate for the 24-hour data sets were 90.52% and 70.41%, respectively with corresponding standard deviations of 5.08 and 17.97. Future studies can control aeration rate or remove aeration and use a different electron acceptor in order to recover higher concentrations of ammonium and consequently produce more struvite.

### 3.4 Struvite Precipitation

Based on the amounts of nutrients recovered, ammonium more specifically since it was the limiting reagent, the amount of struvite that could be produced was calculated. The concentrated solution showed to collect both ammonium and phosphate; therefore, this solution may be collected and magnesium could be added based on the amount of ammonium present, since magnesium ammonium and phosphate react in equimolar ratios to form struvite. It has been found that pH is a key factor in the formation of struvite, forming best at high pH values near 9 and 10 (Acelas et al., 2014). Struvite may be precipitated in an external bottle to avoid membrane fouling within the reactor which would hinder its performance.

As can be seen in Table 1, for the 16-hour sample, it was calculated that 0.7 g/L of struvite would be formed resulting in a production rate of 1g/L/day, the average amount for the 24-hr samples was 2.5 g/L and a daily production rate of this amount. The amount of energy consumed by this system was calculated based on equation 4, resulting in  $1.8 \times 10^{-4} kWh$  for the 16-hour system and an average of  $2.9 \times 10^{-4} kWh$  for the 24-hour data sets. And the MDC system's efficiency was calculated using equation 5, resulting in 3.9 kg/L/kWh for the 16-hour data set and an average of 8.8 kg/L/kWh for the 24-hour data sets. It can be inferred that since higher concentrations ammonium were recovered in the 24-hour setup, it produced better results, meaning more nutrients can be removed and recovered to form struvite.

**Table 1. Feasibility Calculations for Struvite Production.**

	16-hr HRT	24-hr HRT (average)
Struvite Precipitation	0.7 g/L	2.5 g/L
Production Rate	1 g/L/day	2.5 g/L/day
Energy Consumption	1.8E-4 kWh	2.9E-4 kWh
Efficiency	3.9 kg/L/kWh	8.8 kg/L/kWh

#### 4. Conclusions

In this study, the feasibility of the recovery of nutrients in the form of ammonium and phosphate by concentrating ions using a four-chamber microbial desalination cell to precipitate struvite was tested. It was found that this system can effectively remove nutrients from synthetic wastewater and recover them in the concentrated catholyte fluid. This indicates that struvite precipitation is feasible and that MDCs are a promising technology for the future developments of nutrient recovery from wastewater. However, future studies should aim to further improve the harnessing of wastewater's resource potential. There is still a need for improvement studies to be performed to improve the amount of nutrients recovered as well as a need for the optimization of influential parameters.

## 5. Acknowledgements

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# Evaluating Growth Potential of Legionella in Simulated Glass Water Heaters

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## Abstract

Opportunistic pathogens (OPs) are now the leading cause of drinking water related disease outbreaks. Hot water systems have been linked to disease occurrence and are likely the primary location for OPs growth. In previous studies, hydrogen evolved from corrosion of water heater sacrificial anode rods has been hypothesized to support growth of OPs; however, further understanding is needed about the mechanism(s) for growth. Bench-scale reactors were used to evaluate growth potential of *Legionella* growth in two different hot water system designs, standard water heater tanks and tanks with recirculation. Standard systems were simulated in reactors with no agitation while recirculating systems were simulated by continuous agitation. Conditions tested included a control reactor with breakpoint chlorinated and granular activated carbon filtered Blacksburg tap water with no other alterations for a low carbon, ammonia-free water, reactors dosed with 0.50 mg/L-N ammonia (with and without agitation), 60 µg/L hydrogen (with and without agitation), 5 µg/L copper, and ammonia and hydrogen together. Preliminary data showed that agitation resulted in increased microbial growth (279 more total cells/uL as measured by flow cytometry). In addition, while there were no additive impacts of the combination of hydrogen and ammonia nutrients with regard to total microbial growth, there were additive impacts with regard to fixation of total organic carbon (385-216 µg/L more organic carbon fixed in the condition with both ammonia and hydrogen than either hydrogen or ammonia alone). These data suggest there is variable microbial regrowth potential with respect to the nutrient available.

**Keywords:** *Legionella pneumophila*, water heaters, anode corrosion, hydrogen oxidizing bacteria, ammonia oxidizing bacteria

## 1. Introduction

### 1.1 *Legionella Pneumaphelia*

The first outbreak of Legionnaires' disease first affected the attendees of an American Legion Convention in Philadelphia in July 1976. During the outbreak 29 out of the 182 cases were fatal (Tsal et al., 1985). *Legionella* is an opportunistic pathogen (OP) that is the leading cause of waterborne diseases in developed countries. It is the causal agent of Legionnaires' disease, which is a severe pneumonia and has been estimated by the CDC to cause between 8,000 to 18,000 hospitalization cases per year (CDC 2008a).

Waterborne disease outbreaks associated with OPs can be attributed to microbes that grow in building plumbing, in shower heads, faucets, along pipe walls, and in water heaters. (Hilborn, Arduino, Pruden, & Edwards, 2015). Features that make building plumbing suitable for OP growth include high surface-to-volume ratio, intermittent stagnation, low disinfectant residual concentrations, and warming cycles (Hilborn et al., 2015). Water heaters can grow OPs due to their warm temperature environment.

The conventional approach to biological control in water distribution systems, which includes maintaining a disinfectant residual, is often ineffective at controlling microbial regrowth in building (as opposed to distribution) plumbing systems (Lechevallier, Welch, & Smith, 1996). Resistance to disinfectant residuals can be attributed to multiple factors. For *Legionella*, encysted amoeba hosts, whose

cells are relatively chlorine resistant, can shelter *Legionella* from up to 50 mg/L chlorine (Kilvington & Price, 1990).

## *1.2 Anode Rod Corrosion-Explaining Hydrogen and Ammonia*

### *1.2.1 Nitrifying Bacteria:*

Chloramine is generally more persistent compared to free chlorine and is well-suited to maintain disinfectant residuals to consumer's tap (Nguyen, Elfland, & Edwards, 2012). However, autotrophic nitrifying bacteria convert the ammonia associated with the chloramine residual to nitrite and nitrate, fixing organic carbon in the form of biomass and soluble microbial products (Edwards, Marshall, Zhang, & Lee, n.d.). Then the nitrifying bacteria create sufficient levels of organic carbon to support the growth of heterotrophic bacteria (Edwards et al., n.d.).

### *1.2.2 Hydrogen Oxidizing Bacteria*

Deposition of copper ions in water, either from copper pipes or added as a disinfectant, can catalyze the evolution of hydrogen as a byproduct of corrosion of the water heater sacrificial anode rod. As a result, autotrophic hydrogen oxidizing bacteria can use the hydrogen evolved to fix organic carbon and provide an ecological food web or OPs, similar to the mechanism described above for nitrifying bacteria when chloramine decays (Kilvington & Price, 1990)

## *1.3 Copper variability*

Copper has been shown to have variable effects in respect to pathogen growth. It can act as a biocide, nutrient, and have no effect on the growth of *Legionella*. First, copper ions released from copper pipes or added to water as a disinfectant can hinder *Legionella* growth. In one building, copper levels above 50 µg/L were correlated to with lower *legionella* growth in taps (Borella et al., 2004). However, in another study, as the copper pipes aged and released fewer Cu<sup>2+</sup> ions to the water overtime, the inhibitory effect of copper was not observed long-term (van der Kooij et al., 2005). In addition, other factors can affect the bioavailability of copper such as pH and temperature, impacting the speciation of copper compounds formed and potentially rendering the copper harmless. On the other hand, copper can facilitate *Legionella* growth (Buse, Lu, Lu, Mou, & Ashbolt, 2014). However, there is no experimental data to-date that explains these discrepancies.

## *1.4 System Design*

OP growth factor can also be impacted by the type of hot water system. For instance, in a study by Brazeau and Edwards it was seen that that the total chlorine residual was 1.5-10 times higher in standard systems as compared to recirculating systems (Brazeau & Edwards, 2013). In the same study, it was observed that a recirculating system anode rod corroded 1.72 time faster and evolved 4-6 times more H<sub>2</sub> than a standard design system. Since certain types of opportunistic pathogens maybe be able to utilize H<sub>2</sub> directly as a nutrient source leading to potential increased pathogen growth from anode decay (Brazeau & Edwards, 2013; Gomila, Ramirez, Gascó, Lalucat, & Jorge Lalucat jlalucat, n.d.).

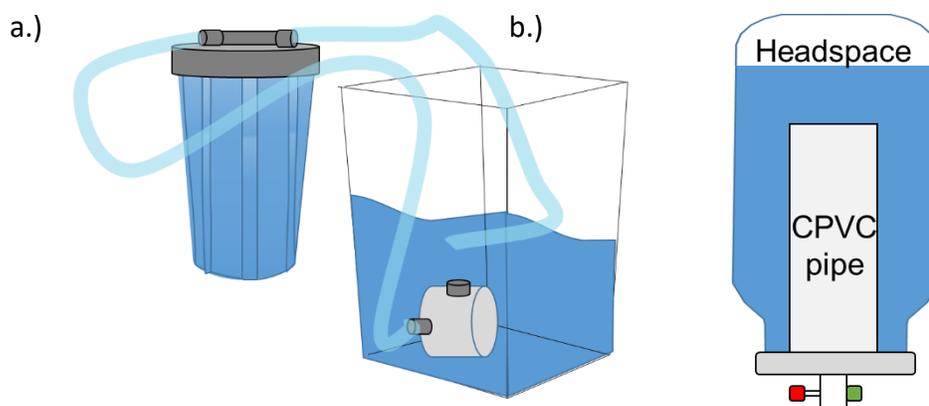
## *2.0 Objectives*

The objective of this experiment was to use bench-scale reactors to evaluate growth potential of *Legionella* in two different hot water system designs, standard water heater tanks and tanks with recirculation. Ammonia and hydrogen are going to be used to identify if growth is facilitated when agitation and excess nutrients are present.

## 2. Research Methods and Experiment Setup

### 2.1 Simulated Glass Water Heaters (SGWHs)

Simulated Glass Water Heaters comprised of 500 mL French square glass bottles with polytetrafluoroethylene (PTFE) caps. Prior to experimentation, all bottles and caps were acid-washed, rinsed in reagent grade nanopure water, and glass bottles were baked in a muffle furnace at 550° C to combust residual organics. CPVC pipe extracted from an on-going experiment (harvested for its mature biofilms) was epoxied (BRNAND) to the interior of the cap to provide a growth surface. SGWHs were incubated at 37°C inverted. A syringe septum sampling port was installed to the exterior of the cap to facilitate headspace hydrogen dosing and analysis. SGWHs were operated in triplicate, and received one of seven influent waters (Table 1). Control water was breakpoint chlorinated and granular activated carbon filtered Blacksburg, VA tap water to achieve a low ammonia, low organic carbon control water (Fig 1).



**Figure 1. a.) Influent water from Blacksburg, VA tap water that was break-point chlorinated and then GA filtered to attain a low nutrient and organic water. b.) The various components of the simulated glass water heaters; the headspace, pipe, and syringe septum.**

#### 2.2.1 Conditions

The following were the conditions dosed and summarized in table 1. Conditions tested included a control reactor with breakpoint chlorinated and granular activated carbon filtered Blacksburg tap water with no other alterations (with and without agitation). Other reactors were dosed with 0.50 mg/L-N ammonia (with and without agitation), 20 mg/L hydrogen (with and without agitation), 5 ug/L copper, and ammonia and hydrogen dosed together.

**Table 1 – Summary of conditions tested (William Rhoads 7/26/16)**

Table 1. Summary of conditions tested
1. Control water, stirred
2. Control water, not stirred
3. Control water + 60 ppb H <sub>2</sub> , stirred
4. Control water + 60 ppb H <sub>2</sub> , not stirred
5. Control water + 0.5 ppm NH <sub>3</sub> , not stirred
6. Control water + 0.5 ppm NH <sub>3</sub> + 60 ppb H <sub>2</sub> , stirred
7. Control water + 5 ppb cupric ions

Hydrogen was dosed directly to the headspace of inverted SGWHs to achieve target aqueous hydrogen concentrations estimated by Henry's Law. Water changes were executed with an 80% water volume dump-and-fill protocol 3X/week. SGWHs were incubated at 37° C and agitated at 150 rpm shown in figure 2a.

a.)



b.)

**Figure 2. a.) The shaker table where the stirred samples were located was set at 150 rpm kept at 37C. b.) The not stirred samples were located in the same incubator room (ICTAS 385a) on a shelf. (Mariana Martinez, 22/07/2016)**

### 2.2.1 Physical and Chemical analyses

General physical and chemical water quality parameters were characterized through routine measurements. Parameters were well characterized through repeat measurement immediately after operational changes in experimental design and monitored for changes. Ammonia was measured NH<sub>3</sub>-N using Standard Method 5310-NH<sub>3</sub> using a DR5000 spectrophotometer (HACH Company, METHODS 5480-NH<sub>3</sub>). Hydrogen gas (H<sub>2</sub>) was measured using Helium ionization detector packed column on a gas chromatograph. Henry's Law was used to back calculate aqueous concentrations. Moreover, Pure hydrogen produced from reacting 12N HCL in deionized water with solid zinc and the hydrogen was dosed directly into the reactors via a syringe septum (Fig. 1b). Total organic carbon was measured by persulfate-ultraviolet detection (Sievers Model 5300C) according to Standard Method 5310C. For microbiological analysis, Total cell counts were conducted using a fluorescence nucleic acid stain (SYBRs Green I) with quantitative flow cytometry (BD ACCURI) using previously developed methods (Hammes et al., 2008)

### 3. Results and Discussion

#### 3.1 Ammonia Nutrient Usage

Ammonia consumption was determined by comparing the influent ammonia concentration to each effluent condition. The amount of ammonia oxidized was used as an indicator for nitrification. The average influent concentration was 0.49 mg/L NH<sub>3</sub>-N (Table 2). Average effluent concentration for the reactors with only ammonia was 0.49 mg/L and was 0.11 mg/L for ammonia and hydrogen combination reactors. This suggests that the nitrifying bacteria, if present, were not able to consume the ammonia in the reactors with ammonia only. On the other hand, the ammonia and hydrogen condition oxidized an average of 0.38 mg/L NH<sub>3</sub>-N. The activity of the ammonia oxidizing bacteria can theoretically fix 0.073 mg organic carbon per 1 mg NH<sub>3</sub>-N oxidized (get ref from poster). Thus, 27.7 ug/L of organic carbon could theoretically be fixed (Table 3).

**Table 2 – Ammonia consumption in the reactors dosed with ammonia  
(Mariana Martinez 7/26/16)**

Sample	NH <sub>3</sub> (mg/L)
Inffluent	0.49 ± 0.12
Ammonia Effluent	0.49 ± 0.08
Ammonia & Hydrogen Effluent	0.11 ± 0.05

**Table 3 – Ammonia consumption in the reactors dosed with ammonia  
(Mariana Martinez 7/26/16)**

Sample	Ammonia Consumed (mg/L)	Theoretical Organic Carbon Fixed (ug/L)
Ammonia	0	0
Ammonia & Hydrogen	0.38	27.7

Total organic carbon levels of above 10 ug/L have been suggested as a threshold for supporting *Legionella* growth in unchlorinated, oligotrophic drinking water. Therefore, the ammonia and hydrogen combination reactors could support additional microbial growth, including OPs.

### 3.2 Hydrogen Nutrient Usage

Total aqueous hydrogen in the influent water was 60.2 ug/L. In the reactors with only hydrogen, 55.8 ug/L of H<sub>2</sub> was consumed in the reactors with agitation and only 15.8 ug/L of H<sub>2</sub> was consumed in the reactors without agitation (Table 4). Agitation in these reactors simulates the continuous supply of H<sub>2</sub> that would be evolved continuous anode rod corrosion. The 3.5 times more H<sub>2</sub> consumed in the reactors implies that recirculating systems would facilitate carbon fixation relative to a standard designed water heater. The most H<sub>2</sub> was consumed in the reactors with ammonia and hydrogen together (60 ug/L H<sub>2</sub>). Hydrogen oxidizing bacteria can fix organic carbon at 0.19 mg organic carbon/mg H<sub>2</sub>. Therefore, theoretical total organic carbon fixed could be up to 11.4 ug/L in the hydrogen and ammonia condition, 10.6 ug/L in stirred hydrogen (Stirred), and only 2.9 ug/L for hydrogen (not stirred) (Table 4). The theoretical organic carbon fixed due to hydrogen oxidation was also was greater than 10 ug/L, and therefore could also be important for understand OPs growth in building plumbing.

**Table 4 – Hydrogen measured in each of the triplicate conditions  
(Mariana Martinez 7/26/16)**

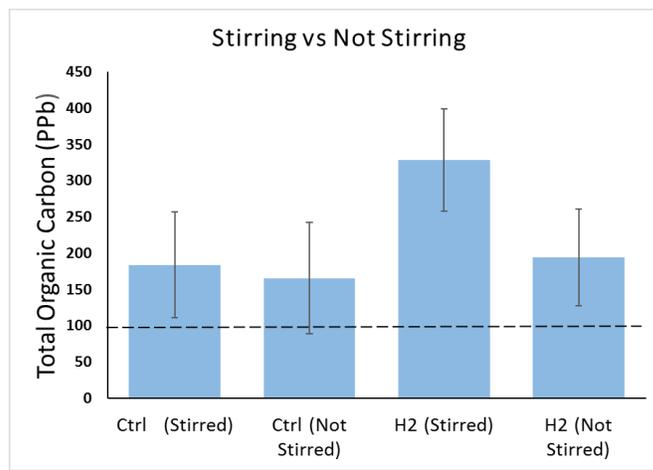
Sample	Hydrogen Measured (ug/L)	Hydrogen Consumed (ug/L)	Theoretical Organic Carbon Fixed (ug/L)
Influent	60.2		
Hydrogen (Stirred)	4.4	55.8	10.6
Hydrogen (No stirred)	44.8	15.4	2.9
H <sub>2</sub> +NH <sub>3</sub> (Stirred)	0.23	60	11.4

### 3.3 Total Organic Carbon

As mentioned previously the total fixed organic carbon found in two of the hydrogen conditions and the ammonia and hydrogen condition were above the suggested amount of 10 ug/L that can support *Legionella* growth. Total organic carbon was measured in each of the following reactors.

#### 3.3.1 Stirring vs Not Stirring

There was more organic carbon fixed when agitation was present compared to no agitation. All the conditions fixed a significant amount of total organic carbon compared to influent (Figure 3, dashed line at 94.0 ug/L). In addition, each condition with agitation had more carbon fixed than the condition without agitation. For the control condition without excess nutrients added, however, the stirred condition only had 18.5 ug/L more total organic carbon fixed than the no stirring condition. However, in the reactors with dosed H<sub>2</sub>, the stirred condition fixed 328.49 ug/L total organic carbon compared to 194.41 ug/L fixed by hydrogen condition that was not stirred.



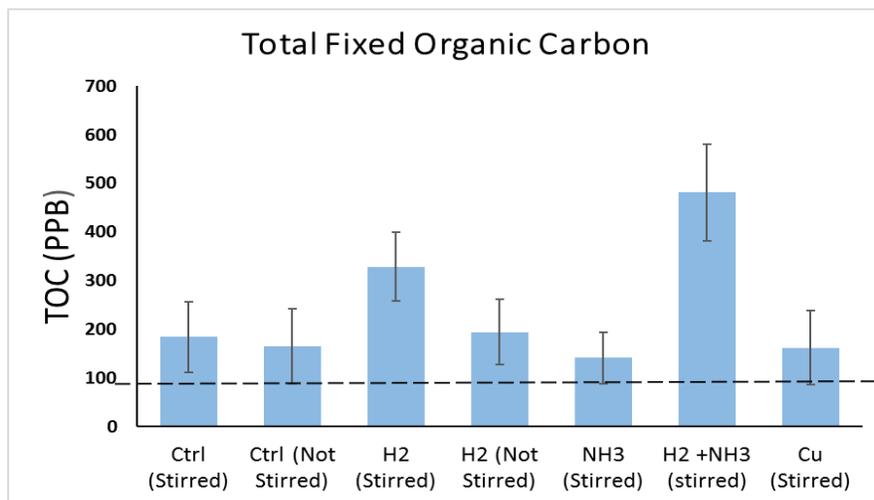
**Figure 3. Total organic carbon (ppb) fixed in the conditions that compared stirring and not stirring**

Therefore, the hydrogen stirring condition fixed 132.08 ug/L more organic carbon than the condition with no stirring. We hypothesize that the stirring (and analogously, recirculation hot water systems compared to standard designed systems) facilitates growth of microorganism by increasing the mass transfer of nutrients to attached growth (Liu et al., 2006). In addition, stirring also physically increased the rate of delivery of the excess H<sub>2</sub> in the headspace of the reactors to the water column.

### 3.3.2 Total Organic Carbon in Conditions

The other conditions (with stirring) fixed a similar amount of carbon as the control with stirring (Figure 4), except the condition with ammonia and hydrogen dosed together. There appeared to be a cumulative impact with respect the amount of organic carbon fixed in the condition with ammonia and hydrogen together over the hydrogen condition alone. The reactors with ammonia and hydrogen together fixed 152 ug/L more organic carbon than stirred hydrogen condition alone. The stirred ammonia condition had the least amount of carbon fixed at 141 ug/L. Because there was no ammonia oxidation in these reactors (Table 3), there was no increase in total organic carbon fixation. It remains unclear if there was a true additive effect in the hydrogen and ammonia reactors because it is unknown how much carbon the reactors with ammonia alone would fix if the nitrifying bacteria had been able to oxidize the ammonia present.

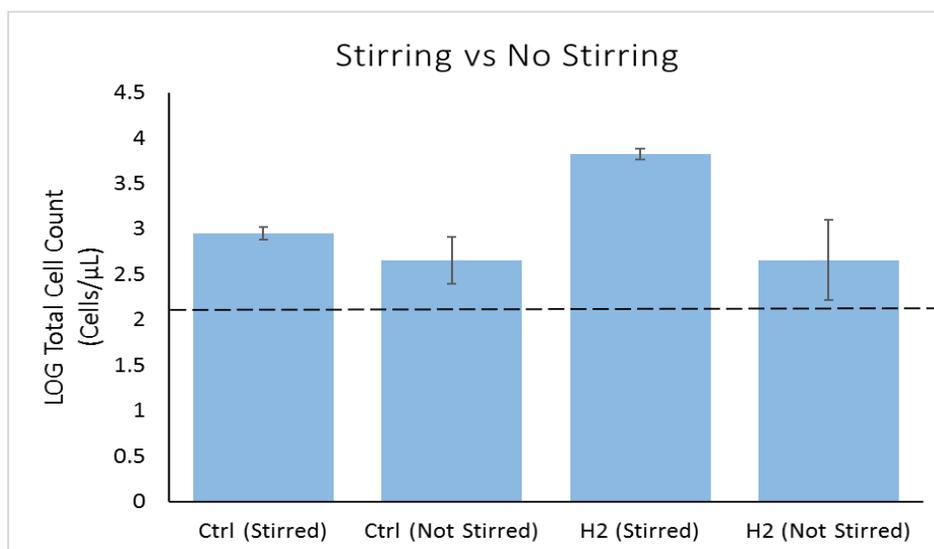
The copper (stirred) condition had low levels of fixed organic carbon (162 ug/L), which is comparable to the levels found in the stirred control (184 ug/L). This suggest that there was similar microbial activity in the presence of low level of copper. Thus, copper neither acted as a critical nutrient nor a biocide for general microbial growth.



**Figure 4. Total organic carbon (ppb) fixed in all conditions tested**

### 3.4 Total Cell Count – Stirring vs Not Stirred

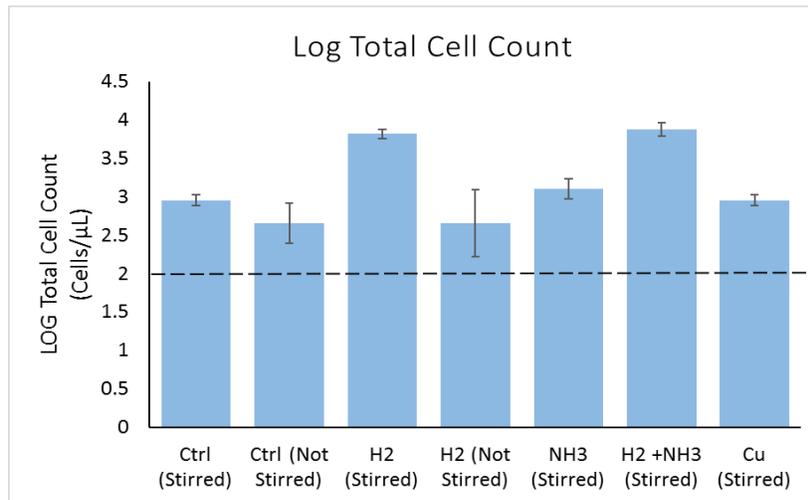
Microbial growth was quantified using total cell counts. There was an increase in cell count in both the stirred and not stirred conditions compared to the influent line (149 cells/uL). The stirred control had 270 more cells/uL than the not stirred control. Therefore, without any extra nutrients present, stirring facilitated microbial growth. Moreover, when excess nutrients were present, the total cell count had a significant increase in total cell count. The stirred hydrogen reactors had more 5,380 more cells/uL than the not stirred hydrogen reactors on average. Comparing both stirring conditions without and with excess hydrogen, there were 6,014 more cells/uL in the condition with hydrogen than the control with H<sub>2</sub> (Figure 5).



**Figure 5. Total cell counts comparing the conditions that were stirred and to those that were not stirred**

#### 3.4.1 Total Cell Count – Difference in Type of Nutrients Added

The stirred ammonia and hydrogen had significant increase in total cell count when compared to the influent (8,108 cells/uL versus 149 cells/uL). Interestingly, the stirred ammonia and hydrogen only had 1,270 more cells/uL than the stirred reactors with hydrogen alone. Therefore, the semi-additive effect observed in the ammonia and hydrogen reactors with respect to TOC was not present with respect to total cell counts. This suggests there was a microbial shift and while the size of the microbial community (total cell counts) was not different, the members of the community were able to more efficiently use the combined ammonia and hydrogen nutrients relative to hydrogen alone to fix organic carbon (Figure 6). Similar to the TOC trends, it remains unclear how many cells would have been present in the stirred ammonia reactors had the nitrifying bacteria been active. This should be investigated in follow up work.



**Figure 6. Total cell counts for all the conditions tested**

#### 4. Conclusion

Bench-scale reactors were used to evaluate growth potential in two different hot water systems, the recirculation hot water system would more likely support the growth of *Legionella* due to the increased microbial growth and organic carbon fixation observed in the presence of agitation and extra available nutrients. Moreover, combined nutrients hydrogen and ammonia could facilitate growth of more OPs due to their ability to fix additional carbon (387 ug/L). This condition is analogous to a recirculating system with a chloramine residual. Therefore, it is likely that hot water systems using chloraminated water, would be more likely to support *Legionella* and other OPs when the disinfectant residual decays. Finally, future tests will examine culture and molecular quantification methods of *Legionella* to further understand the nutrient and agitation mechanisms.

#### 5. Acknowledgements

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# Natural Attenuation Of Groundwater Contaminant Plumes In The Hyporheic Zone

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## Abstract

This study looked at the reaction of resazurin to resorufin used for laboratory simulations of the hyporheic zone, a zone where groundwater and surface water mix in areas such as a riverbed. Little is understood about the rate which pollutants are remediated in this zone and the controlling factors. To establish a method for studying this topic we studied the resazurin to resorufin reaction to be used as a reactive tracer. In this reaction dissolved oxygen (DO) is consumed as well as a charged oxygen atom on the resazurin by sodium sulfite and this transforms the solution from dark blue to pink. This reaction will also be used to mimic attenuation of contaminants during naturally occurring reactions. To study the reaction we measured the rate of DO consumption as well as the time for the solution to change color. Additionally, we used a permeameter to record the time difference of color change when the reaction occurs in sediment and if it is visible enough to be used as a tracer. Next, we also preformed tests on samples using a spectrofluorometer to understand intensity of color versus concentration during the reaction. Lastly, these results were used in computer models to mimic the hyporheic zone. These numerical models will be used to understand flow paths and chemical concentrations of DO and sodium sulfite throughout an experimental tank.

**Keywords:** hyporheic zone, resazurin, dissolved oxygen, numerical models

## 1. Introduction

The hyporheic zone is a region where surface water and groundwater mix. This zone would likely occur in a place such as a river bottom where surface water is flowing and upwelling groundwater can combine with it in the sediment. Surface water flows into the ground due to natural phenomenon such as a barrier<sup>1</sup>. The two water sources often have varying properties such that when they mix, can cause chemical reactions to occur which could break down pollutants otherwise known as mixing-dependent reactions<sup>2, 3</sup>. Mixing-dependent reactions are reactions that can only occur when two solutions mix with each other. The different chemical compositions will allow for a chemical in one of the solutions to react with a chemical in the other solution when they mix. For example, groundwater is unlikely to have dissolved oxygen while the surface water will. Specific pollutants in the groundwater could possibly react with dissolved oxygen in surface water when the two water sources mix in the hyporheic zone<sup>7</sup>. Moreover, bacteria and other microorganisms reside here and can provide a way for pollutants to be naturally remediated in a river or stream<sup>4</sup>. In this case pollutants can be transformed by bacteria into a different compound which may be less toxic or not at all. In addition, the zone can even act as a sink for pollutants like heavy metals keeping it from the stream or river<sup>5, 7</sup>. However, in certain cases such as with methylmercury, the problem with this toxic compound can be intensified which is significant because methylmercury can be passed on in the food chain and accumulate over time<sup>6</sup>. Overall, the hyporheic zone needs to be studied more to understand all the processes that occur in this zone and how effective in treating pollutants it is or any negative side effects from a reaction that may occur here.

The problem we work to solve in this study is to quantify the amount of which a pollutant can be remediated in mixing-dependent reactions in the hyporheic zone as there is not very much data on this

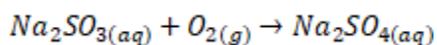
topic<sup>7</sup>. Our method for accomplishing this problem was to first find an abiotic reaction to simulate mixing-dependent hyporheic reactions in the lab that could mimic those that might realistically happen in the field. Furthermore, in this study only a chemical process—the resazurin to resorufin—is investigated compared to a different type of process that could take place in the hyporheic zone such as one by bacteria<sup>8</sup>. Next, data from the reaction was used in computer models to predict concentrations of dissolved oxygen in a large experimental tank (mesocosm) among other variables by the software such as flow paths of the solutions while in the tank. Lastly, using optodes and samples taken from the mesocosm the models will be confirmed in future laboratory experiments. These models in later studies could possibly be used for other chemical or microbial processes that occur in the hyporheic zone.

## 2. Research Methods and Experiment Setup

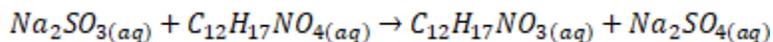
### 2.1 Resazurin to Resorufin Reaction Using Sodium Sulfite

To determine the reaction properties including time for dissolved oxygen consumption and for the solution to turn color from dark blue to pink, we set up a simple beaker reaction. The reactants used were resazurin (Acros Organics), sodium sulfite (certified ACS Fisher Scientific, 98%), and cobalt chloride (certified ACS Fisher Scientific, 99.2%).

The reaction has two parts as follows:



Here sodium sulfite consumes DO producing sulfate.



In this chemical reaction the sodium sulfite consumes an unstable oxygen off of the resazurin transforming it into resorufin, while also producing more sulfate.

For the beaker test 1.5 mL of resazurin from a stock solution of 0.5 g/L were used. The resazurin stocks along with the solutions made were kept in a cold room and out of light to keep the chemical from undergoing photochemical decay<sup>9</sup>. Then 148.5 mL of either tap or distilled water was added for the overall 150 ml solution to get a resazurin concentration of 5 mg/L. A stir plate continually mixed the solution throughout the reaction. A YSI Pro-Plus probe was used to begin measuring dissolved oxygen (mg/L) to get initial DO. Stock solutions of cobalt chloride at 0.001 M and sodium sulfite at 0.1 M were also made. Recording was then started at every one second for dissolved oxygen of the solution followed by 20 mL each of the sodium sulfite and cobalt chloride solution<sup>10</sup>. The DO continued to be recording until it neared zero and this data was analyzed to get reaction rates. Furthermore, the time for the solution to turn from blue to pink was also noted for each experiment, although this is partially variable because of the different shades of pink based off of resorufin concentration. The spectrofluorometer data are clearer on intensity of color versus concentration of both the resazurin and resorufin then what can be easily observed. This reaction was run multiple times to ensure accurate results.

### 2.2 Resazurin to Resorufin Reaction Without Cobalt Chloride

We also ran the resazurin to resorufin reaction with sodium sulfite but without the catalyst cobalt chloride that is meant to speed up the reaction. The reason this test was run is because using cobalt chloride as well as the other reactants adds to the waste of large experiments and past research has shown cobalt chloride to be a probable carcinogen<sup>11</sup>. Cobalt chloride also interferes with the spectrofluorometer reading of the resazurin and resorufin mostly likely due to its slight blue hue. If it can be determined cobalt chloride does need to be used this is the best outcome for the project and future studies that will use this reaction. The experiment was the same as detailed above, but only 20 ml of sodium sulfite was added

to the 150 ml solution. Tests were also run on simply 150 ml of tap or distilled water with 20 ml sodium sulfite being added. The rate of DO consumption was recorded to compare reactions rates to the resazurin to resorufin tests.

### *2.3 Permeameter Tests*

A permeameter is a device that measures the ability of sediment to allow liquid to pass through. By controlling the hydraulic head on the device we can have liquid pass through it at a specific flow rate. The permeameter first had water run through it continuously for at least 3 days to ensure the sand settled and that all the air was pushed out of the column. Additionally, the sand used for the permeameter was also the same type of sand to be used in the large mesocosm experiments to eliminate sand type from being a variable in the experiments (DO-50 white sand with an average diameter of 0.53 mm). Multiple tests with tap water running through the device were then run to find the right hydraulic head for a flow rate that would allow enough time for the resazurin to resorufin reaction to occur and to ensure the permeameter was giving steady readings. Once the flow rate was deemed constant a solution of resazurin at 5 mg/L was ran through the permeameter immediately followed by a solution of 1:1 mix of cobalt chloride (0.001 M) and sodium sulfite (0.1 M). Where these two solutions met is where the resazurin should start to transform into resorufin and pink should be seen in the sediment. This process will not only show that this reaction could most likely be replicated in the mesocosm experiments, but that the blue and pink colors can actually be seen in the sediment so photographs of the tracer can help calibrate computer models of the mesocosm.

### *2.4 Spectrofluorometer Tests*

The spectrofluorometer (FluoroMax®-4 Spectrofluorometer, Horiba Scientific) was used to measure the light intensity emitted by resazurin and resorufin during the reaction. These values will be used to get concentration from samples during mesocosm experiments to see if the data matches the concentration we would expect based off of the computer models. This also shows how intensity can be matched to concentration when using a similar method to get concentration of resazurin in the mesocosm by analyzing pictures from a camera. The camera will have a fairly similar calibration process as detailed below, but still separate than the process done with the spectrofluorometer.

First, using a UV spectrophotometer (UV-1800, Shimadzu), the excitation wavelengths for the both resazurin and resorufin were determined. The samples used for this were from 1 mg/L to 5 mg/L of both resazurin and resorufin to confirm different concentrations would have the same excitation wavelength. Once the excitation wavelength was known the emission was found using single point tests on the spectrofluorometer. Now to know intensity per concentration calibration curves had to be determined. We measured resazurin at 8, 16, 24, 32, 40  $\mu$ L for 1, 2, 3, 4, 5 mg/L solutions respectively at a total 4 mL each (cuvettes hold up to 4mL). The dilution to get to 4 ml was half DI water (1.992-1.960 mL) and 1.5 mL sodium sulfite (0.1 M). Using an excitation of 607.5 nm and 675 nm for emission, the intensity of each solution was recorded to get the calibration curves. Once calibration curves were established a resazurin to resorufin reaction was then performed (one without cobalt chloride) as outlined in Section 2.1. Samples were taken every 1-minute for 40 minutes and then additionally every 5 minutes at 55 minutes until 80 minutes had passed. This is approximately how long it takes to see a full color change using this particular solution. Samples were run in the spectrofluorometer to get the intensity at each time reading. Using the calibration curves we can determine intensity versus concentration to use for mesocosm models.

## 2.5 Ion Chromatography

Preliminary results indicated that the time for the solution of resazurin to resorufin reaction to turn from blue to pink took longer when tap water was used instead of distilled water. Although it is not completely clear why this occurs, one hypothesis is that perhaps sulfate or other compounds in tap water are interfering with resorufin production. Furthermore, the theoretical amount of sulfate produced using stoichiometry is much higher based off of preliminary data when using ion chromatography to measure the sulfate production. Using ion chromatography we can determine the differences in sulfate production when tap water is used compared to distilled water. We used an Ion Chromatography System (Thermo Scientific) and the samples had no headspace to keep the sulfite from reacting with oxygen preventing additional sulfate production after the initial reaction had occurred. The samples were also kept refrigerated as a precaution.

## 2.6 Future Mesocosm Experiments

This mesocosm (HM 169, Gunt Hamburg) has been altered to obtain desired residence times based off of how long the reaction will take to occur. Dimensions of the mesocosm are used in the numerical models of the hydraulic head throughout the tank.



**Photo 1. Picture of the mesocosm that will be used in experiments.  
(Katherine Santizo, 06/02/2016)**

The planar optodes to be used in later experiments were provided by Dr. Glud and Dr. Larsen at the University of Southern Denmark<sup>12</sup>. Blue LED lights, and computer software (Look@RGB, Fish N' Chips) are also needed for analyzing the data from the optodes. The optodes will confirm DO concentrations predicted by the computer models. Syringes (Sigma Aldrich) will be used to draw samples for sulfate concentration.

## 2.7 Numerical Models

The softwares used were MODFLOW and SEAM3D. They were used to come up with models of hydraulic head throughout the mesocosm and concentrations of chemicals at different locations at steady state. MODFLOW used the dimensions of the mesocosm while the SEAM3D models were a combination of the hydraulic head information from MODFLOW, and the reaction rate of dissolved oxygen consumption. Images of the mesocosm using the optodes and samples from the mesocosm will be used to verify the models or indicate needed adjustments.

### 3. Results and Discussion

#### 3.1 Reaction Rates for Dissolved Oxygen Consumption

Shown below in Figure 1 is raw data of dissolved oxygen versus time when using distilled water, the reactant sodium sulfite, and with the addition of cobalt chloride. The reaction time for resazurin to resorufin reaction to deplete the dissolved oxygen in the water occurs in about 12 seconds. A similar reaction time was also shown during tests using tap water and another run without the addition of the catalyst cobalt chloride. Shown in Figure 2 and 3 respectively is the natural log of the DO versus time and 1 divided by DO versus time. Based off of the three graphs you can determine what order reaction it is. The graph with the most linear slope determines this. Concentration versus time indicates a zeroth order reactions. The natural log of concentration versus time indicates first order. 1/concentration versus time indicates a second order reaction. The natural log of DO graph has the most linear slope and indicates the resazurin to resorufin reaction is a first order reaction. The rate coefficient as shown on Figure 2 is  $0.2845 \text{ s}^{-1}$ . This rate coefficient along with type of reaction (first order) is used in the computer models to show the concentration of dissolved oxygen at steady state in the mesocosm.

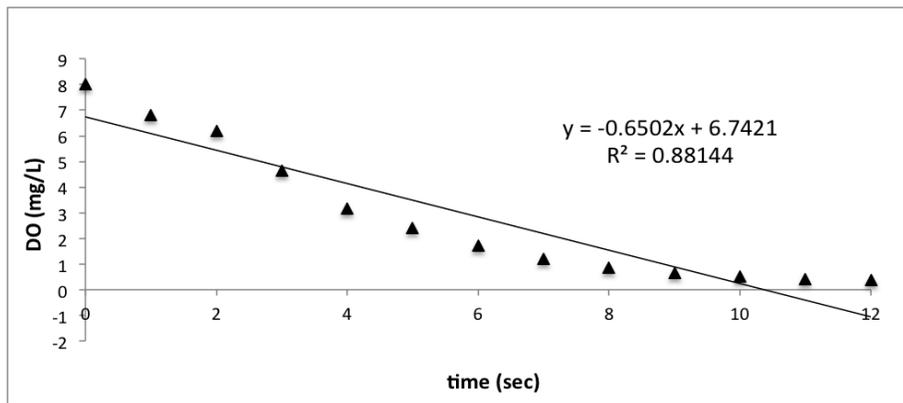


Figure 1. Raw data from YSI ProPlus of DO vs time.

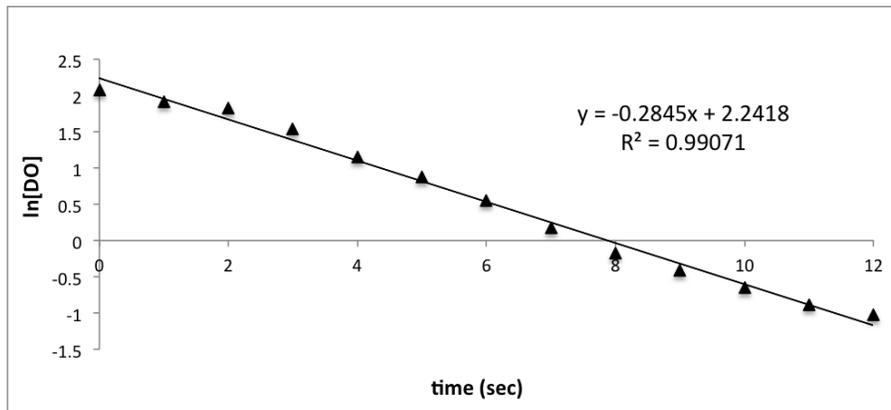
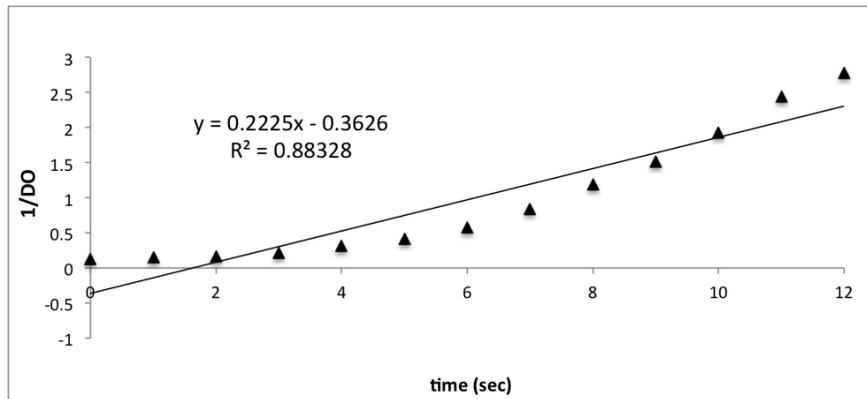


Figure 2. Natural log of DO vs time.



**Figure 3. 1/DO vs time.**

### 3.2 Color Change

Although the time for almost complete dissolved oxygen consumption by sodium sulfite is less than fifteen seconds, it takes about 35-40 minutes for the resazurin to be converted sufficiently to resorufin to make a complete color change (Photos 3-4). Additional results from the reaction without using cobalt chloride and a distilled water solution indicate the time to take slightly longer at about 45 minutes to 1 hour. However, when doing this same test with tap the solution never makes a full color change. This needs to be investigated further to know if tap water can be used in the large mesocosm experiments without using cobalt chloride.



Time = 0 min

Time = 35 min

**Photo 2 and 3. (Aubrey McCutchan, 06/01/2016)**

### 3.3 Permeameter Results

The results as shown in Photo 4 and 5 demonstrate the resazurin to resorufin reaction will be useful as a tracer reaction in the mesocosm because there is a noticeable pink hue that can be seen in the sediment column. Furthermore, the color change happened in about 12 minutes, which is much faster than the time observed in beaker experiments. This result indicates that when mesocosm results are performed the reactions could take a shorter amount of time to occur than expected and the models will need to be adjusted accordingly. Moreover, when the reaction takes place sodium sulfite consumes the dissolved

oxygen first and because the beaker experiments are open, reoxygenation is occurring. In sediment experiments the solution is not open to the air, therefore the sodium sulfite reactant will not be continually consuming DO and will produce resorufin from resazurin much more quickly. This would result in the faster color change that we observed.

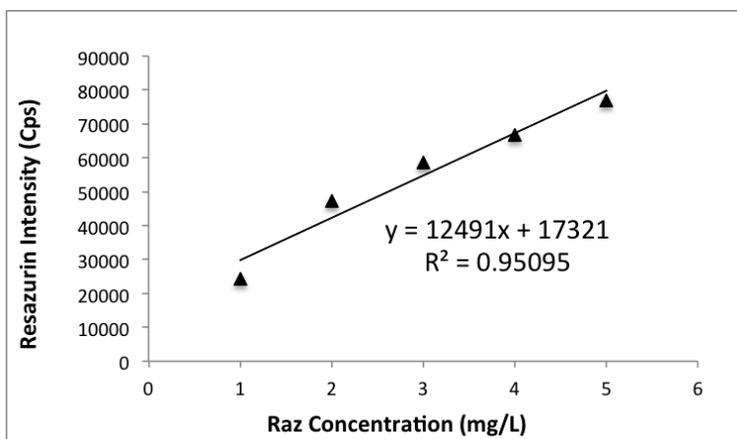


Before reactants are added. Reaction inside of the column.  
Time = 0 min Time = 12 min  
**Photo 4 and 5. (Aubrey McCutchan, 06/09/2016)**

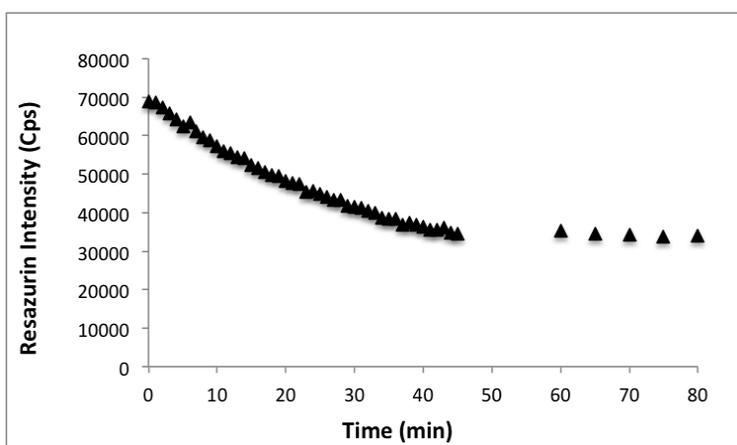
Towards the bottom of the columns note the darker color seen is not the blue resazurin solution but precipitate of the cobalt chloride from sodium sulfite and cobalt chloride mixing. This is another reason to avoid using cobalt chloride in the reaction because the precipitate sticks onto the sand and would most likely interfere with pictures trying to determine resazurin concentration later on.

### 3.3 Spectrofluorometer Results

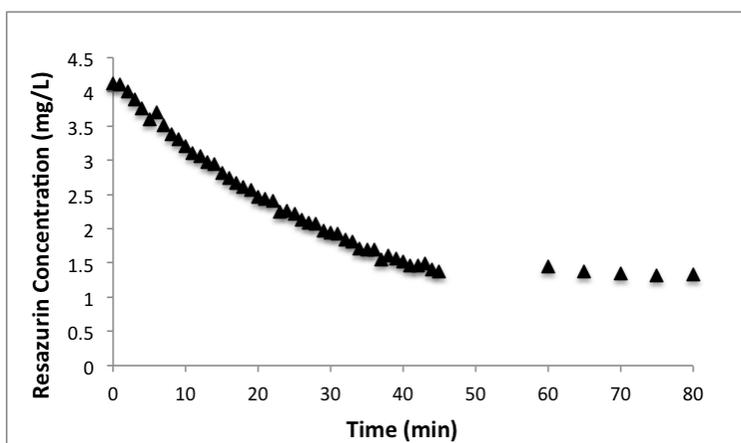
Figures 4 through 6 show the results for the calibration curve, intensity versus time during the reaction, and concentration versus time using the calibration curve equation  $y = 12491x + 17321$  where  $y$  is the intensity and  $x$  is the concentration. Only resazurin values are shown as the resorufin data, although recorded, is not expected to be as accurate because the excitation and emission wavelengths of resorufin to use for the spectrofluorometer were not coming out precisely enough. The data shown will be used for samples taken from future mesocosm experiments to determine the concentration of resazurin.



**Figure 4. Resazurin calibration curve. Raw data from spectrofluorometer of intensity vs concentration.**



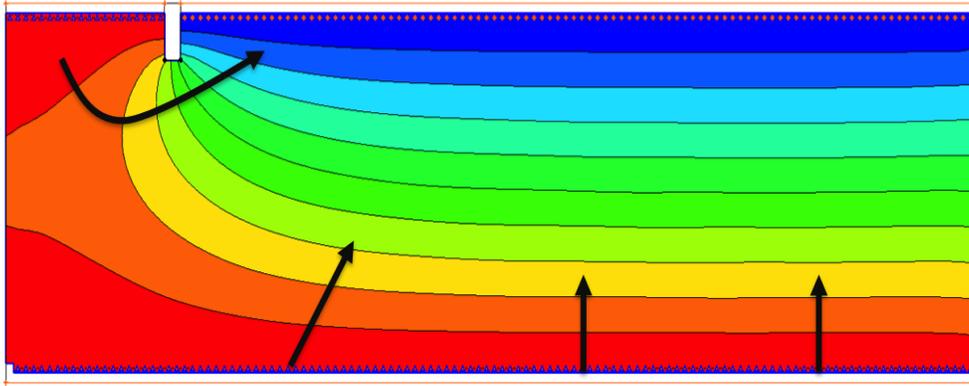
**Figure 5. Resazurin intensity curves. Raw data from spectrofluorometer of intensity vs time.**



**Figure 6. Resazurin concentration curve. Graph using resazurin calibration and intensity curves to show resazurin concentration vs time during the reaction.**

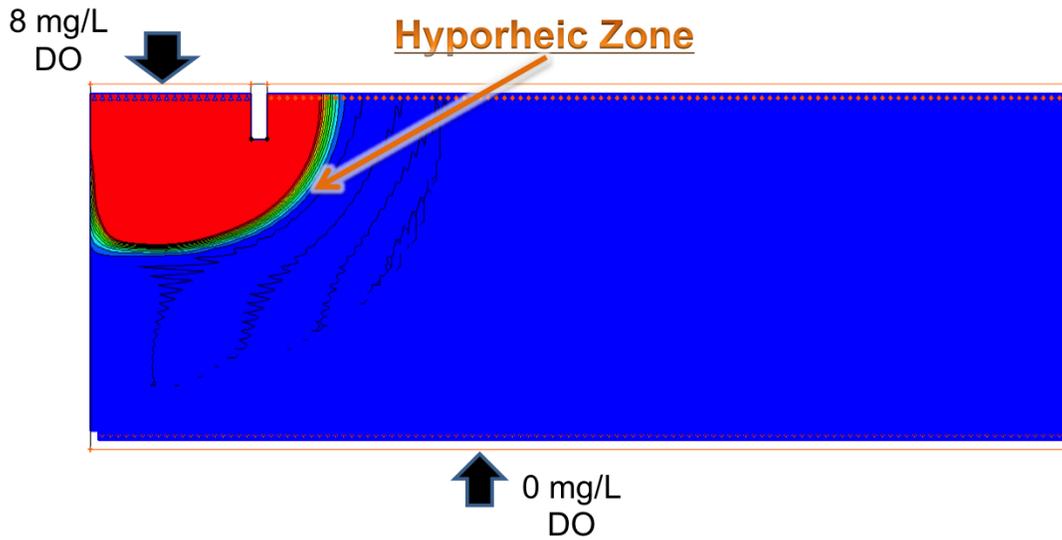
### 3.4 Numerical Models

The three figures below are models of the mesocosm showing the hydraulic head distribution, and the dissolved oxygen and sodium sulfite concentrations at steady state. In these models red indicates a higher value while blue represents lower values.

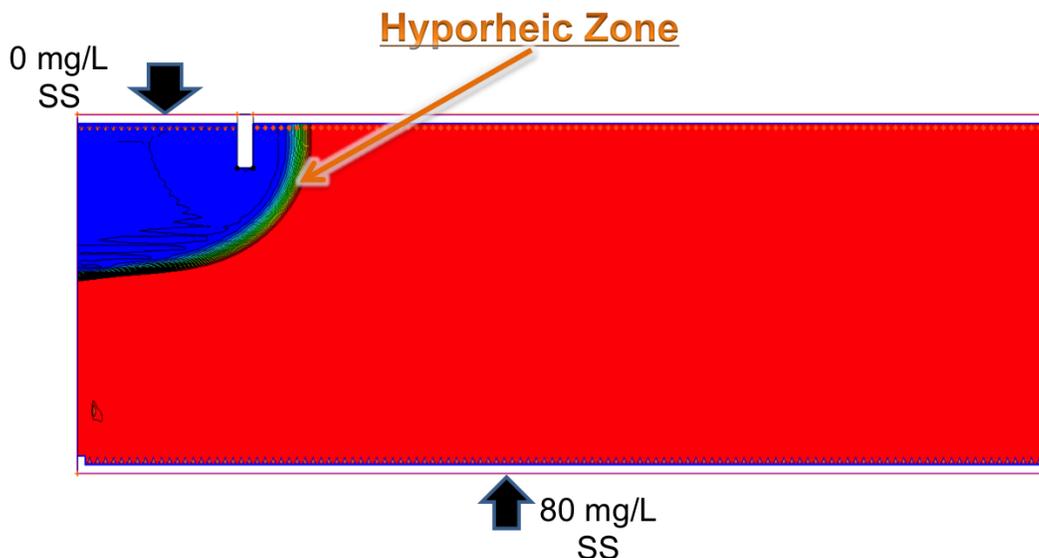


**Figure 7. Hydraulic head distribution of the mesocosm using MODFLOW.**

As indicated by the arrows on Figure 7 this model shows the flow paths of the solutions inside the tank.



**Figure 8. Dissolved oxygen concentration in the mesocosm at steady state using SEAM3D.**



**Figure 9. Sodium sulfite concentration in the mesocosm at steady state using SEAM3D.**

Figure 8 and 9 show the concentration of two of the main components in the resazurin to resorufin reaction. Upwelling water coming in at the bottom of the tank will mimic groundwater. This source will have no dissolved oxygen but will have a sodium sulfite concentration of 80 mg/L. The water flowing in from the top left corner will act as surface water being pushed into the sediment, therefore mixing with the groundwater to create a hyporheic zone as labeled in the models. This source will have a dissolved oxygen concentration but no sodium sulfite. Additionally, in Figure 8 and 9 the hyporheic zone is labeled. Based off of data used for the numerical model this is where we expect the laboratory simulated hyporheic zone to occur. The small multicolored band in each figure represents varying high to low concentrations of dissolved oxygen or sodium sulfite.

### 3.5 Ion Chromatography – Preliminary Results

Data from the ion chromatography tests have not provided meaningful results and more testing is needed. Data showed sulfate production was still much higher than the values we should have received based off of stoichiometry calculations.

## 4. Conclusion

Altogether, this project has made significant progress in determining a method to quantify the amount of which a pollutant can be naturally attenuated in the hyporheic zone. As shown by the data additional testing is still needed to see if the resazurin to resorufin reaction will work in experiments as a reactive tracer for our purposes. Although by the results in this paper, specifically the permeameter data, it can be hypothesized that the reaction will work as a tracer for large laboratory experiments. Furthermore, we have established a reaction rate for dissolved oxygen consumption during the resazurin to resorufin reaction. Moreover, tests on the spectrofluorometer have helped us determine kinetic parameters for the reaction. Through the spectrofluorometer work we can now also estimate resazurin concentration using intensity of samples taken during mesocosm experiments. In future experiments this data and methodology will be used to verify numerical computer models and to make adjustments to them. Later a bacterial component to the mesocosm will be added to understand bacterial remediation that occurs in the hyporheic zone.

## **5. Acknowledgements**

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**NSF REU Interdisciplinary Watershed Sciences and Engineering**  
Virginia Tech, Summer, 2016  
*Assessment Report*  
**John Muffo**

The following is an independent assessment of the level of success of the program conducted during the summer of 2016. As in 2007 through 2009 and in 2011-2015, my role was mainly to develop the entry and exit survey, to conduct the surveys, and then to conduct the focus group at the end of the summer. I had no contact with the faculty and students during the rest of the time when the students were at Virginia Tech.

Abstract

Overall the experience was a positive one for the students involved; they report liking the balance of social and academic activities that the program provides. My impression is that the students took this opportunity to explore whether or not they wanted to go to graduate school. They generally said that they learned a lot and were encouraged by what they learned, i.e., those who were leaning towards graduate school confirmed those leanings even more by gaining experience and those who had doubts about the fit gained confidence in learning that they in fact had the ability to do the work. The one who had the most doubts coming in had those doubts confirmed and is not likely to seek a full-time graduate program after earning a bachelor's degree.

The students in the program on average reported the greatest gains in understanding how to conduct scientific research independently, followed by being able to conduct research in the library and knowing how to communicate those findings in a research paper. Other areas of growth include awareness of the ways in which scientists serve their communities, being able to communicate scientific concepts effectively to a scientific audience, and being aware of the many ways in which scientists in different fields interact with each other in conducting research in water sciences.

In addition, the students seemed to genuinely enjoy each other's company, though I sensed that the lone male might have been isolated from some of the social activities over the summer. The suggestions for improving the program were modest and generally reflected communication and logistical matters, e.g., the inability to deposit large checks locally. On the positive side, they found a number of the presentations interesting, mentioning Dr. Marc Edwards' and the water and wastewater treatment plant site visits in particular.

Entering Survey

There were nine students who completed the pre-test during the summer of 2016. Their responses are below, in order of the highest to lowest average responses. (The questions were developed in cooperation with the faculty who are the Principle Investigators for the project. They were revised in 2015 by deleting some of the questions used during 2011-2014 that were not yielding useful results and adding three others. This allowed for some comparability to the earlier surveys.)

Using the following scale:

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

The entering students provided the following responses upon entry:

- I have an appreciation for the role of faculty in advising students in research work. – 4.56
- I am considering attending graduate school as one of my career options after I graduate. – 4.44
- I have an appreciation for the role of faculty in research work. – 4.44
- I need help from a peer/faculty member/mentor to conduct scientific research. – 4.44
- I have an appreciation for the role of graduate students in research work. – 4.33
- I plan on attending graduate school soon after I graduate. – 4.11
- Water research can be challenging. – 4.11
- I am aware of many ways in which scientists serve their communities. – 4.00
- I have a good understanding of the role of ethics in scientific investigations. – 3.89
- I am aware of many opportunities for employment in the water field. – 3.78
- I am aware of the many ways in which scientists from different fields interact with each other in conducting research in water sciences. – 3.67
- I can communicate scientific concepts effectively to a scientific audience. – 3.33
- I know how to communicate my research findings orally and by documenting it in a research paper. – 3.33
- I plan on joining industry soon after I graduate. – 3.00
- I am confident that I understand how to conduct scientific research independently. – 2.78
- I know everything that I need to know to conduct research in the library. – 2.44

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

- What suggestions do you have for improving the application process for this NSF/REU program?
- Do you think that we should advertise our program on social networking sites like Facebook, MySpace, etc.? Pl. explain your answer.
- Do you have any concerns about the program that you are beginning now? If so, what are they?
- List the top three things that you would like to learn/experience (academically and professionally) during this 10-week long NSF/REU program.

### Exiting Survey

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

- I have an appreciation for the role of faculty in advising students in research work. – 4.89
- I have an appreciation for the role of faculty in research work. – 4.89
- I have an appreciation for the role of graduate students in research work. – 4.78
- I am considering attending graduate school as one of my career options after I graduate. – 4.67
- I am aware of many ways in which scientists serve their communities. – 4.67
- Water research can be challenging. – 4.56
- I have a good understanding of the role of ethics in scientific investigations. – 4.33
- I am aware of the many ways in which scientists from different fields interact with each other in conducting research in water sciences. – 4.22
- I plan on attending graduate school soon after I graduate. - 4.11
- I need help from a peer/faculty member/mentor to conduct scientific research. – 4.11
- I am aware of many opportunities for employment in the water field. – 4.00

- I know how to communicate my research findings orally and by documenting it in a research paper. – 4.00
  - I am confident that I understand how to conduct scientific research independently. – 3.89
  - I can communicate scientific concepts effectively to a scientific audience. – 3.89
  - I know everything that I need to know to conduct research in the library. – 3.44
  - I plan on joining industry soon after I graduate. – 3.29
- 
- I was able to integrate different disciplinary perspectives into my research work. – 4.78
  - I was engaged with interdisciplinary research – 4.67
  - I was engaged with a disciplinary research. – 4.00

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

- Please comment on social activities during the 10-week program. Your suggestions for next year are most welcome.
- Please comment on the weekly seminars you attended during the past 10 weeks. Feel free to list the topics you liked and didn't like. Suggestions for next year are most welcome.
- Please comment on the merit and frequency of presentations you made during the last 10 weeks.
- List the things you learned/experienced (academically and professionally) during this 10-week long NSF/REU program.
- Please comment on any other concern/suggestion/fact you might have related to the 10-week experience that you might want to share.

### Change Over the Summer

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

the time that they began and exited the program. (Note that the numbers in parentheses are negatives.)

- I am confident that I understand how to conduct scientific research independently. – 1.11
- I know everything that I need to know to conduct research in the library. – 1.00
- I know how to communicate my research findings orally and by documenting it in a research paper. – 0.67
- I am aware of many ways in which scientists serve their communities. – 0.67
- I can communicate scientific concepts effectively to a scientific audience. – 0.56
- I am aware of the many ways in which scientists from different fields interact with each other in conducting research in water sciences. – 0.55
- I have an appreciation for the role of graduate students in research. – 0.45
- Water research can be challenging. – 0.45
- I have an appreciation for the role of faculty in research work. – 0.45
- I have a good understanding of the role of ethics in scientific investigations. – 0.44
- I have an appreciation for the role of faculty in advising students in research work. – 0.33
- I know how to communicate my research findings orally and by documenting it in a research paper. – 0.23
- I am aware of many opportunities for employment in the water field. – 0.22
- I plan on attending graduate school soon after I graduate. – 0.00
- I plan on joining industry soon after I graduate. – (0.11)
- I need help from a peer/faculty member/mentor to conduct scientific research. – (0.33)

To summarize, the students reported the greatest gains in understanding how to conduct scientific research independently, followed by being able to conduct research in the library and knowing how to communicate research findings orally and documenting those findings in a research paper. Other areas of reported growth include awareness of the ways in which scientists serve their communities, being able to communicate scientific concepts effectively to a scientific audience, and being aware of the many ways in which scientists in different fields interact with

each other in conducting research in water sciences. Once again, the gains were affected to some degree by how high the students rated themselves on each of these items upon entry into the program.

### Focus Group Results

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

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

- Interaction with graduate students and faculty.
- Exposure to things in academia such as papers, presentations, literature reviews, etc.
- Independent research with some guidance
- Good balance between research work and the social activities and the professional development and the Friday meetings with each other.
- Expansion of view of what you can do with water.
- Know better the employment opportunities regarding water within academia but not outside of academia.

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

- Gives person a chance to dive into research within a limited timeframe, experience it in a summer, engage in it fully, learn if research is something that you want to pursue, to get a better understanding of it.
- Helpful in understanding if water research is interesting.
- Helpful to see if you want to get a master's or Ph.D., to get a basis in research.
- Get some experience with the graduate students and faculty at this particular university if you are considering graduate school here.

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

- Learned a lot of chemistry.
- Learned a lot of microbiology.
- Learned a lot about how science is conducted and communicated and interaction among researchers in a discipline.
- Important to teach yourself.

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

- What the relationship between a faculty mentor and a student conducting research is and how to act professionally within that relationship.
- Effectively communicate research concerns and troubleshooting.
- Communicating research results to experts and to interdisciplinary, more general audiences.

- Navigate the balance between independent work and collaboration.

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

- Of 9 students present, a total of 8 expressed an interest in going to graduate school at the end of the summer.
- Most answered that the program did influence their motivation.
- One of those who intends to go to graduate school stated that she came in intending to go directly for the Ph.D. but now intends to get a master's first and then get some industry experience before going on for a doctorate after talking with graduate students in her program.
- Another found to not work well in a lab setting and therefore not seek to go on to graduate school.
- Another had not given much thought to graduate school but had a positive experience and so is now giving more thought to pursuing graduate study, is more able to make an informed decision now.
- Another is more willing to test the waters now after doing this, has more confidence as a result of this experience, having been encouraged by the graduate mentor to pursue graduate education.

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

- Of 9 students present, a total of 7 expressed an interest in going to graduate school at the beginning of the summer.

*5. How do you think that your communication skills improved as a result of this program?*

*[Probing questions – Verbal? Written? Facebook? YouTube? Other?]*

- Had to present results at research group meetings and presentations.
- Asking the right questions when running into problems.
- Reading papers for the literature reviews and writing them as well.
- Knowing how to present PowerPoint presentations effectively and present complex ideas in them, including the use of animation.
- Communicating results to those not doing the same research.
- Observing the faculty mentor and graduate student mentor talk about their research project with each other, debating various points regarding it.

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

- The nanotechnology one showed a different research path that one could pursue in the water field.
- Drinking water plant was interesting.
- Others good such as wastewater treatment plant and Dr. Edward's presentations.
- Seeing the wastewater treatment plant in person was a positive; had studied it in theory.
- Weather service was not as relevant to what they are studying.

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

- Good.
- Too humid inside the dorm for one of the students; had to leave the dorm several times [she's from Arizona and is not used to our humidity]. The residence hall is not air conditioned.
- The summer was cooler than some of them normally experience.
- They did trips or activities of some kind as a group every weekend.
- One student reported that she interacted with undergrads in her lab who were welcoming and invited her to do things with them over the summer.

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

- Wish checks had been direct deposited. The checks can't be deposited here without with no local checking account, and they are too large to be mailed or deposited with an mobile banking app.
- Disorganization and lack of communication regarding check-in and Friday meetings. The finer points are sometimes missed. The students could have been better informed earlier regarding check-in as one example. Another is a lack of communication as to what they needed for the Friday meetings. Thursday may not have been early enough to allow them sufficient time to get together what they needed to get together for the meetings. The finer points of what was expected for presentations also were not communicated far enough in advance so that the students knew what to expect.
- Recommendation: put together a calendar of events and deadlines in advance, even before students come to campus.
- More than one of the students reported that their graduate student mentors did not seem to understand their roles; one was gone much of the time; some experienced communication problems; some did not understand expectations; experiences were mixed, others had good experiences with grad student and faculty mentors.
- There seemed to be variation in the number of hours worked per week among the students. One claimed to work up to 60 hours per week while most reported more like 40-hour weeks.

9. Other comments?

- Overall positive.
- Dr. Lohani tried to make it a good experience.
- One student reported enjoying it so much that she is applying here for graduate school.

### Concluding Comments

The group in 2016 seemed to be committed to graduate study on average upon entry to the program and, after getting the chance to sample the life of a graduate student over the summer, seemed to solidify and strengthen the earlier leanings. The most popular aspects of the program, in addition to the day-to-day work of simulating the graduate student experience, seem to be those where the students can observe the lives of faculty and graduate students up close. The opportunities to improve professional communication skills rank high as well.

The strongest area of concern has to do with communication and logistics. Communication refers to a lack of specific information on entry to campus and just before making presentations. The

housing situation this summer was particularly uncomfortable due to its lack of air conditioning. In addition, several students were not able to deposit their checks due to not having local checking accounts and other limitations on check deposits. Socially the students appear to have liked living and socializing together and formed a generally cohesive group, though it appeared that the lone male in the group may have been unable to participate in some of the group activities.

One other comment of note now that there are two years worth of data from the newer version of the survey is how consistent the rankings are for the various items or questions for both the entering and exiting survey. It is remarkable that two separate groups of individuals from across the country would be so consistent in their rankings and even raw scores for the items for the two years 2015 and 2016 (see Appendix III). Statistical significance cannot be determined due to the small numbers involved, but the year-to-year consistency is impressive nonetheless.

Overall the NSF REU Interdisciplinary Watershed Sciences and Engineering program in 2016 seemed to provide a sound educational experience for the nine undergraduate students involved. They got to test out being graduate students for ten weeks and improved their knowledge of that experience while also improving their disciplinary knowledge, research skills, and scientific communication skills while forming social bonds with a group of other students from around the country. While this does not guarantee that all will be going on to graduate school in STEM disciplines in the future, it does suggest that those who do should have a better idea of what to expect and therefore be better prepared for the experience.

## **NSF/REU Site Announcements**

## Short Announcement

**Summer 2016 (May 22 – July 30, 2016)**  
**Undergraduate Research Fellowships Announcement**  
**National Science Foundation Research Experiences for Undergraduates (REU) Site**  
**INTERDISCIPLINARY WATER SCIENCES AND ENGINEERING**  
**Virginia Tech, Blacksburg, Virginia**  
**Application Deadline March 13, 2016 (5:00 PM, EST)**

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 22-July 30, 2016) 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 22, 2016 (arrival day) at Virginia Tech and end on July 30, 2016 (departure day). The research internship includes a stipend of \$450/week, housing (two persons per room), meals, and travel expenses (limited to a maximum of \$500 per person). We have already graduated **76** excellent undergraduate researchers representing 55+ institutions in the United States from our prior sites during 2007-09 and 2011-15. Application materials, details of Research Mentors along with summer 2016 research projects and other program activities are posted under “NSF-REU SITE” tab on the following website:

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

Applicants are requested to upload their applications along with other required documents by the deadline (March 13, 2016, 5:00 pm, EST). Successful applicants will be informed by March 21, 2016. Please contact Dr. Vinod K Lohani (phone: (540)231-9545; FAX: (540) 231-6903; E-mail: [vlohani@vt.edu](mailto:vlohani@vt.edu)) for questions.

### Titles of Summer 2016 Projects

Project ID#1. Metal Oxidation in a Drinking Water Reservoir; (Drs. Schreiber and Carey)

Project ID#2: Drinking Water Quality; (Dr. Dietrich)

Project ID#3: Effects of Hydrology on Ecosystem Processes and Water Quality of Streams and Rivers; (Dr. Hester)

Project ID#4: Hydrologic Controls on Wetland Function at the Great Dismal Swamp; (Dr. McLaughlin)

Project ID#5: Recovery of Nutrients and Water from Wastewater Using an Integrated Osmotic Bio-electrochemical System; (Dr. He)

Project ID#6 (A&B): Extension of Online Watershed Learning System for Remote Access of High Frequency Environmental Data and Hydrologic Analysis; (Drs. Lohani, Dymond and Knight)

Project ID#7: Investigation of the occurrence and fate of pharmaceuticals and personal care products (PPCPs) in urban-impacted watersheds; (Dr. Xia )

Project ID#8: Water Conservation and Waterborne Disease Nexus of Faucets; (Drs. Pruden and Edwards )

Project ID#9: Citizen Science Applications of the LEWAS Lab; (Prof. Sharma)

Long Announcement

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Successful applicants (hereafter referred to as REU fellows) will join one of the ongoing research projects in interdisciplinary water sciences and engineering and conduct research under the supervision of Virginia Tech faculty and graduate students. Research projects address interdisciplinary issues related to water science and engineering involving field work, laboratory simulations, literature review, and analysis of data. See Appendices 1 and 2 for list of faculty advisors and typical 2016 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 research internship includes a stipend of \$450/week, housing (two persons per room), meals, and travel expenses (limited to a maximum of \$500 per person).

**Application:** The deadline to receive all application materials (i.e., items 1, 2, and 3 below) is **March 13, 2016 (5:00 PM EST)**.

Applications should be submitted online via the website: <http://www.lewas.centers.vt.edu/>. The application must 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 including a brief justification of your choices (see Appendix 2). The justification should be part of your essay. This should be uploaded as a PDF document using the online application form.
2. Unofficial College transcripts, to be uploaded as a PDF document using the online application form.
3. Two letters of reference to be sent by your referees (Referees are requested to upload a pdf document using a URL that will be emailed to them from our application system. Potential candidates are requested to remind their referees about this requirement. *Letters should address candidate's motivation to pursue research, enthusiasm, reliability, team-work skills and personality.* Applicants are advised to submit their applications asap because only after you submit your online application, an email alert will be sent to your referees requesting their recommendation letters.

Applicants are requested to upload their applications along with other required documents by the deadline (**March 13, 2016, 5:00 PM, EST**)

We will begin contacting the successful applicants by **March 21, 2016**. 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

### REU Site Research Mentors

<b>Name</b>	<b>Organization</b>	<b>Responsibility</b>	<b>Academic Discipline and Field of Study</b>
Dr. Carey	Virginia Tech	Research Mentor; Participant Selection	Biological Sciences; Freshwater Ecology
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. Knight	Virginia Tech	Research Mentor; Participant Selection	Engineering Education; Environmental Science
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. Sharma	Virginia Tech	Research Mentor; Participant Selection	Industrial Design, School of Architecture and Design
Dr. Pruden	Virginia Tech	Research Mentor; Participant Selection	Civil & Environ Eng.; Environmental Contaminants
Dr. Schreiber	Virginia Tech	Research Mentor; Participant Selection	Hydrogeosciences; Chemical Hydrogeology
Dr. Dymond	Virginia Tech	Research Mentor; Participant Selection	Civil & Env. Engineering; Hydrology
Dr. He	Virginia Tech	Research Mentor; Participant Selection	Civil & Env. Engineering; Environmental biotechnology
Dr. McLaughlin	Virginia Tech	Research Mentor; Participant Selection	Forest Resources and Environmental Conservation
Dr. Muffo	Independent Assessment Consultant	Evaluation/Assessment	Academic Assessment

## Appendix 2

### Virginia Tech NSF/REU Site: Interdisciplinary Water Sciences and Engineering Summer 2016: Research Projects REU Site Duration: May 22 – July 30, 2016

#### **Project ID# 1: Metal Oxidation in a Drinking Water Reservoir; Mentors: Drs. Schreiber and Carey**

For this project, the REU intern will work with graduate students and faculty to study ecosystem processes affecting manganese (Mn) and iron (Fe) cycling in the Falling Creek Reservoir (FCR), a drinking water reservoir near Roanoke, VA. The objective is to examine the effects of reservoir stratification, hypolimnetic oxygenation, and epilimnetic mixing on Mn and Fe concentrations in the reservoir. To address the objective, the intern will conduct field sampling at FCR (1-2 times per week) with graduate students. The intern will process field and lab samples and arrange for samples to be analyzed for Mn and Fe. If time allows, the intern will use colorimetric methods to quantify speciation of Mn and Fe. The intern may also collect samples to examine the distribution of Mn-oxidizers in the water column. Using the collected data, the intern will conduct mass calculations to examine Mn and Fe mass in the reservoir. In addition to conducting this independent research, the intern will assist in other sampling campaigns at FCR and will be an integral member of the FCR team. We seek an REU student that has experience and interest in field sampling and data analysis, and can work independently while participating in a fun, collaborative team in the field.

#### **Project ID#2. Drinking Water Quality; Mentor: Dr. Dietrich**

Worldwide, the increasing population/agriculture/industry causes increased natural and anthropogenic contamination at the food-water-energy-human interface. Research projects will focus on the effects of water quality and human health. These projects include: 1) modeling human exposure at the air-water interface to contaminants that are volatilized from drinking water and inhaled by humans; 2) assessing reactivity of zero-valent iron and metal-based nanomaterials in aqueous solutions; 3) investigating communication strategies for informing the public about water quality.

#### **Project ID#3: Effects of Hydrology on Ecosystem Processes and Water Quality of Streams and Rivers; Mentor: Dr. Hester**

This research aims to understand hydrologic processes in streams and rivers and the mechanisms connecting human activities with degradation of stream and river ecosystems and water quality. This will allow better informed ecological stream and river restoration design, pollutant attenuation by natural processes, and watershed planning. Current projects entail laboratory and possibly field work as well as associated data analysis to evaluate exchange processes among river channels, adjacent groundwater, and riparian zones with implications for improving water quality through better stream and river management. The REU participant's role will vary but typical laboratory roles may include culturing riverbed microorganisms, assisting with setup and monitoring of water and tracer dye movement through a laboratory sediment tank; or analyzing photographs of dye movement. Field roles may include sampling riverbeds for microorganisms, or measuring water levels or biogeochemical parameters in streams. Students will also assist with data analysis, and present results in a written report or oral presentation.

**Project ID#4: Hydrologic Controls on Wetland Function at the Great Dismal Swamp**

**Mentor: Dr. McLaughlin**

The Great Dismal Swamp (the Swamp) represents one of the largest (ca. 200,000 acres) forested wetlands in the continental U.S., providing functions at local (e.g., water quality and quantity, habitat) and global (e.g., carbon sequestration) scales. However, the Swamp has been hydrologically impaired resulting in shifted habitats, decreased water storage capacity, increased fire frequency, and loss of stored carbon. To help guide current large-scale restoration efforts, this REU project will link wetland ecosystem and hydrologic monitoring to elucidate the hydrologic effects on wetland function and fire vulnerability. The project will include field research at the Swamp to monitor metrics of ecosystem function (e.g., carbon and water storage, habitat, fire resistance) and hydrologic regime, as well as laboratory analysis of field-collected samples.

**Project ID#5: Recovery of Nutrients and Water from Wastewater Using an Integrated Osmotic Bio-electrochemical System; Mentor: Dr. He**

Sustainable wastewater treatment should significantly clean polluted water while minimize energy consumption of the treatment process and decrease the carbon footprint. Wastewater contains more energy contents than what is required for treatment process, and extracting such contents from contaminants will help accomplish sustainable wastewater treatment. In addition, water and nutrients are valuable resource that can be recovered from wastewater. An innovative system based on synergistic cooperation between microbial electrolysis cells (MECs) and forward osmosis (FO) has been developed at Virginia Tech. MECs oxidize organic matters in wastewater for electricity generation, which drives the recovery of ammonia. Ammonia is then used as a draw solute in FO for recovering high-quality water from the treated wastewater from MECs. In this project, the developed system will be examined for treating the effluent from anaerobic digesters. The REU participant will work with a graduate student and obtain hands-on experiences in reactor setup and operation, and chemical analysis. The participant will be a part of multidisciplinary team and learn the knowledge in engineering, electrochemistry, materials, and biotechnology. The results will be presented in major conferences, and a research paper will be highly desired."

**Project ID#6 (A&B): Extension of Online Watershed Learning System for Remote Access of High Frequency Environmental Data and Hydrologic Analysis; Mentors: Drs. Lohani, Dymond, Knight**

A Learning Enhanced Watershed Assessment System (LEWAS) (old name: LabVIEW Enabled Watershed Assessment System) lab was established on Virginia Tech campus for remotely assessing high frequency water quality and quantity data from a creek that flows through the campus. A water quality sonde provides the capability to sense the temperature, conductivity, dissolved oxygen, turbidity and pH of water. A flow meter and an ultrasonic sensor measure the flow in real time. In addition, a weather station has also been integrated into the LEWAS to allow real-time monitoring of weather parameters including precipitation, temperature, humidity, etc. The data is shared with remote clients via a database on a server that is connected to the Raspberry Pi via a Wireless LAN. Raspberry Pi is a single board computer with LINUX environment that is used here to collect and process data from the LEWAS sensors and to store

the data in a database. Ultimately, a user is able to access the high frequency data from this database in real time through an interactive user interface called the Online Watershed Learning System (OWLS). Eleven NSF/REU participants have worked in this lab since 2007. This project will engage two REU participants who will work in the LEWAS lab. Participant one will learn to calibrate the LEWAS sensors and conduct hydrologic analysis of high frequency (every 3-5 min) water data and develop case studies to demonstrate use of high frequency data in environmental monitoring. Please pick project ID: 6A if you are interested in the hydrologic analysis work. Participant two will engage in programming new features for the OWLS web application using python, HTML5, CSS and JavaScript to help users engaged in environmental monitoring, to access environmental data and to perform analysis. This programming will include tracking these users and finding their navigational paths through the user interface. Please pick project ID: 6B if you are interested in the programming work. The REU participants will be mentored by 2 PhD student/s along with Drs. Lohani, Dymond and Knight. Each participant will write a research paper to document her/his research experiences.

**Project ID#7: Investigation of the occurrence and fate of pharmaceuticals and personal care products (PPCPs) in urban-impacted watersheds; Mentor: Dr. Xia**

Due to rapid urbanization in the State of Virginia and nationwide, many watersheds are increasingly affected by urban activities. Nutrient loading and biological indicators have been the focus for water quality monitoring in the affected watersheds. Limited effort has been devoted to assessing the occurrence of organic contaminants associated with urban activities. The objective of this REU project is to assess urban impact on the water quality of Stroubles Creek Watershed and the New River Watershed by monitoring the levels of PPCPs, often used as indicators for urban impact. The REU student will participate in a team effort to assess how leaky sewer systems affect stormwater water quality and Stroubles Creek water quality by investigating the occurrence and levels of PPCPs in the stormwater and receiving surface stream. The REU 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). The REU student will be working with graduate students under Dr. Xia's guidance and is expected to write up the results and present the work at a research conference.

**Project ID#8: Water Conservation and Waterborne Disease Nexus of Faucets; Mentors: Drs. Pruden and Edwards**

*Legionella pneumophila* is an opportunistic pathogen found in building plumbing. *L. pneumophila* can cause Legionnaire's Disease (severe pneumonia) in immuno-compromised individuals, hospitalizing 8,000 to 18,000 people each year and is responsible for a majority of the waterborne disease deaths in the U.S. At the same time, society is striving to become more energy and water sustainable, prompting installation of low (and lower) flow faucets in hospitals, schools, and homes around the country. Limited sampling to date has raised concern about these new "green" faucets, because they seem to have much higher levels of *L. pneumophila* than normal faucets. This research would be the first to systematically study this issue. In addition to operating a plumbing rig to generate samples and explore the relationship between *L. pneumophila*, flow rates, and flow volumes, the REU student would monitor *L. pneumophila* levels via qPCR and agar plating. Temperature, TOC, ICP and 16S rRNA genes (total bacteria) will also be quantified. The overall goal is to determine if the prior sampling results correctly indicated a systemic problem with low flow faucets causing higher *L. pneumophila*, and to

consider what can be done about it. The plumbing rig will be constructed and ready for the REU to lead experiments summer 2015 in collaboration with a graduate student and under the direction of Drs. Edwards/Pruden. The REU student will write up the results and be encouraged to present work at conferences.

**Project ID#9: Citizen Science Applications of the LEWAS Lab; Mentor: Prof. Sharma**

The Learning Enhanced Watershed Assessment System (LEWAS) is a unique real-time water and weather monitoring system that aims to enhance water sustainability education and research at Virginia Tech. The system collects high frequency (1-3 min.) environmental data (water and weather) from an on-campus site. This data can be used as an indicator of stream health for an on-campus impaired stream. All of these environmental parameters can be accessed by remote users in real-time (see: [www.lewas.centers.vt.edu/dataviewer/](http://www.lewas.centers.vt.edu/dataviewer/)) through a web-based interface for education and research. This project proposes to use qualitative research methods to identify, understand and explore possible user experience scenarios so that the scientific data and its analysis can be presented to different user groups in an appropriate format. Some potential user groups can be elementary school kids, patrons in a public library, students with non- engineering background, and citizens in general. The REU student will be responsible for the following: (i) Get certified by IRB for conducting research (routine process), (ii) Learn about qualitative research methods, (iii) Create a plan of research, (iv) Develop research instruments, (v) Find willing participants, (vi) Meet patrons at the local public library, (vii) Get access to talking with elementary school kids, may be make a presentation to a group, (viii) Students attending summer academy on campus in non- engineering programs, (ix) Conduct structured conversations, (x) Collect and analyze data, (xi) Draw conclusions, (xii) Develop guidelines for kiosk interface design of LEWAS data to the selected user groups, and (xiii) Write a paper about this project and submit it to at least one journal.