VIRGINIA WATER RESOURCES RESEARCH CENTER

2009 NSF REU PROCEEDINGS OF RESEARCH

Research Opportunities in Interdisciplinary Watershed Sciences and Engineering

Edited

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VWRRC Special Report No. SR47-2009

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY
BLACKSBURG, VIRGINIA

Acknowledgments

The research program was supported by the National Science Foundation Research Experiences for Undergraduates Program (NSF-REU Grant No. 0649070). The program directors would like to thank REU-NSF site research mentors/advisors for their kind cooperation and excellent research mentoring during summer 2009. We also would like to thanks our graduate student mentors, undergraduate researchers, laboratory staff, and all other individuals who directly/indirectly contributed to the success of the program.

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Summary

The goal of our NSF REU program is to expose future water scientists and professionals to critical research related to the sustainable management of water resources. The program provides opportunities for participants to acquire advanced analytical and field measurement experience, strengthen their computational and scientific communication skills, and stimulate their professional curiosity.

Summer of 2009 was the third year of our program for this funding cycle. During the 10- week program, NSF REU fellows conducted individual research under the supervision of their research advisors and graduate student mentors. Fellows were required to attend structured weekly forums/seminars on Friday afternoons. These seminars offered by Virginia Tech faculty and graduate students were designed to meet several objectives of the NSF REU program: 1) gain a broader view of water issues; 2) learn how to be a good researcher; 2) learn how to communicate research results verbally and through research reports; and 3) learn about ethics in science and research. At these forums, fellow also learned about conflict resolution and graduate student life experiences.

This document is a compilation of the research papers of our 2009 NSF REU program. Upon completion of the program, fellows prepared PowerPoint presentations and presented their research results to research advisors/mentors and guests. Proceedings of research for previous years are available on our NSF REU website: http://www.vwrrc.vt.edu/nsf_reu.html

2009 NSF REU Fellows and Program Directors

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Understanding Arsenic Bioavailability Processes in *Corbicula fluminea*

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ABSTRACT

C. fluminea (Asian clam) has recently been identified as a potential biosensor for arsenic contamination in natural waters. This study sought to improve current understanding of arsenic bioavailability to *Corbicula* by examining its uptake and accumulation of As (III), As(V), Roxarsone, and monosodium methanearsonate (MSMA). Accumulation of all four As species was observed, with the highest levels of accumulation occurring in clams exposed to As(III). This study also sought to determine if detoxification processes occurring within the gut of *C. fluminea* could alter the speciation of the arsenic to which it was exposed. Biotransformation of As(III) to As(V) was observed, although control data suggest that at lower concentrations biotransformation was a consequence of arsenic absorbance to clam shells rather than internal detoxification pathways. No biotransformation was observed in clams exposed to As(V).

Keywords: arsenic, *Corbicula fluminea*, bioaccumulation, MSMA, Roxarsone, biotransformation

Introduction

Arsenic contamination of drinking water is an issue of global concern. A mobile, ubiquitous metalloid, arsenic can be acutely toxic in high concentrations while a lifetime of low-level exposure has been linked to several types of cancer (Mandal & Suzuki 2002). Although arsenic contamination can occur naturally through leaching from rocks and minerals, human activities such as mining, agricultural applications, and fossil fuel combustion have intensified its accumulation in the environment (Gbaruko, et al. 2008).

Unfortunately, reliable, inexpensive arsenic monitoring methods are not accessible to those areas currently most affected by arsenic contamination, including Bangladesh, West Bengal in India, and Vietnam (Chowdhury, et al. 2000, Berg at al. 2001). Colorimetric field kits, while inexpensive and readily available, are neither sensitive nor accurate enough to detect arsenic below 70 ppb (Harms, et al. 2005), which is well above the World Health Organization's recommended exposure limits (WHO 2001). Laboratory techniques are significantly more accurate, yet are too costly and time-consuming to effectively monitor the decentralized drinking water systems characteristic of developing countries. Over the last decade, these inadequacies have piqued an interest in discovering alternative arsenic detection techniques (Diesel at al. 2009).

The use of in-field biosensors as a method for detecting and monitoring arsenic contamination has been the subject of recent investigation. *Corbicula fluminea*, also known as the Asian clam, is a highly invasive freshwater clam species that has been identified as a biosensor organism of potential interest (Doherty 1990; Peltier, et al. 2008; Shoults-Wilson, et al. 2009). Asian clam populations are currently endemic in many parts of the world, and as of 2002 the Asian clam could be found in at least 42 out of 50 states in the U.S. (Figure 1).

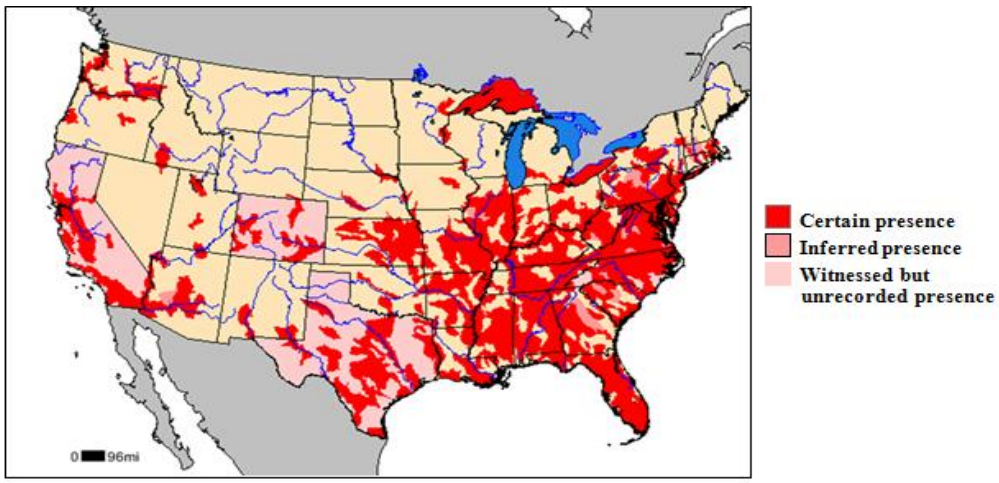


Figure 1. Distribution of *Corbicula Fluminea* in the United States. Adapted from Foster, et al. 2009.

C. fluminea is readily available and could be used to monitor arsenic contamination in natural waters, in addition to serving as a proxy for arsenic accumulation that may be occurring in other aquatic species. *C. fluminea* has recently been shown to accumulate As(III), the most toxic form of inorganic arsenic, in exposure levels of up to 5 ppm in its tissue (Liao, et al. 2008; Santos, et al. 2007). However, no investigation has been made of *C. fluminea's* ability to accumulate other forms of arsenic, including As(V), the other major inorganic form of arsenic, or any organoarsenical compound. Furthermore, it is unknown whether or not any arsenic biotransformation processes occur within the gut of *C. fluminea*. While As(III) is more toxic to humans and is generally the target of public health concerns (Gbaruko, et al. 2008), As(V) is more toxic to algae, which are essential in maintaining the stability of aquatic ecosystems (Pawlik-Skowronska, et al. 2004). Considering established populations of *C. fluminea* can reach densities upwards of 3,250 individuals per square meter in sandy areas (Werner and Rothhaupt 2007), an ability of this species to biotransform existing inorganic arsenic could have a critical impact on human and or ecosystem health. A more complete understanding of arsenic bioavailability processes in *C. fluminea* is necessary if utilization of this organism for arsenic detection is to move forward.

The objectives of this study were two-fold. First, this study sought to understand the bioavailability of four different arsenic compounds to *C. fluminea*: As(III) and As(V), the two major inorganic forms of arsenic, Roxarsone, an arsenic-based chicken feed additive, and MSMA, an organoarsenical herbicide. Roxarsone and MSMA were selected for study as both compounds have been suspected of triggering arsenic contamination of natural waters in the United States (Rutherford, et al. 2003; Dicarlo & Fuentes 2000). As a second objective, this study sought to determine if As(III) and As(V) undergo biotransformation within the gut of *Corbicula*.

Methods

Field Sampling and Acclimation

Clams were collected in three separate batches for the three experiments conducted, with a total of 414 clams collected over an eight week period. All clams were recovered from the same section of the New River flowing through Bissett Park of Radford, Virginia. The New River runs over 320 miles through parts of North Carolina, Virginia, and West Virginia. *C. fluminea* has been present in the New River drainage basin since the late 1970s (Doherty, et al. 1987).

Prior to the start of any experiment, clams were allowed 48-72 hours to depurate in an aerated tank and acclimate to laboratory conditions. A standard, moderately hard, synthetic freshwater was prepared for this acclimation period as well as for consequent experiments. This freshwater was prepared following a formula suggested by the EPA for use in acute toxicity experiments in freshwater organisms, and had the following ionic composition: Cl^- = 1.9 mg L^{-1}, K^+ = 2.1 mg L^{-1}, Na^+ = 26.3 mg L^{-1}, H^+ = 1.2 mg L^{-1}, CO_3^{2-} = 1.2 mg L^{-1}, Mg^{2+} = 12.1 mg L^{-1}, SO_4^{2-} = 81.4 mg L^{-1}, and Ca^{2+} = 14.0 mg L^{-1} (EPA 2002).

Given this composition, the following chemical characteristics were approximately expected: pH = 7.4-7.8, hardness = 80-100 mg $CaCO_3$/L, and alkalinity = 57 -64 mg $CaCO_3$/L (EPA 2002). These ranges were periodically verified in new batch preparations.

Shell sizes and weights were not recorded, as both of these variables have been shown to have no effect on levels of arsenic accumulation in *C. fluminea* (Sebesvari, et al. 2005).

Experimental Setup

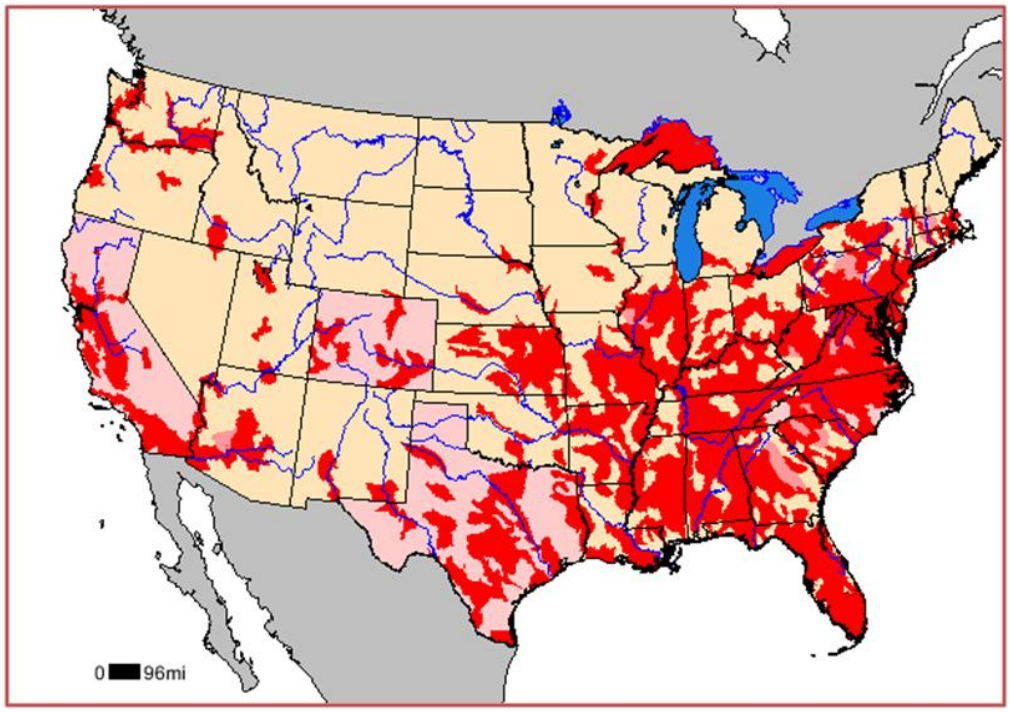
Two separate exposure experiments were conducted examining the accumulation of the four arsenic species of interest in *C. fluminea*.

The first followed the uptake of As(III), As(V), and Roxarsone and was conducted for six days, from June 10-16. For each species five arsenic concentrations were chosen for examination, each differing by a factor of 10: 10 and 100 ppb, and 1, 10 and 100 ppm. Each concentration was examined in triplicates of 100 mL each, with three clams allotted per beaker. Each 100 mL solution was made up from synthetic freshwater and an appropriate aliquot of 1000 ppm sodium arsenite (Na_2AsO_2), sodium arsenate ($Na_2HAsO_{4?}$), or Roxarsone ($C_6H_6AsNO_6$) stock solutions, prepared in the lab. Additionally, a set of triplicate arsenic-free control solutions was included, in order to account for any pre-existing arsenic accumulation in the collected clams. A total of 45 experimental beakers, 3 control beakers, and 144 clams were used in this experiment. In order to monitor changes in the arsenic concentrations of the solutions over time, a 2 mL sample was taken from the first beaker of each of the sixteen triplicates at the start of the experiment, while 1 mL samples were taken from all beakers on Day 2 and Day 6 of the experiment. Samples were preserved with one drop of conc. HNO_3 and refrigerated in opaque bottles.

The uptake and accumulation of As(III), As(V), and MSMA was examined in the second exposure experiment, conducted for five days from June 25-30. Based on the lack of measurable uptake of arsenic at low concentrations observed in the first experiment, higher concentrations were used in the second experiment: 500 ppb, and 1, 5, 10, and 50 ppm As. Experimental setup was much the same as the first experiment, with the MSMA solutions diluted from a 500 ppm stock solution prepared in the lab. In addition to the set of triplicate arsenic-free controls, controls containing only arsenic solution were included for each concentration in order to account for arsenic adsorption to glassware. A total of 45 experimental beakers, 18 control beakers, and 189 clams were required for this experiment. 1 mL samples were taken from all replicates every 24 hours from the start of the experiment to the conclusion. Samples were again preserved with one drop conc. HNO_3 and refrigerated in opaque bottles.

A final exposure experiment was conducted to investigate the ability of *C. fluminea* to biotransform inorganic As species. For the first 71 hours of experimentation, clams were exposed to four different concentrations of As(III) and As(V): 500 ppb, and 1, 5, and 10 ppm. Again, each concentration was examined in triplicate with three clams per beaker. Three sets of triplicate controls were also included for this first half of the experiment: the first containing clams but no arsenic, meant to account for any pre-existing arsenic accumulation in the collected clams, the second containing, zip-tied empty shells, meant to account for any arsenic transformation that may occur on the shell surface and the third containing only arsenic solution and three zip-ties, meant to account for any arsenic transformation resulting from adsorption to the glassware or zip-ties, or resulting from exposure to the atmosphere. The empty-shell controls were obtained from a frozen (-4°C) batch of clams collected May 18, 2008 from the New River. Live clams were not utilized for these controls to avoid the possible secretion of interfering compounds by the clams in response to stress. 24 experimental beakers, 20 control beakers, and 81 live clams were used for this half of the experiment. After 71 hours, all clam-containing beakers were transferred to 50 mL beakers of fresh water and allowed to depurate for six days. 24 experimental beakers and 12 control beakers were required for this half of the experiment. 5 mL samples were taken from the starting solutions of each set of replicates, from each replicate before transferring all clam-containing beakers to fresh water, and at the conclusion of the experiment. Samples were preserved with one drop each of conc. HCl and 0.25 M EDTA and stored in opaque bottles.

All glassware and sample bottles used in these experiments were acid-washed in 0.1 N HCl beforehand to ensure removal of all trace arsenic.



Laboratory Analysis

For the first exposure experiment with As(III), As(V), and Roxarsone, aqueous samples with arsenic concentrations of 10 ppm and below were analyzed for total arsenic using a Graphite furnace atomic absorption spectrophotometer (GFAAS) (Varian, SpectrAA 220Z) with Zeeman background correction. The matrix modifier used for these analyses was a mixture of Pd and $Mg(NO_3)_2$. A detection limit of approximately 3 µg As/L is observed for this instrument while arsenic concentrations of up to 150 µg/L can be analyzed. Due to the high dilution factor necessary for analyzing the higher concentration samples and the loss of accuracy that would have resulted, aqueous samples from 100 ppm replicates were analyzed for total arsenic on an Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES) (Spectro, SpectroFlame Modula Tabletop ICP). The detection limit of this instrument is approximately 20-25 µg As/L while concentrations of up to 10 mg As/L can be analyzed.

At the conclusion of the exposure period, all clams were removed from solution and frozen overnight. Clams were then dissected, rinsed with deionized (DI) water and the tissue removed. Tissue from the three clams in each replicate was combined as one sample and weighed by difference. 5 mL of concentrated HNO_3 was added to each sample and left overnight. Complete digestion of the tissue proceeded the following day in a closed vessel microwave digestion apparatus (CEM, MARS Xpress, 30 min, 1200 W, 200 °C) with an additional 5 mL of HNO_3. 10 and 100 ppb digested clam samples were then diluted to 10% acid and run on the GFAAS, while 1 ppm, 10 ppm, and 100 ppm clam samples were diluted to 5% acid and analyzed on the ICP-AES.

A similar analysis was conducted for samples obtained from the second exposure experiment with As(III), As(V), and MSMA. Aqueous samples with arsenic concentrations of 1 ppm and below were analyzed for total arsenic on the GFAAS, while 10 and 50 ppm aqueous samples were analyzed on the ICP-AES. Clam tissue was also similarly dissected and digested, and 50 ppm digestion samples were analyzed on the ICP-AES while all other concentrations were analyzed on the GFAAS.

For the final exposure experiment examining biotransformation capabilities of *C. fluminea*, arsenic in aqueous samples were speciated on silica-based solid phase extraction columns (Fisher-Scientific, PrepSep SAX) following the method of Le, et al. (2000). Each column was conditioned beforehand with 2 mL of methanol followed by 10 mL of DI water, in order to activate the column bed for maximum efficiency. 1 mL of sample was then diluted to 10 mL and eluted through the column at a rate of 1-2 mL/minute. As (III) was eluted through this first flush while As (V) was released in a second

flush following an injection of 10 mL 0.16 N HNO_3. Each flush as well as 1 mL of the original sample was then analyzed for total arsenic content. 500 ppb and 1 ppm samples were analyzed on the GFAA, as were the 10 ppm samples taken after the 6-day depuration phase. 10 ppm samples taken at the start of the experiment and after the 71-hour exposure period were run on the ICP-AES. 5 ppm samples were not analyzed due to lack of time and the high cost of the extraction columns. A fresh column was used for each speciation.

Results and Discussion

As Accumulation Experiments

Uptake of arsenic by *C. fluminea* was observed for all four arsenic species examined. Uptake proved to be concentration-dependent for As(III), As(V), and Roxarsone-exposed clams (Figures 2 and 3), with the highest rates of uptake occurring at the lowest concentrations. For all four As species examined, uptake of As in the lowest concentration solutions was typically found to be significantly different from uptake in the highest concentration solutions (Appendix). Clams exposed to As (III) had the greatest relative rates of uptake, followed by As(V), then Roxarsone. These data were consistent with tissue concentration data, which found As(III) to be the species most readily accumulated. Uptake of MSMA occurred but was not observed to be concentration dependent (Figure 3). Daily aqueous sampling during the second exposure experiment proved loss of arsenic over time to be non-linear for all arsenic species examined, with the onset of depuration creating an opposing flux of arsenic into solution within 48 hours (data not shown).

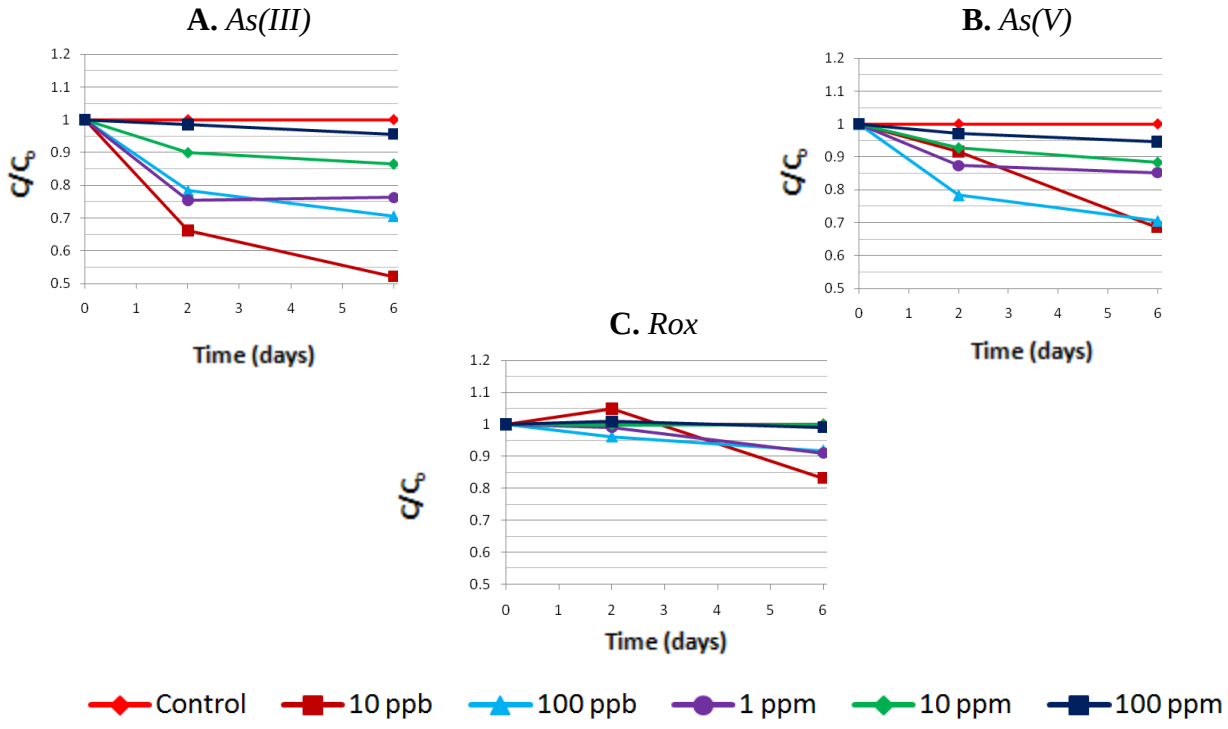


Figure 2. Concentration dependent uptake of As III, As V, and Rox by *C. fluminea* (Exp. 1). Initial As concentrations in experimental solutions were 10 ppb, 100 ppb, 1 ppm, 10 ppm, and 100 ppm. As concentration measurements were taken at 0 days (initial concentration), 2 days, and 6 days. **(A)** Uptake of As III. **(B)** Uptake of As V. **(C)** Uptake of Roxarsone.

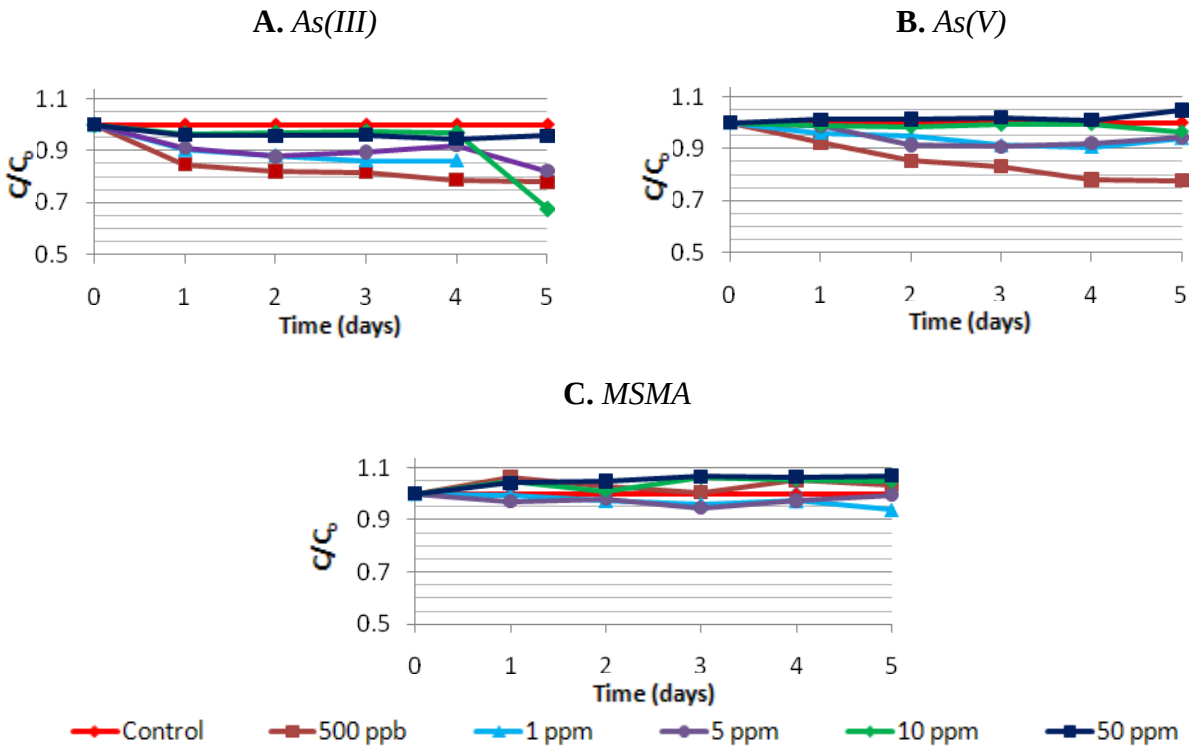


Figure 3. Concentration dependent uptake of As III, As V, and MSMA by *C. fluminea* (Exp. 2). Initial As concentrations in experimental solutions were 500 ppb, 1 ppm, 5 ppm, and 10 ppm. As concentration measurements were taken every 24 hours starting at 0 days (initial concentration). **(A)** Uptake of As III. **(B)** Uptake of As V. **(C)** Uptake of MSMA.

Arsenic accumulation in clam tissue was also observed for all four arsenic species (Table 1 in Appendix). For the first exposure experiment (Figure 4), arsenic accumulation in only the clams exposed to 10 ppm and 100 ppm solutions was significant enough to be quantified, while arsenic in clams exposed to 10 ppb, 100 ppb, and 1 ppm solutions was at or below the detection limit (Figure 4, B). The highest quantities of arsenic were found in clams exposed to As(III), with considerably less accumulation in those exposed to As(V) and Roxarsone. Significantly more arsenic accumulation was seen for each species at higher concentrations, though aqueous sampling found rates of uptake at these concentrations to be lower. In the second exposure experiment (Figure 5), arsenic accumulation in all clams was above the detection limit. At exposure concentrations of 500 ppb, 1 ppm, and 5 ppm, the highest quantities of arsenic were observed in clams exposed to MSMA (Figure 5, B), although differences between species were not statistically significant (Appendix). Similar levels of accumulation were observed in all clams for the 10 ppm As species, while for the 50 ppm As solutions significantly higher arsenic accumulation was found in those clams exposed to As(III).

Overall, the least accumulation of As in clam tissue was observed with Roxarsone and MSMA-exposed clams. Both Roxarsone (MW=263.04 g/mol) and MSMA (MW=161.95 g/mol) are relatively large in comparison to As(III) and As(V), and therefore may have been mostly rejected by *Corbicula's* feeding mechanism. As an alternate explanation, it is possible that each of these compounds was taken up by *C. fluminea*. However, cleavage of As(V) from the molecular structure of each compound may have been rare, and as a result, both Roxarsone and MSMA may have passed through *Corbicula's* gastric system with very little accumulation of As in tissue resulting.

Meanwhile, the highest accumulation of As was seen in As(III)-exposed clams, although the reasoning for this remains unclear. In the biotransformation experiment conducted, it was observed that

clams exposed to 10 ppm As(III) oxidized the As in their environment via an internal detoxification pathway. It is possible that this detoxification pathway necessitates a lengthy As(III) residence time, resulting in the high As accumulation observed in clams exposed to As(III) solutions. Considering this internal detoxification mechanism was only observed at a minimum concentration of 10 ppm As(III), this would also explain why significantly greater As(III) accumulation was only observed at exposure concentrations of 10 ppm and above. However, further study is required to validate this hypothesis.

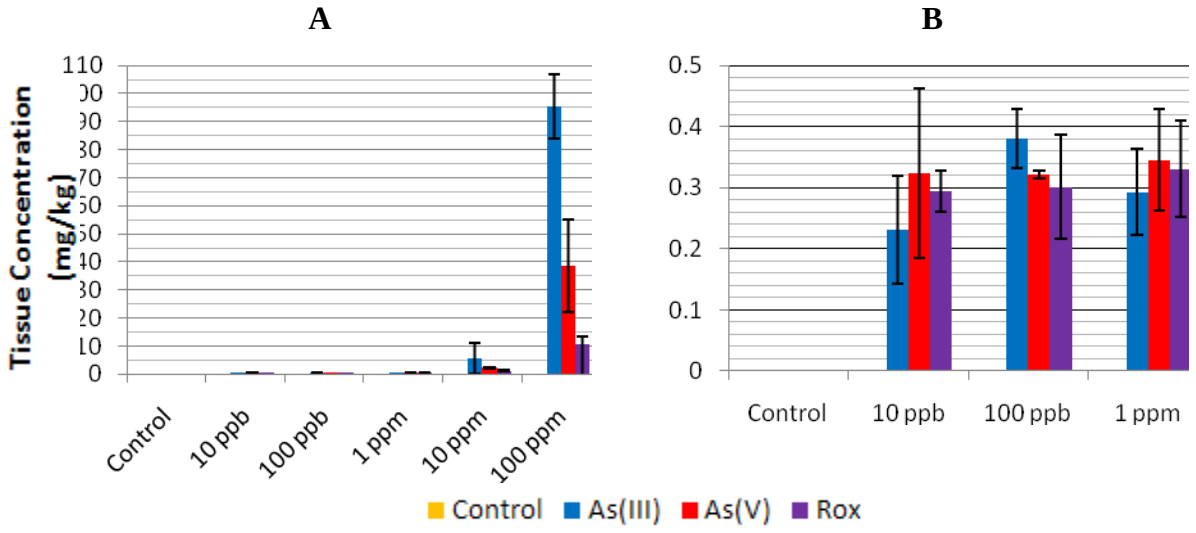


Figure 4. Arsenic Accumulation in *C. fluminea* Tissue after Exposure to As(III), As(V), and Roxarsone (Exp. 1). (A) Arsenic accumulation at all five concentrations. **(B)** Arsenic accumulation (mg/kg) in clams exposed to 10 ppb, 100 ppb, and 1 ppm As solutions was at or below the detection limit of the AA. Controls were analyzed on the ICP, which provided no value for accumulation below the detection limit.

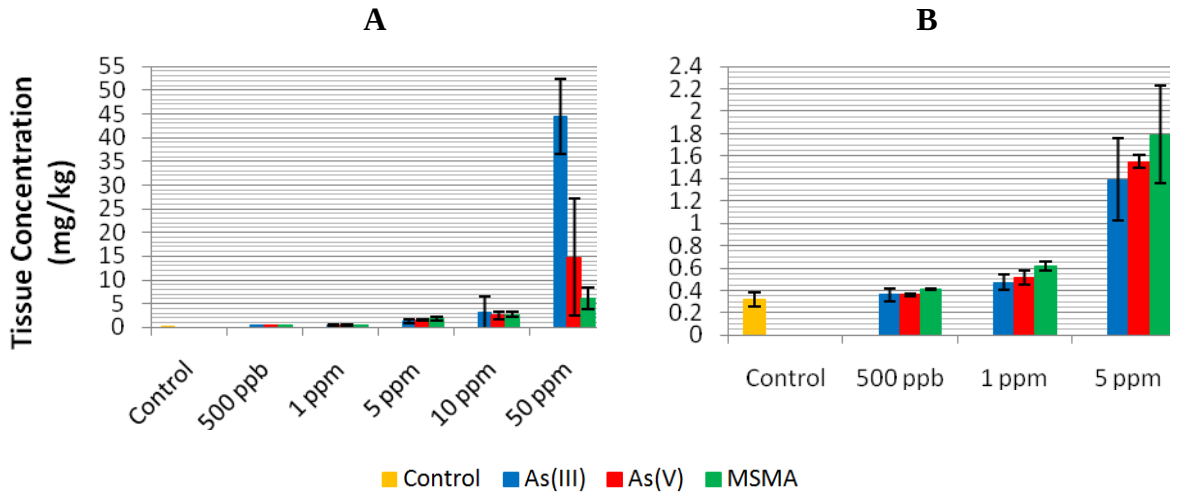


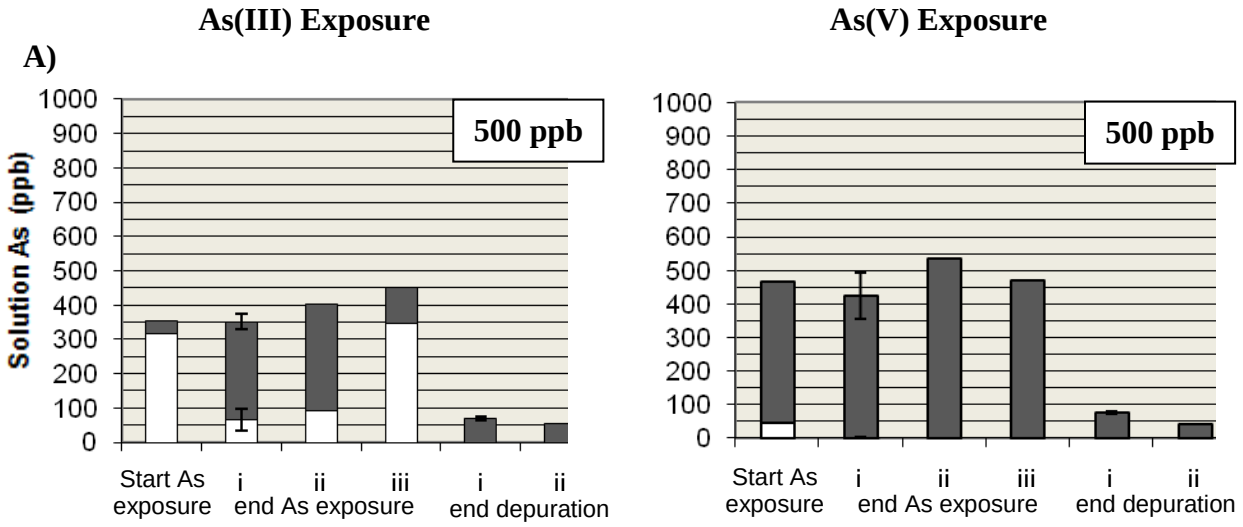
Figure 5. Arsenic Accumulation in *C. fluminea* Tissue after Exposure to As(III), As(V), and MSMA (Exp. 2). (A) Arsenic accumulation (mg/kg) was quantifiable at all five exposure concentrations. **(B)** Arsenic concentration at the three lowest exposure concentrations with controls.

Observations recorded during the duration of these experiments found As(III) to be the most lethal species to *C. fluminea*, with As (V) showing toxicity only at the highest concentrations and after more than four days of exposure. In the first exposure experiment, the majority of clam death occurred in the 100 and 10 ppm As(III) solutions, with only one clam death recorded in a 100 ppm As(V) solution at the very end of the exposure period. Maximum experimental concentrations were lowered for the second exposure experiment to eliminate fatalities, but the 50 ppm As(III) solution still proved lethal. Clams ceased to feed both at toxic concentrations and after a toxic duration of exposure, but such avoidance behavior appeared to have no effect on the levels of arsenic accumulating in their tissue. Roxarsone and MSMA did not appear toxic to *C. fluminea*, even at the highest tested concentrations. These findings are consistent with results typically achieved in toxicity testing of organic arsenic compounds (Abernathy, et al. 1999).

Biotransformation Experiment

Speciation results obtained on the extraction columns were generally consistent with total arsenic readings obtained for original samples on the GFAAS and ICP. For most samples, the sum of their As(III) and As(V) extractions fell within 10% of the total arsenic readings for that solution (data not shown). Many samples had negative percent differences, indicating arsenic content that was in the original sample but was not captured by the As(III) and As(V) extractions. Common organic arsenic metabolites such as monomethylarsonate (MMA) and dimethylarsinate (DMA) may account for this unidentified arsenic content (Oremland and Stoltz 2003). Large positive percent differences were observed when arsenic levels in the speciation samples were at or below the detection limit.

Considerable oxidation of arsenic was observed in the feeding and depuration products of clams exposed to As(III) solutions, while no transformation of any kind was observed with clams exposed to As(V) (Figure 7). Start solutions for 500 ppb and 1 ppm As (III) replicates contained little to no As (V). At the conclusion of the 71-hour exposure period, however, an average of more than 2/3 of arsenic content in replicate solutions had been oxidized to As(V) as a result of feeding activity. Only a small portion of this oxidation was due to atmospheric oxidation and or adsorption to glassware, as illustrated by the 500 ppb and 1 ppm no-clam controls. Interestingly, oxidation observed in the live clam replicates was observed to be similar to oxidation observed in the zip-tied shell controls, suggesting that at these concentrations, transformation of arsenic species during feeding was a function of arsenic adsorption processes occurring on the clam shell rather than a result of internal biotransformation process.



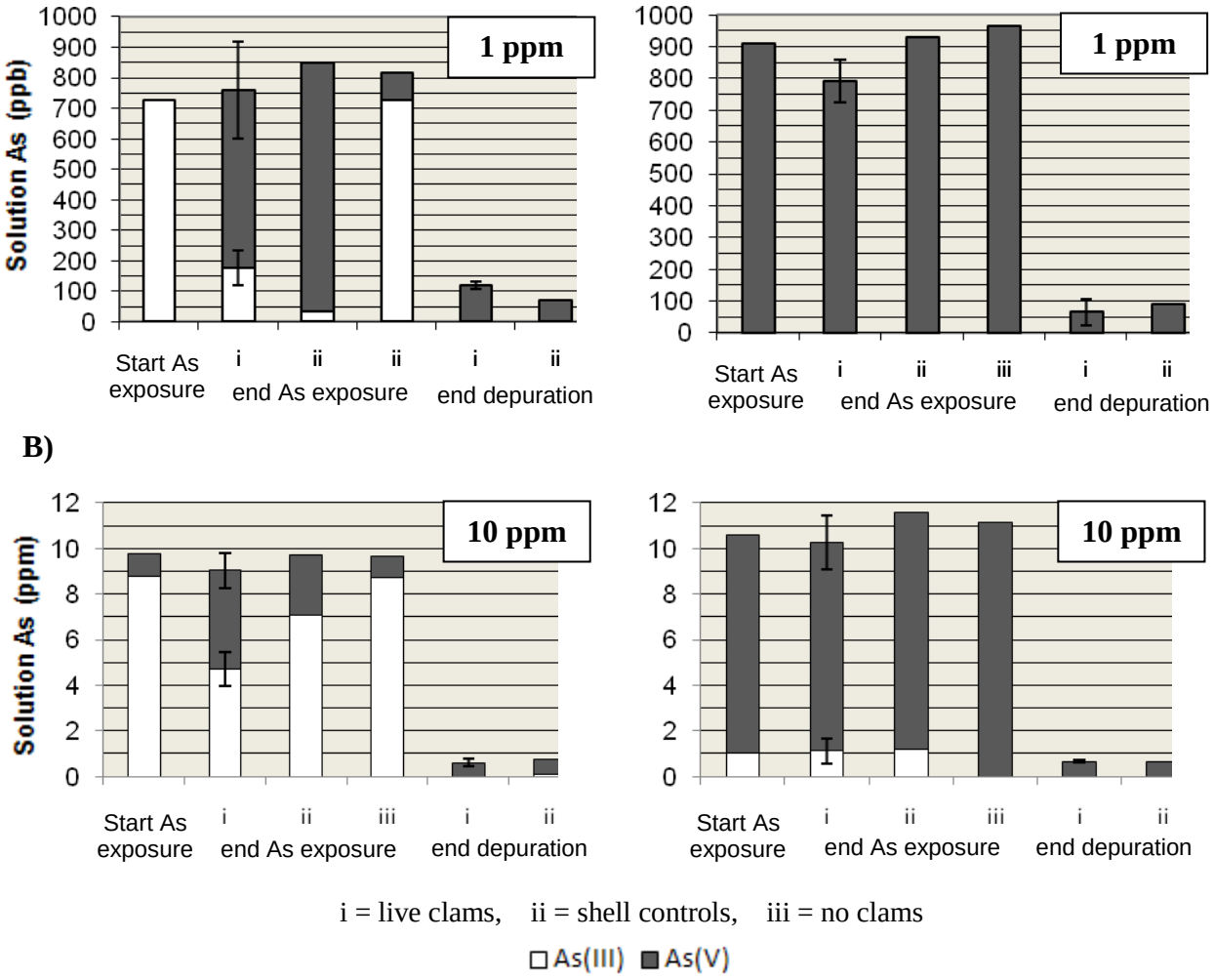


Figure 6. Transformation of Arsenic Species in Solution When Exposed to *C. fluminea*. The x-axis marks the time at which samples were taken. Clam-free controls (iii) indicate the amount of oxidation resulting from exposure of experimental solutions to the atmosphere. All As concentration readings below the detection limit were set to zero.

In the clams exposed to 10 ppm As(III) solutions, however, conversion of As(III) to As(V) after the 71-hour exposure period was significantly greater with the live clams than with the shell controls. Therefore, at this concentration, oxidation observed during *C. fluminea's* exposure to As could not have been a result of simply shell adsorption processes. Instead, these results suggest that internal As(III) detoxification processes do occur in *C. fluminea*, although they may be diminished at lower concentrations or, alternatively, may only engage at high exposure concentrations. Additionally, these results suggest that while shell transformation of As(III) to As(V) is certainly taking place at a range of As concentrations, this potential can be maximized at higher concentrations.

At the end of the six-day exposure period, all arsenic depurated by live clams and released by shell controls was in the form of As(V). No transformation was observed with clams initially exposed to As(V) solutions in any of the three experimental concentrations. As(V) remained in the same form during the 71-hour exposure period, with occasional oxidation of any trace As(III) present in the start solutions, likely through exposure to the atmosphere (Figure 6, A). Only As(V) was depurated by the live clams and released by the shell controls after the 6-day exposure period.

A possible abiotic mechanism for the transformation of As(III) to As(V) observed with the clam shell controls could be the adsorption of As(III) to manganese oxides. Manganese dioxides (MnO_2) have been shown to oxidize As(III) to As(V) at a decreasing rate over the first 2-3 days of initial exposure (Driehaus, et al. 1995). $MnO_{(x)}$ can be directly deposited onto the shells of freshwater invertebrates via the activity of microorganisms, while manganese itself has been shown to accumulate in invertebrate shells and tissue, particularly freshwater invertebrates' (Gordon, et al. 1970; Bourget 1974). It is unknown whether manganese accumulated within C. *fluminea* is oxidized to produce the same effects as MnO_2, but ubiquitous species of bacteria are capable of triggering this transformation (Driehaus at al. 1995). Accumulation of Mn is proportional to its concentration in the aquatic environment (McCorkle and Dietz 1980), and in the New River watershed, where clams used in this study were collected, manganese ore is commonly associated with the limestone deposits prolific in the region (Harder 1910). However, the Virginia DEQ does not sample for manganese at the sampling site.

Alternatively, a biotic mechanism could explain the oxidation of As(III) by clam shells observed in this experiment. Several species of ubiquitous bacteria are capable of oxidizing As(III) to As(V), a chemical reaction by which many of these species derive energy (Oremland and Stolz 2003). Although the shell controls used were obtained from a batch of clams that had been frozen (-4°C) for over a year, which are less than optimal storage conditions for bacteria (Haines 1938; Proom and Hemmons 1949), it is possible that some of the bacterial strains inhabiting the shells were able to survive, and simply remain dormant. If any of these strains were capable of oxidizing As(III) to As(V), thawing of the clam shells for use in this experiment could have allowed these bacteria to resume their activity. However, the possibility of this mechanism's occurrence remains slight.

Conclusion

C. fluminea shows promise as a biosensor organism for arsenic due to its prevalence, typically high abundance, and its relative robustness against a variety of organic and inorganic pollutants (Sebesvari, et al. 2005). Previous studies examining the bioavailability of As to *C. fluminea* have only experimented with As(III) (Santos, et al. 2007; Sebesvari, et al. 2005; Liao, et al. 2008, 2009). However, as this research shows, *C. fluminea* is capable of accumulating a variety of As species, including As(III), As(V), Roxarsone and MSMA. This capability deems it a particularly suitable organism for arsenic monitoring purposes, as arsenic contamination can occur in a variety of forms. Furthermore, this research identified the ability of *C. fluminea* to oxidize inorganic As (III) in its environment through an external shell adsorption mechanism, and, at higher concentrations, an internal detoxification pathway. These results suggest that, in addition to serving as a biomonitoring organism, *C. fluminea* could also play a role in natural remediation of arsenite-contaminated aquatic systems.

Recommendations for Future Research

Much research must be completed before *C. fluminea* can be implemented as an arsenic biomonitoring organism on a wide scale. First, while this research suggests that in-situ monitoring of any of the four As species examined is possible with *C. fluminea*, it remains that there are a variety of environmental factors that could influence each species' accumulation in clam tissue, including the age of the clams sampled, the sampling season, and the chemistry of the immediate ecosystem (Shouts-Wilson, et al. 2009). Further research must be conducted to assess the influence of each of these factors on arsenic bioavailability to *C. fluminea*. Secondly, the applicability of *C. fluminea* as a biomonitoring organism in water bodies where it is not yet present must also be investigated. Currently, the utility of *C. fluminea* as an arsenic biomonitoring organism is limited to those regions where the species is already established. The extent of its utility would be greatly increased if a method could be developed that allows for the transplantation of live clams while preventing the release of their gametes.

Acknowledgements

Special thanks to Dr. Madeline Schreiber and Beth Diesel of the Department of Geosciences for their continued guidance and support during this project. Acknowledgements are also due to Matt Hull, Jason

Jones, Oluyinka Oyewumi, Dr. Tamim Younos and Dr. Vinod Lohani. This research was made possible by the National Science Foundation REU grant (No. 0649070) awarded to Virginia Tech.

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APPENDIX

Table 1: Accumulation of As(III), As(V), Roxarsone, and MSMA in *C. fluminea* after 5-6 Days of Exposure: Summary Statistics.

Exp. 1 Samples	Ave Accumulation As in tissue (mg/kg)	Exp. 2 Samples	Ave Accumulation As in tissue (mg/kg)
Control	BDL[1]	Control	0.32 ± 0.07
10 ppb As(III)	0.23 ± 0.09[2]	500 ppb As(III)	0.36 ± 0.06
10 ppb As(V)	0.32 ± 0.14	500 ppb As(V)	0.36 ± 0.01
10 ppb Rox	0.29 ± 0.03	500 ppb MSMA	0.41 ± 0.01
100 ppb As(III)	0.38 ± 0.05	1 ppm As(III)	0.47 ± 0.07
100 ppb As(V)	0.32 ± 0.01	1 ppm As(V)	0.51 ± 0.06
100 ppb Rox	0.30 ± 0.09	1 ppm MSMA	0.62 ± 0.04
1 ppm As(III)	0.29 ± 0.07	5 ppm As(III)	1.39 ± 0.37
1 ppm As(V)	0.35 ± 0.08	5 ppm As(V)	1.55 ± 0.06
1 ppm Rox	0.33 ± 0.08	5 ppm MSMA	1.79 ± 0.44
10 ppm As(III)	5.64 ± 5.49	10 ppm As(III)	3.07 ± 3.51[3]
10 ppm As(V)	2.24 ± 0.36	10 ppm As(V)	2.54 ± 0.81
10 ppm Rox	1.22 ± 0.27	10 ppm MSMA	2.71 ± 0.51
100 ppm As(III)	95.26 ± 11.53	50 ppm As(III)	44.46 ± 8.01
100 ppm As(V)	38.59 ± 16.49	50 ppm As(V)	14.78 ± 12.41
100 ppm Rox	10.56 ± 2.73	50 ppm MSMA	6.16 ± 2.30

1. BDL = below detection limit.
2. ± = standard deviation from triplicate experiments.
3. Replicate 1 data excluded form ave and stdev.

Statistical Analyses

On Concentration-Dependent Uptake Data

As(III) Uptake – Exp. 1

One-way ANOVA Summary

Source	SS	Df	MS	F	P
Treatment (between groups)	0.363854	4	0.090963	7.24	0.005255
Error	0.125682	10	0.012568		
Total	0.489535	14			

Tukey HSD test

Vs.	M1	M2	M3	M4	M5
M1		nonsignificant	nonsignificant	P<0.05	P<0.01
M2	nonsignificant		nonsignificant	Nonsignificant	P<0.05
M3	nonsignificant	nonsignificant		Nonsignificant	nonsignificant
M4	P<0.5	nonsignificant	nonsignificant		nonsignificant
M5	P<0.1	P<0.05	nonsignificant	Nonsignificant	

Notes: M1= Ending concentrations for 10 ppb replicates, M2= for 100 ppb replicates, M3= for 1 ppm replicates, M4= for 10 ppm replicates, M5= for 100 ppm replicates

As(V) Uptake – Exp. 1
One-way ANOVA Summary

Source	SS	Df	MS	F	P
Treatment (between groups)	0.155867	4	0.038967	15.86	0.000249
Error	0.024564	10	0.002456		
Total	0.180431	14			

Tukey HSD Test

Vs.	M1	M2	M3	M4	M5
M1		nonsignificant	P<0.05	P<0.01	P<0.01
M2	nonsignificant		P<0.05	P<0.01	P<0.05
M3	P<0.05	P<0.05		Nonsignificant	nonsignificant
M4	P<0.01	P<0.01	nonsignificant		nonsignificant
M5	P<0.01	P<0.05	nonsignificant	Nonsignificant	

Notes: M1, M2, M3, M4, M5=Same as Exp. 1: As(III) Uptake

Rox Uptake – Exp. 1
One-way ANOVA Summary

Source	SS	Df	MS	F	P
Treatment (between groups)	0.098331	4	0.024583	6.53	0.007508
Error	0.037664	10	0.003766		
Total	0.135995	14			

Tukey HSD Test

Vs.	M1	M2	M3	M4	M5
M1		nonsignificant	nonsignificant	P<0.05	P<0.01
M2	nonsignificant		nonsignificant	Nonsignificant	nonsignificant
M3	nonsignificant	nonsignificant		Nonsignificant	nonsignificant
M4	P<0.05	nonsignificant	nonsignificant		nonsignificant
M5	P<0.01	nonsignificant	nonsignificant	Nonsignificant	

Notes: M1, M2, M3, M4, M5=Same as Exp. 1: As(III) Uptake

As(III) Uptake – Exp. 2
One-way ANOVA Summary

Source	SS	Df	MS	F	P
Treatment (between groups)	7.009013	3	2.336338	36.79	<0.0001
Error	0.507993	8	0.063499		
Total	7.517006	11			

Tukey HSD Test

Vs.	M1	M2	M3	M4
M1		P<0.01	nonsignificant	Nonsignificant
M2	P<0.01		P<0.01	P<0.01
M3	nonsignificant	P<0.01		Nonsignificant
M4	nonsignificant	P<0.01	nonsignificant	

Notes: M1= Ending concentrations for 500 ppb replicates, M2= for 1 ppm replicates, M2= for 5 ppm replicates, M3= for 10 ppm replicates, M4= for 50 ppm replicates
1 ppm data were contaminated, not included in analyses or graphs

As(V) Uptake – Exp. 2
One-way ANOVA Summary

Source	SS	Df	MS	F	P
Treatment (between groups)	0.056196	4	0.014049	4.33	0.027398
Error	0.032411	10	0.003241		
Total	0.088607	14			

Tukey HSD Test

Vs.	M1	M2	M3	M4	M5
M1		nonsignificant	nonsignificant	P<0.05	P<0.05
M2	nonsignificant		nonsignificant	Nonsignificant	nonsignificant
M3	nonsignificant	nonsignificant		Nonsignificant	nonsignificant
M4	P<0.05	nonsignificant	nonsignificant		nonsignificant
M5	P<0.05	nonsignificant	nonsignificant	Nonsignificant	

Notes: M1= Ending concentrations for 500 ppb replicates, M2= for 1 ppm replicates, M3= for 5 ppm replicates, M4= for 10 ppm replicates, M5= for 50 ppm replicates

MSMA Uptake – Exp. 2
One-way ANOVA Summary

Source	SS	Df	MS	F	P
Treatment (between groups)	0.032437	4	0.008109	6.71	0.006842
Error	0.012091	10	0.001209		
Total	0.044528	14			

Tukey HSD Test

Vs.	M1	M2	M3	M4	M5
M1		P<0.05	nonsignificant	Nonsignificant	nonsignificant
M2	P<0.05		nonsignificant	P<0.05	P<0.01
M3	nonsignificant	nonsignificant		Nonsignificant	nonsignificant
M4	P<0.05	P<0.05	nonsignificant		nonsignificant
M5	P<0.05	P<0.01	nonsignificant	Nonsignificant	

Notes: M1, M2, M3, M4, M5=Same as Exp. 2: As(V) Uptake

On Exp.2: Diff in As Accumulation between 500 ppb-1 ppm species

500 ppb samples
One-Way ANOVA Summary

Source	SS	Df	MS	F	P
Treatment (between groups)	0.0123	3	0.0041	2.3	0.154036
Error	0.014267	8	0.001783		
Total	0.026567	11			

Notes: Sample 1=Controls, Sample 2=500 ppb As(III) replicates, Sample 3=500 ppb As(V) replicates, Sample 4=500 ppb MSMA replicates
No significant diff. between samples, F ratio not significant.

1 ppm samples
One-way ANOVA Summary

Source	SS	Df	MS	F	P
Treatment (between groups)	0.032822	3	0.016411	4.42	0.066092
Error	0.022267	6	0.003711		
Total	0.055089	8			

Notes: Sample 1=1 ppm As(III) replicates, Sample 2=1 ppm As(V) replicates, Sample 3=1 ppm MSMA replicates
No significant diff. between samples, F ratio not significant.

5 ppm samples
One-way ANOVA Summary

Source	SS	Df	MS	F	P
Treatment (between groups)	0.2546	3	0.1273	1.16	0.375045
Error	0.6584	6	0.109733		
Total	0.913	8			

Notes: Sample 1=5 ppm As(III) replicates, Sample 2=5 ppm As(V) replicates, Sample 3=5 ppm MSMA replicates
No significant diff. between samples, F ratio not significant.

All analyses run on online tool provided by Dr. Richard Lowry, Vassar College's Department of Geosciences: http://faculty.vassar.edu/lowry//anova1u.html

Profile of Ferrous Iron Flavor Intensities and Lipid Oxidation Products

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ABSTRACT

The mechanisms and flavor caused by ferrous iron are poorly understood. The goal of this study was to identify any relationships between metallic flavor intensity, levels of lipid oxidation and changes in metallic salivary composition caused by the introduction of ferrous iron at various concentrations into the oral cavity. Panelists were unable to discriminate between 0.2 and .5 mg/L or 1 and 5 mg/L ferrous iron. The introduction of metals had no effect on the salivary pH and electrolyte composition. The change in TBARS from control to metallic sample increase from 2.5 to 5 to 10 mg/L but did not differ between 10 and 20 mg/L Fe^{2+}.

Key Words: Lipid Peroxidation, Ferrous Iron, Saliva, TBARS, F_2-isoprostanes

Introduction

Iron is one of the three most common culprits of metallic flavor in drinking water (Burlingame 2007). Iron can either occur naturally in groundwater or come from the corrosion of pipes in a distribution system (Volk, Dundore, et al. 1967). The flavor generated by ferrous solutions has been described as metallic, sweet, bitter and astringent (Cohen, Kamphake, et al. 1960; Lim & Lawless 2006).

Various studies have been conducted to determine the human threshold for ferrous iron. A study done by Lawless showed significant variation in mean iron thresholds between three iron salts: ferrous sulfate 5.5 mg/L, chloride 3.7 mg/Land gluconate 1.1 mg/L. The $FeSO_4$ was found to have a standard deviation 25.5 mg/L of Fe^{2+} (Lim & Lawless 2006). Others have found threshold values for ferrous sulfate ranging from .04-256 mg/L (Cohen, Kamphake, et al. 1960). A more recent study had the threshold of ferrous iron as .003- 5 mg/L (Omur-Ozbek 2008).

The wide range of threshold values indicated a level of complexity in the causes of metallic flavors and lead to additional studies. The initial studies looked at the effect of nasal occlusion on threshold values (Hettinger 1990). Theses studies showed an increase in the threshold values for ferrous iron when the retronasal response was excluded (Lawless, et al. 2004). The values showed an increase ranging from 3.5 to 5.3 times for ferrous chloride and sulfate respectively in individual thresholds (Epke & Lawless 2007).

The effect retronasal occlusion had reducing metallic flavor perception led to attempts to isolate and identify specific volatile compounds linked to metallic flavor. In this attempt, a study was conducted by placing copper or iron on the skin and collecting the volatilized compounds produced in a small head space. Solid Phase microextraction (SPME) and GC/MS analysis was then performed on the head space samples. The samples showed the production of aldehydes and ketones, including 1-octen-3-one. The carbonyl compounds were the lipidperoxides created by the metal oxidized lipids within the skin (Glindemann, et al. 2006).

Metallic flavors are caused in part by lipid oxidation. Metals act as a catalyst in the free radical processes that breakdown polyunsaturated fats (Spanier 1991). There are numerous pathways and products that are produced in the lipid oxidation process. One of the major compounds is malonaldehyde

(MDA) which is a secondary byproduct of lipid oxidation (Figure 1) (Marnett 1999). An indirect measurement of MDA can be made colorimetrically after its controlled reaction with thiobarbituric acid in a procedure called thiobarbituric acid reactive substance (TBARS). Since TBARS is not a direct measurement of MDA and therefore lipid oxidation, other methods must be used to accurately measure lipid oxidation. Paralleling lipid oxidation byproducts such as F_2-isoprostanes have been measured using Enzyme Linked Immunoassay (ELIA) procedure (Lykkesfeldt 2007).

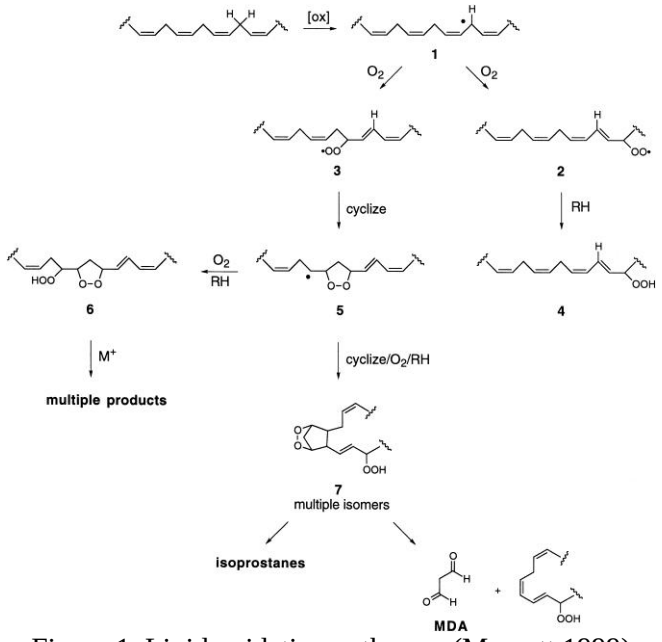

Figure 1. Lipid oxidation pathways (Marnett 1999)

Saliva plays an important role in taste sensitivity as it is the solvent in which taste substances are dissolved and transported to the taste receptors where a signal is generated and transmitted to the brain (Plattig 1988). Saliva is composed of fluids from three major and multiple lesser salivary glands; each fluid has varying electrolyte compositions. Whole saliva is more commonly studied due to the difficulty of isolating each oral fluid. The electrolyte profile can vary between individuals and the time of day (Tenovuo 1989). Average data for whole saliva is available from multiple sources giving slightly varying concentrations (Hong 2006).

Table 1. Profile of whole unstimulated saliva (Table 1(Hong 2006)).

Electrolyte	Mean ± s.d.	Range
sodium (mmol/L)	12.03 ± 8.9	.93-31.15
Potassium (mmol/L)	21 ± 4	.02-40
Calcium (mmol/L)	2.16 ± 1.11	.001-2.8
Magnessium (mmol/L)	.83 ± .33	.13-1.78
Copper (μg/L)	19.5	6.3-460
Zinc (μg/L)	49.2	46.9-1627
Chromium (μg/L)	230 ± 30	

Studies have been conducted examining changes in the metallic profile of saliva and corrosion of dental alloys. In one study the salivary profiles show an increase in metal species in salivary composition

for: silver, iron, chromium, nickel, copper and zinc (Garhammer, et al. 2004). There has not been a study looking at changes in saliva for oral metallic exposure.

The aim of this study was to identify any relationships between metallic flavor intensity and lipid oxidation in the oral cavity as well as changes in saliva composition with respect to metallic constituents when human subjects are exposed to varying concentrations of metallic tasting water utilizing sensory and biochemical measurements.

Methods and Materials

Nine human subjects from Virginia Tech's Department of Civil and Environment Engineering took part in this study over the course of one week. Before the subjects started the study they signed an informed consent from explaining the study and the risks of participation. This study was approved by the Institutional Review Board (IRB) at Virginia Tech. Afterward subjects were asked to fill out a survey to collect basic information such as: age, gender, health, nutritional supplements and dental appliances. The subjects varied in age from 20-53 years old and a mean of 32. The group consisted of five males and four females. The group was composed of one Asian, two Europeans and six Americans.

Three concentrations of ferrous iron were tested (5, 10 and 20 mg/L) with at least six hours between samples. The subjects were asked to refrain from eating or drinking for one hour before each session. To begin, the subjects were asked to rinse their mouths with nanopure water, after which a base-line oral pH was taken using 5.0-10.0 pH strips (EMD Chemicals Inc., Gibbsown NJ). Following a two minute rest period, the subjects swished in their mouths a 2 ml control sample consisting of nanopure water for 15 seconds without swallowing. Then they expectorated the control in addition to two additional ml of saliva into a clean test tube. Next the oral pH was taken. The procedure was then repeated using a 2 ml metal sample containing ferrous iron. Afterward the subjects were asked to rate the metallic flavor on a twelve point scale: none (0), weak (4), moderate (8) and strong (12). The samples were taken to the libratory where one ml was removed for metals testing before being placed in a -50º C freezer for storage. The ferrous iron solutions were prepared daily using iron sulfate heptahydrate ($FeSO_4 \cdot 7H_20$, Fisher Scientific, Fair Lawn, NJ). Solutions were prepared to be 5, 10 and 20 mg/L Fe^{2+}. After sample preparation the concentrations were verified using Flame Atomic Adsorption Spectroscopy (Perkin-Elmer 5100 PC, Waltham, MA).

Metals were determined with the one ml saliva sample set. Each sample was treated with an acid digestion using the EPA 846-SW Method 3010 A (USEPA 1992). Five 5 ml of nitric acid (trace metals grade 67% pure, Fisher Scientific, Fair Lawn, NJ) were added to each sample and then diluted to 20 ml with nano pure water. The samples were heated until they had evaporated to less than 10 ml. Then an additional 3 ml of nitric were added. The evaporation process was repeated, after which the samples had 3 ml of hydrochloric acid added (trace metals grade 34% pure, Fisher Scientific, Fair Lawn, NJ). The samples were diluted 1:20 with nanopure water for ICP measurement. Afterward the samples were analyzed by ICP-MS (Thermo Electronic Corporation, X-Series ICP-MS, Waltham, MA) in accordance with EPA 846-SW Method 6020 A (USEPA 2007). The samples were diluted 1:20 with nanopure water for ICP measurement.

The saliva samples were tested for lipid oxidation byproduct production using the TBARS procedure and an Isoprostane EIA Kit (Oxford Biomedical, Oxford, MI).

Samples were thawed and analyzed by both tests within the same day. The TBARS method was modified from Spanier's to work with liquid samples and to enhance readings at low concentrations(Spanier 1991; Wang 2009). Two reagent solutions were prepared; Solution I was prepared from 1.88 g of 2-thiobarbituric acid (MP Biomedicals, Solon, OH), 2.53 g sodium doedecyl sulfate (MP Biomedicals, Solon, OH), 59.5 ml acetic acid (glacial, Acros, Greel, Belgium), the solution was then topped off to 500 ml with nanopure water and had the pH adjusted to 3.4 with sodium hydroxide. Solution II was prepared by mixing n-butanol (Acros Organics, Greel, Belguim) and pyridine (Fisher Scientific, Fair Lawn, NJ) of 15:1 respectively. Two ml of solution I was added to one ml of each sample and MDA standard mixed and then placed in a 95º c water bath for one hour. Afterward the samples were cooled in an ice bath. Next two ml of solution two was added to each sample; then the

samples were centrifuged at 3000 rpm for fifteen minutes. One ml of the supernatant was placed in a cuvette and the absorbance was measured at 532 nm using Spectronic 21D spectrophotometer (Milton Roy, Ivyland, PA) (Wang 2009).

A standard calibration curve (absorbance versus concentration) was developed using the data from the known standards. The concentration of TBARS in saliva samples was calculated using the standard curve and measured absorbance values. The dilution effect was taken into consideration in the calculations. The concentration of TBARS in saliva samples fell in the range of standards in the linear calibration curve.

Saliva samples for the isoprostane test were left untreated. The procedure in the ELIA kit instruction manual was followed for the testing of free isoprostanes.

Since the protein content of saliva can vary among individuals, the total protein content of the saliva in each sample was measured. The results were used to report the lipid oxidation byproducts produced per gram of protein. All samples had the protein levels measured using the Bradford Assey (Bradford 1976). Standard curve was obtained by using Bovine Serum Albumin (BSA) at 1mg/mL concentration; BSA is a known standard for protein. To perform the analysis, 1 mL of Bradford reagent was mixed with 6 μL of saliva. The samples were mixed by vortex and 200 μL of the mixture was transferred to the well plates to be read by spectrophotometer at 595 nm.

Results and Discussion

Flavor Ratings

The metallic flavor intensity rating give by each panelist were assigned values according to the flavor profile analysis rating system (0 none, 4 weak, 8 moderate and 12 strong). The mean panelist rating for each metallic solution was progressively stronger (Table 2). All intensity data was plotted using box plots with R 2.9.1(Figure 2). The group could not distinguish between 0.2, 0.5 and 1 and 5 mg/L. The flavor rating increased for each sample above 5 mg/L.

Table 2. Metallic Flavor Ratings

Iron (mg/L)	Mean Rating	Standard Deviation
0.2	2.00	0.00
0.5	1.80	1.48
1	4.29	0.76
5	5.00	2.14
10	5.67	1.51
20	8.00	2.53

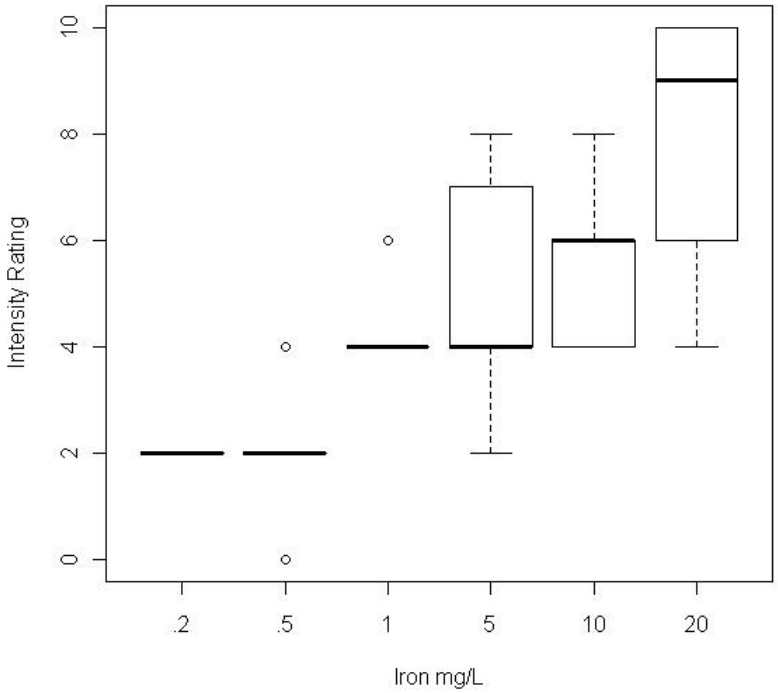

Figure 2. Metallic Flavor Ratings

Change in Salivary Composition

The pH of the panelists showed an insignificant change from after the control sample to after the metallic sample (Table 3). The pH showed an increase from the baseline to control to the metal. The change replicates the change shown by Tenovuo between unstimulated 6.7 and simulated 6.8-7.5 saliva (Tenovuo 1989).

Table 3. Oral pH before and after iron exposure

	Baseline	Control	Metal	Δ Metal to Control
5 mg/L	6.52	6.95	7.05	0.10
10 mg/L	6.65	6.79	6.87	0.08
20 mg/L	6.76	7.01	7.09	0.08

Saliva profiles showed little or no change for the major of salivary electrolytes (Figure 3). All electrolytes fell within the normal ranges outlined in by Hong (Table 1).

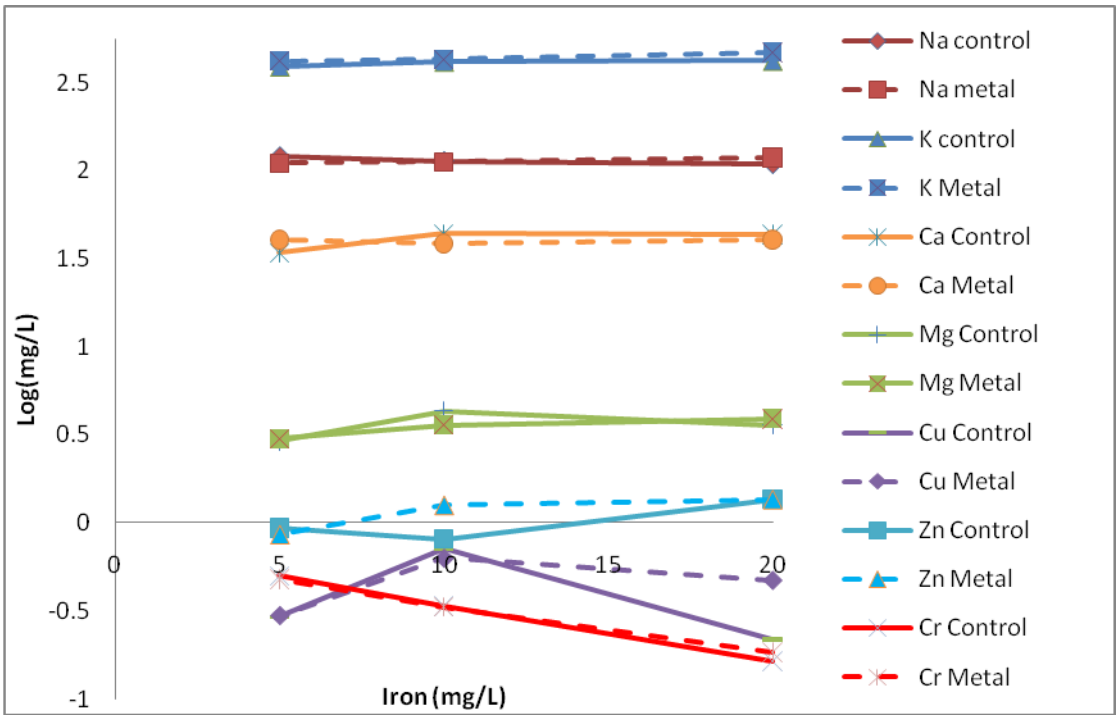

Figure 3. Major salivary electrolytes and metals before and after exposure to ferrous iron

A notable difference was seen in the oral iron concentrations. The increase in total iron was less than that of the amount added by the metallic solution (Table 4). The loss of iron was the greatest in the 20 mg/L sample but only differed by 0.5 mg/L from the losses in the 10 mg/L sample this was while the iron added increased by 5 mg/L. The 5 mg/L samples lost about 50% of what the 10 mg/L samples had. Hong showed that copper would bind to and precipitate proteins from saliva in a test tube (Hong 2006). Given that other oxidizing metals bind to proteins it is reasonable to assume iron would behave in a like fashion. The missing iron is thought to be bound to the proteins of oral tissues. The flat trend seen starting at 5 mg/L is likely due to the iron reaching the binding compactly of the available oral proteins.

Table 4. Change in ferrous iron in salivary samples

	Increase (mg/L)	Projected Increase (mg/L)	Iron missing (mg/L)
5 mg/L	0.95	2.50	1.55
10 mg/L	1.70	5.00	3.30
20 mg/L	6.20	10.00	3.80

TBARS

The mean level TBARS produced in the oral cavity increased as the level of iron exposure increased (Figure 4). The significance of the trend was analyzed using box plots (Figure 5). The 2.5, 5 mg/L samples were distinct and showed a small increase. The 10 and 20 mg/L samples were not differentiable. Indicating that the level of lipid oxidation starts to plateaus above 10 mg/L Fe^{2+}, this is likely caused by the limited quantity of lipids available for oxidation.

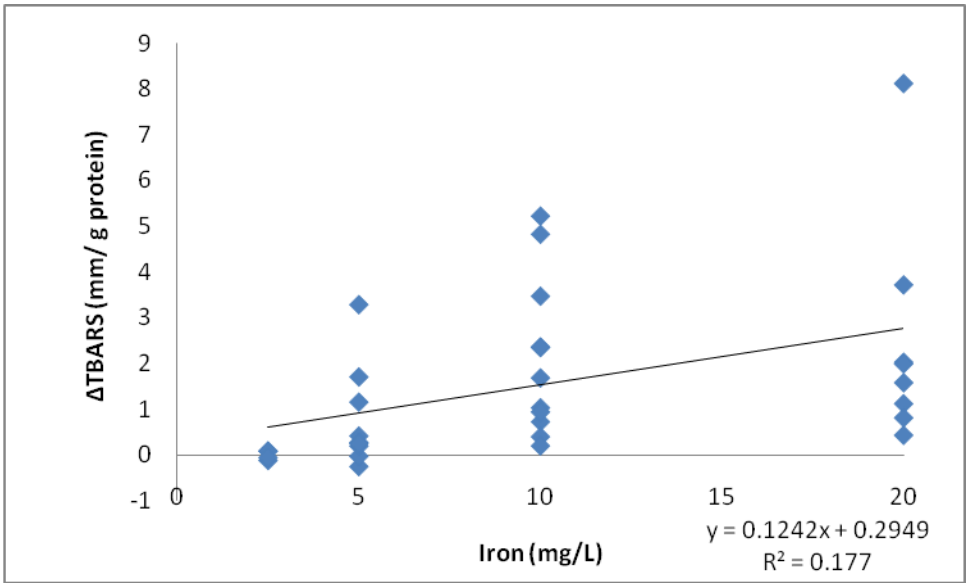

Figure 4. Change in TBARS as concentration of iron exposure increased

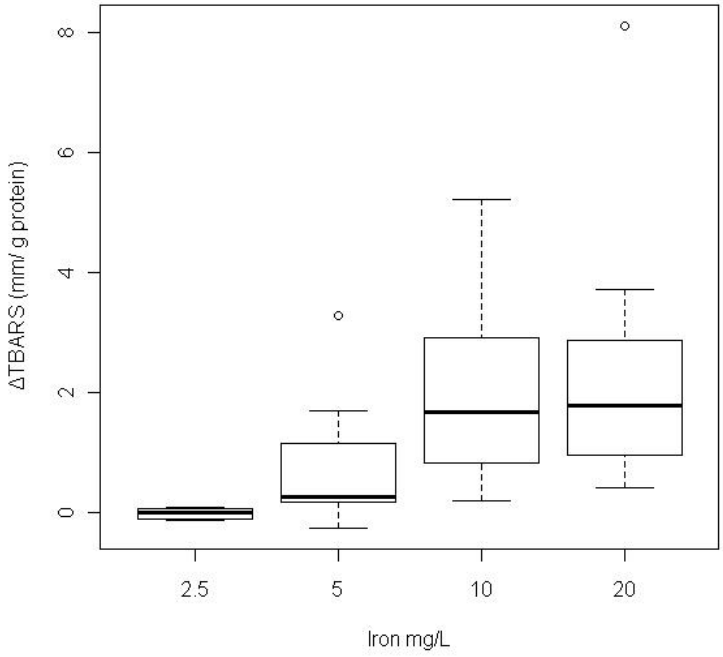

Figure 5. TBARS variation with increasing iron exposure

F_2-Isoprostanes

The isoprostane analysis showed a small overall decrease in free F_2-isoprostanes between control and metallic in the majority of samples. This trend was found for all ferrous iron concentrations tested. The trend is in contrast with the increase shown by TBARS (Figure 6). The lack of change between control and metal samples could indicate that the isoprostanes are bound to salivary proteins or trapped in esterified membrane phospholipids (Roberts & Fessel 2004). Following the total isoprostane test procedure which is designed to break these bonds may yield a larger difference. It is also possible that isoprostanes are bond or trapped to oral tissues and are not collected. Recent work has shown that the pathway that was thought to only produce isoprostanes also produces isofurans. Isofuran formation is favored over isoprostane in oxygen rich environments like the oral cavity (Roberts & Fessel 2004).

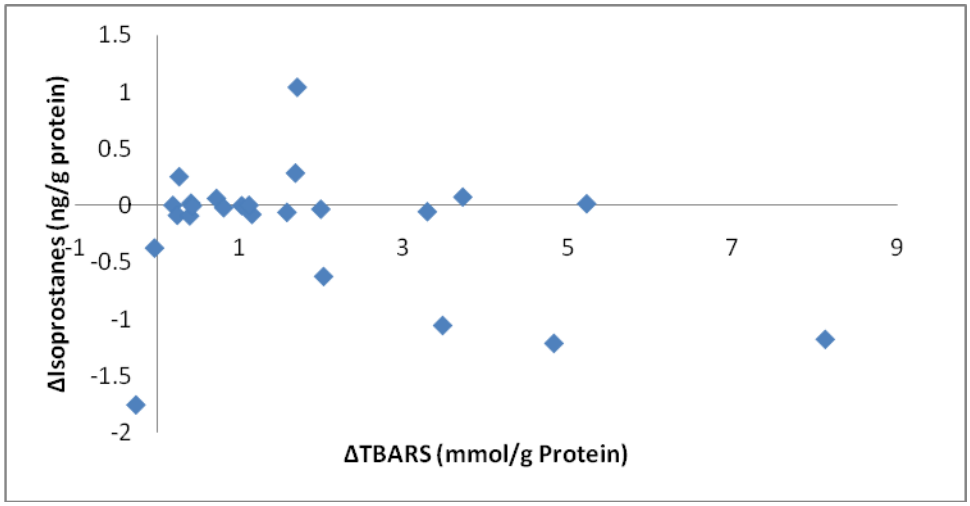

Figure 6. Comparison of the change in TBARS and F_2-Isoprostanes before and after metal exposure

Conclusions

The panelists could not distinguish between 0.2, 0.5 mg/L and 1, 5 mg/L ferrous iron. The panel was able to perceive an increase in concentration from 5 to 10 and 10 to 20 mg/L (Figure 7). The addition of iron into the oral cavity had no effect on the oral pH and concentration of oral electrolytes. It was found that not all the iron that entered the mouth was recovered. Levels of lost iron decreased as a percent of iron present as the level of exposure increased above 5 mg/L which maybe caused by iron binding to oral proteins. TBARS increased as the iron concentration was increased from 2.5 to 5 to 10 mg/L. TBARS did not increase from 10 to 20 mg/L which may indicate that the supply of lipids available for oxidation is limited (Figure 4). Free F_2-isoprostanes did not increase with exposure to metals.

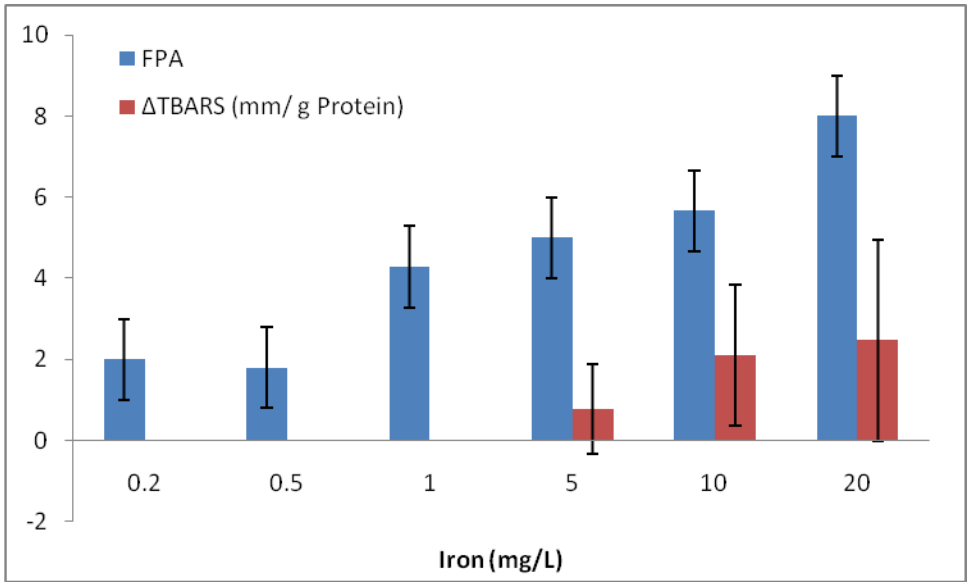

Figure 7. Comparison of Flavor intensity and TBARS

Acknowledgements

The 2009 Interdisciplinary Watershed Sciences and Engineering REU at Virginia Tech was funded by NSF and organized by Dr. Younos and Dr. Lohani. The Authors gratefully acknowledge the Institute for Critical Technology and Applied Science (ICTAS) for partial funding of laboratory space and supplies in

support of this research. Thank you to Jody Smiley for all her help and patience throughout the summer. Thank you to Dr. Lee and Dr. Wang for use of their labs for this project. Also Laëttia Maillet for providing flavor profile training and help collecting data. Finally to all the panelists who took part.

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The Sensory Perception of Drinking Water Hardness

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ABSTRACT

The complexity of the taste of divalent cations such as calcium and magnesium, the major constituents of water hardness, requires better characterization. Sensory sensitivity to calcium and magnesium was explored using ranking tests on taste-testing panels. Calcium, magnesium, and 1:4 magnesium to calcium solutions were tested at six concentrations ranging from 25 to 800 mg/L hardness as $CaCO_3$. Concentrations between 25 and 100 mg/L hardness showed very little statistical perceptual difference across the testing population.

Keywords: aesthetics, calcium, magnesium, hardness, water taste

Introduction

Water hardness is a water quality parameter which measures the presence of polyvalent cations, correlating highly to the concentration of calcium and magnesium cations (Clevay & Combs 2005). The most notable physical effects of water hardness are scaling on pipes and dishes and an inability of formation of soap suds. However, research within the last several decades, beginning with a study by Kobayashi in 1957, has produced evidence of beneficial effects of hardness on health (Kozisek 2003). In addition, significant experimentation has been done on the taste and corrosion potential of drinking water hardness. The ideal levels are a current area of research and the main focus of this paper.

Table 1: Classification of Water Hardness

Classification	mg/l as $CaCO_3$	grains/gal
Soft	0 - 17.1	0 - 1
Slightly hard	17.1 - 60	1 - 3.5
Moderately hard	60 - 120	3.5 - 7.0
Hard	120 - 180	7.0 - 10.5

Calcium is the most abundant mineral found in the human body, and sufficient dietary intake of calcium is necessary to maintain and replenish its essential functionality. Nearly all of the calcium (99%) is found in the bones and teeth, but calcium is also vital for muscle contraction, hormone and enzyme secretion, and transporting sensory information message sending in the nervous system (Abrams et al. 2009). The dietary intake of calcium has been epidemiologically linked to an inverse relation with the occurrence of osteoporosis in aging women (Azouly, et al. 2001). However, nutritional surveys have revealed that more than half of North Americans do not intake the recommended daily amount (RDA) of calcium. An estimated 75% of men and 90% of women do not meet the recommended calcium daily intake levels (Cotruvo 2006)

The three most vital sources of naturally bioavailable calcium are milk, dairy products, and water (Azouly, et al. 2001). In fact, drinking water can provide significant amounts (several % of RDA) of calcium intake, but extreme values may adversely affect the flavor and acceptability of the water. High levels of calcium create a bitter or salty taste and a slimy mouth feel, depending on the associated anion concentrations (Lawless, et al. 2003). The presence of calcium in water has a 0.96 correlation with water

hardness and primarily originates from limestone, dolomite, gypsum, and other minerals (Clevay & Combs 2005).

Magnesium is also a vital mineral in the body for good health. It is essential to hundreds of enzymatic reactions, calcium absorption, and cardiac health (Kozisek 2003). Magnesium deficiency has been linked to heightened risk of cardiac arrhythmia, myocardial infarction, ischemic heart disease, cardiovascular disease, hypertension, eclampsia in pregnant women, and osteoporosis, and 75% of men and 50% of women do not reach daily recommended levels (Cotruvo 2006). However, magnesium is usually found in smaller quantities in source water as calcium is much more abundant in the earth's crust. In fact, the large majority of natural sources have a magnesium to calcium ratio of less than one, except saline waters as shown in Table 2. A common ratio found in ground and surface waters is 1:4, and it is also common with in the human bodily fluids (see Table 2) (Kozisek 2003).

Table 2: Magnesium to Calcium Ratios of Source Waters

Source	Mg:Ca Mass Ratio
Spring*	0.05-0.4
Limestone Aquifer*	0.5-0.9
US Tap Water Median*$^{+}$	0.238
Typical Seawater**	3.2
Arabian Gulf at Kuwait**	3.5
Average in Human Body^{+}	0.239

** (Saad, et al., 2004),*
*** (Expert Consensus, 2005), + (Freidman & Rubin, 1955),*
**+ (Van der Leedan, et al., 1990)*

Drinking water, whether it is tap water or bottled water, describes a consumer product with expectations not only for safety and sanitation but also for aesthetics and tastiness. In fact, producing an acceptable product is a large determiner of public trust and confidence in a drinking water utility (Burlingame, et al. 2007). For this reason, it is important to understand the sensory perception of drinking water to determine what composes a desirable taste versus an unacceptable taste. Several regulatory organizations, including the USEPA, have already placed some aesthetic standards on a number of drinking water constituents but there have been no values set for hardness, though it is often constrained by total dissolved solids (TDS) limits. Within the last century the science of sensory testing has evolved, searching for qualitative laws of human perception (Moskowitz 1983). However, that research has not expanded much into the drinking water industry.

When evaluating the taste of water hardness, the concentration of cations is the major determinant of taste. Taste is stimulated when molecules interact with chemoreceptors in the taste buds (Fox 2008). However, the taste of divalent cations such as in calcium and magnesium salts has proven to be a complex science. Conversely, the tastes of monovalent salts follow trends correlated with the corresponding atomic masses (Lawless, et al. 2003). Therefore, the taste of divalent salts needs more descriptive studies to search for qualitative relationships with perceived sensation. Good tasting waters have been found to have a hardness between 10 to 100 mg/L due largely to calcium hardness (Burlingame, et al. 2007). The growing importance of the taste of tap water is evidenced by the budding bottled water industry which primarily seeks to fill the market niche of a quality consistent aesthetic product.

Similarly, as desalination and membrane technologies grow in prevalence, the remineralization of deionized water is vital to make healthy, palatable, and minimally corrosive water (Hasson & Bendrihem 2006). The high purity processes necessary to remove unwanted salts also remove minerals which are experimentally linked to good taste and good health in drinking water. In addition, the supplementation of minerals to deionized water is essential to prevent the corrosion of distribution pipes. The water distribution industry often uses corrosion indices to predict the corrosivity of source waters (Imran, et al.

2005). The most commonly used indices are the Larson Ratio (LR), Langlier Index (LI), Ryznar Index (RI), and Calcium Carbonate Precipitation Potential (CCPP), which account for the effects of scaling, anions, alkalinity, and other factors (Imran, et al. 2005).

The mineral content of water may be able to be honed to optimal cation and anion concentrations for a balance of aesthetics, health benefits, corrosion reduction, and cost effectiveness. Finding this level is a difficult task, so a better understanding of water hardness, one of many constituents, will help to better characterize water quality as a whole.

Materials and Methods

The primary means of data collection for the sensory perception of drinking water hardness were taste testing panels. The taste testing sessions were held with individual panelists or in separated groups to attain subject isolation. All test subjects were Blacksburg residents or Virginia Tech students, faculty, research fellows, or professors. The testing procedure was approved by the Instituitional Review Board (IRB) at Virginia Tech. Each test subject signed a written consent form and completed a demographics questionnaire before testing began. The ages of the population sample ranged from 19 to 67, and no subjects were below 18 as it was the minimum age for consent.

Figure 1. A Taste Testing Session

Panel Desciption

The mean ($\pm$ standard deviation) panelist ages for the calcium, magnesium, and combination tests were 35.9 $\pm$ 15.5, 37.0 $\pm$ 15.0, and 36.1 $\pm$ 15.8 with medians of 33, 37, and 30 and sample sizes of 41, 48 and 36, respectively. The age ranges for the test populations were 19-60, 19-67, and 19-67, for the same tests. The panels were 58.5, 58.3, and 51.4 percent female, respectively. All subjects were healthy individuals with varied drinking water preferences and were regular consumers of varied hardness drinking waters. 26 of the panelists were participants in all three taste tests.

Concentration Preparation

Stock solutions of calcium chloride, magnesium chloride, and a combination were prepared at 1600 mg/L hardness as $CaCO_3$ to be diluted for desired concentrations. The water used for the stock solutions and dilution was deionized water from an Aries column unit and a Barnstead Mega-pure organic removal system. The deionized water exhibited a chemical resistivity of above 16 MΩ/cm and a pH of approximately 5.45. The stock solutions and all other concentrations were tested using atomic absorbance spectroscopy or inductively coupled plasma mass spectroscopy (ICP-MS). The shelf life was tested three weeks after preparation, and the concentrations were stable with any changes being less than 5%. All tests were given within three weeks of the preparation. Likewise, the concentrations of uncovered samples were tested after 3 hours of exposure to the laboratory atmosphere. After 3 hours the samples were still well within 10% of their original concentrations, so concentration changes due to evaporation were negligible. All samples were given within 3 hours of being poured or covered if kept any longer.

Calcium, magnesium, and combination solutions were prepared from the stock solutions at 6 different concentrations varying by a factor of 2. The concentrations used were 25, 50, 100, 200, 400, and 800 mg/L hardness as $CaCO_3$ prepared using A.C.S or FCC certified Fisher Chemical calcium chloride dihydrate and magnesium chloride tetrahydrate. All three taste tests were administered at the same molarities (and hardness) so as to keep the atomic concentrations constant for the sake of comparison. The quantity of atoms was kept constant because taste intensity is correlated to atomic interaction with the chemoreceptors in the taste buds. The values can be seen in Table 3 below.

The combination calcium and magnesium solutions were prepared using calcium and magnesium chloride at a mass ratio of 1:4 magnesium to calcium. This ratio was selected to best emulate the levels in natural source waters according to literature values (Kozisek 2003).

Table 3. Values for Calcium, Magnesium, and Hardness Ranking Tests

Moles/L	*Hardness (equivalent $CaCO_3$)	*Ca^{2+} as $Ca(Cl)_2$	*Mg^{2+} as $Mg(Cl)_2$	*Mg^{2+} / Ca^{2+}	*TDS for $Ca(Cl)_2$	*TDS for $Mg(Cl)_2$	*TDS for (Mg / Ca)Cl_2
0.00025	25	10	6.075	1.77 / 7.08	27.75	23.825	26.57
0.0005	50	20	12.15	3.54 / 14.17	55.5	47.65	53.15
0.001	100	40	24.3	7.08 / 28.34	110	95.3	106.3
0.002	200	80	48.6	14.17 / 56.67	220	190.6	212.6
0.004	400	160	97.2	28.34 / 113.35	440	381.2	425.2
0.008	800	320	194.4	56.67 / 226.71	880	762.4	850.4
0.016**	1600	640	388.8	113.35 / 453.42	1760	1524.8	1700.8

* in mg/L **stock solution

Taste Testing Procedure

The taste testing panels were given a randomized complete block design simple ranking test in which the six different concentrations of a solution were presented to the panelist in a balanced, random order (Meilgaard 2007). After the panelists rinsed their mouths with taste free (nanopure) water, they tasted a moderately high concentration of the mineral(s) to train or calibrate their sensory perception. The panelist then rinsed with taste free water again and began tasting the six different solution concentrations. The panelists were asked to wait 15-30 seconds between samples to avoid aftertaste effects and highly encouraged to retest samples as needed. The panelists then recorded their answers when confident in their ranks chosen for each of the six cups. Each white 3 oz. sample cup had a three digit code so as to limit preference bias in ranking selections.

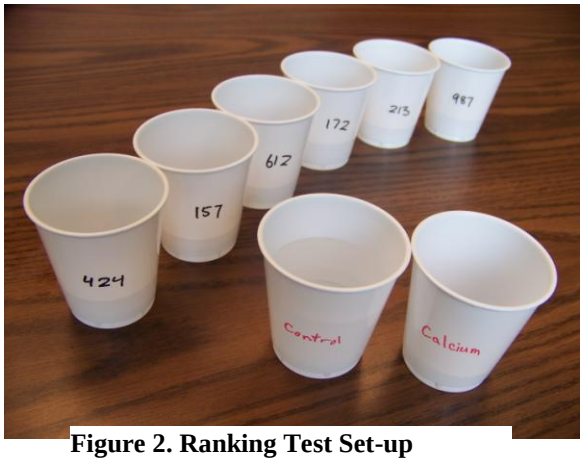


Figure 2. Ranking Test Set-up

The simple ranking test was used to compare the samples according to a single attribute, which was "mineral content" in the study (Meilgaard 2007). The test is well suited to provide a large amount of data about a sample set using a relatively small panel size. The apparent disadvantage to the testing method was that no quantification was given to the differences between samples as adjacent samples were only separated by one unit rank value. Thus the test and analysis reveals statistically significant perceptual differences but not how significant the differences are.

Data Analysis

Analysis of the collected data was carried out using a Friedman-type statistic and a multiple comparison procedure. The Friedman's test assigns a T-value (1) to the data which must be greater than the table chi-squared value for the prescribed confidence interval (0.95) and the degrees of freedom (5) in order for the null hypothesis (no significant differences or type I error) to be rejected. If the null hypothesis is rejected, a multiple comparisons test assigns a least significant difference between samples based on the parameters of the data. The difference is assigned using a non-parametric equivalent of the Fisher's least significant difference (LSD) for rank sums (2) (Meilgaard 2007):

$$T = \frac{12}{bt(t+1)} \sum_{j=1}^{t} X_{.j}^{2} - 3b(t+1) \quad (1)$$

$$LSD_{rank} = z_{\alpha/2} \sqrt{\frac{bt(t+1)}{6}} \quad (2)$$

b = number of panelists, t = number of samples, z = z score of α, x term = sum of squared rank-sums.

For example, with the data for the magnesium ranking test,

$$T = [12/\{48*6*(6+1)\}] *193524 - 3*48*(6+1) = 143.9$$

$$LSD_{rank} = 1.96 \sqrt{48*6*(6+1)/6} = 36.3$$

Since T is much greater than the chi-squared of 11.1 for the given alpha and degrees of freedom, the null hypothesis is rejected and a least significant difference can be calculated. This difference can then be used to detect statistically significant differences between the concentrations tasted in the ranking test. The difference between the rank sums, the total sums of ranks assigned to each concentration by the panelists, must be greater than LSD in order for two concentrations to be considered statistically different. The LSD describes a statistical value valid for the population that may or may not be correct on an individual basis.

The type II error or false negative (a false similarity between samples) was tested with a post hoc power analysis using a bootstrapping method in the statistical software, R version 2.9.1. The method compared each concentration to each other for each test. The powers between the first, second, and third concentrations were low, but this confirms the sensory perception of "no difference" though there is a known physical disparity in concentrations. The powers between the lower and higher concentrations were high enough to confidently reject a type II error (greater than a power of 0.8). Therefore, enough panelists were used to produce statistically significant results and the power analyses are consistent with expected results. Figure 3 shows two different power graphs for the magnesium test data comparing between the first and second concentrations and the first and third concentrations.

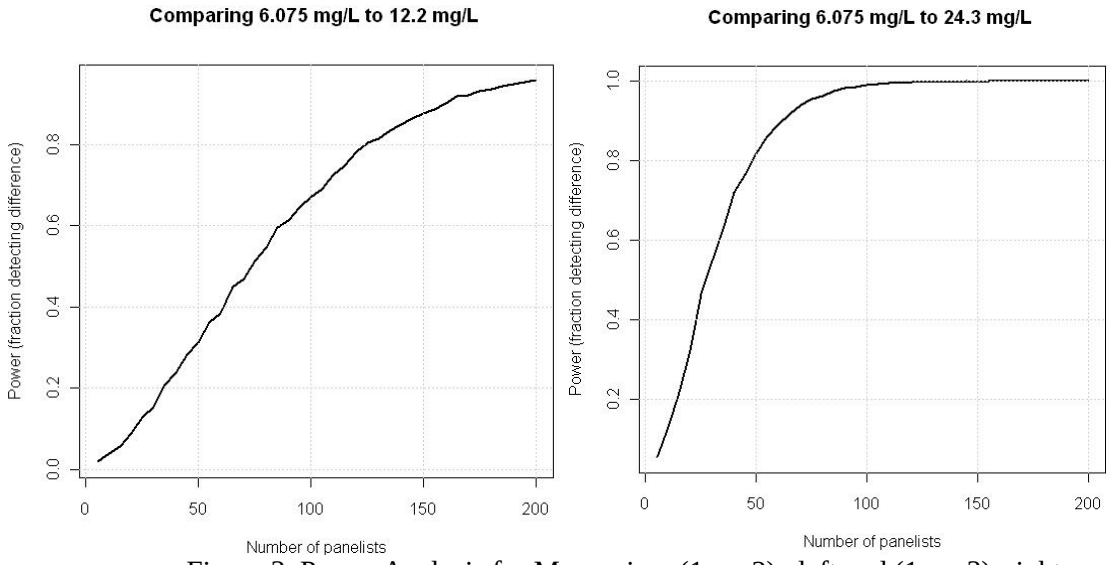


Figure 3. Power Analysis for Magnesium (1 vs. 2)., left and (1 vs. 3), right

Results and Discussion

Small differences at low concentrations were difficult for the panelists to distinguish across all three ranking tests. In all three tests, the highest concentration was significantly different than all of the other samples. Concentrations between 25 and 100 mg/L hardness were the most difficult to discriminate for the population as a whole.

The calcium taste was most commonly described as bitter, salty, and astringent, with additional comments of a tongue coating sensation. The results indicate that the first three concentrations showed no significant difference for the population. However, the fourth and fifth concentrations were both significantly different from all of the first three concentrations but not significantly different from each other as shown in Figure 5. The highest concentration was significantly different from all of the other concentrations. The bars underneath the concentration boxes signify a statistical equivalence between concentrations as determined by the difference between concentration rank sums (the numbers below the boxes) being less than the LSD.

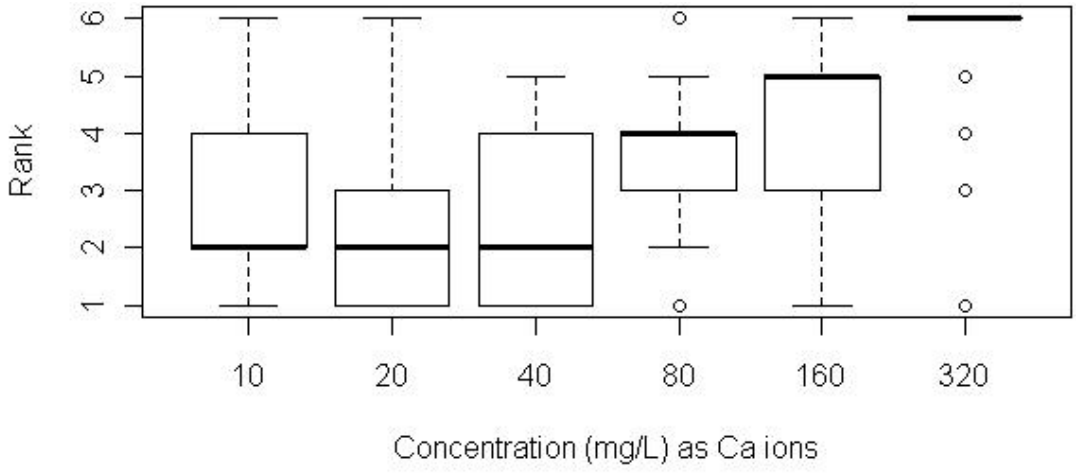
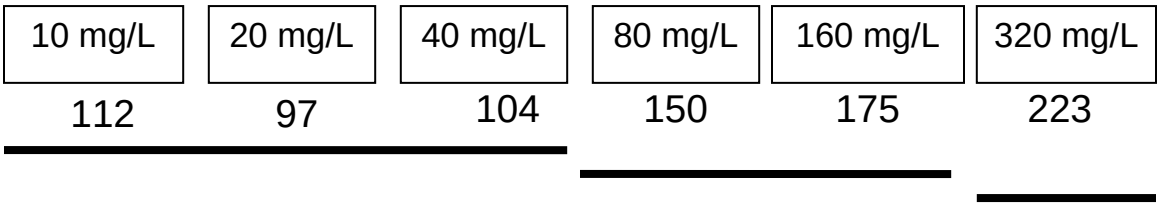


Figure 4. Boxplot Summary of Assigned Ranks for Calcium Only



n = 41 p-value = < 2.2e-16 LSD = 33.2

Figure 5. Statistically Significant Differences for Calcium Only

The taste of magnesium was most commonly described as bitter, astringent, and possessing a drying aftertaste. Differing from calcium, only the first two concentrations were not significantly different, as the third was significantly different from the first yet not the second as shown in Figure 7. All other concentrations were significantly different from their adjacent concentrations and all others as well.

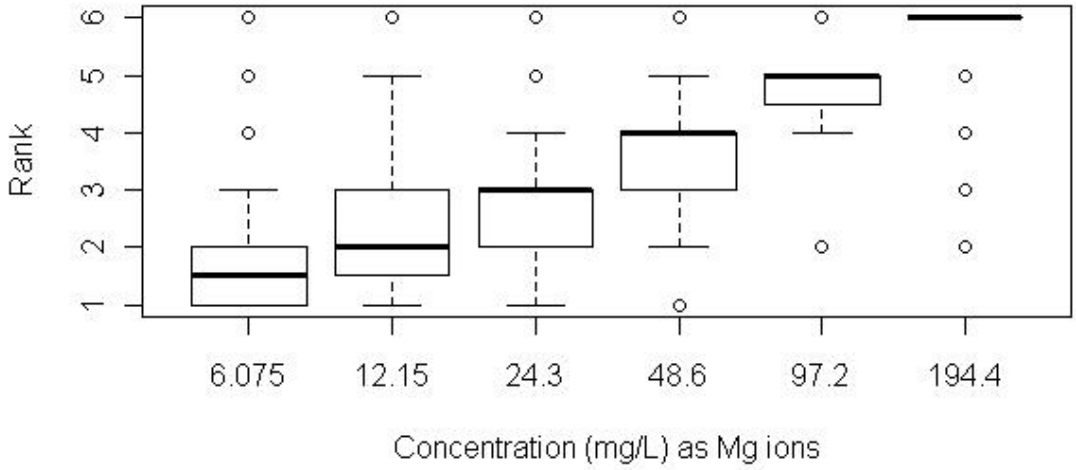
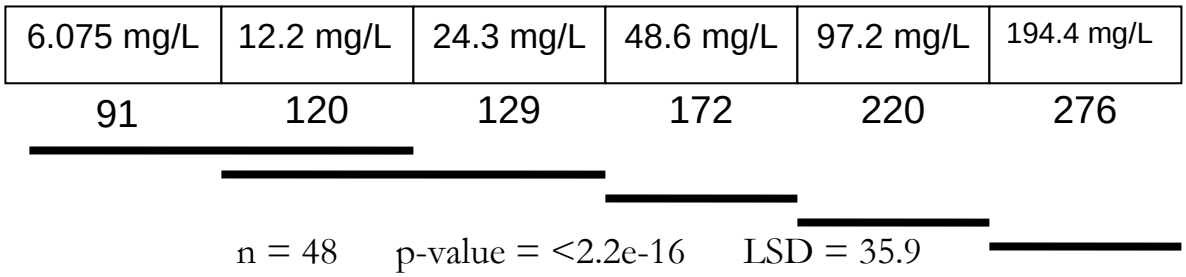


Figure 6. Boxplot Summary of Assigned Ranks for Magnesium Only



n = 48 p-value = <2.2e-16 LSD = 35.9

Figure 7. Statistically Significant Differences for Magnesium Only

The taste of the combination of calcium and magnesium was described as salty, slightly metallic and sour, and "mineral" while others cited the "chalky" or drying mouth feel as the largest sensory perception of mineral content. The lowest three concentrations were not statistically different. The fourth concentration was significantly different from all concentrations except for the second and third concentrations as shown in Figure 9.

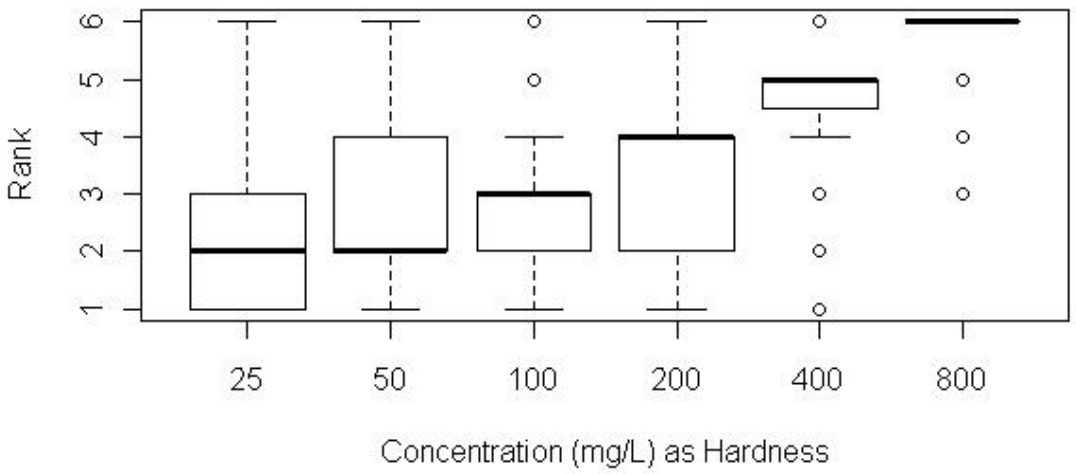


Figure 8. Boxplot Summary of Assigned Ranking for Combined Hardness with 1:4 Mg:Ca

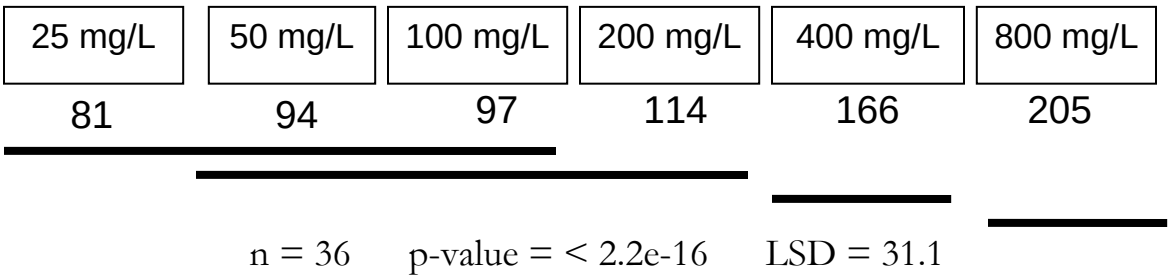


Figure 9. Statistically Significant Differences for Combined Hardness with 1:4 Mg:Ca

The taste threshold for chloride is 200-300 mg/L, depending on the associated cation, while the taste threshold for calcium is between 100-300 mg/L and magnesium is most likely somewhat lower, though consumers have been known to accept hardness levels up to 500 mg/L (WHO, 2004). Therefore, the ranking tests results are consistent with threshold values published by the World Health Organization (WHO) and complementary to the taste intensity results found by Lawless in a study on the taste of calcium and magnesium (Lawless, et al., 2003). Though the primary focus of Lawless' study was food-typical levels of calcium and magnesium, the lowest concentration (0.01 M) was close to the highest concentration (0.008 M) for the ranking test. At these levels, the taste intensity of calcium and magnesium was about equivalent for the sensations of salty and bitter, but differences came at levels (>0.06M) above typical drinking water concentrations (Lawless, et al. 2003).

Magnesium, chlorides, and carbonates are believed to have negative impacts on taste while calcium and bicarbonates can have positive effects on taste (Burlingame, et al. 2007). However, many panelists described the taste of calcium as similar to magnesium within the concentrations of this test. Therefore, at typical drinking water levels calcium and magnesium hardness have similar effects on sensory perception. Both calcium and magnesium were indistinguishable in the slightly/moderately hard level of drinking water hardness, which is valuable as changes in water quality are a main source of customer complaints (Whelton, et al. 2007).

Conclusions and Future Work

The population as a whole had trouble distinguishing between concentrations between 25 and 100 mg/L hardness. However, at lower levels the population seemed to detect magnesium with a slightly better accuracy than calcium as demonstrated in Table 4. The combined calcium and magnesium ratio solution seemed to have a sensitivity in between the sensitivities of the solutions of calcium and magnesium alone. Therefore, the necessary remineralization of desalinized water should focus on the

corrosion potential and cost of the treated water, as there is evidence that fluctuations in taste between 25 and 100 mg/L hardness will not significantly affect the taste for the large majority of the population. In addition, these findings provide evidence for easier quality control for utilities with highly variant source water and/or need for softening.

Table 4. Comparison of Tests

Concentration Comparison	Calcium	Magnesium	1:4 Magnesium: Calcium
A to B	X	X	X
A to C	X		X
A to D			
A to E			
A to F			
B to C	X		X
B to D			
B to E			
B to F			
C to D		X	X
C to E			
C to F			
D to E	X		
D to F			
E to F			

X = statistically equivalent
(A, B, C, D, E, F = 25, 50, 100, 200, 400, 800 mg/L hardness, respectively)

Much more research is necessary to better characterize the taste of divalent cations such as calcium and magnesium, especially at the moderately hard to hard levels (see Table 1). The taste of water hardness will be especially important as desalination remineralization becomes more prevalent. Therefore, the tastes of different ratios of magnesium to calcium should be explored as well as the taste effects of the associated anions at different concentrations of hardness. Also, the effect of personal drinking water habits on the perception of drinking water hardness could be explored. Lastly, remineralization techniques should be explored for solution preparation to better simulate municipal drinking water.

Acknowledgements

The authors would like to acknowledge the 2009 Virginia Polytechnic Institute and State University Watershed Science and Engineering REU for the opportunity to conduct research and the National Science Foundation for providing essential funding. Thanks to Dr. Younos, Dr. Lohani, and the Virginia Water Resources Research Center for making this opportunity possible. Thanks also to the Virginia Tech Department of Environmental and Water Resources Engineering and its faculty and staff: Jody Smiley- for lab support, Dr. Parks – for running countless ICP-MS samples, and Dr. Gallagher – for statistical support. In addition, the very willing taste testing panelists were essential to data collection and immensely appreciated. The authors gratefully acknowledge the partial support of the Institute for Critical Technology and Applied Science (ICTAS) for research supplies and the financial support of Mr. Snyder-Beattie.

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In-Field Procedure Development for Measuring Biofilm Uptake Rates of Ammonium and Phosphate in Regional Headwater Streams

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ABSTRACT

Nutrient uptake and remineralization dynamics among multiple compartments in streams is an important aspect of lotic ecology with applications in public planning and water quality. Epilithon biofilms are assemblages that have a major role in nutrient dynamics, potentially an especially significant one in summer headwater streams. A developing method was explored by which to measure uptake of ammonium and phosphate of these biofilms at in-field sites using small, unpowered microcosms and representative biofilm on tile substrates from unrestored headwater streams and restored coal mining streams in the Southern Appalachians.

Keywords: biofilm nutrient assimilation nutrient cycling stream restoration

Introduction

Nutrient movement and processing through lotic systems is of great importance to downstream stakeholders, especially in watersheds where impaired water quality and eutrophication are common problems. The dynamics of nutrient movement in rivers and streams is not simply a matter of transport; significant nutrient processing occurs in the downstream direction (Triska, et al. 1989). Biotic processes, collectively resulting in "nutrient spiraling," take up and remineralize nutrients of concern out of and back into solution (Webster & Patten 1979; Newbold, et al. 1981). This assimilation of nutrients (from abiotic flow to storage in a biotic compartment) and remineralization (from biotic storage back into flow) is a normal function carried out by a number of different assemblages of organisms. These assemblages, depending on both temporal variables (Francouer, et al. 1999) and spatial heterogeneity (Dent & Grimm 1999; Baldwin, et al. 2006)), can slow or decrease nutrient load carried downstream (Peterson, et al. 2001).

Biofilms are just one potential assimilation compartment in streams. Generally, biofilms can be found on any surface/water interface in a stream within favorable light, temperature, and nutrient conditions. Biofilms are microbial assemblages of diatoms, bacteria, algae, fungi, or any combination of the above (Lock, et al. 1984). These surface communities carry out both assimilation and mineralization processes within the same matrix (Mulholland, et al. 1991), but seasonality, available organic matter, and ambient concentrations can result in a net effect of one of processes dominating (Hoellin, et al. 2007).

Regionally, fall is the most productive season for biofilm uptake of available nutrients in wooded headwater streams ("closed" reaches) in the Southern Appalachians. High sunlight and carbon inputs from leaf fall contribute most to biofilm growth and aggradation on leaf and other available surfaces (Mulholland, et al. 1985; Chenier, et al. 2003). It has been hypothesized that in summer, as often the season when remineralization dominates stream nutrient processing, biofilms could be net mineralizers regionally (Cheever 2008). The severely depleted organic inputs from the previous fall combined with the shade of heavy forest cover (Hill, et al. 2001) often correlates to a decrease in the biomass of biofilms.

Due to the lack of available leaf derived organic matter, epilithon assemblages make up a large portion of total stream biofilm in these headwater streams in summer. Therefore, epilithon and animal compartments should account for the vast majority of total stream biotic nutrient cycling in summer.

Although the extent (and, therefore, nutrient processing) of these assemblages is heterogeneous both in time throughout the summer as well as location in the stream, a comparison of total stream uptake (calculated via a stream drip enrichment of the nutrients of concern) to uptake of representative biofilm samples (scaled up to the reach size) could shed light on the magnitude of the animal compartment in summer nutrient cycling. This scenario is the subject of ongoing research of regional headwater streams in Southern Appalachia. But in order to explore this hypothesis, a procedure for easily and effectively measuring the uptake rates of representative biofilm samples needs to be developed.

The method needs to be effective at quickly and successfully cultivating representative samples, have a relatively simple and cheaply repeatable procedure, as well as be robust enough to yield clear and evident results. Most constraining of all, although previous studies of biofilms have used in-lab "chamber methods" (O'Brien & Dodds 2007; Teissier, et al. 2007) this method needs to be used streamside and overcome certain access and disturbance limitations. Streamside testing allows for minimal disturbance of biofilms by avoiding the removal of their substrates from the in-stream incubation location for any length of time prior to the sampling/testing period. Previous studies that utilized streamside channels or microcosms for incubation and sampling required either large amounts of bank space and associated property access or a consistent power source for recirculating pumps (Mulholland, et al. 1991; Romani, et al. 2004; Rier & Stevenson 2006) The procedure to be developed must account for having none of these allowances. Also, these previous studies used the side channels or microcosms in order to introduce new nutrient regimes over a long period in order to study the change in biofilm assemblage composition, not the uptake dynamics of the original in-stream biofilm assemblage. Therefore, long term introduction of a new nutrient regime for which many of these micro- and mesocosms were designed is unnecessary in a developing in stream method.

Mountain-top removal coal extraction is a method of mining used most prevalently in the Appalachians. Coal mining companies are required by law to carry out environmental remediation in the form of "contour restoration" (USEPA 2005) but are sometimes compelled by outside pressure to carry out further remediation. Due to the extreme nature of topographical and hydrological disturbance associated with mountain-top removal mining (Pond, et al. 2008), this mitigation can take the form of stream restoration. The merits of this process are hotly debated, both in general among the scientific and engineering communities towards the developers of form based stream restoration (Simon, et al. 2007), as well as in specific towards these coal mining companies.

The most commonly applied stream restoration practice (Malakoff 2004) is that of the Rosgen Stream Classification System (Rosgen 1994) and Natural Channel Design (NCD; Rosgen 1996). Outside of widespread criticism for these processes never having been submitted for scientific review, common scientific assessments of NCD point to a number of problems with Rosgen's and all "form-based" systems for assessing future stream forms (as well as governing active processes) based solely on simple current measurements of channel geometry in unstable systems (Miller & Ritter 1996, Simon, et al. 2007). Planners or corporations that utilize Rosgen Stream Classification and NCD practices are often striving for different goals that the system is designed to deliver: NCD strives to achieve a return of stability to an unstable system while planners are often striving for a more functional or aesthetic metric of "restoration." (e.g. USEPA 2006) It is uncertain if there is accuracy in the thought process that if a stable form is restored to a stream, then organisms and function of normal natural streams are quickly restored as well (Schoenholtz et al. 2009). Therefore, a metric of the success of stream restoration based on the restored stream function would be beneficial in assessing NCD and Rosgen Stream Classification. Rosgen's restored stream forms should also restore stream function. If this is true then in established reaches in which NCD was applied, functions such as natural, regionally normal biofilm uptake and behavior should be restored.

The objectives of this project were twofold. First, develop a procedure by which to easily and successfully measure biofilm uptake of two common nutrients of concern, ammonium and phosphate (NH4-N and PO4), using in-field sampling. Second, use this procedure to determine biofilm uptake in restored coal mining streams as well as representative regional unrestored reference streams to form a rough comparison of this normal stream function.

Methods

Five streams were already under study by several research projects within the Department of Biological Sciences. These streams were chosen for previous study as an available cross section of both background nutrient regimes/ratios as well as land uses representative of headwater streams of Southern Appalachia. Also, as reaches of these streams were already under study for overall uptake, summer biofilm uptake measured with this procedure could be used in future tests of the predictions that these "closed-" or "mixed reaches" (Cheever 2008) should have uptake/remineralization governed primarily by epilithon and animals in summer. In-stream access disputes with local landowners would not be problematic, as students had been previously sampling within these reaches. However, access had not been granted for any larger, recirculating streamside microcosms or side channels and procedure design had to take this limitation into account.

Colonization substrates were prepared by using silicone adhesive to attach $2in^2$ unglazed, impervious porcelain tiles to 18lbs concrete pavers. These substrates were then placed on riffles or bars in five regional representative headwater streams and three streams restored using NCD within the last three years. Substrates were placed at water depths (above the tile surface) between ¼ - 6in and left to incubate for at least 30 days (longer if required to obtain representative biofilm growth on the tile substrates).

After assemblages representative of natural biofilms (growing on rock surfaces in ambient stream conditions) had colonized the tiles, 100mL microcosms of stream water spiked to 40ppb above background level for ammonium, phosphate, and both nutrients, as well as microcosms containing un-spiked stream water, were prepared and floated in a basin of stream water in order to standardize temperature. With minimal disturbance of the tile growing surface and exposure to the air, tiles were removed from the paver and placed in the microcosms. 5ml water samples were removed from the microcosms at five increasing time standards over a two hour sampling period; 1ml was used to rinse the filter and 4ml were collected as sample. All sampling periods were begun under initial conditions of fair weather and direct sunlight. Water samples were placed on ice and returned to the lab for nutrient analysis using a Lachat Quickchem 8500 Flow Injection Analyzer. Tiles were put in sterile bags and placed on ice in the field at the conclusion of water sampling. The tiles were scraped of biofilm, which was filtered, dried for 24 hours at 50^oC, ashed at 550^oC, and analyzed for ash free dry mass (AFDM) in order to determine the rate of uptake per unit epilithon biomass.

Repeated laboratory tests of the microcosm sample cups, tiles, and cured caulk adhesive strongly suggest that abiotic adsorption of nutrients from these concentrations of spiked solutions is below detection level (4-5ug/L). However, uncured caulk adhesive did sometimes cause unpredictable and large fluxuations in measured nutrient levels over the two hour sampling time in lab tests of the procedure. All microcosm sample cups, vessels used to prepare spiked solutions, and syringes were acid washed as per laboratory procedures to best attempt an experimental environment free of background nutrients. A stringent rinsing process using Milli-Q water was utilized to minimize increasing contamination of nutrient samples by the filters as the two hour sampling period proceeded. Samples of Milli-Q water collected through the experimental filters used at the end of the two hour sampling period registered concentrations almost always at or below the limit of detection. Samples that showed higher concentrations than the limit of detection were evaluated for inclusion in results based on the always greater magnitude of uptake measured compared to the filter bias.

Results and Discussion

Substrates were placed in Little Back Creek and Stonecrop (basins with low agricultural and high forested land uses), Smith Creek (high agricultural land use with riparian forested and grass buffers), and Little Stoney and Hugh White Creeks (exclusively forested land use). The headwaters of Little Stoney Creek ran dry prior to the colonization of the tile substrates with biofilm, so no method testing could be completed at one regional site. Hugh White Creek at Coweeta Hydrologic Laboratory remained as a basin of purely forested character in which to incubate substrate.

Of nine streams under current study located in the coalfields of extreme southwestern Virginia, three were chosen in which to place substrates. These three (Laurel Branch, Left Fork, and Stone Coal) had all become established after restoration using Rosgen Stream Classification and NCD within the last three years. Their riparian zones were more open and grass dominated than those of the five unrestored regional streams. However, they are subject to similar climates, seasons, and topography as the five regional headwater streams. Rain and the physical loss of the substrate prohibited the method testing at the Laurel Branch site.

Of the eight streams in which substrates were placed, six substrates could be recovered with sufficient biofilm growth to attempt uptake measurements: four regional streams and two coal mining streams. Of these six streams, four returned uptake measurements that could be deemed statistically significant (recognizable trends that researchers could confidently distinguish as being different from zero over the period of the sampling window): Smith Creek, Hugh White Creek, Left Fork, and Stone Coal. The total uptake measurements are highlighted in Table 1.

When comparing the ratio of uptake of NH4-N alone and the uptake of P alone to the NH4-N:P uptake ratio in samples which received elevated levels of both nutrients, only Smith Creek experienced a jump in ratio in the presence of elevated concentrations of both nutrients (from 1.57 to 3.85). Hugh White Creek experienced dramatic remineralization of NH4-N, while Laurel Branch and Stone Coal did not experience a meaningful change in this ratio.

The degree of remineralization in Hugh White Creek draws questions about the accuracy of these measurements. But there are few answers for what could be the source. The most likely culprit would have been contamination of samples had these high remineralization rates not been registered in only all trials where NH4-N levels had been elevated and risen steadily over the sampling period. Cross trial contamination in these sample basins could have come from the caulk adhesive, as this was determined in laboratory testing to be the only likely abiotic source of NH4-N. However, these same tests also showed the adhesive to cause a correlated rise in the concentration of PO4 and both nutrient levels to fluctuate wildly, not rise logistically over time as occurred in the Hugh White Creek NH4-N sampling (Figures 1 and 2).

The two coal mining streams, Left Fork and Stone Coal, developed the thickest biofilm of all the streams in which substrates were placed. Tiles in these streams even developed filamentous streamers within 30 days of incubation. The developing method measured relatively low uptake for these biofilms compared to the quantity of biomass available to take up nutrients. This could be a product of having insufficiently raised the nutrient levels in the sample basins to register the full potential fall in ambient concentrations of nutrients. However, Battin, et al. (2003) states that with a thickening of biofilm, anoxic (and unproductive) voids can develop while mass transfer rates of nutrients into and out of the biofilm can limit the rate of assimilation.

The stream-side, nutrient uptake sampling method developed here can only be evaluated based on 1) its ability to return repeatable results and 2) logical and reasonable hypotheses can be formulated in order to explain the patterned or trending results.

Table 1: Average Total Uptake of Representative Biofilm using the Streamside Method

Site Name	**Nutrient(s) Elevated**	**Average Uptake (n; Range)** (ug/L-g AFDM-min)
Smith Creek	**Average "N" Uptake (alone):**	6.94 (n=3; 4.59-8.95)
	Average "P" Uptake (alone):	4.41 (n=4; 1.84-9.80)
	Average "N" Uptake (combined):	16.24 (n=3; 13.90-18.58)
	Average "P Uptake (combined):	4.21 (n=3; 3.13-5.52)
Hugh White Creek	**Average "N" Uptake (alone):**	-4013.26 (n=4; -887.08-8280.81)
	Average "P" Uptake (alone):	1.51 (n=4; 0.88-2.12)
	Average "N" Uptake (combined):	-4595.94 (n=4; -598.48-7824.00)
	Average "P Uptake (combined):	1.85 (n=4; 0.975-2.70)

Left Fork	**Average "N" Uptake (alone):**	2.54 (n=3; 2.03-2.81)
	Average "P" Uptake (alone):	0.29 (n=4; 0.22-0.44)
	Average "N" Uptake (combined):	5.95 (n=3; 1.90-12.05)
	Average "P Uptake (combined):	0.70 (n=3; 0.31-1.17)
Stone Coal	**Average "N" Uptake (alone):**	1.66 (n=4; 1.28-2.31)
	Average "P" Uptake (alone):	0.80 (n=4; 0.62-1.00)
	Average "N" Uptake (combined):	1.99 (n=4; 1.50-2.46)
	Average "P Uptake (combined):	0.85 (n=4; 0.72-1.02)

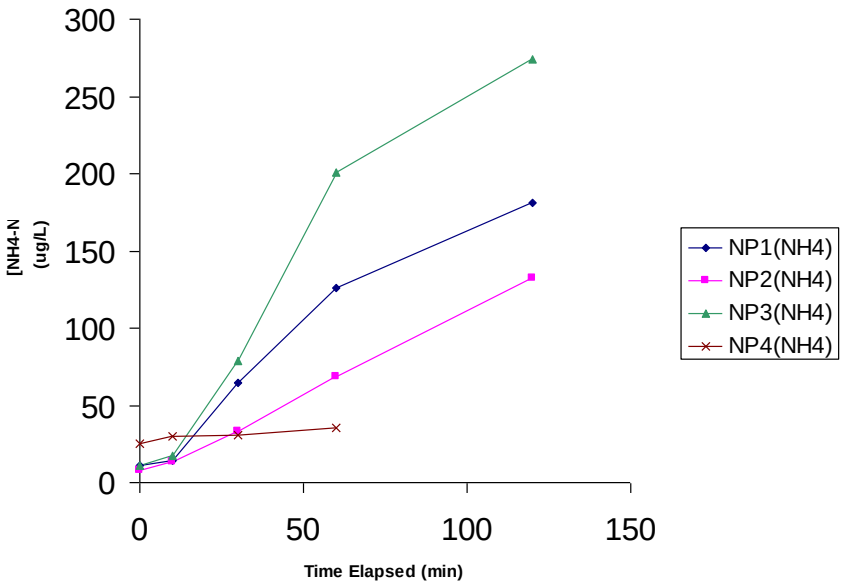

Figure 1: Hugh White Creek NH4-N Remineralization in "Combined" Elevated Nutrient Cups

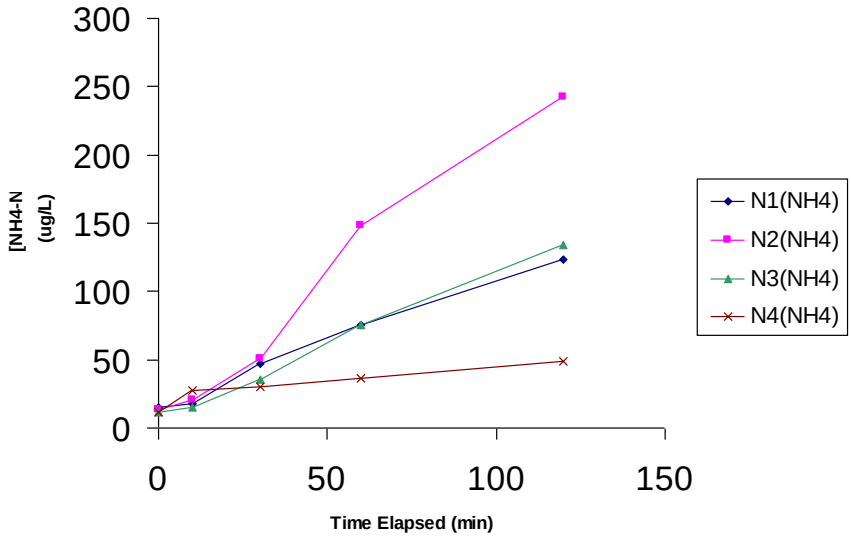

Figure 2: Hugh White Creek NH4-N Remineralization in "Alone" Elevated Nutrient Cups

On the method's ability to return results, the reasons for failure must be examined in two streams where forces of nature were not cause. Failure to return significant results during Stonecoal sampling could be derived from a variety of factors, but the most likely culprit was the lack of direct sunlight. Stonecoal's substrate was placed in an area of heavy leaf cover. Laboratory tests (and later analysis of field results from testing) strongly suggested that even moderate disturbance of the tile substrates resulted in a release of NH4. Lab tests of this method also suggested that diffuse light could result in no net trend in uptake could be detected with this streamside method over the two hour sampling window. To avoid this disturbance as much as possible but balance the need for overhead light, the Stonecoal tests were carried out at the sunniest available site under tree cover. Unfortunately, skies became overcast as the test proceeded. The issue of minimizing tile disturbance is one described in previously cited literature as always being of concern in biofilm testing methods. However, the need for direct overhead light is a necessary factor in just this stream side sampling procedure. Little Back Creek failed to return meaningful results most likely because of an isolated problem (to this stream) with the caulk adhesive. There was frequent material failure in the form of still uncured caulk during the sampling period that contaminated several of the samples noticeably and an untold number below the threshold of visual detection.

In the streams that did return significant trends, there were a number of different NH4-N and PO4 concentration behaviors that recurred over many trials in all streams. These diagnostic trends are shown in Figure 3.

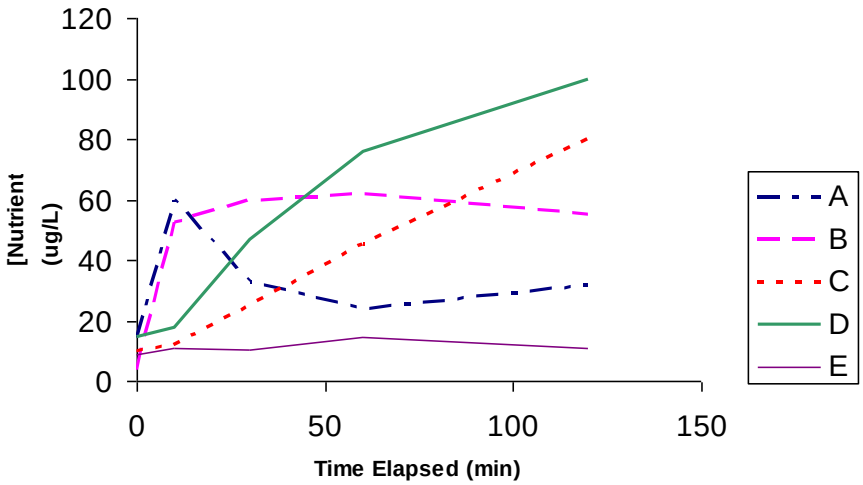

Figure 3: Common Trends in Uptake using the Streamside Method

Trend A and B were common curves to NH4-N spiked samples when subjected to either elevated or only background PO4 concentrations. These elevated initial spikes, followed by either a flattening or an exponential decay in the concentration of the nutrient, could be explained by a disturbance of the biofilm matrix resulting in an expulsion of nutrient into the sample when the tiles were lowered into the trial basins of spiked solution. Battin, et al. (2003) describe how the dominant process in thickening biofilms can be mass transfer of the reagents and products of mineralizing and assimilating reactions across the biofilm matrix. Even in thinner biofilms, as sometimes could only be grown in the limited 30 day window of incubation for these tile substrates, disturbance of this matrix could cause this initial elevation in nutrient levels. Laboratory trials of the method using only free algae in acid washed sample basins with spiked stream water reproduced this initial elevation without the presence of the tiles or caulk adhesive. The difference between the flattening off or dropping of the nutrient levels after this initial jump could be a product of the quantity or species makeup of biomass on the tiles. Tiles with low levels of AFDM registered both Trends A and B, but only tiles with higher biomass levels caused a drop in nutrient levels over time after an initial elevation in concentration. Different predominant species in each biofilm sample, especially across different streams, could result in different patterns of uptake kinetics; a decrease in concentration over time could take longer with some assemblages over others. No species diversity analysis was performed on the sample biofilm assemblages during this testing. This, plus the facts that no laboratory test of just abiotic controls could repeat either of these trends and both trends occurred in multiple streams, support this behavior as natural and accurately captured by the sampling process.

Trends C and D were remineralization patterns registered in multiple streams using this method of sampling. Trend C often appeared logistic in nature, while Trend D appeared more linear. A logistic increase in the quantity of nutrient in the sample basin is logical and expected in a closed system considering the mechanism of biofilm nutrient mineralization. Biofilms mineralize and assimilate nutrients concurrently, with multiple factors (including ambient concentrations) governing which process dominates. A buildup of ambient nutrient levels in the sample basins over the two hour sampling period could build logistically; in future applications of this procedure, a longer sampling period could capture the asymptotic equilibrium (or even subsequent drop) in nutrient levels at the point of saturation. These data points would add further support to this hypothesis, but were not collected during these tests of the sampling process.

Trend E is a simple maintenance of constant nutrient concentrations in the sample basins. This could be explained for a variety of reasons. A previous attempt to use a form of this field procedure failed because insufficient algal growth compared to basin volume caused short term uptake to be undetectable in the sample basins. Incubation time, or spike magnitude to initiate uptake as a dominant process, could have been insufficient in these trials.

None of these hypotheses are extraordinary or lack preliminary support from this test or the literature. However, there is insufficient data to support any of them as naturally recorded phenomena over simply being a product of the testing method. The trends in nutrient uptake became tighter and more replicable in the final two streams tested: Left Fork and Stone Coal (Figures 4 & 5). This could be a product of greater precision in the method application with practice or be a result of their similar character as restored coal mining streams.

Conclusion

In order to determine if this in-field technique for measuring biofilm uptake accurately gauges the degree of uptake, further in-field testing and replication must be carried out. Statements about comparing the uptake between different stream classes and types cannot occur until the method has been sufficiently verified. Future replication of this method in the streams already tested could help verify the apparent patterns observed in short term biofilm uptake mechanics. Further method development into a finer resolution time scale, especially in the early minutes of the sampling period, could produce a better picture of what is going on in the sample basins. The procedure must be applied in different seasons, conditions, and stream reaches to determine its usefulness as a robust and low cost alternative to existing incubation and mechanized methods.

Perhaps the greatest difficulty to overcome in applying this procedure is that of the need for precision. The nutrient sampling and data analysis techniques used to collect and process the data are simple and straightforward, but relatively unforgiving towards collection errors and must be precisely timed. There are no other standards for making in-field measurements, in the short term, of uptake of the existing biofilm community. The long growing time required to incubate biofilm substrate makes errors in the application of the method a potentially long term prospect to correct.

Until future statements about the accuracy and precision of the method can be more confidently made, stream-to-stream comparisons of uptake rates (such as those between restored and unrestored headwater streams) cannot be made with any reasonable degree of confidence.

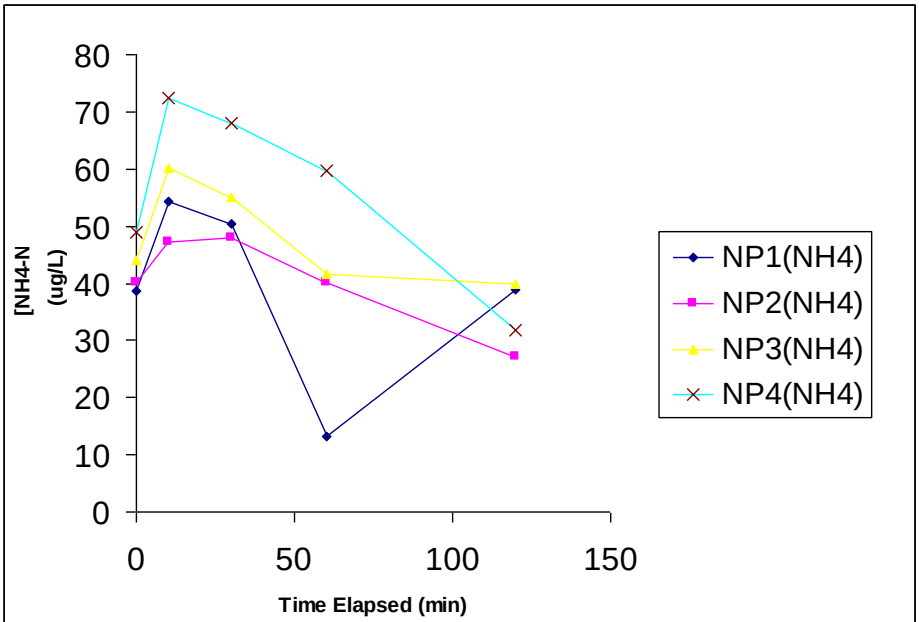

Figure 4: Diagnostic Coal Mining Stream NH4 Uptake Trend (Stone Coal)

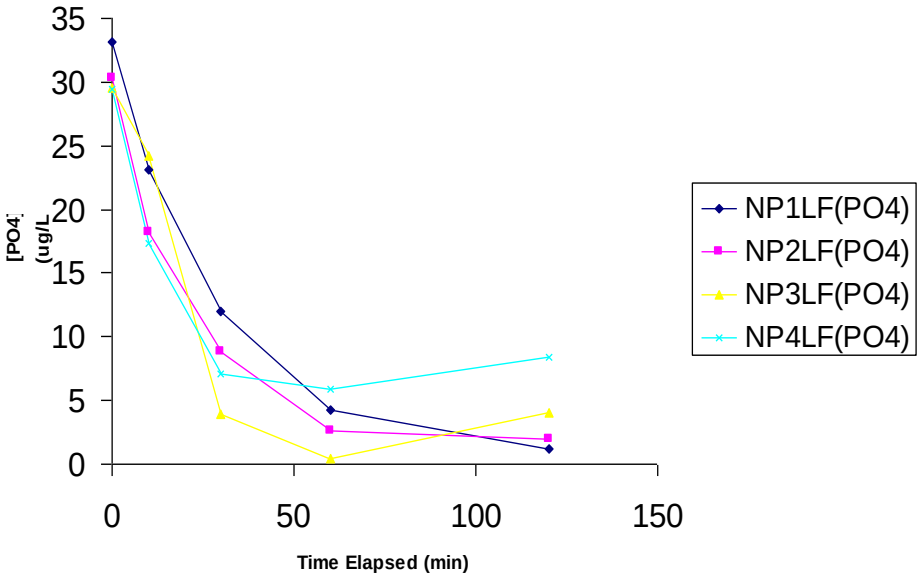

Figure 5: Diagnostic Coal Mining Stream PO4 Uptake Trend (Left Fork)

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Effects of Hypolimnetic Oxygenation Systems on a Drinking Water Reservoir

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ABSTRACT

Hypolimnetic anoxia occurs when the oxygen demands of the environment are unable to be met. Anoxia is particularly common in eutrophic lakes and reservoirs that are subject to thermal stratification. The raw water received from such reservoirs is often of poor quality and requires expensive treatments before distribution. Hypolimnetic oxygenation is commonly implemented to remediate this condition while not significantly disrupting the natural thermal stratification. Water column velocities were measured and recorded during the course of an experiment that controlled the flow rate of the oxygenation system in Carvin's Cove Reservoir. The velocities were correlated with the flow rate and wind velocities of a nearby location showing no significant correlation between either the flow rate or wind velocity.

Keywords: Anoxia, Oxygenation, Water Velocity, Stratification

Introduction

When the dissolved oxygen (DO) demand is greater than the available DO supply within a water body, then anoxia occurs. Lakes and reservoirs are subject to seasonal anoxic conditions in the hypolimnion due to the biological and chemical oxygen demands of algal and aquatic respiration, aerobic decomposition of organic matter, and oxidation of metals (Gantzer, et al. 2009a). Eutrophic lakes and reservoirs with high nutrient content, and subsequently high primary production, often exhibit DO depletion (McGinnis, et al. 2004). The anoxic conditions in the hypolimnion can result in the release of reduced forms of manganese (Mn), iron (Fe), hydrogen sulfide, ammonia, and phosphorus from the sediment into the water column. The increased phosphorus may lead to increased DO demand if it reaches the productive surface zone where it can stimulate algal growth. The hydrogen sulfide, ammonia, Mn, and Fe cause poor water taste, clarity, and odor if hypolimnion water is treated for drinking water (Singleton & Little 2006). These anoxia-related problems can result in increased treatment costs for drinking water authorities and consequently the consumers.

Hypolimnetic oxygenation systems can remediate and prevent anoxia by replenishing the DO in the hypolimnion (bottom water) of a stratified lake, where DO-depleted conditions usually exist. These systems use pure oxygen gas, supplied via a variety of methods, to reoxygenate the water. The three most common systems used are the bubble plume diffuser array, linear diffuser, and submerged contact chamber (Beutel, et al. 2007). In the bubble plume diffuser array and linear diffuser low flow rates are maintained to preserve the natural thermal stratification of the lake (Singleton & Little 2006).

Methods

Study Site

Carvin's Cove Reservoir (CCR) is a water-supply reservoir for the City of Roanoke, Virginia and the surrounding areas. It is located on private lands in a heavily-forested watershed and supplied by two natural tributaries that flow through agriculturally-dominated lands and two creeks from an adjoining

watershed that are routed through diversion tunnels. The maximum depth of CCR is 23 m, with a surface area of 2.5 km², and volume of 24 hm³ at full pond (Gantzer, et al. 2009b). CCR has a linear bubble-plume diffuser installed in the two deepest trenches supplying the water column with pure oxygen. Each of the two diffuser lines is ~60 m in length (Figure 1). The purpose of this oxygenation system is to 1) increase and maintain DO levels throughout the hypolimnion as well as the upper layers of the sediment and 2) minimize the transport of soluble species from the sediment to the bulk water, therefore reducing chemical demand and cost during the treatment process. The characteristics of CCR are summarized in Gantzer, et al. (2009a). The location and schematic of CCR are pictured in Figure 1.

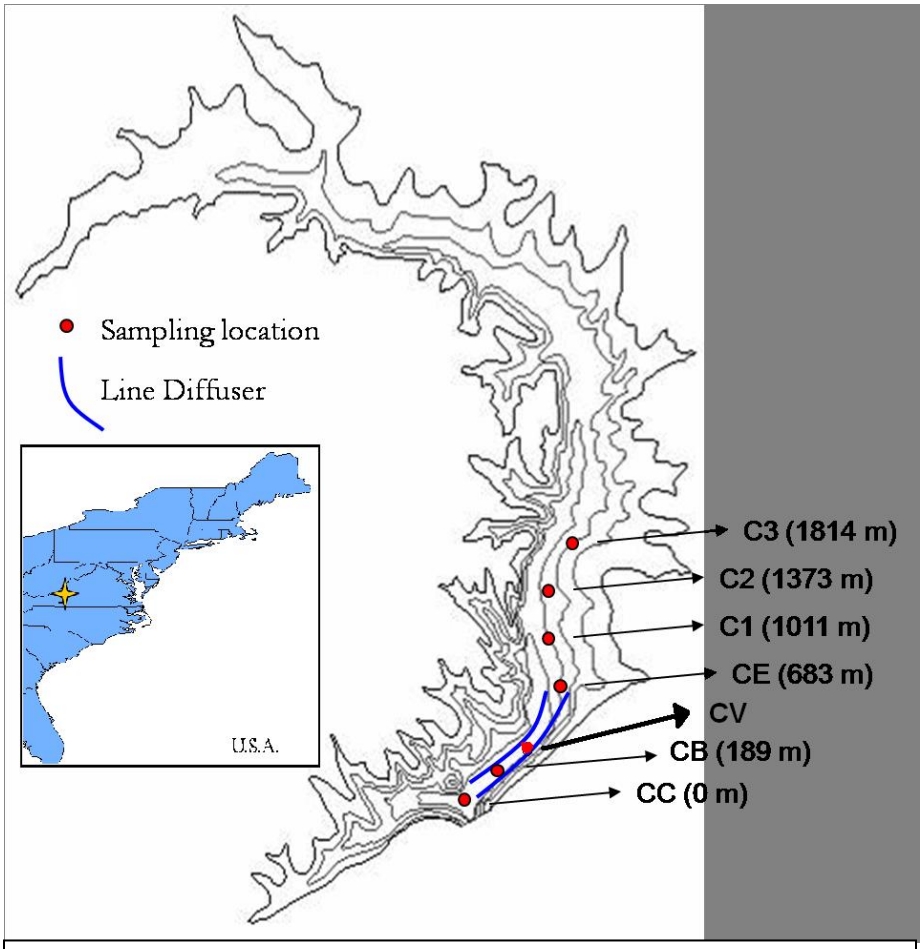


Figure 1. Carvin's Cove Reservoir, Roanoke County, Virginia
Two diffuser lines are installed near the dam. Notice the seven DO profile sites: CC, CB, CV, CE, C1, C2, C3.

Velocity Analysis

Velocity profiles were collected during the summer of 2008 using a Workhorse Rio Grande 1200 kHz ADCP to characterize water-column velocity during diffuser operation. The Workhorse Rio Grande is manufactured by Teledyne RD Instruments, Inc. The accuracy of this particular unit is +/- 0.25% of water velocity when the boat is stationary.

The ADCP functions by emitting an acoustic signal into the water column. This acoustic signal is reflected off of "scattering particles" and returns to the ADCP, which determines the velocity of the

scattering particles. Since the majority of the scattering particles are moving with the water within the reservoir, if their velocity is known then the velocity of the water can be determined.

A disadvantage of the ADCP system is that it cannot collect data over the entire water column. There are two zones in which data is not collected, at the surface and along the reservoir bottom. The zone at the surface is caused by the ADCP unit sitting a couple centimeters below the surface of the water and the small blanking distance present just below the unit. The blanking distance occurs because the ADCP both produces sound and records the backscatter. Once it produces and sends the sound out into the water column it cannot record the backscatter until the vibration produced lessens enough that it will not contaminate the backscatter. Accordingly, the blanking distance is the distance the sound travels during this period. The zone along the bottom of the reservoir is a result of the fact that most of the energy produced is contained in the main beam. The side lobes that are produced have a lower energy that does not cause a problem for most of the water column but does cause a problem for the near-sediment region. This problem is attributed to the angle at which the beams are set, some of the side lobe beams strike the sediment and are reflected back at the same time as the main beam is reflecting back from the scattering particles near the sediment. The reflected energy from the main beam is weaker than the reflected energy from the side lobes and is therefore contaminated. The immeasurable distance is about six percent of the total depth from surface to sediment. Therefore, we were unfortunately unable to acquire near-sediment velocity data (which would have been beneficial from a sediment-flux perspective); however, we were able to attain valuable water column data.

Velocity profiles were collected from three sites on CCR. The first site, CBCV, is located between the two diffuser lines near the dam. The second and third sites, C3 and CR, are located up stream of the diffuser lines 760 meters and 1500 meters respectively. These locations are shown in Figure 2. Profiles were collected at each site on June 12th, 2008, July 15th, 2008, August 12th, 2008, and September 14th, 2008. During the campaign, diffuser flow was systematically increased, with the exception of August 12th, which had the same flow as July 15th. Weeklong campaigns were performed at CBCV and C3 from June 20th, 2008 to June 26th and August 18th, 2008 to August 31st respectively. During the weeklong campaigns the diffuser flow was controlled manually and varied throughout.

The profiles collected were used to determine if there was a correlation between water velocities at specified depths, 5 and 10 meters below the surface and 3, 2, 1 meter above the sediment water interface (SWI), and diffuser flow rate. The velocity profile data were using analyzed using ADCP-supported software (WinADCP) and Microsoft Excel.

DO Monitoring

Weekly DO profiles were taken on CCR. They were taken at seven locations shown in Figure 1. The DO profiles were taken using a SBE 19plus SEACAT Profiler produced by Seabird Electronics. The SEACAT was operated in profiling mode which runs continuously, sampling at four scans per second (4Hz). Data collected by the SEACAT was automatically averaged before being stored. The sample locations were found using a handheld GPS that had the locations previously stored in its memory. Once at the sampling location, the SEACAT was lowered manually from the boat using a hand winch with a small boom to measure a vertical profile. As the SEACAT reached the sediment, the winch operator would feel less weight and would begin to crank in the opposite direction to raise the SEACAT. After all of the profiles were taken the data was uploaded to a computer at the Western Virginia Water Authority's Spring Hollow site and analyzed by one of their employees.

Sediment Core Extraction

A laboratory sediment core experiment was performed to estimate how oxygenation affected DO penetration into the sediment, and the subsequent sediment oxic zone. These experiments were performed in a controlled-temperature environment with a mini-diffuser installed in the sediment core water column (to establish oxygenation effects on a lab, rather than reservoir, scale).

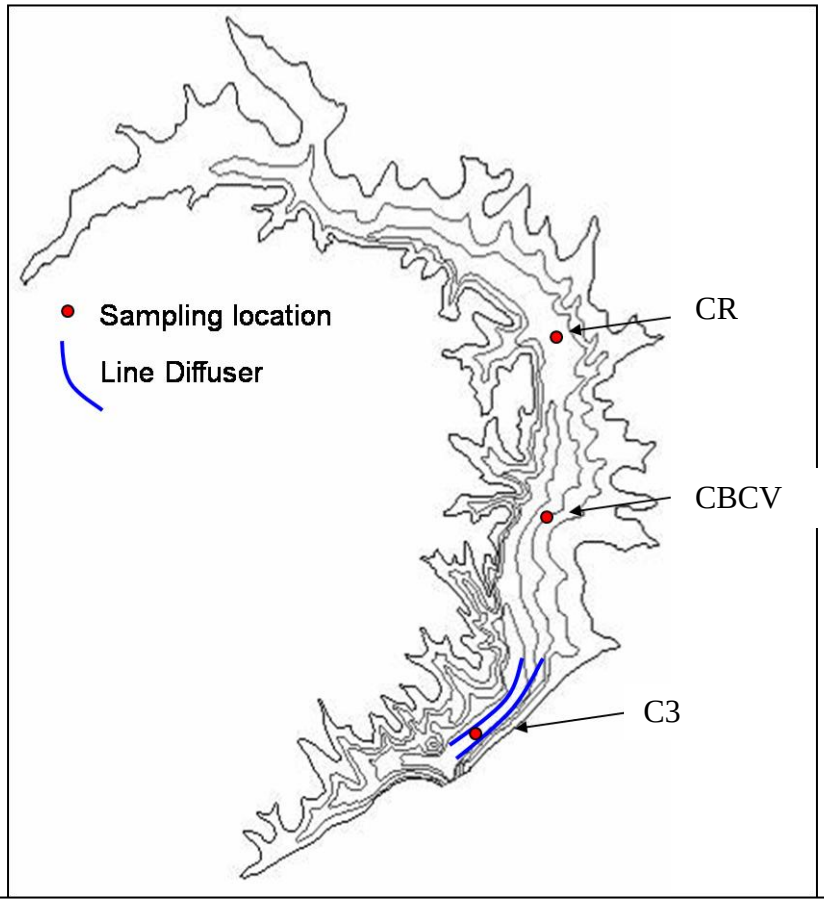


Figure 2. Carvin's Cove Reservoir, Roanoke County, Virginia
Two line diffusers and ADCP sampling locations.

Sediment cores were taken from location CBCV (Figure 2) in 9cm diameter plexiglass tubes. They were extracted using an Uwitec ball corer and cut to proper size using an Uwitec core cutter. The cut cores had approximately 10cm of water column above the SWI. The four cores that were retained had largely undisturbed sediment surfaces. The cores were covered and stored on ice to block light, prevent photosynthetic respiration, and reasonably maintain in-situ reservoir temperatures. The cores were then transported to Virginia Tech's Blacksburg Campus for diffuser simulation.

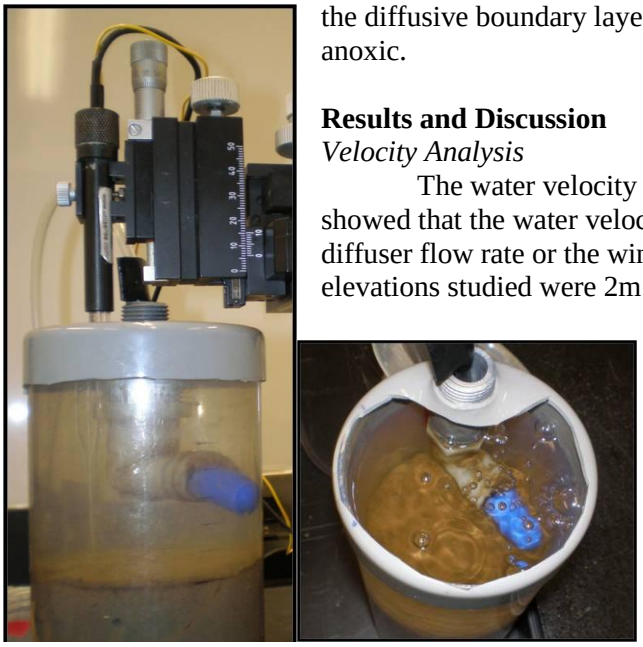
Sediment Core Profiling

Six sediment core samples were obtained in July 2009. The most undisturbed sediment core sample was set up in a controlled environment at 10 degrees Celsius. The core was allowed to go partially anoxic overnight then was brought to DO saturation and allowed to go anoxic. To bring the core to DO saturation a sandstone mini-diffuser (2cm height, 1cm diameter) was suspended about 2cm above the SWI. To control the flow rate through the mini-diffuser we used a low-flow rotameter (Aaalborg model P) set at 50 mL/min. DO microprofiles were taken periodically throughout the transient

stages. Unchanging DO concentrations in the water column and across the SWI indicated that the core had reached DO saturation.

Profiles were taken using a glass DO microsensor (100µm diameter tip; Unisense OX-100) connected to a picoammeter (Unisense PA2000). The method used is similar to that used in House (2003). The sensor was lowered manually in 1mm increments into the water column and sediment using a micromanipulator attached to a magnetic stand. Measurements were recorded using the Unisense microprofiling computer program Profix and were calibrated daily using Winkler titrations.

The core was incubated in the dark except when the profiles were being obtained. Efforts were made to keep the core as motionless as possible to avoid transport of DO into the diffusive boundary layer (DBL) during the transition from DO saturation to anoxic.



Photographs of Diffuser Simulation Set-Up

Results and Discussion

Velocity Analysis

The water velocity profiles did not show the expected results. They showed that the water velocities were not significantly influenced by the diffuser flow rate or the wind velocities at the elevations studied. The elevations studied were 2m above SWI for location CBCV and 1m above SWI for locations C3 and CR. The average velocities and diffuser flow rate are shown in Figure 3, along with wind velocity. The wind velocity data were obtained from the National Oceanic and Atmospheric Administration (NOAA) site at the Roanoke Airport approximately 8 km from CCR. Based on the correlations observed in Figure 3 data, it seems that wind has a much stronger influence on water column velocity than diffuser-induced turbulence.

Sediment Core Profiling

Figure 4 shows the DO profiles of the core as they progressed from normal (pre-aeration state) to O_2 saturation. The profiles did not show a defined DBL during this phase of the experiment. A DBL may have been present but compressed to less than 1mm due to the turbulence caused by the mini-diffuser; thus, it would not have been captured by the mm-scale profiles. Saturation was reached in ~10 hours, which was indicated by unchanging DO gradients across the SWI. The initial DO concentration at the SWI was <1 mg/L DO and reached saturation at ~11.5 mg/L.

Figure 5 shows the DO profiles as the core was allowed to go anoxic from the previously established DO saturation state (Figure 4). Once the aeration was removed, the core began to show a visible DBL extending between 1 and 2 mm above the SWI. The core did not reach a complete state of anoxia in the time allowed, which may be attributed to the DO supplied during the DO-saturation phase of the experiment met the DO demand of the sediment and overlying water column. The final DO concentration reached was 1.8 mg/L DO. The anoxic phase of the experiment was performed for 192 hours after the mini-diffuser was removed. The DO concentration in the DBL after 72 hours was nearly the same as 192 hours which decreased from 2mg/L to about 1.8 mg/L DO.

The times required to take the core from pre-aeration state to O_2 saturation and from saturated to anoxic may have been affected by fluctuations in the temperature of the core. The controlled temperature room that was utilized initially malfunctioned during our experiment, which caused the temperature to fluctuate from approximately 6°C to 12°C during the pre-aeration to saturation phase. The temperature during the saturation to anoxic phase fluctuated from 6°C to 11°C with a peak of 17°C.

Conclusions

Anoxia in lakes and drinking water reservoirs is effectively remediated by hypolimnetic oxygenation systems. These systems replenish DO in the bottom waters without mixing the bottom and surface waters, thereby not disturbing the thermal stratification. By not disturbing the thermal stratification, oxygenation systems tend to only minimally disturb the aquatic ecosystems. Aside from minimally disturbing the ecosystem the oxygenation systems increase the DO concentration at the SWI. This increase at the SWI results in lower flux rates of metals from the sediment to the water column. Overall hypolimnetic oxygenation systems can be beneficial to lakes and reservoirs if properly designed.

Acknowledgements

The author would like to thank Tamim Younos, Vinod Lohani, and the Virginia Tech facilities for organizing this research opportunity. Special thanks to Lee Bryant, Dyan Lorge, and John C. Little for their guidance and support. In addition, thanks to the Western Virginia Water Authority staff at the Spring Hollow treatment plant. This work was supported by the National Science Foundation under Grant number 0649070. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author and do not necessarily reflect the views of the National Science Foundation.

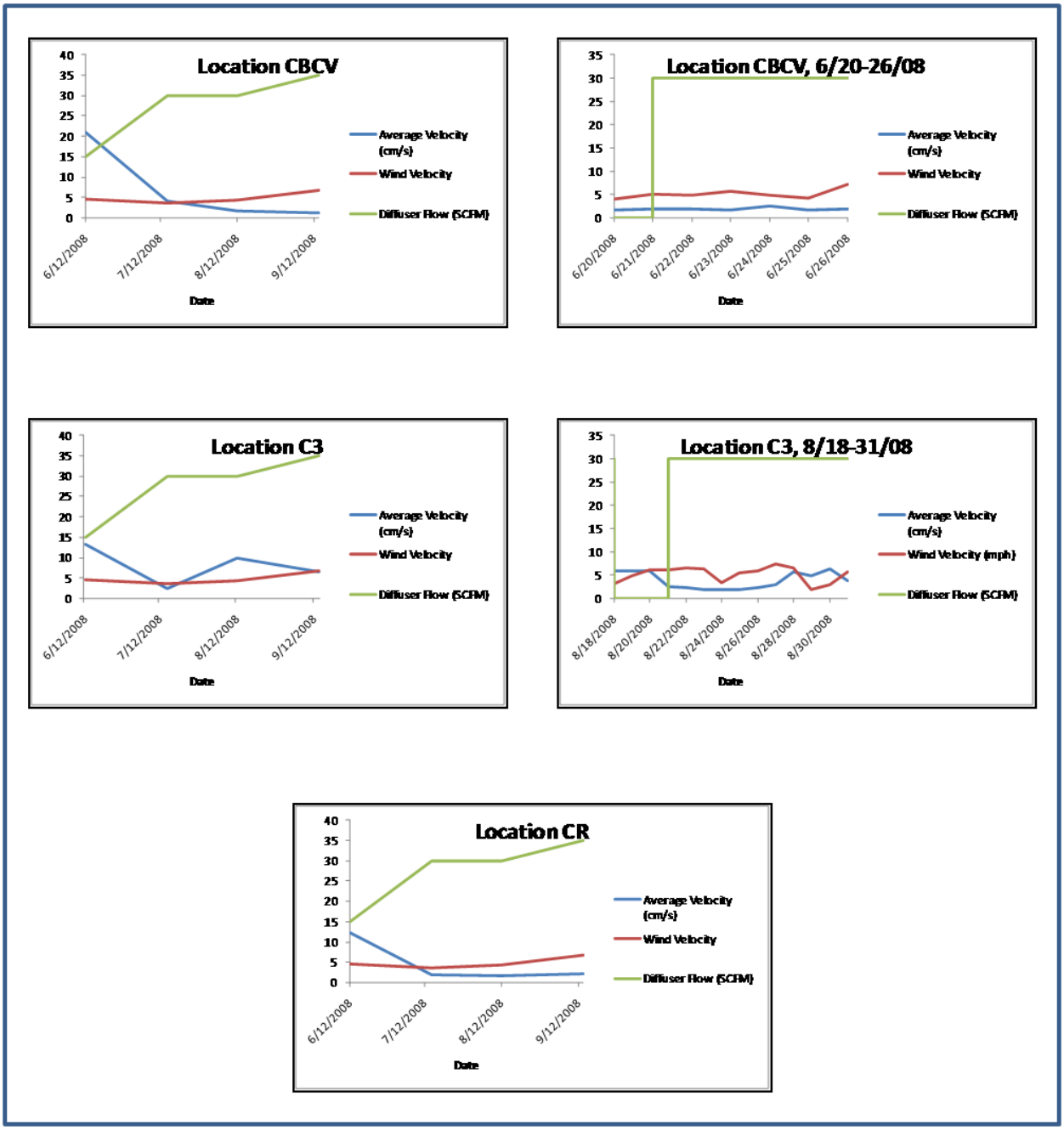


Figure 3. Velocity Analysis Results

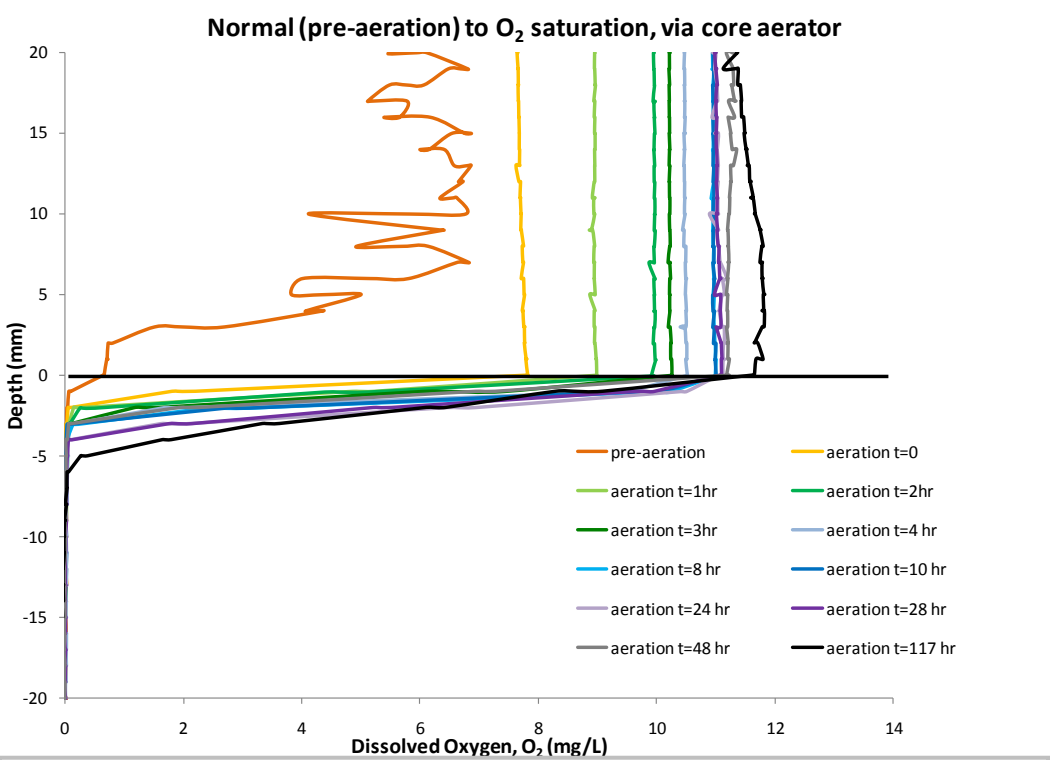


Figure 4. Diffuser Simulation: Pre-aeration to O_2 Saturation

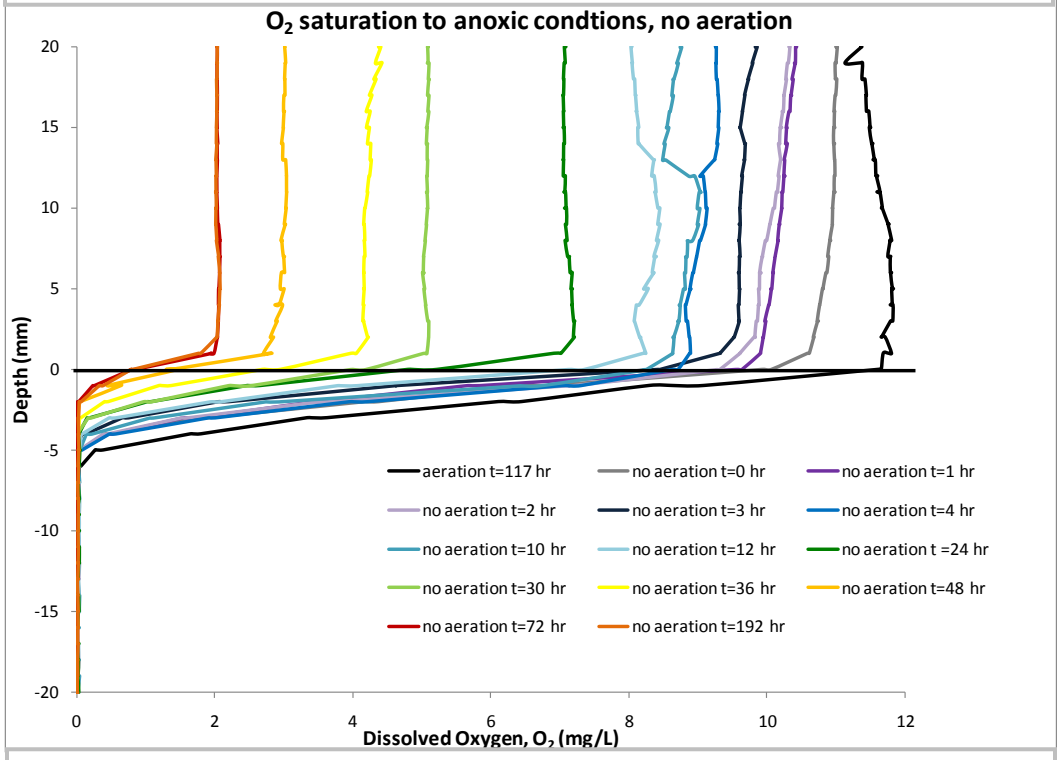


Figure 5. Diffuser Simulation: O_2 Saturation to Anoxic

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Fate and Survival of Known-Source Fecal Indicator Bacteria in Chesapeake Bay Sand and Water

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ABSTRACT

A major emerging issue with current water quality assessments is that large numbers of fecal indicator bacteria (FIB) are often found by disturbing the sand or sediments where water samples are taken, especially at recreational beaches (Haller *et al.* 2008). Sediment FIB populations have become a priority issue for the USEPA because anyone using such waters for recreational purposes is exposed to the sediment populations, and higher numbers of FIBs are well correlated with greater risk from water-borne pathogens (USEPA 2007). By better understanding the fate and survival of FIB, it may be possible to determine what constitutes safe and acceptable levels for FIB in beach sand and sediment. In this study, the current FIB, *Escherichia coli (E. coli)* and *Entercoccus sp.*, from known sources (humans, dogs, and birds) were added separately to microcosms and monitored for 21 days. The microcosms were made to simulate both a favorable (stagnant) and natural (aerated) environment, with the sand and water obtained from the lower Chesapeake Bay (Festival Beach, Matthews County, VA). In the microcosms, water samples were collected from the water column, the water-sediment interface, and the sediment. Dissolved oxygen and electrical conductivity were measured before every sampling. Both the general *Bacteroides* and human-specific *Bacteroides* genetic markers were measured as well, to assess the performance of this DNA-based source tracking method. When exposed to lower dissolved oxygen (stagnant) *Enterococcus* does not thrive as well as *E. coli*. In most cases, the added FIB settled into the sediments by day 10, and colony counts grew from that point on. The aerated microcosms experienced either rapid FIB die-off or a temporary spike in the sediment, followed by a rapid die-off. The genetic markers were not detected in the aerated microcosms after one week, but were detected in the stagnant tanks for the entirety of the 21-day experiment .

Key Words. Fecal Indicator Bacteria, Microbial Source Tracking Methods

Introduction

Much of our wells and recreational water, and in many cases even water already treated, can contain trace amounts of potentially harmful bacteria. This is because the United States' sewage collection infrastructure is not only aging and in need of replacement, but it is also running at a much higher capacity than it was intended for (Hathaway 1998). This means that our wells, recreation, and potable waters are subject to pollution through leaks and breakages in the infrastructure. In order to prevent serious illness from this contamination, the USEPA created the Clean Water Act. After that, the nation adopted a system known as the National Point-Source Discharge Elimination system (NPSDES) to eliminate pollution from known sources of contamination (Stoeckel 2005). Although reasonably successful, there were still many areas of the country that were unable to meet the Clean Water Act's guidelines. In such cases it became necessary to eliminate nonpoint source contamination, or contamination of water from other locations that contain a variety of bacteria from several different sources. The identification of nonpoint pollution sources is known as Microbial Source Tracking, or MST.

Due to the variance in bacterial strains from state to state, each state constructed a library to reference when trying to properly implement MST. These libraries were very difficult to use, because as more bacterial strains were being discovered, they continually needed to become more location specific. In order to reduce this trouble, indicator bacteria, or bacteria more universally associated with fecal pollution, began to be utilized as a way to determine if the water source was polluted. Indicator bacteria are still considered to give the most consistent results (Hagedorn 2009). Methods used at this time mostly called for consulting said libraries. The most popular of these methods is Antibiotic Resistance Analysis (ARA). In a report submitted to the Florida Stormwater Association in 2005, ARA is described as "[using] the antibiotic resistance patterns of the microbial isolates as fingerprints for distinguishing human from animal sources" (Bitton 2005). Other methods often used are the BIOLOG System (another library system), Ribotyping (a genotypic method), and Pulsed Field Gel Electrophoresis (a molecular method).

It was soon realized that these methods were all too broad to be used alone to assess the quality of water. Multiple MST methods were tested together in the hopes that the confirmation in the origins of fecal contamination through many different lines of evidence would solidify the specific origin of the pollution.

MST still utilizes multiple methods at a time to derive reliable results. This route of source tracking is becoming tedious, expensive, and outdated. New methods or indicators are needed to simplify and update pollution source identification. Methods using genetic markers are currently being tested, however MST cannot advance without understanding fully the most reliable asset to source tracking: the FIB. Particularly in the state of Virginia, *Escherichia coli* (*E. coli)* is used as the indicator for fresh water pollution, and *Enterococcus* is used for salt water. These indicator bacteria were mandated by the USEPA in the 2001 National Beach Act (Stoeckel 2005). Little is noted about the behavior of these bacteria as a whole. Many strains of each FIB have been studied and determining their similarities to one another will make the use of these indicators more effective.

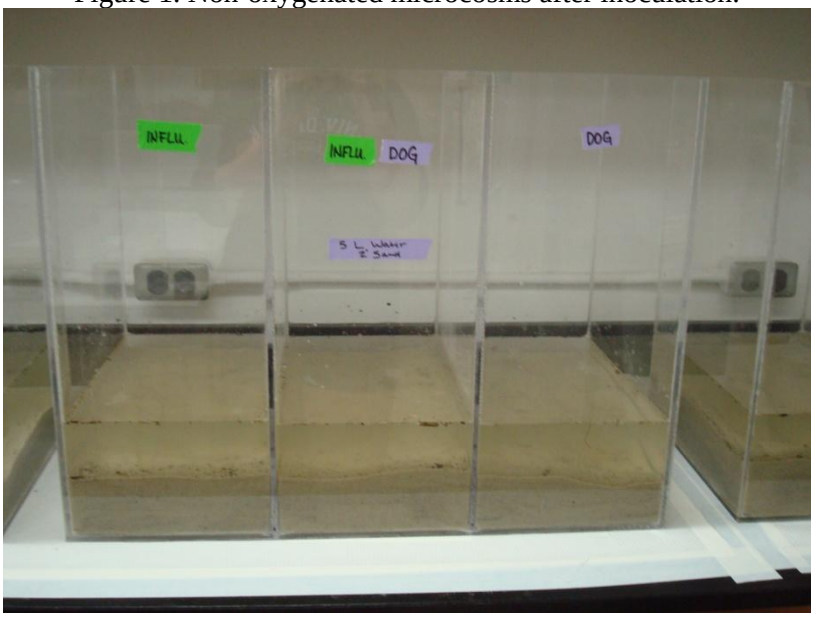
E. coli is a type of fecal coliform, which is usually found in the feces of warm-blooded animals. It is also currently the most popularly used indicator bacteria (Bitton 2005). *E. coli* is so common because it is thermotolerant, it indicates the presence of warm-blooded feces, and – most importantly – its lifespan and survival patterns are very similar to most bacterial pathogens (Bitton 2005). *E. coli* is also known to survive well in coastal sediment and is subsequently subject to resuspension due to strong tides. *Enterococcus* is a robust gram-positive bacteria, that also follows the life patterns of harmful bacterial pathogens. Less is known about *Entercoccus*, as it is less widely used.

Materials and Methods

Microcosm Set up

First, the 304X914X457 mm plexiglas tanks were cleaned and dried. Sand and ocean water were collected from Fairview and Festival Beaches off the coast of Virginia. The sand was dried in an oven at 100˚C, and poured evenly into all the tanks until it was 5 mm tall. 5 L of the beach water was poured over the sand, and the sand was allowed to settle for several hours. The day that the water was poured into the tanks, the water, water-sediment interface, and sediment were all sampled and filtered according to the protocol listed below. The filters were placed on mEI and mTEC agar to test for initial *E. coli* and/or *Enterococcus.* All tanks were covered with saran wrap. The aerated tanks were fitted with a small bubbler, and tubes inserted into the column. The top of each tube releases air constantly and was resting at the water-sediment interface.

Figure 1. Non-oxygenated microcosms after inoculation.



Tank Inoculation

After the initial filtrations, the tanks were inoculated with the fecal samples. The stagnant tanks were inoculated in the following way: three tanks contained mixture of phosphate buffer solution (PBS) and dog fecal matter, three tanks contained a PBS and bird fecal matter solution, three tanks held an influent and PBS solution, and there was one tank left clean as a control.

There were also four aerated tanks – one control tank and three tanks inoculated with the same dog fecal matter and PBS solution.

Sampling

Prior to sampling, the percent dissolved oxygen and the electrical conductivity of the water was assessed. Water samples of 10 mL or less are removed from the individual microcosms from midway in the water column, the water-sediment interface, and midway through the sediment layer. To take the sample, a serological pipette is utilized. Depending on the amount of growth from a particular tank, the water sample is either placed directly into the membrane-filtration apparatus or diluted accordingly. The dilutions used in this experiment are shown in Table 1. Samples were diluted with sterile water in 15 mL Corning tubes.

Membrane-Filtration and Agar Incubation

One hundred mL graduated cylinders, sterile filter towers, and a manifold are used for filtration along with MicronSep, cellulosic, 47 mm filters. The filtration apparatus is cleaned with ninety percent ethanol, and then rinsed with sterile water until the ethanol is visibly washed away. The filter is inserted after cleaning. The proper amount of either direct or diluted sample is placed into the top of the apparatus, and vacuumed through the filter using a small motor. The filters are removed using sterile forceps and placed on 50 mm plates of either mTEC (for *E. coli* identification) or mEI (for *Enterococci* identification) agar (Myers 2007). The mTEC agar is placed in a water bath at 44.5˚C (112.1˚F), and bacterial colonies are counted after 24 hour incubation. The mEI agar is placed in a water bath at 41˚C (105.8˚F), and colonies are counted after 24 and 48 hour incubations.

Table 1. Dilutions of samples and the amount filtered.

Pollution Type	Aerated/Stagnant	Sample Dilution	Amount Filtered
Control	Stagnant	None	5 mL
Dog	Stagnant	.1 mL/9.9 mL	3 mL
Bird	Stagnant	None – *E. coli* .5 mL/9.5 Ml – *Enterococcus*	5 mL
Influent	Stagnant	.5 mL/9.5 mL	5 mL
Control	Aerated	None	
Dog	Aerated	.5 mL/9.5 mL	5 mL

Table 2. Chart of antibiotics used in ARA and various concentrations.

Antibiotic	Various Doses (µg/l)
Rifampicin	60, 75, 90
Oxytetracycline	2.5, 5, 7.5, 10, 15
Streptomycin	2.5, 5, 7.5, 10, 15
Cephalothin	15, 25, 35
Erythromycin	70, 90, 100
Tetracycline	2.5, 5, 7.5, 10, 15
Neomycin	2.5, 5, 10
Control	0

DNA Extraction from Filtered Samples

A 5:50 mL dilution of the sample was filtered according to the protocol listed above. The filter was removed with sterilized forceps and rolled to fit into a 15 mL Corning tube. The following protocol was used directly from the instruction manual provided in the Quigen QIAmp Fecal DNA Extraction Kit. One and four tenths mL Buffer ASL was placed into each sample (regardless of tube size) and vortexed for 1 minute. The tubes were then heated in a water bath pre-heated to 70˚C for 5 minutes or 6 minutes if samples in ASL were refrigerated. One and two tenths mL of the supernatant was pipetted into a 2 mL tube and the remainder discarded. One InhibitEX Tablet was added to each sample and vortexed immediately and continuously until the tablet was completely dissolved. This was centrifuged for 3 minutes and vortexed to create a pellet. Fifteen µl of Proteinase K was pipette into a new 1.5 mL tube, also 200 µl of the supernatant from step 6 was added and 200 µl of Buffer AL. This tube was vortexed for 15 seconds and incubated in the water bath for 10 minutes. Then, 200 µl of ethanol (96-100%) was added to the lysate the DNA and mixed by vortexing. The lid of a new 1.5 mL QIAmp Spin Column was labeled, the EtOH combination from step 9 was placed inside, and centrifuged for 1 minute. A new tube was taken out and the QIAmp spin column was replaced, while 500 µl Buffer AW1 was added. That was centrifuged for 1 minute. The QIAmp spin column was placed in a new 2 mL tube and the remaining fluid from the filtration was discarded. Five hundred µl of Buffer AW2 was then added to the QIAmp Spin Column and centrifuged at full speed for 3 minutes. The collection tube containing the filtrate was discarded and the QIAmp spin column transferred into a new, labeled 1.5 mL tube. Finally, 200 µl Buffer AE was pipette directly onto the QIAmp membrane, the cap closed, and the unit incubated for 1 minute at room temperature. Then it was centrifuged at full speed for 1 minute to elute DNA. The product was either used immediately, or frozen for future use.

Polymerase Chain Reaction (PCR)

First, a master mix was created using 125 µl of Promega Master Mix, 70 µl of nuclease-free water, 10 µl BSA (diluted 10 µl BSA to 30 µl sterile water), 10 µl of the backward primer (Bac708), and 10 µl of the forward primer (either Bac32F or HF183 depending on whether *Bacteroides* or *Human*

Bacteroides PCR product was to be obtained). 22.5 µl of the master mix was placed in a PCR tube along with 2.5 µl of DNA extraction product. The PCR tubes were labeled appropriately and placed in the PCR machine. The PCR protocol chosen by Dr. Books Crozier for the two primer sets is shown in Table 3.

The final step in the PCR process was to set the machine to incubate at 4˚C, so the PCR product can be left in the machine until needed.

Table 3. PCR protocol for the PCR machine for the two primers.

Bac32F/Bac708		**HF183/Bac708**		**Cycles**
T ˚C	**Time**	**T** ˚C	**Time**	
95	5 min	95	5 min	1
94	1 min	95	30 sec	35
53	1 min	59	1 min	
72	1.5 min	72	2 min	
72	5 min	72	10 min	1
4	Continuous	4	Continuous	1

Gel Electrophoresis

A Biorad Gel Electrophoresis apparatus is used for this process. First, a 1% Agarose gel was made from 0.5g of Fisher Agarose powder and 50 mL of 1XTris Acetate EDTA (TAE). A stir bar was added to this mixture, and it was heated and stirred on a hot plate until it became transparent. The Agarose was then poured onto an align tray with a 20-well comb inserted. This was placed in the refrigerator until hardened. Five µl of the PCR product or control ladder was added to 2 µl of 6X blue running dye over a piece of parafilm. After setting the pipet to 7 µl, the PCR product-dye mixture was individually placed in each well of the gel. The gel was then run at 120 V for 45 minutes in cold 1X TAE.

While the gel was running, 2 µl of 10,000X SYBR green was mixed in 20 mL of 1X TAE, and placed in a light sensitive jar. When the gel finished, it was removed from the electrophoresis machine and put in a rig that allowed the SYBR green to be poured directly over top. The gel in the SYBR green was protected from the light and allowed to sit for 20 minutes. Finally, the gel was removed from the rig and looked at and photographed over a UV light.

Results/Discussion

Samples were taken before the tanks were inoculated yielded 7 *E. coli* colonies (an insubstantial amount) and zero *Entercoccus* colonies. After the initial sampling, the microcosms were inoculated once (10% by volumeand sampled seven times from the water column, the water-sediment interface, and in the sediment. Two filtrations were done from each tank in each location so the filters could be incubated on both mEI and mTEC to properly grow the *Enterococcus* and *E. coli* respectively. The colonies on the mTEC plates were counted 24 hours after incubation began. Figure 2 is graphs of average numbers of colonies at each sampling point in all three tanks for the bird, dog, and influent. The graphs were reinforced by the graphs of the dissolved oxygen readings taken before each sampling. Figure 3 shows the correlation between the graph of the dissolved oxygen percent and the colony counts in the sediment, specifically. It is easy to tell in these graphs that mostly where the counts rise, the dissolved oxygen percent drops and vice versa. These graphs show the averages of the *E. coli* colony counts in the sediment. However, since dissolved oxygen is measured in percents, the counts were divided to be less than 100. So, the bird counts were divided by 10^{-1}, the dog counts were divided by 10^{-4}, and the influent counts were divided by 10^{-2}. The aerated dog counts did not need to be altered to fit the requirement.

E. coli in all cases peaked by the second sampling on day 5. Generally, after day 5, the colony counts in the water and interface tapered off until they could no longer be detected even undiluted samples. It is easy to see that the counts in the sediment stayed relatively constant, but toward the end of the experiment the numbers began slowly rising. By the fifth sampling, the fourteenth day, the sediment

colony counts have surpassed that of the water and interface in all cases. This is demonstrating *E. coli*'s natural tendency to grow in the sediment. It has been hypothesized that the sediment holds more nutrients for the bacteria to grow (Craig 2004). Aside from that, *E. coli's* tendency to be found predominantly among the sediment is noted in many current journal articles. Seeing this trend in a 21 day experiment, using both aerated and stagnant tanks, could confirm another current idea tested at Nova Southeastern University in Davie, Florida that *E. coli* may not be the best indicator. During a storm or other weather event, the sediment is moved the most. It is also kicked up and often beached by recreational swimmers, usually to be picked up again by a high tide. It is possible that it may not be indicating current pollution, but only past pollution (Hartz 2008). By day 21 (sample 7), all *E. coli* counts were zero except for the sediment in the influent (human) microcosms.

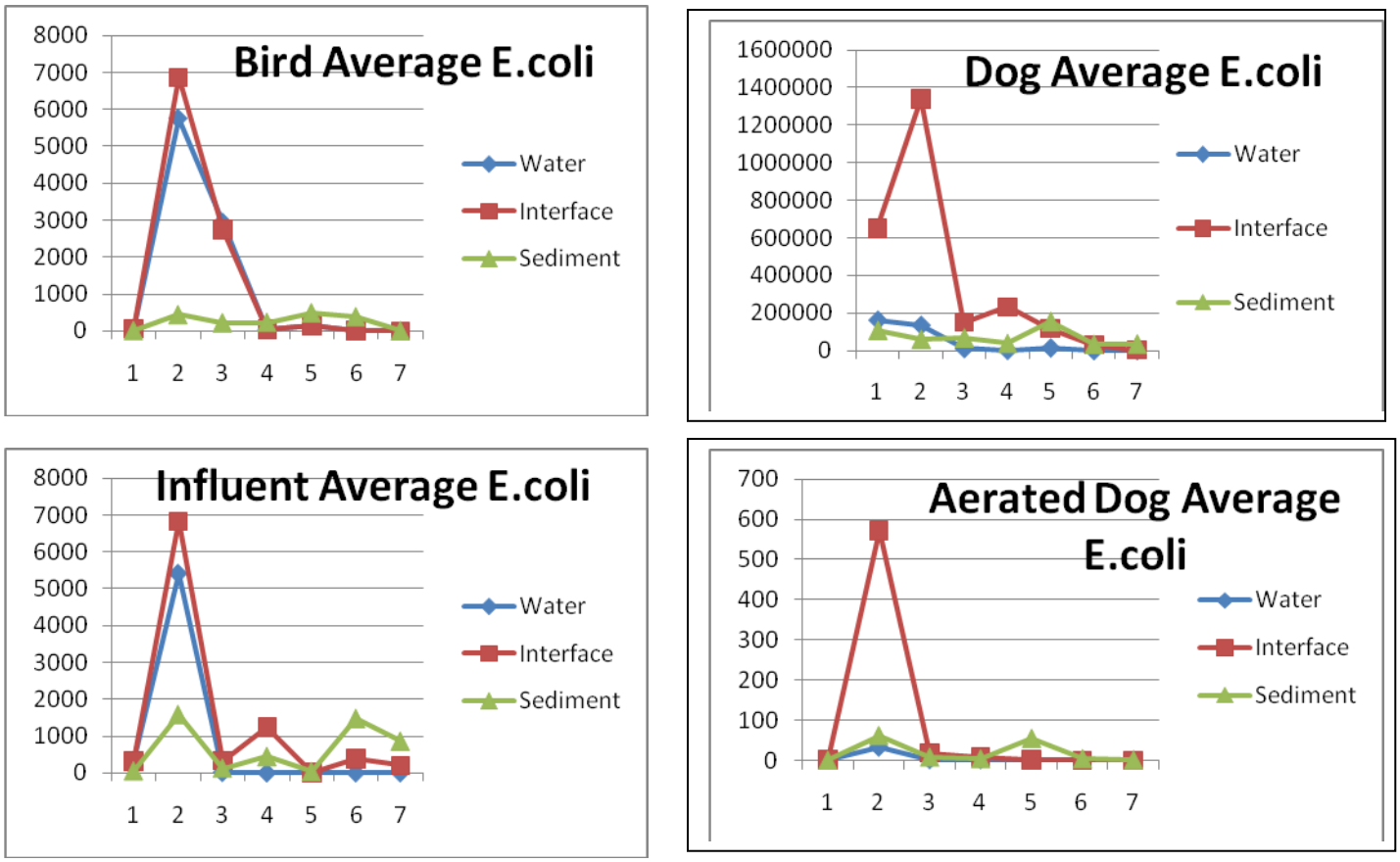


Figure 4. *E. coli* population averages over all microcosm sampling points

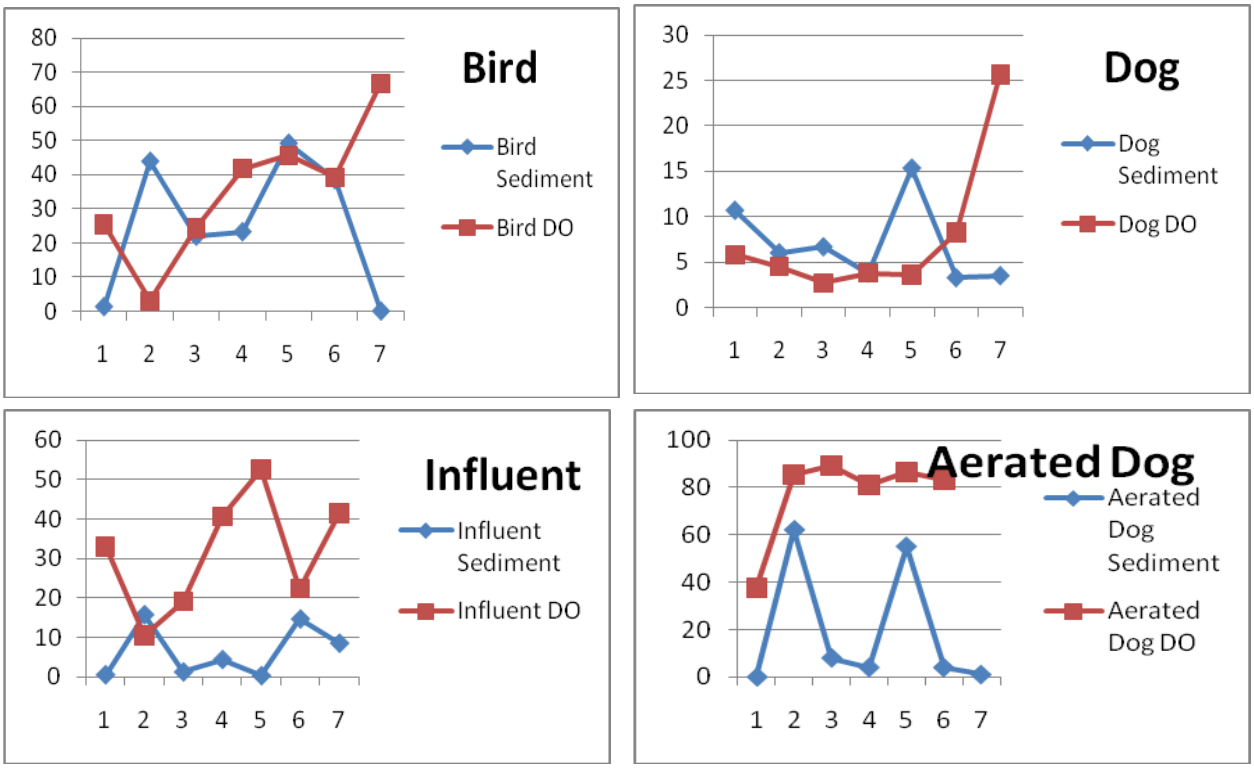
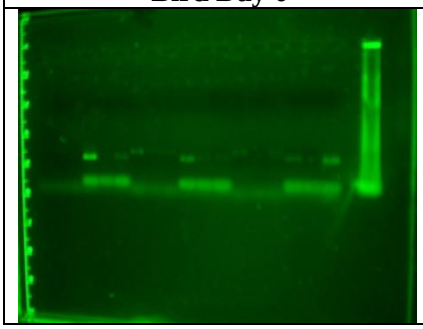
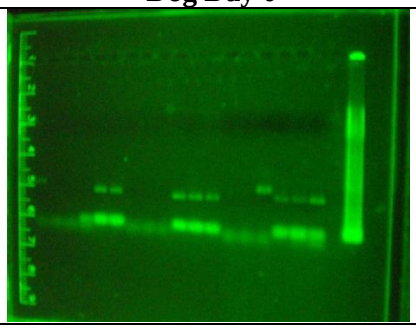


Figure 5. Comparison between *E. coli* colony count averages in the sediment to dissolved oxygen percentages

Even more interesting are the patterns created by the *Enterococcus*. The rise of colony growth in the sediment as the die off in the growth in the water and interface happens is even clearer in these graphs. It is also easy to see the correlation of the dissolved oxygen percents to the growth and die off of colonies in the sediment. The graphs in Appendix A show the average colony counts at each sampling point for *Enterococcus*. It should be noted that, although the initial die off is much quicker than that of *E. coli* the *Enterococcus* becomes stable at lower volumes and never completely dies off. Although it is subject to resuspension just like *E. coli*, the *Enterococcus* proves to be longer lasting, and more consistent in its counts. It is also clear to see, that if there are high numbers of *Enterococcus* in water being examined, the pollution is very recent.

After the filtrations were completed, DNA extractions, PCR, and gel electrophoresis were run on samples from day 0, day 14, and day 21 to see if there was a correlation between the detection of the *Bacteroides* genetic marker and the growth and decay of the fecal indicator bacteria. Day 14's samples yielded the best PCR results. The gels were clear and bands were easily visible. Bird was consistently positive for human *Bacteroides* in about half of all the samples, and dog consistently looked very similar. As expected, influent tested positive for human *Bacteroides* about ninety percent of the time. For the generic *Bacteroides* maker, however, the majority of all the wells were negative. This could indicate that this marker is more difficult to detect than expected, or that the primer used was not replicating in the PCR machine. Interestingly, the strongest results (from day 14) correlate almost exactly with the median of the colony count numbers. This information should be noted. It shows that these genetic markers, although effective, are very sensitive and can be deterred by having too much or too little bacteria to work with. This should be further explored to determine exactly the sensitivity of the *Bacteroides* genetic markers. Figure 6 shows the gels obtained through gel electrophoresis.

Bird Day 0	Dog Day 0	Influent Day 0
		This gel broke in the process of electrophoresis, and there was not enough PCR product remaining to duplicate it.

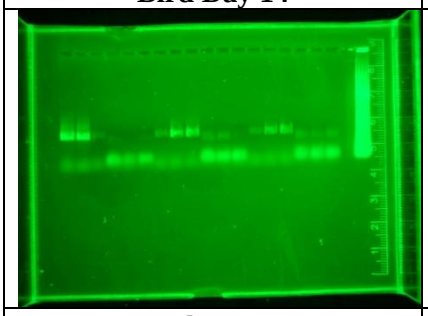
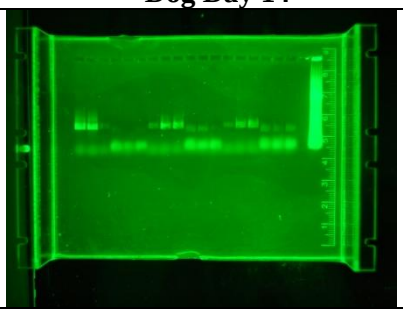
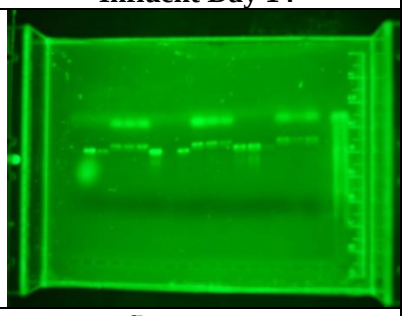
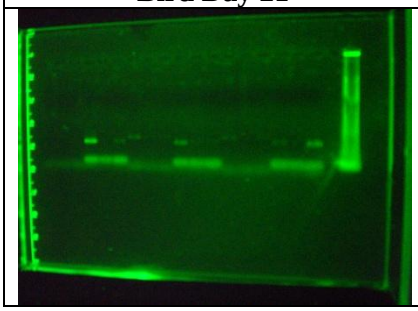
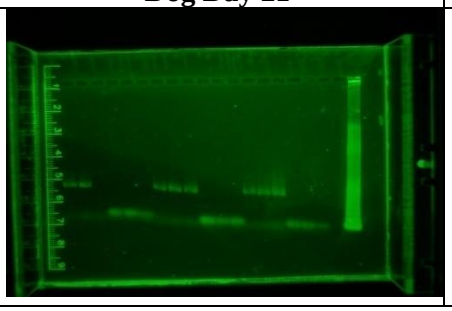
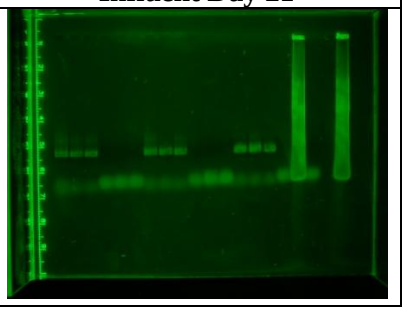
Bird Day 14	Dog Day 14	Influent Day 14
		
Bird Day 21	**Dog Day 21**	**Influent Day 21**
		

Figure 6. Photos of gels for DNA Extractions, PCR, and Gel Electrophoresis.

Acknowledgments

I would like to acknowledge the National Science Foundation for the opportunity given to me, by them, through Virginia Tech. Virginia Tech's Watershed Resource Research Experience for Undergraduates program should also be acknowledged, specifically Dr. Tamim Younos. Finally, I would like to thank the Hagedorn lab for allowing me to use their space and helping me learn and understand Microbial Source Tracking.

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Myers, D.N., D.M. Stoeckel, R.N. Bushon, D.S. Francy, & A.M.G. Brady. (2007) Fecal indicator bacteria: U.S. Geological Survey Techniques of Water-Resources Investigations. Book 9.

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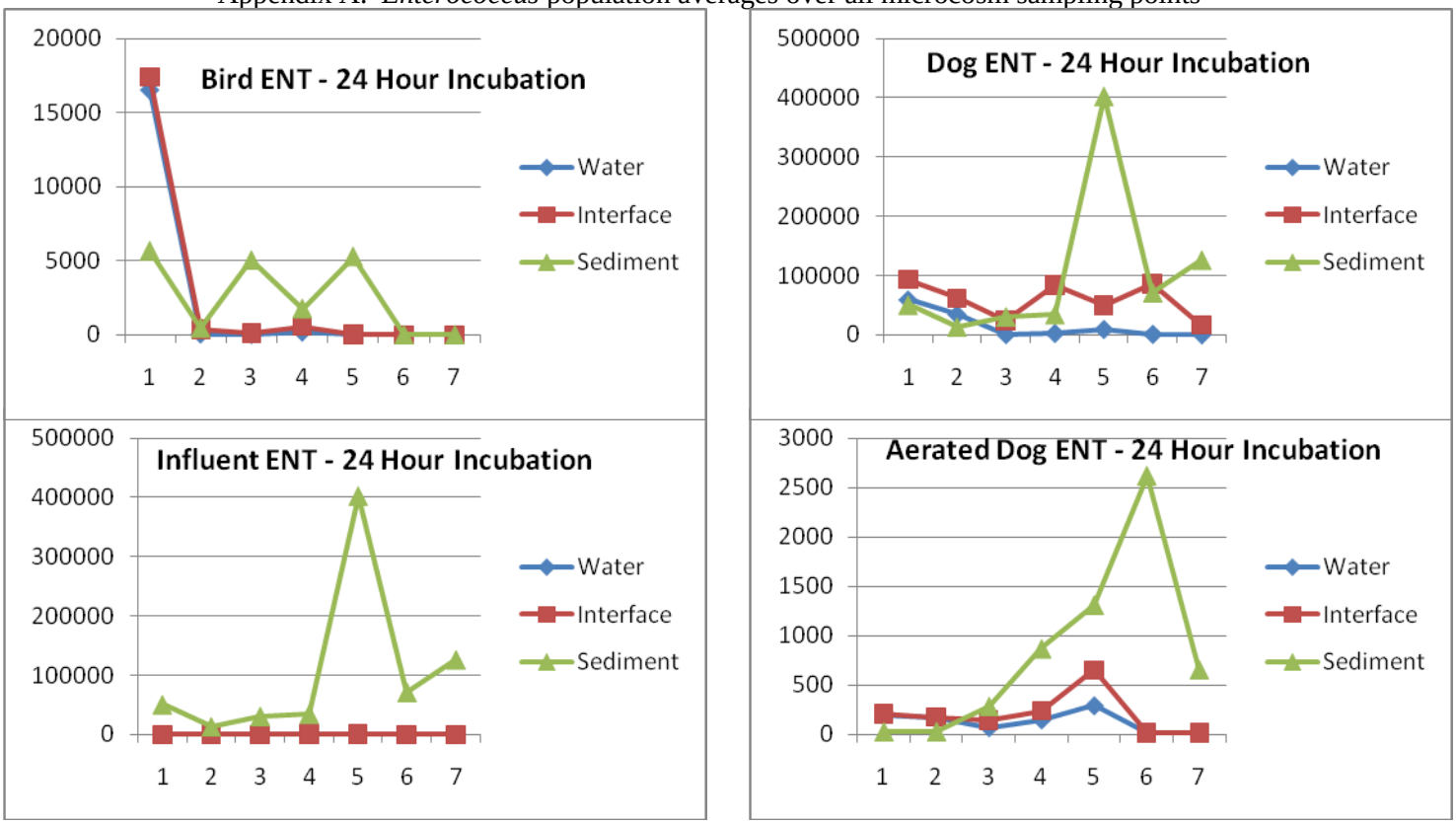
Seurinck, S., T. Defoirdt, W. Vertraete, & S.D. Siciliano. (2005) Detection and quantification of the human-specific HF183 Bacteroides 16S rRNA genetic marker with real-time PCR for assessment of human faecal pollution in freshwater. *Environmental Microbiology*, *7*, 249-159.

Tennant, S.M., M. Tauschek, K. Azzopardi, A. Bigham, V. Bennett-Wood, & E.L. Hartland. (2009) Characterisation of atypical enteropathogenic E. coli strains of. *Biomed Central Microbiology*, 1-11. http://www.biomedcentral.com/1471-2180/9/117.

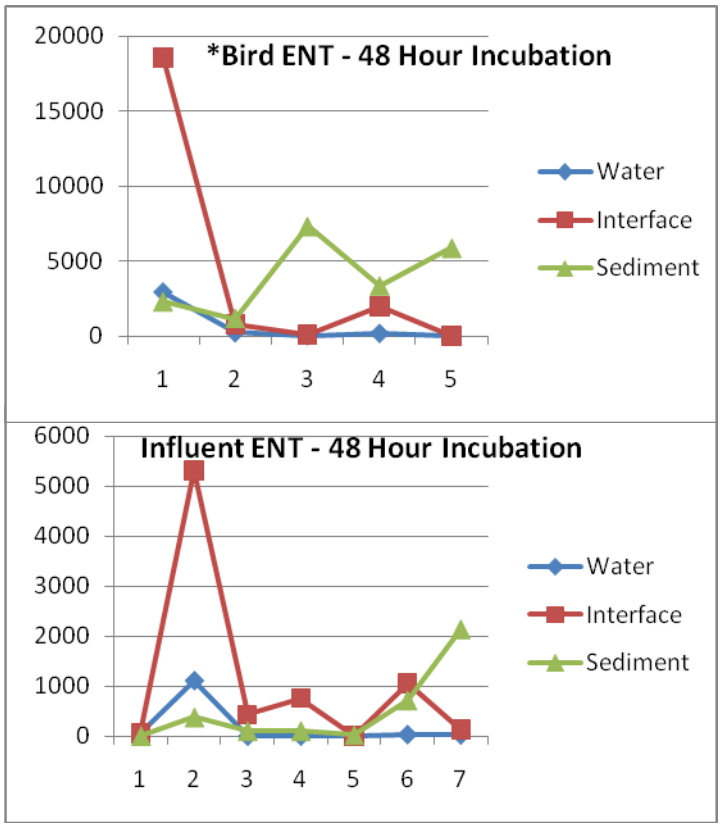
University of Southern Mississippi. (2008) *Contribution of Sediment to High Enterococcus counts along the northern gulf of Mexico*` [Brochure]. Hattiesburg, MS: Jason Townsend. Poster.

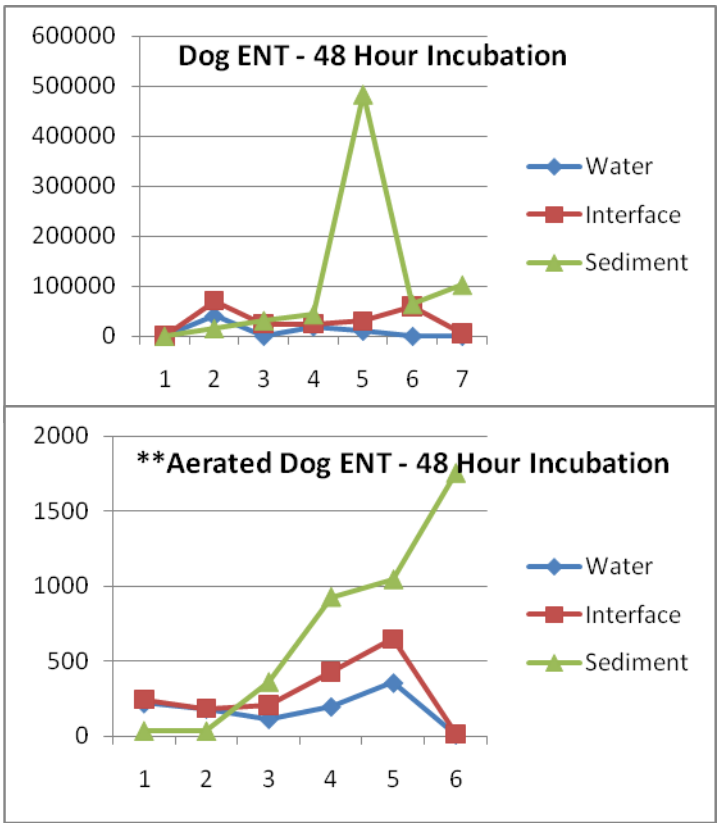
Appendix A. *Enterococcus* population averages over all microcosm sampling points



The above graphs represent the average Enterococcus counts at each of the seven sampling points. These counts were taken after the Enterococcus samples were incubated for 24 hours as explained in the Methods section. These graphs show that the *Enterococcus* in the sediment follows the same general growth pattern regardless of the fecal samples used. The bird, is the only sample that did not demonstrate this growth pattern. It is clear the *Enterococcus* in the sediment yields about the same counts until the fifth sampling (Day 14), when the counts spike. It is seen in the Dog and the Influent graphs, that this spike is followed by, what appears to be regrowth in the bacterial counts. Future studies could reveal the same result using the Aerated Dog feces, which is a closer simulation to natural waters. This could potentially indicate pollution problems lie in the sediment of recreation waters.

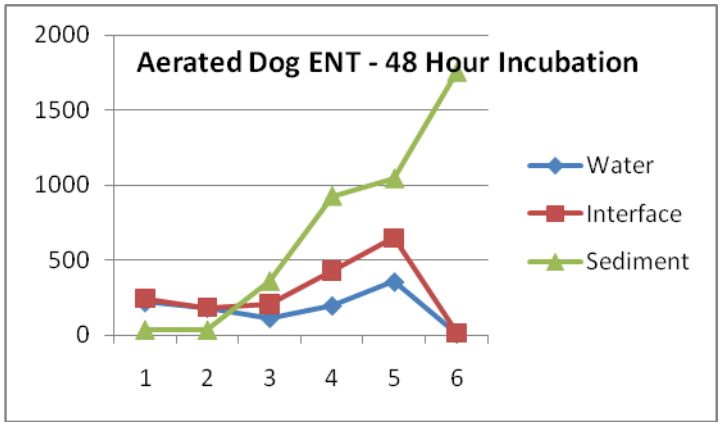
Appendix A Continued





The above graphs depict the average *Enterococcus* colony counts at each of the seven sampling points. *Enterococcus* regrowth in the sediment is demonstrated in all of the above graphs.

**The 48 hour incubation for the Bird Enterococcus continually yielded unreadable results for the first two samplings. As a result, only the last five samplings of the Enterococcus incubated for 48 hours is shown above.*
***Only six samplings of the Aerated Dog Enterococcus were taken during this experiment due to time constraints.*



Water Conservation: Rainwater Harvesting in the Dominican Republic

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ABSTRACT

Most of the world's population is concentrated in coastal areas. Freshwater scarcities and saltwater intrusion into coastal aquifers are two of the problems that plague many coastal systems around the world. The island nation of the Dominican Republic builds much of its economy on the tourism sector throughout the northern and eastern coastlines. The need to preserve natural resources and study the impact of water demand in coastal areas is crucial to tourism economy and social welfare in the Dominican Republic. While tourism industry for this small island is booming, availability of adequate water resources are now coming into question. There is a significant need for developing water supply management plans and accountability for water use or water consumption. This study provides an opportunity to evaluate and design a rainwater harvesting system within the tourist district of Punta Cana, Dominican Republic. This system design and feasibility study could provide other resorts and areas in the region to consider rainwater harvesting as a way to conserve groundwater resources and practice water conservation. The goal of this study is to investigate the feasibility of rainwater harvesting as an alternative water source that will complement traditional centralized and groundwater supplies in Punta Cana. An analysis of decentralized rainwater harvesting and the potential benefits in terms of water conservation and preventing saltwater intrusion will be discussed.

Key Words: Rainwater harvesting, water conservation, Dominican Republic tourism.

Introduction

Water is crucial to the existence and welfare of human populations. It is often the limiting resource in a particular settlement and some believe that freshwater will be the first resource to become widely unavailable (Furumai 2008). With a human population that is continuing to explode, the management of water resources will become of vital importance. The human population is expected to grow nearly 30% over the next 44 years from 6.7 billion people to 9.2 billion by 2050 (U.S. Census Bureau 2009). Most of this growth will happen in developing countries. In order to accommodate more growth, resource management, especially of freshwater supplies, will need to be included in future development plans.

The Dominican Republic is no exception from increasing water demand and limited freshwater resources. The province of La Altagracia in the eastern-most region of the island nation has seen significant growth throughout recent years. Tourism continues to boom and brings significant economic growth to the area and to the entire country of the Dominican Republic. Because of tourism and the increase in available jobs, there has been a growth of local migrant populations in the form of many "shanty-towns," most which lack basic infrastructure and have unknown population rates. The majority of the water use throughout this region relies heavily on groundwater aquifers with some water coming from surface water in the mountainous regions of this province.

Utilizing captured rainwater has potential in reducing the water demand on other water sources. The amount of possible water conservation is highly dependent on particular site use and climate of the

region. The Dominican Republic has varied rainfall amounts across the country but in all localities there is potential for captured rainwater to reduce water demand on conventional sources, even if only slightly. The eastern region of the Dominican Republic experiences rain throughout the entire year and rainwater harvesting potential should be evaluated to help reduce the strain on the groundwater aquifers. If water consumption is not monitored or factored into policy and development throughout this region, saltwater intrusion and contamination of freshwater aquifers could become a very realistic problem in the province.

Project Objectives

The goal of this research is to determine the potential for implementing a rainwater harvesting system at Punta Cana resort area. The site of study in Punta Cana is the Punta Cana Ecological Foundation. Specific objectives include:

(a) Estimate the water use for the building (indoor use) and irrigation demand in the building vicinity
(b) Calculate monthly available rainwater that will meet the water demand estimated in step (a) above
(c) Determine the building retrofit needs (roof, gutters, plumbing, etc.)
(d) Determine the approximate costs of the system and identify value of potential benefits

The ultimate objective is to develop the rainwater harvesting implementation plan and recommendations for the building.

Approach

This project will be completed by 1) reviewing current literature regarding rainwater harvesting technologies; 2) Evaluating the efficiency and potential for implementing rainwater harvesting in Punta Cana; 3) Designing a potential rainwater harvesting system if rainwater collection is beneficial to the site

Literature Review

Dominican Republic Water Resources

As an island nation, the Dominican Republic boasts 1,288 km of coastline stretching north, east, and south around the country (Factbook 2009). The freshwater withdrawal is estimated at 3.81 m^3/year per capita (Factbook 2009). This per capita water withdrawal has seen a significant increase over the past decade as the economy grew (Factbook 2009). Because of the climate, with significant yearly rain and several large bodies of surface water, the Dominican Republic may seem to have ample water resources. Unfortunately, however; even with increasing infrastructure, government, and private sector investment, undernourished people still account for nearly 35% of the population and over 42% of people still live below the poverty line (FAO 2009; Factbook 2009).

The province of La Altagracia is the easternmost province of the Dominican Republic. This province includes the three large tourist areas of Bavaro, Punta Cana, and Cap Cana as well as a small island off the south coast called Isla Saona, among other inland, cities, villages, and towns. The geology of the area is made of predominately reef limestone which is very permeable (Harlan, et al., 2002). This very permeable rock type allows for quick infiltration of the surface water to below ground freshwater aquifers. The majority of this region's aquifers has ample water and can be found at depths ranging from 5 to 25 meters in the low-lying areas and between 100-200 meters in the mountainous areas (Harlan, et al. 2002). The groundwater throughout the region is very clean and very hard with the exception of the coastal areas such as Punta Cana and Bavaro where most of the groundwater is considered brackish or completely saline (Harlan, et al. 2002).

Tourism Economy and Water Use

Water use throughout the tourism sector is varied by region, province, and by each particular resort. The policies regarding water use by resorts and tourism are lacking and very lenient. The Secretariat of Environment and Natural Resources (SEMARN) was formed in 2000 after the passage of an environmental framework law. This law was a great start in establishing very basic principles regarding things such as the penalty for polluting and effluent limits (Werbrouck 2004). This new body of decision makers also mandated that every new hotel needs to have a wastewater treatment plant (Werbrouck 2004). Unfortunately, the environmental legislative body has not yet addressed any regulations on water consumption. The estimation of overconsumption leading to saltwater intrusion on the coastline of the Dominican Republic has speculated that saltwater is already reaching 20 to 50 km inland from the shoreline (Werbrouck 2004).

While environmental degradation is beginning to be addressed on both the national and the individual basis, water resources management will need to be immediately implemented into the decision making process for the tourism sector if this sector would like to continue to increase in development (Werbrouck 2004).

Rainwater Harvesting Technology

In ancient times, rainwater harvesting was achieved using channels and diversion systems (Boers & Asher 1981). Today however, rainwater harvesting for personal consumption most often utilizes rooftop collection. There are still agriculture systems which continue to utilize past civilization techniques as well. The efficiency of a particular rainwater harvesting system can be altered by many different attributes but the most common efficiency markers are the roof material and the first filter system (LaBranche 2009).

Rainwater systems are extremely adaptable according to the needs of a particular site. The components for a rainwater system today include a roof area or catchment area, a gutter system which allows water to flow by gravity to a storage tank. In addition to the rooftop, gutter system, and storage tank, filters and pumps can be included in design according to the needs of the particular site.

Case Study Site

PuntaCana Resort and Club

The Punta Cana Resort and Club, on the eastern coast of the Dominican Republic, includes hotel accommodations, golf courses, villa rentals, and even privately owned real estate. GRUPO PUNTACANA owns the resort & hotel as well as the ecological foundation. Grupo Punta Cana was founded in 1969 on unique beliefs that contributing to the local community and the environment would benefit the tourism economy as well. This group owns and operates the Punta Cana Resort and Club, Punta Cana Ecological Foundation, the Punta Cana Community Foundation, and the Punta Cana International Airport, along with several private real estate developments and much of the infrastructure in the Punta Cana region. The Grupo Punta Cana employs over 1,700 people with the overwhelming majority of the employees from the Dominican region (tourismfortomorrow.com).

Punta Cana Ecological Foundation

In 1994 the Grupo PuntaCana established the PuntaCana Ecological Foundation in order to help protect the natural environment of the Dominican Republic eastern region. One of the main goals of The Ecological Foundation is to help address the growing problem of coral reef degradation and to help maintain a healthy coastal ecosystem.

The Punta Cana Ecological Foundation also established at 1,500 acre ecological reserve that is home to native plant and animal species, organic gardens, a petting zoo, and “los ojos,” or the eyes, which are twelve natural freshwater ponds. The Foundation has a housing capacity of 24 people in the main Biodiversity Center building and is often a place of study for universities including Harvard, Colombia, Cornell, Miami, and Virginia Tech (Kheel 2009).

The Ecological Foundation became a site of study because of the commitment to the environment that the Grupo Punta Cana expresses throughout their work. The site evaluated the potential to implement rainwater harvesting as a water conservation tool on the buildings within the Ecological Foundation grounds. This project could also be a stepping stone for other resorts in the area to begin to practice sustainable tourism as a measure to conserve resources and preserve the natural environment.

Table 1. Ecological Foundation Building Rooftop Area

	Size-m^2
Biodiversity Center Building	672
Environmental Management Building	195
Storage Building	84.10
Gardener Bathrooms and Dining	92.40

In order to evaluate the potential for rainwater harvesting, Jake Kheel, the Ecological Director for the Foundation, calculated the roof size of each building. Because of various factors including roof size, roof material, water use, and distance between buildings, it was concluded that the most efficient rainwater system would be a single system for the Biodiversity Center Building, instead of all four buildings. Rainwater harvesting is evaluated from not only roof size, but available rain and efficiency of the rainwater system. In order to calculate the harvesting potential, rainwater data was collected by Jake Kheel and Jamna Polanco (Table 2). This rainwater data was the monthly precipitation for three years.

Table 2. Precipitation in Punta Cana 2006-2008 (mm)

	2006	**2007**	**2008**	**Average**
Jan	137.3	39.6	102.6	93.2
Feb	34.9	82.3	25.7	47.6
Mar	76.1	218.3	23.4	105.9
Apr	98.6	58.7	96.2	84.5
May	40.2	70.4	29.3	46.6
Jun	200.3	108.1	145.3	151.2
Jul	52.2	36.8	50.5	46.5
Agu	159.4	46.1	123.7	109.7
Sep	59.6	33.4	590.2	227.7
Oct	178	282	90.4	183.5
Nov	102.7	168.9	57	109.5
Dec	104	197.8	77.4	126.4
Total	1243.3	1342.4	1411.7	1332.5

The averages of the three years of data were used to evaluate the harvesting potential. The precipitation data and the roof size and site data allowed for the beginning of the rainwater harvesting system to be designed.

Rainwater Harvesting Design

Methods

In order to design the rainwater harvesting system for the Punta Cana Ecological Foundation climate data as well as data regarding the building design and use were collected. This data was then analyzed to produce information regarding potential harvestable rainfall as well as information regarding water use within the main Ecological Foundation Building. From this data, system components were outlined and constructed into a form readable for possible future contractors.

Rainwater Availability

Based on the roof size and the rainfall data, the harvestable potential was evaluated by multiplying the roof size, the rainfall, the efficiency coefficient and a coefficient for unit conversion. Table 3 shows each buildings' harvestable potential in m^3 and shows the sum of the possible rainwater reuse.

Table 3. Harvestable Potential in m^3

	Biodiversity Center	**Environmental Management Building**	**Storage Building**	**Gardener Bathrooms**	**ALL BUILDINGS**
Ene	49.8	9.5	5.4	5.8	70.5
Feb	25.2	9.7	2.9	3.8	41.7
Mar	56.7	23.8	5.9	8.5	94.9
Abr	45.3	11.0	5.6	5.9	67.8
May	25.4	8.9	2.9	3.7	40.9
Jun	80.6	18.5	8.8	9.9	117.8
Jul	24.9	6.3	3.1	3.3	37.5
Ago	58.9	11.2	6.6	6.9	83.5
Sep	121.3	33.9	27.0	20.8	203.1
Oct	98.2	33.9	10.4	13.7	156.2
Nov	57.9	20.2	6.2	8.1	92.5
Dic	67.5	24.4	7.8	9.8	109.5
Total	715	211.4	92.8	100.0	1115.8

The total amount of rain can not only dictate the feasibility of the rainwater harvesting based on water use, but this information also helps decide the size of the rainwater harvesting system components, especially the storage tank.

The water use for the Ecological Foundation is not specifically known. Given information is only an estimate of water usage. Water use for the entire area (Biodiversity Center, gardens, office, and bathrooms of the gardens) is estimated between 33m³/day and 80m³/day (Kheel, 2009). At this rate of water consumption, the potential harvestable rainfall is not nearly enough to provide for all water needs. In addition, because of the distance between buildings, two separate systems would need to be designed in order to optimize water re-use. Based on this information, it is assumed that the majority of the water used is from the garden and irrigation system. Also, a leak in the water storage tank throughout the past three months has skewed the given estimate of water usage. An evaluation of estimate water usage for the Biodiversity Center Building was constructed based on general water use per person guidelines from both the United States Geological Survey (USGS) and the Rainwater Harvesting in the United Kingdom. These estimates give a general guideline for water use of the Biodiversity Center Building and this is compared to harvestable water on the rooftop of only the Biodiversity Center Building because of the distance between the Biodiversity Center Building and the other garden buildings.

Table 4. Water Use Estimate

Building Information		
Roof area	**672**	square meters
Annual rainfall	**133.1**	Centimeters
Annual rain harvest	**715,546**	Liters (including .8 efficiency coefficient)
	715	m^3
Water Use		
A washing cycle (50 liters assumed)	**8**	washing cycles per day (2 washing machines, 2 meals of dishes 4 wash cycles in Lab
Toilets (10 liters assume)	**93**	flushes per day (11 employees/12.2 residents, 3 flush/5 flush day)
Shower use (8L/min assumed)	**915**	liters per day (12.2 people daily, 1 shower each)
Total daily use	**2,245**	liters per day
Year use	**819,425**	liters across the year
Will rainwater provide more than 75% of all water use?	**yes**	Rain can provide 87%

This table displays the estimated water use for the Biodiversity Center Building. The table, as shown, explains that nearly 90% of the total water demand could be obtained through the use of rainwater. If the rainwater was only used for non potable water use i.e. toilet flushing, irrigation, clothes washing, vehicle washing, the rainwater could provide all indoor use and some irrigation demand. Water quality and plumping would have to be explored further in order to decide the ability of the water to be used for all water uses, potable and non potable.

System Components

The capital investments involved in implementing this rainwater harvesting system include several components. In addition, a filtration system would need to be included in the design if the rainwater is to be used for all potable and non-potable use. The single largest and most expensive part of the capital costs is the storage tank. Storage tanks can come in all shapes, sizes, and prices. Because of available supplies and need for a belowground tank, constructing a concrete tank is the most logical for this study site. A number of local construction companies could pour the necessary storage tank.

The other components include; first flush filters, intake filter, water pump, and new pipelines to connect the current downspouts to the water storage tank. The first flush filters allow the system to function more efficiently by removing debris and organic matter through gravity and a vortex motion. The brand recommended for this project is the WISY filters made in Germany (LaBranche 2009). Also recommended from the WISY company is the intake filter which allows for the cleanest water to be used when drawing water from the tank. This filter rests just below the surface and intakes the best water to be reused. Many various water pumps can suffice in order to redistribute the water within the building. Because of the height and distance from water tank to distribution, the system would require a 1HP pump. Pumps, depending on availability and company, can range in price.

Cost Analysis

Rainwater harvesting systems are relatively inexpensive in comparison to other systems of water, for example well drilling. They do however require a large capital cost. Operation and maintenance costs are very marginal and depend on the type of components with each system. All prices are from the Virginia and Texas rainwater harvesting manuals and may be different based on supplies as well as labor.

The storage tank is the most expensive component with a concrete tank costing between $0.30- and $1.25/gallon USD. This tank would, if cost was averaged at $0.77 would cost $5400 USD. New pipes to

move the water from the downspouts are estimated at 120m. These pipes are estimated at $0.30/ft or $1.00/meter so the cost of new pipes would total $120USD. The water pump on the current system may be used to pump the water into the building however, if a new pump is required, a Grundfos MQ Water Supply System would cost between $385-600 USD. The two WWF 150 Filters from WISY are priced at $1050 USD plus shipping. The 2" coarse floating filter with 7' of hose is $320 USD.

Table 5. Cost Estimate

Components	Size	Cost Estimation
Storage Tank	$27m^3$	$5,400 USD
Pipes	120m	$120 USD
Water Pump	1hp	$500 USD
First Flush Filters	2 WWF150 filters	$1,050 USD
Intake Filter	1 2"filter	$320 USD
Total Cost		$7390 USD

A notable difference in the cost will also be the availability of these supplies in the Dominican Republic. While labor and simple construction such as the concrete tank will most likely cost less than the cost of the same thing in the United States, the components from the WISY company would cost more because of the need to import them from Germany or the United States (LaBranche 2009). Also, it is estimated that the excavation and implementation of the tank below ground will roughly double the cost of the system costing nearly $7,000 USD (LaBranche 2009).

Design Sketch

The design components, after tank size, water use, and water collection were evaluated, were sketched in order to provide a future contractor with a design idea. This design involves new plumbing fixtures at the end of each of the six downspouts on the building. It also involves this new plumbing to drain downward into a storage tank with a capacity to hold $27m^3$ of water.

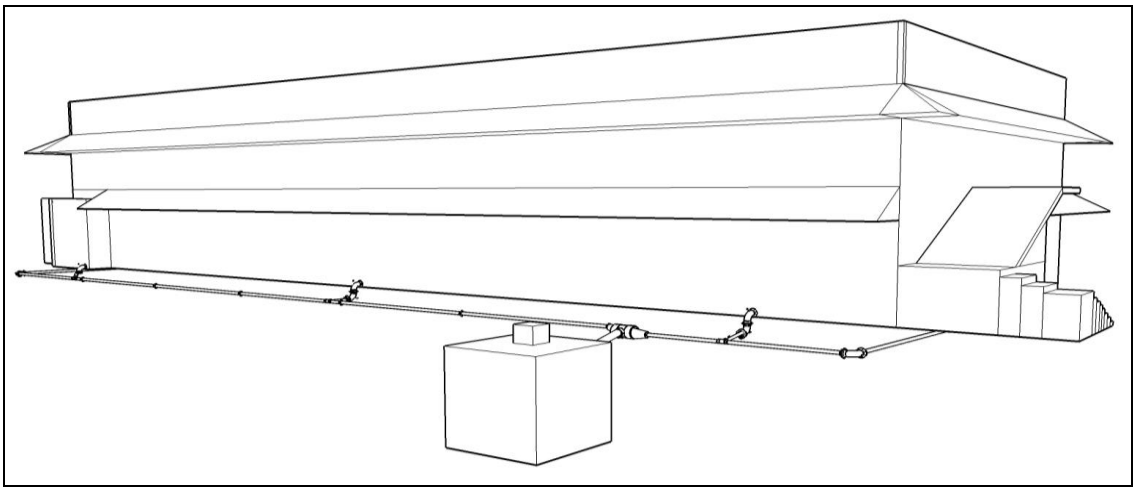

Figure 1. Back View of Ecological Foundation Rainwater System

Discussion and Conclusions

Within the climate of Punta Cana, ample rainwater is available for reuse. This rainwater could provide as much as 87% of the total water supply for the Biodiversity Center Building. This study provides information on the feasibility of rainwater versus water use. In order to continue to develop this plan, the quality of rainwater would need to be investigated over a period of time. This rainwater harvesting system has extreme potential since it can collect enough rainwater to provide nearly all of the

water needs for the Biodiversity Center Building. While the system has an initial investment cost, conserving water in this way, and potentially on other buildings throughout the Punta Cana Resort and Club, could save money and energy in the long run. Although it was not calculated, the Punta Cana Resort and Club will save a small amount of money if the system was implemented because there would be less electricity used to pump water from the current water treatment plant. In addition, the rainwater will save the Punta Cana Resort and Club money because it could possibly be much less expensive to treat rainwater on site, than the current treatment process in the water treatment plant.

Decentralizing water systems has the potential to reduce energy use and promote water conservation. While exact numbers vary by location, the energy that contributes to centralized water systems primarily involves treatment and distribution. Distribution of water can greatly increase energy demands. Many distribution systems require 70% of total energy needs for centralized water while treatment only requires 30% of energy (Younos). It is difficult to evaluate the energy savings of decentralization when each decentralized system can be different. For rainwater harvesting, the main component requiring energy is the water pump (Grady & Younos 2008).

As water consumption continues to rise and as more resorts are being constructed and opened, the groundwater table as well as the water collected from the mountains will become strained. The possibility of salty seawater contaminating the entirety of the freshwater below ground aquifer is extremely probable and realistic in the upcoming years. It is nearly impossible to say when this saltwater intrusion, as the contamination process is called, could occur because it very hard to model and estimate the process unless a very rigorous research study is performed regularly in the particular area of concern. While the exact time and severity of a possible water drought is unknown, it is extremely important to begin to address water shortages now so that when they become a reality the resorts and local economy of the area can be prepared. If the groundwater table is completely contaminated, desalinization may be the only treatment process available to treat local water. This process is very expensive and energy intensive. Collecting rainwater and reusing wastewater are two very good conservation practices that could greatly reduce the consumption stress currently placed on the natural fresh water.

Recommendations

Although this study showed the feasibility of rainwater harvesting in one location in the Dominican Republic, future work could include a study regarding water conservation through rainwater harvesting in the entire region. Even if only a small percentage of buildings within each resort implemented rainwater harvesting, a possible reduction in consumption could be evaluated. It is also important to study the rate of saltwater intrusion in the area so the severity of the water situation can be understood. A future study could also involve constructing a water budget and water conservation plan for the entire resort region.

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Water guidelines:
http://ga.water.usgs.gov/edu/sq3.html
http://www.rainwaterharvesting.co.uk/index.php

A Longitudinal Analysis of the Impact of Urbanization on Stroubles Creek: Historical Perspective

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ABSTRACT

Watersheds are often studied for alterations resulting from urbanization. Research is usually conducted to understand urbanization's impacts and if these impacts are adverse. Research goal and approach varies by researcher, location within the watershed and in parameter selection. These analyses are independent of each other although in most cases reference each other to show time period and parameter gaps and methodology differences. Documenting urbanization's impact overtime is an important step to understanding these impacts. From a review of several decades of research physical, chemical and biological parameters are analyzed to determine urbanization's effect on the Upper Stroubles Creek Watershed in Blacksburg, Virginia. Town history, land use changes, macroinvertebrate studies and chemical parameters from archival sources were used to evaluate these changes overtime.

Key Words: Stroubles Creek, urbanization impact, watershed, archival, water quality

Introduction

That increased urbanization and land development results in increased impervious surface cover, reduced infiltration and groundwater recharge, increased surface runoff and increased stream flow is well known. The increase in water volume often results in floods, which can cause harm to human life and economic loss from property damage (Town of Blacksburg 2008). Increased stream flow changes the stream path, erodes the stream bank, and changes the stream bed. These changes alter the way the stream functions as a whole (Wildrick & Kuhn 1976).

In urbanized areas, runoff volume increases in direct proportion to increases in impervious surface cover; therefore pollutant loads from surfaces entering streams increase as well. Increased impervious area also leads to reduced stream habitat and biodiversity loss over long periods of time (Schueler 1994). In general, stream drainage areas with 10- 25% impervious surface cover are considered negatively impacted (Center for Watershed Protection 2003). Urbanization occurs over a period of time gradually impacting the watershed characteristics, the streams in developed area and the groundwater system. However, less is known about gradual and long-term impacts of urbanization on stream water quality. The Upper Stroubles Creek watershed provides an opportunity to document the impact of gradual urbanization on stream water quality.

Research Goals

The goal of this research was to analyze the impact of gradual urbanization on stream water quality by documenting available historical data from the early 20th century to present day in the Upper Stroubles Creek watershed in Blacksburg, Virginia.

Methodology

The study focused on the urbanized area of the Upper Stroubles Creek watershed that encompasses a portion of the Town of Blacksburg and Virginia Tech, specifically above the Virginia Tech Duck Pond (Figure 1).

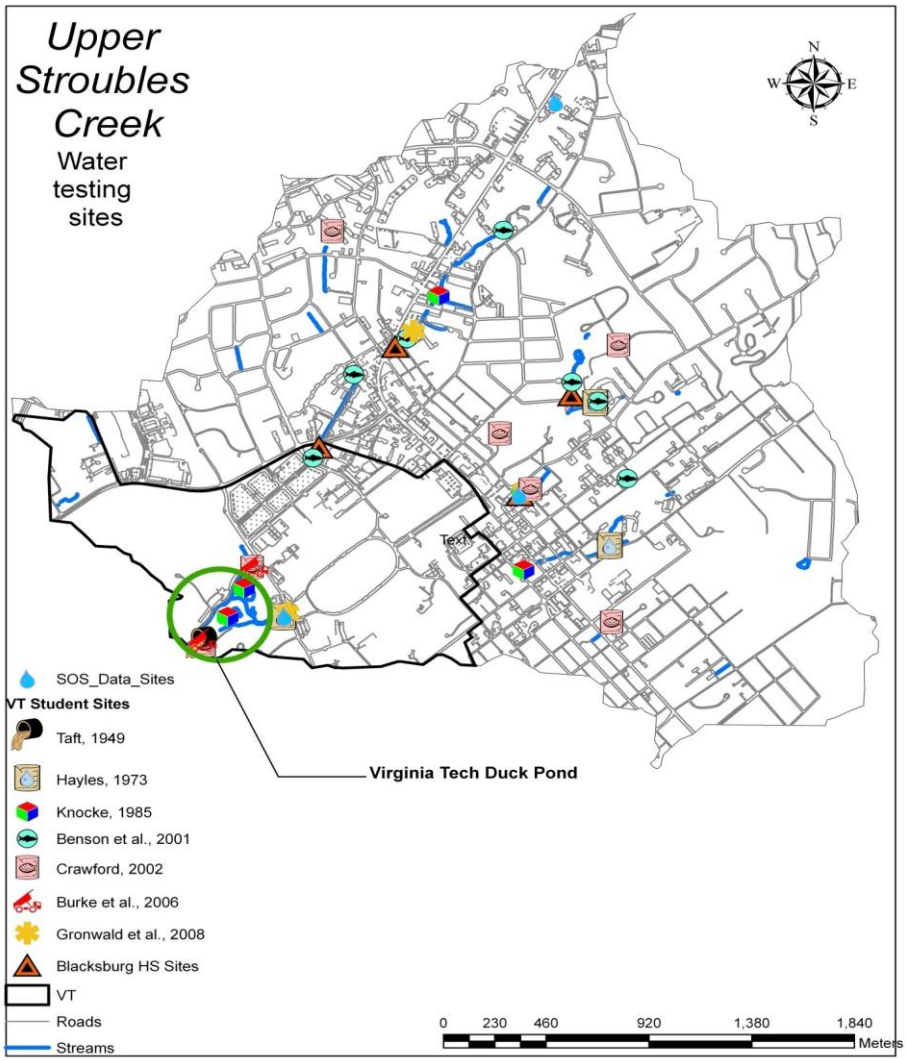

Figure 1. Upper Stroubles Creek Watershed and Study Site Locations

Documents were obtained through literature review, searching Internet databases, library searches, and finding unpublished reports. Reports related to land use and historical town changes and water quality data were obtained from Virginia Tech professors, Virginia Save Our Streams and student reports (Blacksburg High School and Virginia Tech). Interviews were conducted to gather information and for clarification purposes. The water quality data was categorized by chemical, biological, and physical parameters and then organized into Excel spreadsheets in chronological order. ESRI's ArcMap was used to create a map of the study area to show roads, waterways, and specific data collection sites as shown in Figure 1. Basemap information was obtained from the Town of Blacksburg and Virginia Tech GIS databases.

Results and Discussion

Results of this study are organized in two parts. Part 1 documents the historical development of the Town of Blacksburg and Virginia Tech within the Upper Stroubles Creek watershed. Part 2 documents the gradual impacts of urbanization on stream water quality using historic and contemporary research reports and other documents.

Stroubles Creek: The Historical Perspective

Stroubles Creek flows through the Town of Blacksburg, Virginia Tech and Montgomery County before emptying into the New River. The stream has been experiencing human impact since 1740 when the area was settled by the Draper's Meadow Community. A 38-acre square grid consisting of 16 blocks was established in 1798, forming the original Town of Blacksburg. Three natural springs, i.e., Town Spring, Keister- Evans Spring and Spout Spring, form the headwaters of Stroubles Creek, and were the major sources of water supply serving the growing community. As the town gradually expanded, more than one half of Blacksburg's streambeds were diverted to underground pipes (Dunay 1986). At present, Spout Spring, located near Clay and Wharton Streets, is the only spring not paved over.

In 1860, the population of Blacksburg was 460 people (Hedgepeth 1998). Until the early 1900s, urbanization's impact was not an issue because of a low population density, small town area and few college buildings. In 1872, the Virginia General Assembly purchased the Preston and Olin Institute located in Blacksburg to establish the Virginia Agricultural and Mechanical College. In 1896, this school was renamed the Virginia Agricultural and Mechanical College and Polytechnic Institute (VPI) and became a four- year land-grant education system. At that time, the land area known today as the Drillfield was used for agricultural experiments to grow wheat, hay, orchards, and horticulture gardens. Stroubles Creek ran along the edge of this experiment station and often flooded during spring months (Kinnear 1972).

In 1937, the drillfield area was expanded to a 1.2million ft^2 exercise field for the school's Corps of Cadets, creating the Drillfield existing today. Creation of the Drillfield greatly changed the two major tributaries of Stroubles Creek i.e., the Central and Webb Branches. The Central Branch was diverted and became invisible under the Drillfield (Roanoke Times 1928). At the same time, a dam was built on a small pond that existed where Central and Webb Branches merged, creating a larger pond for winter recreation. Students eventually named the enlarged pond, the Duck Pond (date unknown) perhaps due to the migrating duck population. At present, the Duck Pond is a popular recreation spot of Virginia Tech's Campus and also serves as a stormwater management facility for urban runoff from portions of the Town of Blacksburg and the university (Younos & Walker 2002).

The Town of Blacksburg experienced a population boom after World War II, as returning soldiers settled in the area. In late 1940s, due to increased population water supplies from the springs were not meeting increased demand and for several years the town and VPI experienced water shortages. In 1950, a special act of the Virginia General Assembly established the Blacksburg Christiansburg and VPI Water Authority. The Authority was chartered by the State Corporation Commission on September 15, 1954 (Water Authority). Since early 1950s, the New River replaced the springs as the source of water supply for Blacksburg and Virginia Tech.

Changes occurred with sanitation systems as well. Until 1948, a VPI septic system was directly releasing wastewater into Stroubles Creek in a location below the Duck Pond dam. In 1948, a sewage treatment plant was established about 4.2 miles downstream from the Duck Pond on Stroubles Creek to treat wastewater from Blacksburg and the university. The treated wastewater was discharged to Stroubles Creek. From 1960 to 1970, the university size doubled, resulting in high demand for off-campus housing, apartments, restaurants and other businesses (Dunay 1986). The existing Blacksburg- VPI Sanitation Authority was established in 1979 (State Water Control Board). The treated wastewater from this new plant was discharged into the New River.

Urbanization of Blacksburg and Virginia Tech has contributed to the changing quality of Stroubles Creek. Stormwater runoff inflow from urban areas is major cause of sediment deposition in the Duck Pond. The Duck Pond was dredged in 1950, 1960, and 1986 to remove the accumulated sediment

(Hoehn & Woodside 1988). In 1986, the Duck Pond maximum depth after dredging was 12- 14ft. However, when the Duck Pond was drained in 2007 after the tragic incidence of April 16, the observed maximum depth in the pond center was only a couple of feet (Younos 2009).

Other direct and point source factors have contributed to Stroubles Creek contamination. From approximately 1970 to 1978 chemical waste generated in Davidson Hall (chemistry laboratories on Virginia Tech Campus) was directly discharged into the Duck Pond. Therefore, deep sediment within the Duck Pond may still pose a potential risk of toxic waste. Also, a kerosene spill incident in January 1985 in downtown Blacksburg reached into the Duck Pond (Knocke 1985). On December 15, 2006, 50 to 80 gallons of fuel oil were released into the stream due to a 275 gallon above-the-ground storage tank spill at Heavener Hardware (Steering Committee 2007).

In 1972, the Virginia Department of Environmental Quality (DEQ) established a monitoring station near the discharge point of the old wastewater treatment plant as a mandated by the Clean Water Act (1972). In 1996, the DEQ designated 4.98 miles of the Stroubles Creek below the Duck Pond as impaired because of benthic degradation and Stroubles Creek was put on the 303(d) list requiring the development of a total maximum daily load (TMDL) report. A TMDL report for the impaired sections was developed in 2003 (BSE 2003). Subsequently a TMDL implementation plan (IP) for watershed restoration was developed in 2006 (DEQ 2006). The segment of stream designated as impaired is below the Duck Pond, while problems contributing to impairment originate mostly above the Duck Pond, i.e. the Upper Stroubles Creek watershed.

Urbanization Impact on Stream Water Quality

Stroubles Creek has been experiencing land use changes over the past 100 years from urban and urbanizing areas in Blacksburg and on the Virginia Tech main campus. Water quality studies on Stroubles Creek have been conducted since the early 1900s (Fowle 1913; Sutton 1914). However, most of the studies were conducted below the Duck Pond. Studies above the Duck Pond are mostly conducted since early 1970s. Table 1 shows a summary of research and data collection conducted in the Upper Stroubles Creek watershed since 1971.

Table 1. Study Sites Located in the Upper Stroubles Creek Watershed

Report	Site Description	Year(s) Tested, detail
"Biological and Chemical Monitoring of Three Streams in the Area of Blacksburg, VA" (Hayles 1973)	Clay St., 175'N of Corner of Clay St. and Wharton St. (Site 1) Central Branch	1971- 1972
	Behind Georgetown Apartments on Owens St. (Site 3) Central Branch	
	Corner of Greenhouse Rd. and South Gate Dr., Virginia Tech, stream underground .25mi. before site (Site 7)	
	Bridge on Greenhouse Rd. crossing Stroubles Creek at end of drill field, Virginia Tech, stream underground .75mi. before site (Site 8) Central Branch	
"Assessment of Pollutant Loads to the Virginia Tech Duck Pond: (Knocke 1985)	Fire station Central Branch	1981
	Ewald Clark Central Branch	

	Hoy Funeral Central Branch		
	Northview Drive/ Apartments Webb Branch		
Save Our Streams	Longitude	Latitude	1992*-2005*, Webb Branch
	-80˚24' 45"W	37˚14'56"N	
	-80˚25' 32"	37˚13'33"N	1992*-2005*, Central Branch
	-80˚24' 50"	37˚13'53"N	1992*-2005*, Central Branch, Fire Station
"Fish Survey of Webb and Central Branches of Upper Stroubles Creek, Blacksburg, Virginia" (Younos et al. 2001)	Behind 809 Giles Rd. below area with heavy machinery, exposed dirt and erosion. Muddy and silt substrate, vegetated banks		2001, Webb Branch (W1)
	Main St. behind Wade's Supermarket (under building), no riparian buffer, fish barriers and concrete/ gabion banks. Silt and gravel substrate		2001, Webb Branch (W2)
	Webb St. between Papa John's and Hardware Store. Heavily urbanized, little riparian buffer, trash, erosion, pipe outfall. Silt and gravel substrate		2001, Webb Branch (W3)
	Virginia Tech Campus, Webb branch before flowing under parking lot, some riparian buffer and pipe outfall. Mostly muddy and silt substrate		2001, Webb Branch (W4)
	Owens Rd., grassy park across from apartments with a lot of aquatic vegetation, some trash and muddy substrates		2001, Central Branch (C1)
	Behind Owens Rd. apartments, some trash, a lot of aquatic vegetation. Muddy substrate		2001, Central Branch (C2)
	Next to fire station, gabion banks, gravel and pebble substrate		2001, Central Branch (C3)
"Biological Physiochemical Assessment of Stroubles Creek: Winter Condition" (Crawford 2002)	Longitude	Latitude	2002, Webb Branch Headwaters (Site 1)
	-80˚24' 53.8"	37˚14'34.7"N	
	-80˚25' 25"	37˚14'2.8"N	2002, Webb Branch Urban landscape (biological testing) (Site 2)
	-80˚25' 38.2"	37˚13'40.7"N	2002, Webb Branch Entering the duck pond (Site 3)

	-80˚24' 32.6"	37˚14'17.3"N	2002, Central Branch Headwaters (Site 4)
	-80˚24' 48"	37˚13'54"N	2002, Central Branch Urban Landscape (biological testing) (Site 5)
	-80˚24' 32.7"	37˚13'33"N	2002, Central Branch Entering Duck Pond (Site 6)
E.Coli and Sediment Monitoring for the Virginia Tech Duck Pond in Blacksburg, VA (Burke et al. 2006)	Central Branch (before the duck pond)		2006
	Webb Branch (before the duck pond)		
Blacksburg High School	Owen's St. Park Central Branch		2007- 2009
	Progress St. at Rescue Squad Central Branch		
	Webb Branch at YMCA		
	Prices Fork Rd. at Turner St., Virginia Tech Campus		
"Water Quality Assessment of a Mixed Land Use Watershed" (Gronwald et al. 2008)	Longitude	Latitude	2008, Webb Branch behind YMCA (Site 1)
	-80 25.164	N37 14.315	
	-80 24.818	N37 13.895	2008, Central Branch behind Fire Station (Site 2)
	-80 25.526	N37 13.558	2008, Central Branch above Duck Pond (Site 3)
	-80 25.638	N37 13.675	2008, Webb Branch above Duck Pond (Site 4)

*Not all parameters and/or years are tested for the same number of years

Pollutants carried off urbanized surfaces and into Stroubles Creek have impacted macroinvertebrate life. In Hayles' (1973) results, all locations tested within the upper watershed were described as moderately polluted though one site was classified as heavily polluted. A 2001 fish survey (Younos & Benson 2001) found low species diversity. Again, the water was classified as polluted and unable to support healthy and diverse species. Central Branch was found to be too narrow and shallow to contain life. As urbanization continues throughout Blacksburg, water quality in the Stroubles Creek depletes and as is seen when analyzing macroinvertebrate conditions. SOS data collected from 1998 to 2005 shows the most consistent biotic index rating to be fair, although in 2005 (the latest testing) was found to be unacceptable based on the benthic macroinvertebrate sampling. Central Branch was tested three times throughout 2005 and 2006 and was found unacceptable each time.

A visual assessment was done on 33 sites in the Upper Stroubles Creek watershed in 2001 (Porter & Roessler 2001). Of the 33 stream segments tested, 267 environmental conditions were observed. The conditions include channel alteration, erosion, exposed pipes, pipe outfall, fish barriers, inadequate buffers, in or near stream construction, and trash. The greatest problem was channel alteration occurring 47 times on the Webb Branch. The most severe condition was in or near stream construction (De Leon et al. 2001), although it only occurred twice throughout the survey and only on the Webb Branch. Pipe outfall is the second most occurring problem happening 46 times on the Webb Branch, although pipe

outfall discharge on the Central Branch is not evident. In general, physical conditions are more prominent on the Webb Branch than the Central Branch. Urbanization impacts and anthropogenic forces likely cause all physical disturbances occurring on the watershed.

Levels of bacteria (Sutton 1914) and coliform organisms (Taft 1949), in past testing on Stroubles Creek, have shown high concentrations resulting from faulty sewage treatment, agricultural and farm runoff. In more recent chemical testing, relatively high levels of E. coli and fecal coliform bacteria were found in the Upper Stroubles Creek. E. coli and fecal coliform findings from reports done in 2002 to 2008 have been averaged by year and branch to analyze contamination (Figures 2a., 2b., 3a., 3b.). The Virginia standard for E. coli in surface water is 235 cfu/ 100mL and fecal coliform is 200cfu/100mL (State Water Control Board), though it is hardly ever met. In general, the Webb Branch contains greater amounts of fecal coliform and E. coli than the Central Branch, although, recent testing shows both branches have increased. The highest amount of E. coli and fecal coliform in the Webb Branch are found in the daylighted area just before the branch flows into the duck pond. Possible reasons that high amounts of E. coli and fecal coliform exist are from sewage overflow, and domestic pet or wildlife wastes entering the stream. Fecal matter on urban surfaces can also be carried into the stream during storm events.

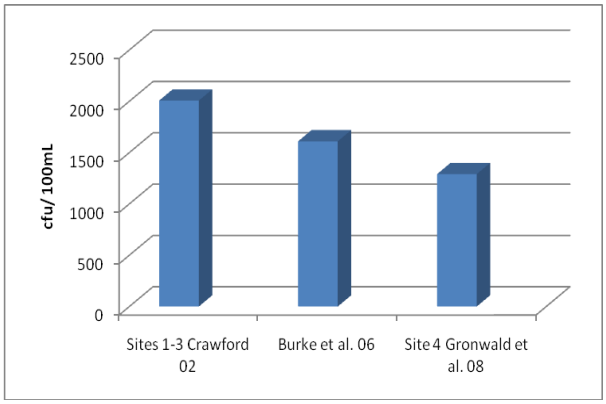

Figure 2a. E. Coli in Webb

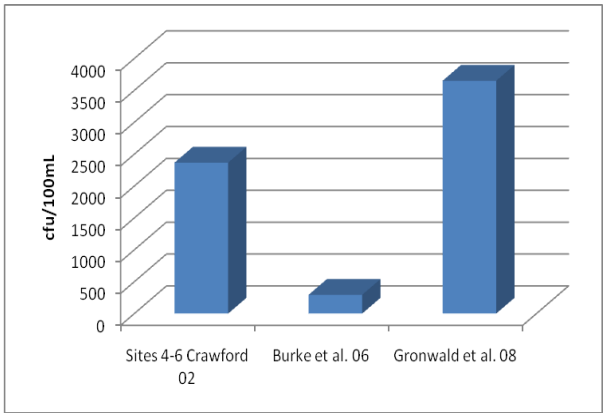

Figure 2b. E. Coli in Central Branch

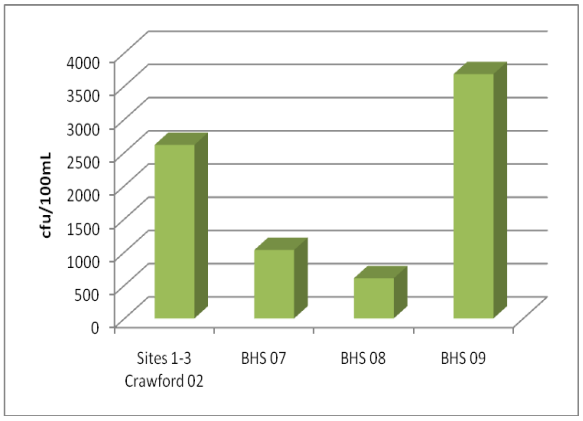

Figure 3a. Fecal Coliform in Webb Branch

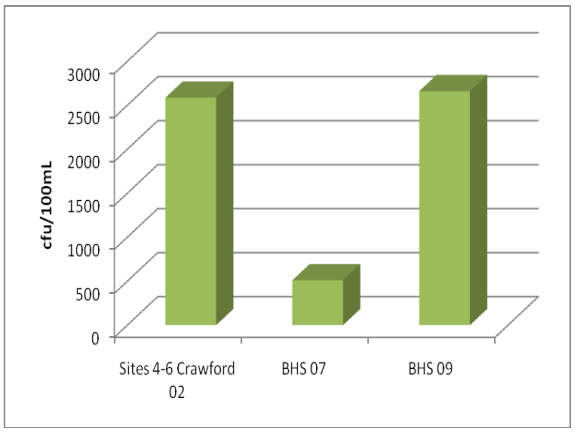

Figure 3b. Fecal Coliform in Central Branch

Conclusions

Continuing urbanization in the Town of Blacksburg allows for Stroubles Creek's water quality to remain poor. Upper Stroubles Creek experiences more urban development, which is depleting the health of macroinvertebrates and fish species (if existent) and increasing areas of physical disturbance. The

Webb Branch experiences higher levels of fecal coliform, E. coli and greater environmental conditions because of the greater impacts of urbanization felt on this branch. Central branch is almost entirely underground due to past town development and degradation is caused by land use changes. Stormwater runoff caused by impervious surface continues to input pollutants and sediment into the stream. As impervious surface cover becomes more significant in the town, these pollutants become more extreme. Without daylighting more stream area and implementing pervious surface cover (constructed wetlands), the stream will only suffer more severely. By performing a longitudinal analysis of Stroubles Creek done from a historical perspective, evidence shows that water quality in Stroubles Creek is gradually becoming more disturbed as the town becomes more urbanized.

Recommendations

A visual survey has not been done since 2001, and would be beneficial in observing urbanization changes occurring presently. Chemical data, though up to date is not done as consistently in the Upper Stroubles Creek watershed. The DEQ only tests Stroubles Creek downstream, below the duck pond. The pollutants flowing into the duck pond are predominantly originating upstream of testing locations. E. coli and fecal coliform sources of pollution should be identified and minimized to the acceptable surface water standard level for the state of Virginia. Biological data, especially SOS testing, should be made more uniform and consistent. All volunteers should be asked to test for the same parameters and use the same unit of measurement, along with entering all data into the online database.

Acknowledgements

We would like to thank the NSF (grant number 0649070) and the VWRRC for making this REU possible. Also, we would like to greatly acknowledge Dr. Gene Yagow (VT BSE Department), Llyn Sharp (VT Geosciences Department), Patricia Colatosti (Blacksburg High School), all of the Geography Department, and Tiffany Sprague.

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Analyzing the Upper Stroubles Creek Watershed using Geospatial Technologies

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ABSTRACT

Urbanization alters physical characteristics of watersheds and, in many cases, changes the stream's natural flow and impacts the stream quality. To document land cover and elevation changes caused by urbanization, a study was conducted in the Upper Stroubles Creek Watershed in Blacksburg, Virginia. Methodologies for studying land cover and urbanization changes through time using geospatial technologies were used in this investigation. A comparative analysis between the field data, electronic and archival sources indicates that urbanization is affecting this stream's water flow and movement.

Key words: urbanization, watersheds, Stroubles Creek, geospatial technologies

Introduction

Within all disciplines, researchers are interested in the effects of urbanization on society and the environment. Characteristic of the United States, urban and suburban sprawl has begun to dominate the landscape of many towns. The advent of interstate highways after World War II increased mobility throughout the nation. As a result, sprawl, the heavy land-consumptive feature of suburbia, flourished and to this date, continues to dominate urbanization patterns in the U.S. (Civco, et al. 2000). As increased urbanization has altered the natural landscape, research has become focused on quantifying these effects on the health of the environment.

Urbanization's effects on watersheds are varied and potentially detrimental to the natural state of a watershed's stream, flora and fauna. General urbanization trends result in a decrease of natural canopy cover and an increase in impervious surface cover. This alteration of landscape produces a decrease in groundwater recharge and storage, leading to a low base flow of groundwater into streams (Rose & Peters 2001). As a result of these low summer base flows and increased flooding frequency, urbanization not only damages stream health and stability, but also negatively affects aquatic life, especially fish spawning and development (Finkenbine, et al. 2000). Furthermore, studies have shown a close relationship exists between a stream's natural fluvial process and its riparian vegetation (White & Greer 2006). As White and Greer (2006) point out, decreased summer base flows can alter germination patterns of riparian plants, and the increase in frequency and intensity of flooding reduces the bank stability required by riparian plants in order to thrive. Research has indicated that an impervious surface cover greater than ten percent in a watershed can affect water quality from increased pollutant runoff (Dougherty, et al. 2004). Doughtery, et al. (2004) also indicate that reduced canopy cover increases water temperature and decreases bank stability, and the overall increase of impervious surface cover in an urbanized area can decrease biodiversity within a watershed.

Urbanization's effects on watershed health have been studied through many mediums and methods. Utilization of geospatial technologies and remote sensing has proven to be effective in studying

increased patterns of urbanization. Geospatial technologies allow local land use planners and residents the ability to understand land cover within their town, community, and local watersheds (Civco et al. 2000). While geographic information systems (GIS) and remote sensing (RS) are becoming widespread technologies for understanding landscapes on a small-scale, grasping environmental changes on a watershed level has become a particular focus in recent years.

Research at the watershed level is considered an appropriate scale for understanding complex environmental problems (Tim & Mallavaram 2003). Through the use of digital elevation models (DEMs), drainage networks and flow paths of waterways can easily be traced. Additionally, GPS assessments can be combined with GIS programs to gain a more in-depth understanding of watershed conditions. Proper delineation of watershed boundaries and full understanding of the land use within those boundaries allows for suitable management practices and policy regulations (Tim & Mallavaram 2003).

Increasing research at the watershed scale using GIS and RS is valuable in understanding land cover patterns, especially those of expanding urbanization. In 2004, an analysis of satellite imagery of the Cub Run watershed in Occoquan, VA concluded that studying satellite imagery was an effective means of determining impervious surface cover changes over time (Dougherty, et al. 2004). This research revealed that from 1990 to 2000, urbanization increased by 30% and impervious surface cover increased 4%. Research by White and Greer in 2006 on the Los Peñasquitos Creek watershed used RS applications and aerial photo interpretation; their investigations revealed that an increase in urban cover from 9% to 37% resulted in runoff increases of 4% per annum.

The Upper Stroubles Creek Watershed in Blacksburg, VA is a notable site for analyzing urbanization's effects on watershed health. Urbanization is prevalent on Virginia Tech's campus and the surrounding Town of Blacksburg. Increasing development is a cause of concern for Stroubles Creek. To date, research conducted presents a snapshot of the conditions at a single point in time and none has been conducted to analyze and quantify urbanization changes in the Upper Stroubles Creek watershed over time.

Using remote sensing and geospatial analysis, this research intends to track urbanization from the 1930s until the present day as a way to understand and communicate the expanse of development within the watershed. The three specific goals of this investigation are to (1) track changes in urbanization through manual imagery interpretation, (2) derive a present-day DEM using GPS points collected in the field, and (3) evaluate changes in flow paths and movement based on historical and field-derived records.

Research Methods

Study Site

Upper Stroubles Creek flows through Virginia Polytechnic Institute and State University (Virginia Tech) and the Town of Blacksburg, Montgomery County, Virginia (see Figure 1). The boundary of the Upper Stroubles Creek watershed includes a man-made pond (hereinafter referred to as the Duck Pond), which is located on Virginia Tech's central campus, and the land area upstream. The Stroubles Creek watershed is a sub-basin of the New River Watershed and encompasses approximately 1,975 acres. Three natural springs feed the stream: Town, Keister-Evans, and Spout and two main branches flow through Virginia Tech's central campus, the Webb Branch and the Central Branch. As town and campus development has progressed, major portions of the stream tributaries have been diverged underground (SCWI).

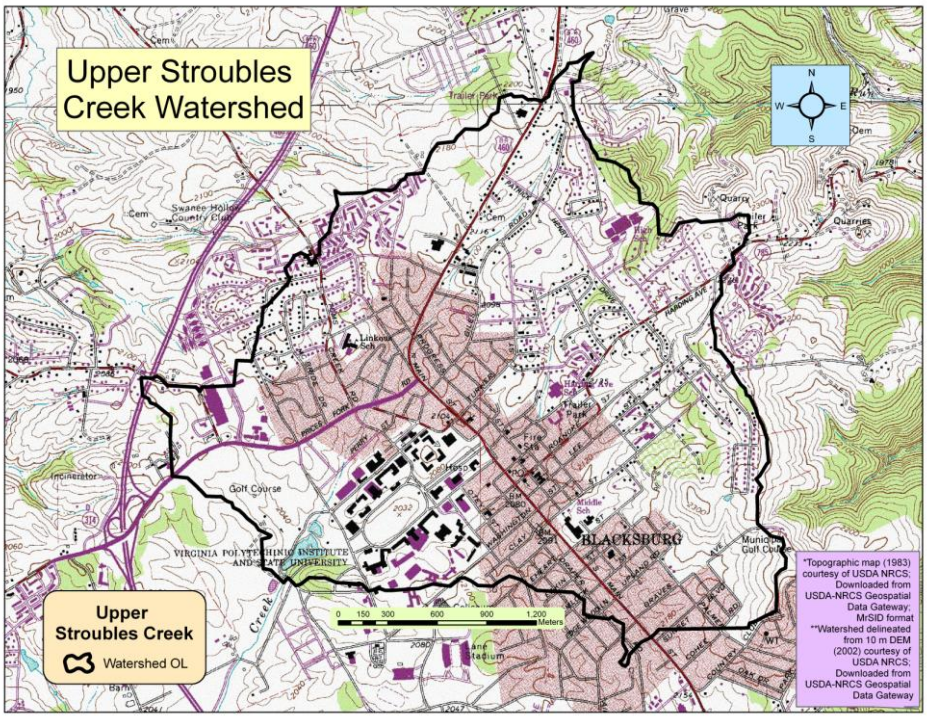

Figure 1. Map of the Upper Stroubles Creek Watershed.

Watershed Delineation from Historical DEMs

ESRI's ArcGIS version 9.2 was used in the GIS analysis. A ten meter DEM was downloaded for Montgomery County, VA (USDA 2009) and projected in North American Datum 1983 (NAD 83) and Universal Transverse Mercator Zone 17 North (UTM 17N). A 1:24 000 1983 topographic SID mosaic of Montgomery County, VA (USDA 2009) was used to guide the vector shapefile pour point directly below the Duck Pond on Virginia Tech's campus. Using the Spatial Analyst/Hydrology tools in ArcToolbox, the Upper Stroubles Creek Watershed and flow accumulation paths were delineated. This watershed delineation was used as the Upper Stroubles Creek Watershed boundary for the remainder of the study. Light Detection and Ranging (LiDAR) data (Town of Blacksburg 2005) was processed in the provided projection, NAD 83, Virginia StatePlane South Zone. Using ArcGIS, a DEM was created from the LiDAR elevation points using the Natural Neighbor interpolation method. A flow accumulation layer was generated from this newly created DEM by the same process as previously described.

Field-Derived Watershed Delineation from GPS Readings

Using Garmin E-trex® GPS units, elevation readings were collected throughout the town of Blacksburg and on Virginia Tech's central campus. To maintain consistency in the comparative analysis, each GPS unit was spatially referenced to NAD 83, UTM 17N. Collecting points was accomplished by randomly walking the watershed. In some instances, due to safety issues, elevations readings were obtained by driving, and in those instances, one meter was subtracted from the elevation readings prior to analysis in GIS. Using the MapSource® program provided with the E-trex® unit, the files were downloaded onto a laptop and saved as a .gdb file. The MapSource® program was set to the NAD 83 reference system. Using GPS Utility, which was also set to NAD 83 UTM 17N, each waypoint .gdb file was converted to a vector shapefile (.shp, .shx, and .dbf) to allow for analysis in GIS. To eliminate 'over-weighting' of points that were close together, points within five meters of each other were integrated. A DEM and flow accumulation layer was created by the same methodology as used to process the LiDAR data.

Utilizing GPS technology to develop a DEM is done so with uncertainty, as vertical accuracies of civilian GPS units can range from ±77 meters 95 percent of the time (Thuston, Moore & Poiker 2003). Therefore, the field-derived DEM and flow accumulation layers should be used as only a guide to understanding the current watershed conditions.

Orthoimagery Interpretation

Aerial photographs of Blacksburg for 1937 and 1971 were compiled and digitally scanned by Dr. J.B. Campbell. Additionally, spatially referenced (NAD 83 UTM 17N) orthoimagery for 2000 and 2008 were downloaded for Montgomery County, VA (USDA 2009).

The historical aerial photographs did not include spatial reference information, and were thus referenced by collecting ground control points (GCPs) using the 1:24 000 topographic SID mosaic of Montgomery County, VA as a guide. Using road intersections as the main basis for GCP collection, twenty to thirty control points were originally collected. The twelve to fifteen control points possessing the lowest residuals were used in the final analysis to ensure a root mean square error (RMSE) below three pixels. Additionally, GCPs were collected throughout the entire image to ensure an even distribution of georeferencing "weight". Georeferencing was accomplished by manually editing the Link Table of control points for the aerial imagery with the appropriate X map and Y map coordinates recorded from the spatially referenced topographic SID. A first-order linear transformation was used to georeference the aerial photographs to reduce distortion in areas distant from GCPs,

For this analysis, the land cover classes used were based on the principals of Anderson Level I classification (Anderson et al. 1976). Only four of the nine Anderson Level I land cover classes were required to classify the land within the watershed boundary: urban land, agricultural land, forest land, and water. Anderson Level II classification was used to define the characteristics of each land cover type (Anderson et al. 1976). In addition to these definitions, agricultural related building structures (farm houses, barns, etc.) were considered agricultural, not urbanized, and patchiness levels were used to discern forested areas from urbanized tree cover.

Using GIS, land cover was digitized into polygon shapefiles for each year. For the 1937 and 1971 historical aerial photographs, the watershed boundary extended beyond the edges of the scanned imagery, so the land cover classes were digitized in these gap areas based on the visual observations of the aerial photograph hard copies. While not typical, this method was possible due to the broad definitions of the land cover classes and the relatively simple outline of the watershed boundary. Uncertainty also exists in the photographic interpretation process, as determining areas within a specific land cover class are at the discretion of the photographic interpreter. Therefore, land cover results should be used as an indication of land cover changes through time.

Results and Discussion

Urbanization in Upper Stroubles Creek

The Upper Stroubles Creek Watershed is dominated by urban land cover to such an extent that the amount of land cover dedicated to forest and agricultural lands is virtually nonexistent. Furthermore, omniscient urbanization is not a recent development in this region. Analyzing the NLCD Retrofit Change Product (MRLC 2008), from 1992 to 2002, 90% of land cover remains urbanized, 3% remains forested, 4% remains agriculture; 2% changed from forested to urban, and 1% changed from agricultural to urban. In this product, urbanization includes grassed lawns, recreational fields, and urbanized vegetative cover (MRLC 2008). According to the 2004 NASS survey (USDA 2009), 85% of the land cover in Upper Stroubles Creek Watershed is agri-urban, and the other 15% is ranges from fifteen to fifty percent cultivated. These two data sources confirm that urbanization is wide spread in this watershed, yet a fair majority of the urbanization is open space, low and medium development.

Impervious surface cover and canopy density cover products of the NLCD reveal widespread urbanization trends in the Upper Stroubles Creek Watershed (USGS 2008). According to the canopy density product, 85% of the 30 meter by 30 meter grid cells within the watershed contains fifty percent or less canopy density cover. 74% of these grid cells contain zero percent canopy density cover. Only 35%

of the grid cells contain greater than fifty percent impervious surface cover, once again indicating the effects of low and medium intensity development within the watershed (USGS 2008).

The trend of urbanization within this watershed has continued into the new century. Over the past ten years, a portion of the golf course on Virginia Tech's campus progressed from open space development to high development and impervious surface cover. Even in a watershed region of high urbanization, more intense development is possible on areas of open space and low urbanization.

Land Cover Changes over time

Settled initially as an agricultural area, Blacksburg and the Upper Stroubles Creek Watershed have seen an increase in lands dedicated to urbanization (see **Figures 2 & 3**).

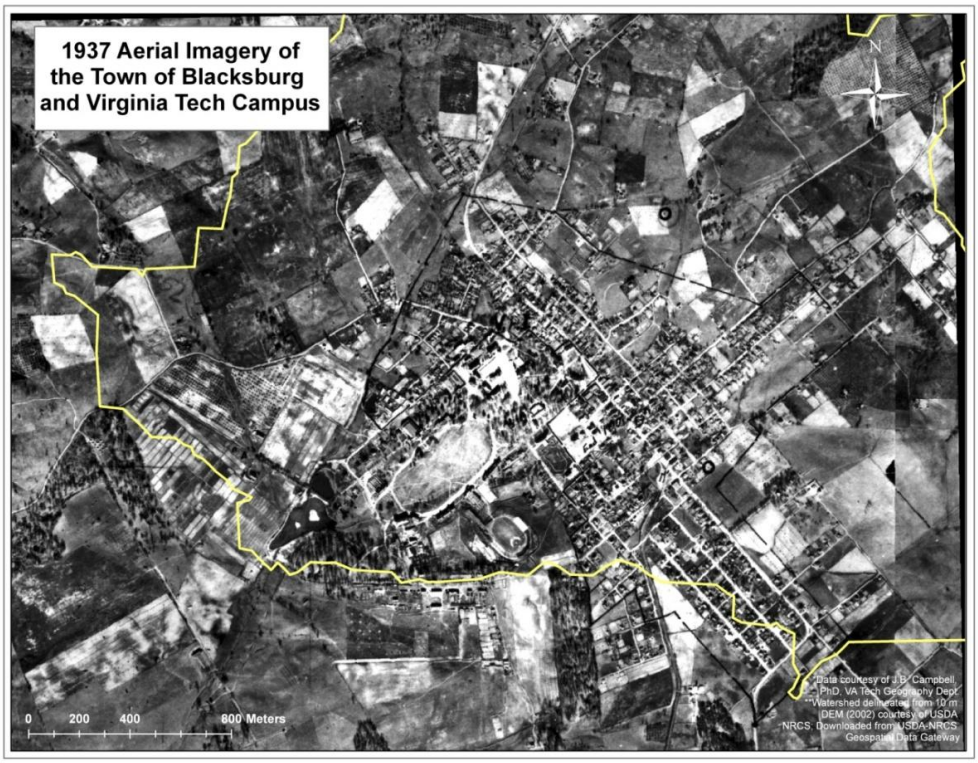

Figure 2**.** 1937 Aerial image of Blacksburg and Virginia Tech's central campus.

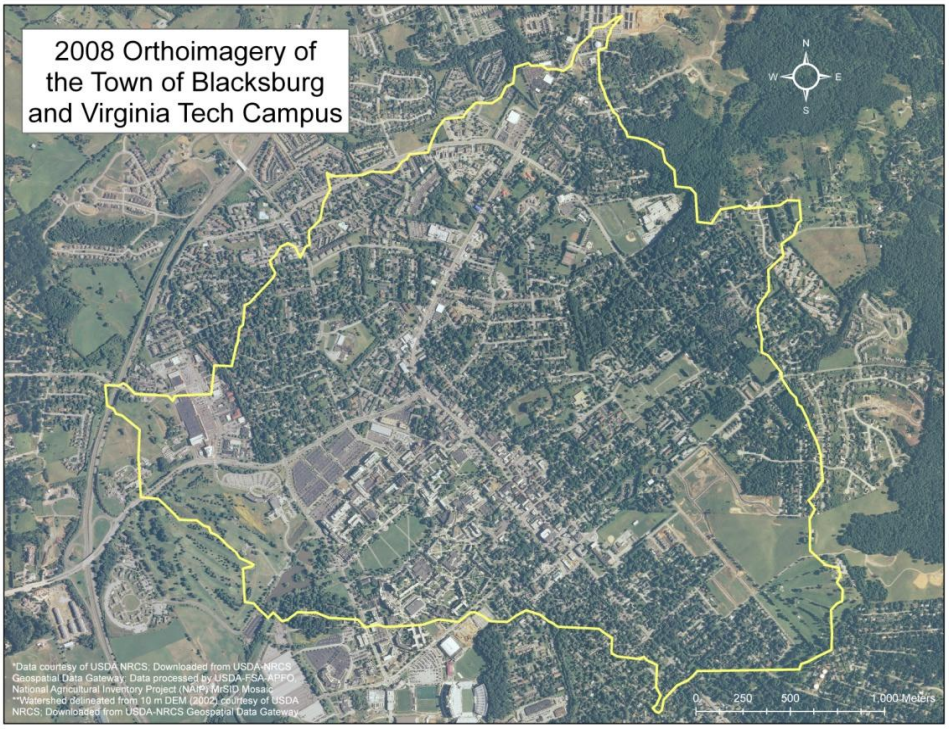

Figure 3. 2000 Orthoimage of Blacksburg and Virginia Tech's central campus.

Digitization of land cover in the Upper Stroubles Creek Watershed reveals that urbanization is increasing at the expense of agricultural and forested land cover (see Table 1). Between 1937 and 1971, urban land cover increases 194%, while agricultural lands decrease by 60%. Urbanization expansion continues into the 2000s – urban land cover increases 41% between 1971 and 2008, while agricultural land cover decreases 93%. Within approximately the past ten years, urbanization has expanded 5% and agriculture has reduced 60%, indicating the increasing trend in development in an already urbanized watershed.

Table 1. Land cover area (acres) of Upper Stroubles Creek Watershed over time.

Year	Urban	Agriculture	Forest	Open Water
1937	459	1508	0	8
1971	1348	597	24	7
2000	1815	118	35	8
2008	1900	40	30	9

Future of Stroubles

As development has become widespread in the Upper Stroubles Creek Watershed, the land area dedicated to agriculture has decreased. Recently, a portion of one of the remaining plots of agricultural land within the watershed has been converted to a housing development, Fiddler's Green. A conservation easement in proximity to Fiddler's Green has the potential to preserve the few remaining acres dedicated to agriculture in the watershed. According to Andrew Warren, Zoning Administrator for the town of Blacksburg, Katherine Hoge placed thirty-two acres of her land into a conservation easement in 1991. Warren indicated that the easement would be violated if the thirty-two acres were abandoned or allowed to fallow. A *Collegiate Times* article discussing the easement reported that the land was recently purchased by Jim and Heather Cowan. The new owners intend for the area to remain ninety percent

agricultural with organic farm management practices (November 19, 2008). Ironically, one of the selling points of Fiddler's Green housing development is the close proximity to the easement.

Comparison of Digital Elevation Models

Comparison of the flow accumulation layers of the 2002 DEM and 2005 LiDAR data layers reveals similarities (see Figure 4). This is important for a few reasons: (1) these results support the argument that land cover changes such as urbanization are not significant enough to change the movement and flow path of water in Upper Stroubles Creek between 2002 and 2005; (2) abnormalities in the data are not present, giving credibility to the data layers used in this study; and 3) this interpolation is a defensible method for establishing a DEM.

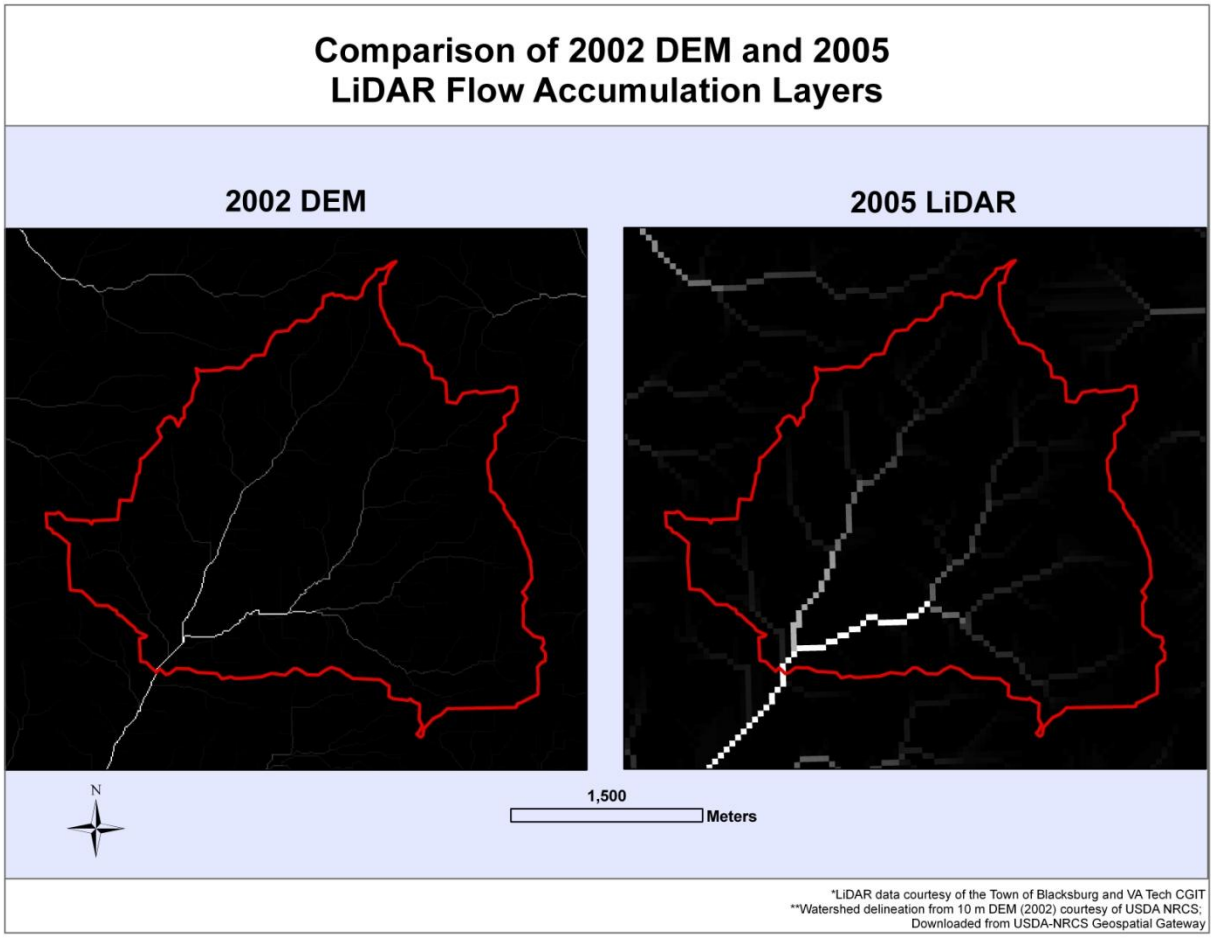

Figure 4. Flow accumulation comparison of 2002 DEM and 2005 LiDAR derived DEM.

A disparity does exist between the GPS and LiDAR derived flow accumulation layers (see Figure 5). This disparity can be attributed to (1) developmental changes altering the flow and movement of water within the watershed, (2) error associated with the elevation readings of the GPS device, and (3) an unsystematic collection of GPS points throughout the watershed area. Due to time limitations, inability to get onto private property, other inaccessible areas and safety, GPS readings were not collected uniformly throughout the entire town and campus. This resulted in "weighting" issues in developing a surface raster and flow accumulation layer.

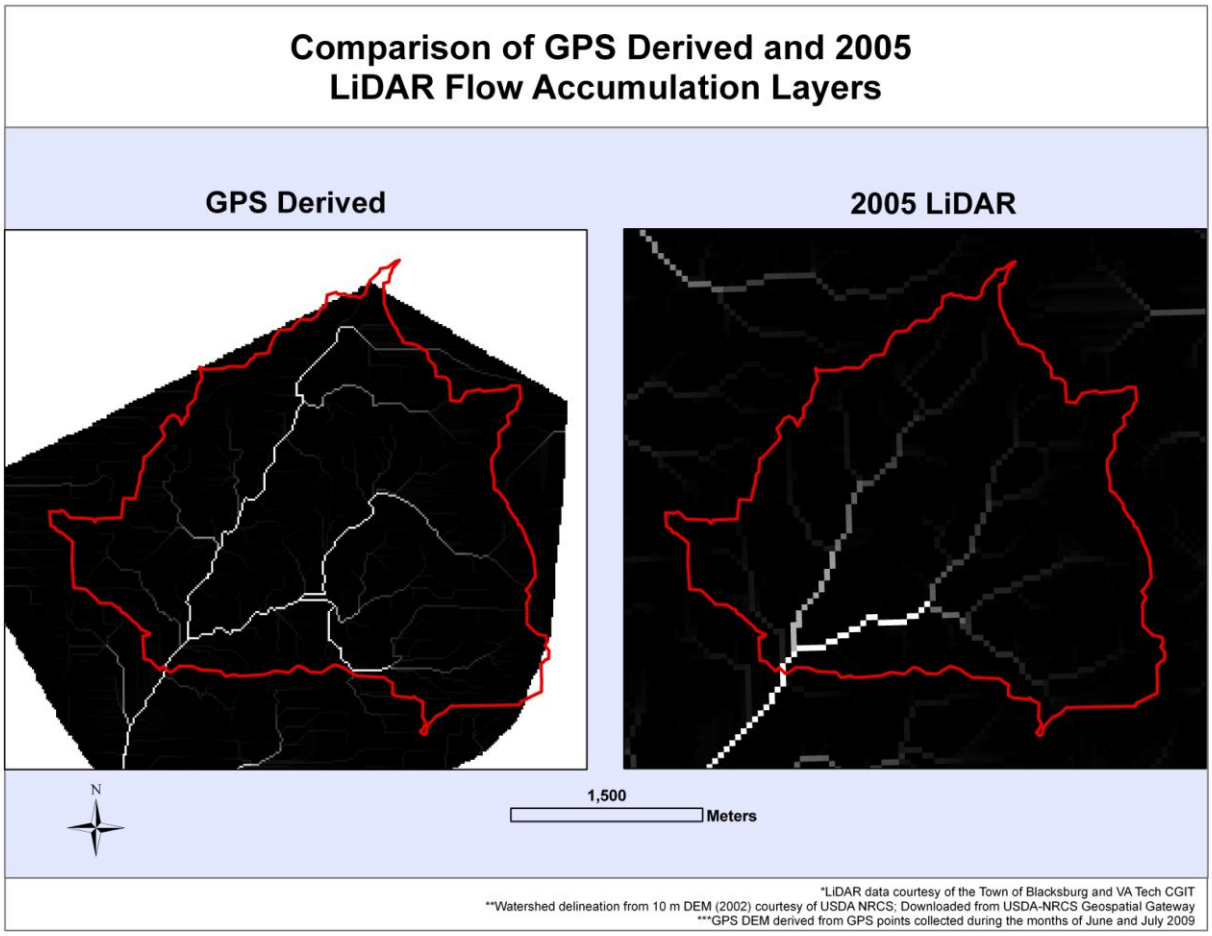

Figure 5. Flow accumulation comparison of GPS derived DEM and 2005 LiDAR derived DEM.

Conclusions

Urbanization is currently the leading land cover type in the Upper Stroubles Creek Watershed. Due to sprawl, open space, low, and medium development dominate the types of urbanization found throughout the watershed. Large expanses of open space development on Virginia Tech's central campus, along with the large number of recreational parks throughout the town of Blacksburg, reveals low impact and open space development throughout the watershed. Recently, growth has progressed in the already urbanized watershed by converting open space areas to medium and high development buildings and housing communities. As revealed in the orthoimagery analysis, mass urbanization began as early as the 1970s and has continued into the 2000s. Less than 40 acres of land cover in the watershed is currently dedicated to agricultural lands, whereas agriculture predominated in the past.

Geospatial technologies provide a method of analyzing, understanding and communicating the impacts of development in a watershed. As previously mentioned, each technique carries a degree of uncertainty. Relying on low accuracy GPS technology to develop a present day DEM of the Upper Stroubles Creek area indicates the need for up-to-date high resolution elevation models in Montgomery County, VA. On the other hand, simple remote sensing techniques are sufficient to trace land cover changes over time.

Future Recommendations

Locating additional historical aerial photographs for the 1940s-60s and 1980s-90s time periods would provide the opportunity to document land cover changes for a more detailed timeline scale

Additionally, this project could be expanded in the future by a researcher skilled in remote sensing programming and advanced computer analysis. Utilizing a researcher who is proficient in these programs, or computer programming, would result in the ability of in-depth urbanization analyses. A researcher skilled in photographic interpretation could analyze land cover on a finer scale, and thus the different types of urbanization (open, low, medium, high, industrial, etc.) could be followed over time. More comprehensive field investigations should be employed. Walking the stream's daylighted sections and obtaining GPS readings would produce a more accurate surface model and flow accumulation layer. Finally, the field-derived DEM and subsequent flow accumulation layers need to be supported by other field methodologies such as surveying.

Acknowledgements
I would like to thank the NSF/REU program for funding and support (Grant No. 0649070). Recognition also goes to Dr. J.B. Campbell in the Geography Department for Remote Sensing guidance, Stephanie DiBetitto from the University of Vermont and Erika Karsh from Oberlin University for assisting in GPS collection.

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Data Sources

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