Nevada Water Resources Research Institute
Annual Technical Report
FY 2015

Introduction

Success and dedication to quality research has established the Division of Hydrologic Sciences as the recognized "Institute" under the Water Resources Research Act of 1984 (as amended). A total of 54 Institutes are located at colleges and universities in the 50 states, the District of Columbia, Puerto Rico, and the U.S. Virgin Islands.

The primary mission of the Nevada Water Resources Research Institute is to inform the scientists of Nevada.

Research Program Introduction

Nevada is the most arid state in the United States and it is experiencing significant population growth and possible future climate change. With competing water demands for agricultural, domestic, industrial, and environmental uses, issues surrounding water supply and quality are becoming more complex, which increases the need to develop and disseminate sound science to support informed decision making.

As the NWRRI, the continuing goals of DHS are to develop the water sciences knowledge and expertise that support Nevada's water needs, encourage our nation to manage water more responsibly, and train students to become productive professionals. Therefore, DHS has chosen to make a valuable contribution to water research and education in Nevada by judiciously distributing its Section 104 research funds among numerous subject areas. Projects must be of significant scientific merit (as determined by the review process) and relevant to Nevada's total water program to be considered worthy of funding.

To ensure collaboration and coordination among water-related entities throughout the state, DHS maintains a Statewide Advisory Council on Water Resources Research composed of leading water officials who may be called upon to assist in selecting the research projects that will be supported by Section 104 funds.

Optimization of ozone-biological activated carbon treatment for potable reuse applications

Basic Information

Title:	Optimization of ozone-biological activated carbon treatment for potable reuse applications
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Descriptors:	None
Principal Investigators:	Daniel Gerrity

Publications

1. Selvy, A., D. Gerrity. 2014. Optimization of Ozone-Biological Activated Carbon Treatment for Potable Reuse Applications. Nevada Water Resources Association 2014 Annual Conference, Las Vegas, NV, Jan. 2014.
2. Selvy, A., D. Gerrity. 2015. Impacts Of Ozone Dose And Empty Bed Contact Time On Total Organic Carbon Removal Through Ozone-Biological Activated Carbon Treatment. 19th Annual Water Reuse and Desalination Research Conference, Huntington Beach, CA, May 2015.

Final Report

1.0 Problem and Research Objectives

In the face of climate change, pollution, and population growth, water scarcity has become a global threat. Many populations have witnessed their drinking water sources dwindle to an unsustainable level. These severe conditions have sparked interest in potable reuse as an increasingly viable alternative to typical 'pristine' drinking water sources. Although potable reuse has been practiced for decades, the public has become more supportive of the concept over the past few years based on the historical success of several benchmark facilities in the United States and abroad (Gerrity et al., 2013). Many municipalities are considering implementing their own projects, but there is considerable debate as to the level of treatment needed to ensure protection of public health.

Among existing potable reuse guidelines and regulations, the California Division of Drinking Water (DDW) provides the most stringent requirements for water quality (CDPH, 2014). Currently, the best way to meet these standards is through the use of full advanced treatment (FAT), which consists of reverse osmosis (RO) and an advanced oxidation process (AOP). Although extremely effective, RO is energy intensive and produces a concentrated brine solution that is both difficult to dispose of and an ecological concern in coastal regions (Gerrity et al., 2014). Alternative treatment trains composed of ozone and biofiltration, particularly biological activated carbon (BAC), have been employed in several locations throughout the world, but these systems have not yet been optimized and are unable to compete with RO-based treatment trains on the basis of total organic carbon (TOC) removal and reductions in total dissolved solids (TDS). While RO-based treatment trains have been known to remove TOC to the μg/L level, ozone-BAC trains have yet to achieve this threshold.

With the exception of TOC and TDS, which are generally more relevant to aesthetics rather than public health, ozone-BAC is capable of producing a water quality similar to that of RO-based treatment trains on the basis of pathogen reduction, trace organic contaminant mitigation, and a variety of other parameters. There are also significant energy and cost savings for the ozone-BAC alternative so there is an incentive to optimize such treatment trains to achieve greater TOC removal. This process requires up to 70% less in capital costs and 80% less in operation and maintenance (O&M) costs in comparison to FAT (Gerrity et al., 2014).

The objective of this study was to identify the necessary operational conditions needed for ozone-biofiltration treatment trains to compete with FAT on the basis of TOC reduction. The original hypothesis was that by coupling greater effluent organic matter transformation via increased ozone doses with longer empty bed contact times, ozone-biofiltration systems could achieve better TOC removal and possibly approach the 0.5-mg/L threshold for TOC set by the California DDW.

2.0 Methodology

2.1 Pilot-Scale Reactor

2.1.1 Construction and Operation

A 0.6 liter-per-minute (lpm) pilot-scale reactor was constructed at a water recycling facility in the Las Vegas metropolitan area. It consisted of 12 ozone contactors and six biofilters, which were used to treat full-scale membrane bioreactor (MBR) filtrate. The flow rate through the system was measured with an in-line flow meter. The addition of sodium chloride for a tracer study, which was used to determine the hydraulic retention time of the ozone contactor, was achieved through a sample injection port followed by a static mixer. Ambient air, oxygen, or ozone was introduced through a Venturi injector downstream of the static mixer. Concentrated oxygen was achieved with a portable medical system equipped with molecular sieves (AirSep, Denver, CO). The oxygen was generated at a flow rate of between 0.5 and 2 lpm and a pressure of 20 psig throughout the study. After passing through an air filter to remove particulates, the oxygen traveled to a Magnum-600 air dryer (Ozone Solutions Inc., Hull, Iowa) to remove any moisture from the oxygen prior to reaching the Nano dielectric ozone generator (Absolute Ozone, Edmonton, AB, Canada). The output from the ozone generator traveled either through a bypass line to a catalytic destruct unit or to the Venturi injector, where the ozone was injected into the process flow. The bypass line was controlled by a standard gas flow meter. In addition to check valves, the feed gas line was equipped with a water trap that prevented water from entering the feed gas tubing and backing up into the generator, as well as a pressure gauge to monitor feed gas pressure entering the Venturi injector.

The ozonated water then traveled to the 12 ozone contactors connected in series (four were one inch in diameter and eight were two inches in diameter); samples were collected from sample ports located at the bottom of the contactors. Ozone off-gas was collected in Teflon tubing at the top of each contactor and was sent to a catalytic destruct unit. The ozone off-gas line was also protected by a water trap that prevented water from reaching the catalytic destruct unit. Ozone doses were estimated by measuring differential UV_{254} absorbance after ozonation and then estimating the ozone to total organic carbon (O_3/TOC) ratio based on established correlations in the literature (Gerrity et al., 2012) and an independent site-specific correlation (*Section 4.3.1*).

The effluent from the final ozone contactor fed five parallel, 1-inch diameter biofilter columns. At various times throughout the project, these biofilter columns were filled with either 1.2-mm diameter anthracite provided by the San Jose Creek Water Reclamation Plant, 0.95-mm diameter exhausted GAC (Norit 820, Cabot Corporation, Alpharetta, GA) from the F. Wayne Hill Water Resources Center (Gwinnett County, GA), or a proprietary denitrifying biocatalyst (hereafter referred to as “BC”). The column-to-media diameter ratios were approximately 21:1 and 27:1 for the 1.2-mm BAC and 0.95-mm BAC, respectively. The BC was manufactured as a porous polyvinyl alcohol (PVA) bead containing denitrifying microorganisms. The BC has historically

been used in suspended growth (i.e., activated sludge) systems so this was the first evaluation of the BC in a packed-bed configuration. A separate control biofilter (containing 1.2-mm anthracite) received non-ozonated pilot influent (i.e., MBR filtrate) to allow for the evaluation of organic matter removal with and without the synergistic effects of ozonation. An experimental anthracite column, the biocatalyst column, and the control anthracite column were all operated at the same EBCT during long-term operation to allow for direct comparisons of treatment efficacy. Biofilter sample ports for treated water were located at the bottom of each column, and the flow rates (and EBCTs) were controlled by independent needle valves. Samples of biofilter media were also collected periodically from dedicated sample ports to evaluate the development of the microbial community. The microbial community in the biofilters will be discussed in *Section 4.4*.

Figure 1 illustrates the layout of the pilot-scale reactor, and corresponding photos of the ozone contactors and biofilter columns are provided in Figures 2A and 2B, respectively. In Figure 1, the pink asterisks mark the sampling locations for treated water from the biofilters, the white "X's" represent the lower media sampling locations at a 17.5-inch filter media depth, and the blue "X's" signify the upper media sampling locations at a depth of 5.5 inches. The green circles denote the non-filtered water samples. The influent sample port was located prior to ozone injection into the water stream, and the effluent sample location was located after the ozone contactors. The effluent samples consisted only of ozonated water. The BC column did not have any media sample locations; the samples had to be collected by backfilling the column with water and expanding the media to the top where it could be collected.

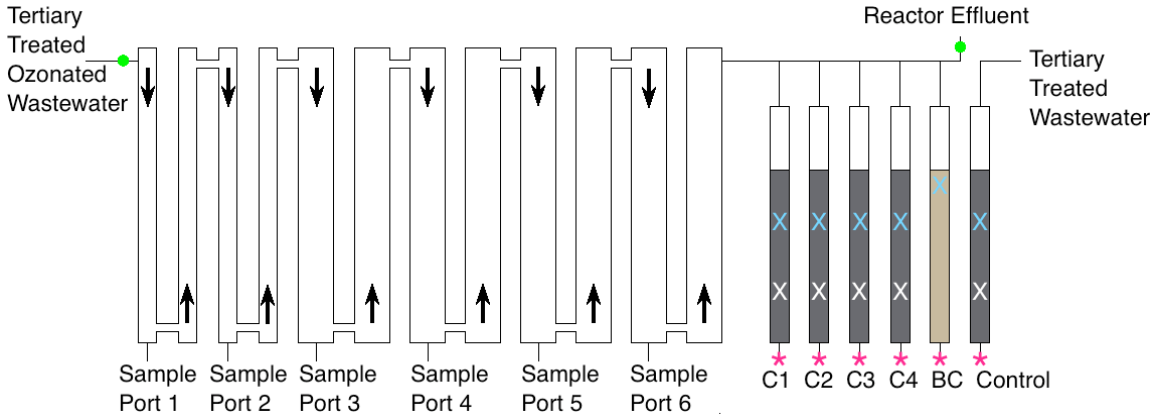


Figure 1. Schematic of pilot-scale reactor.
During the initial long-term operation of the pilot, C1-C4 and the Control were filled with 1.2-mm diameter anthracite. During later phases of the project, C3 was switched to the 0.95-mm diameter exhausted GAC (i.e., BAC). The column containing the biocatalyst is denoted as BC.

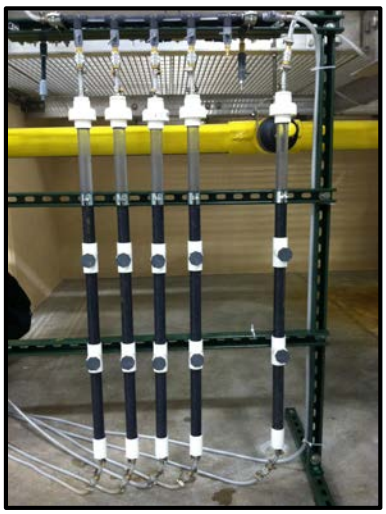
A.  B. 

Figure 2. Photos of the (A) ozone contactors and (B) biofilters.

2.1.2 Backwashing

Backwashing of the pilot-scale biofilter columns was performed based on performance observations. When accumulation in the filters was too high, the flow rate would drop significantly. This was used as an indication of the need for backwashing. This method was chosen (as opposed to regular time intervals) because of the variability of the influent water quality and flow rate, which would impact the filter run time.

The biofilter effluent tubing was detachable making it possible for the backwash tubing to be attached at the bottom of the filter. The top of the filter was also detachable. Membrane bioreactor effluent was used as the backwash water and was pumped through the bottom of the filters using a MasterFlex peristaltic pump (Cole Palmer, USA). Backwashing flow rates varied between each filter and over time. A bed expansion of 34% was targeted and the flow rate adjusted accordingly. A 34% bed expansion was chosen because it was within typical values found in literature. Backwash duration was ten minutes. No air scour or chlorination was used.

2.1.3 Start-up

Initially, five columns were filled with the 1.2-mm anthracite and fed membrane bioreactor effluent without ozonation (C1, C2, C3, C4, and control). The total organic carbon was monitored for indications of microbial growth. Initially, the goal was to develop the microbial community without the use of ozone. This would allow for the identification of a TOC removal baseline from which the synergistic impacts of ozone could be quantified. However, limited TOC removal and biological growth (based on the adenosine triphosphate (ATP) measurements described later) were observed during long-term operation of the pilot prior to start-up of the ozone generator.

Initially, it was hypothesized that there was an insufficient seeding of bacteria because of the membrane component of the MBR, which is essentially an impermeable barrier to larger microorganisms. To supplement the bacteria already attached to the media prior to start-up, the columns were seeded with secondary treated wastewater effluent from a separate full-scale facility for 24 hours each. After observing minimal improvements in system performance, it was then hypothesized that the MBR filtrate may have been overly recalcitrant, thereby limiting the supply of a suitable carbon source for any attached bacteria. Potential solutions included seeding the reactors with an alternative carbon source, such as acetate or methanol, or implementing ozonation to transform the recalcitrant effluent organic matter (EfOM) into a more bioavailable supply. Continuous ozonation was identified as the preferred alternative. Four months after start-up, the ozone system was initiated to enhance biological growth in the filters.

A proprietary biocatalyst containing denitrifying bacteria was provided as an alternative biofilter media for this study. It is made of a porous material, which allows for the passage of water and dissolved constituents and subsequent interaction with the bacteria. The BC was installed two months after ozone start-up but did not receive ozonated water until three months after installation. During this time, the control biofilter was receiving ozonated effluent to promote biological growth. After the biological community stabilized in the control column, the control and BC column positions were switched so that the control and BC were receiving non-ozonated and ozonated effluent, respectively. The empty bed contact times were then adjusted and maintained at C1 (ozone+anthracite) = 5, C2 (ozone+anthracite) = 10, C3 (ozone+anthracite) = 10, C4 (ozone+anthracite) = 15, Control (anthracite only) = 10, and BC (ozone+biocatalyst) = 10 minutes for a three-month period. During this time, the impact of EBCT on TOC removal was monitored.

During testing, several issues were observed with the BC, all of which were related to using the material in a configuration for which it was not designed. For example, the BC beads would not fluidize during backwashing. This could be attributed to the low density of the BC beads and/or its material properties coupled with the peristaltic pump used for backwashing. A flow rate of approximately 56 mL/min (the lowest setting on the pump) resulted in full bed expansion, but there was insufficient agitation for fluidization to take place because the BC consisted of a soft material that would cause the beads to stick together and form a thick cake. For this reason, the BC column was never effectively backwashed throughout the study. Also, the BC beads began to compact after about five months of operation, which drastically inhibited flow through the column. It was difficult, and sometimes impossible, to achieve the desired flow rates through the BC column. After initial signs of compaction, the BC column was backfilled with water, which allowed time for the beads to expand to their original form. However, the beads would slowly compact once filtration was resumed, and after several cycles, the beads began to compact instantly. Again, the BC is typically used in suspended growth systems where compaction is not a concern. Finally, the true purpose of the BC is denitrification, which generally requires anoxic conditions (i.e., nitrite/nitrate serve as the electron acceptor in the absence of dissolved oxygen). Ozonation leads to supersaturation of treated water with dissolved oxygen, which inhibits the

denitrification process. As will be described later, the BC initially performed well for the removal of bulk organic matter, presumably due to the growth of bacteria on the outside of the beads, but modified process configurations would be necessary for future BC implementation.

Seven months after ozone initiation, the media in C3 was replaced with exhausted 0.95-mm Norit 820 GAC. This was done to provide a direct comparison between anthracite and exhausted GAC as potential surfaces for biofilm development. Due to the pore structure and high specific surface area of virgin GAC, it is often used as an adsorbent material for the removal of trace organics, bulk organic matter, and other water contaminants. GAC eventually loses its adsorptive capacity, which results in contaminant breakthrough and ultimately exhaustion. If adsorption is required to achieve specified operational criteria, the GAC is then replaced or regenerated, which increases operational costs. In a biofiltration application, the adsorptive capacity is less important because the intent of the media is to serve as a surface for biofilm attachment and growth. Although the pores are generally too small for bacteria to penetrate, the exhausted GAC, or BAC, still has a relatively high specific surface area, which is advantageous for development of the microbial community.

After examining the impact of EBCT on reactor performance, all columns were maintained at an EBCT of ten minutes in preparation of the kinetics tests discussed in *Section 2.2*.

2.2 Methodology for Performing Kinetics Tests

Three kinetics tests were performed, each at a different ozone dose. The ozone dose was held constant while the EBCTs of the filters were adjusted in small intervals. After adjustments to the EBCTs were made, a time equivalent to three hydraulic retention times was allowed prior to sampling. This was done to allow sufficient time for stabilization. The range of hydraulic loading rates employed during the kinetics tests was 0.51 – 119 cm/min (0.12 – 29 gpm/ft^2).

The first kinetics test was performed at a mass-based ozone to total organic carbon ratio (O_3/TOC) of 0.35. The EBCTs were increased step-wise from 1.75 minutes to 10 minutes. Ten sample events were performed during this test. The second kinetics test utilized an O_3/TOC ratio of 1.12. The EBCTs were varied between 2-30 minutes; the order of the EBCTs was random to determine if any systematic error occurs from increasing the EBCT step-wise. For the third test, an O_3/TOC of 0.62 was applied. Again, 10 sampling events were performed at EBCTs between 2 and 14 minutes.

2.3 Methodology for Ozone Demand Decay Testing

An ozone demand decay study was performed on the source water using the indigo trisulfonate colorimetric method for dissolved ozone. Potassium indigo trisulfonate is dark blue in color but will quickly decolorize in the presence of ozone as the chemical is oxidized. A spectrophotometer is used to determine the absorbance of the indigo trisulfonate solution at 600

nm, which is directly related to the strength of the blue color. The extent of decolorization, or bleaching, during ozonation is directly correlated with the dissolved ozone concentration.

For this study, an ozone demand decay test was performed in a batch configuration. Five gallons of source water were collected and ozonated at the following O_3/TOC ratios: 0.25, 0.5, 1.0, and 1.5. Then, 10 mL of potassium indigo trisulfonate test solution were added to several 100 mL volumetric flasks that had been previously weighed. The ozonated source water was added to a single flask at specified time steps (every 30 seconds for the first two minutes, every minute for the next eight minutes, and then every two minutes thereafter). A sufficient sample volume was added to each flask to induce a noticeable color change due to the combined effects of oxidation and/or dilution. The flasks, which now contained indigo trisulfonate plus sample, were weighed to determine the mass of sample added, which was later converted to volume. The absorbance of each sample was then measured with a spectrophotometer, and the absorbance of each sample was converted to a dissolved ozone concentration using Eq. 1:

$$O_3(mg/L)=\frac{V_{blank+indigo} \times Absorbance_{blank} - V_{sample+indigo} \times Absorbance_{sample}}{f \times V_{sample} \times b} \quad (1)$$

where, f represents the proportionality constant (0.42) and b is the cell path length (1 cm) (Rakness et al., 2010). The dissolved ozone residual data were then modeled as a first order decay process (Eq. 2), which could then be used to calculate the corresponding ozone exposures (i.e., CT values) using Eq. 3.

$$O_3 \text{ residual [mg/L]} = ((O_3/TOC)*TOC - IOD)*e^{-kt} \quad (2)$$

$$CT \text{ [mg-min/L]} = \frac{(O_3/TOC)*TOC-IOD}{k}*\left(1-e^{-kt}\right) \quad (3)$$

where, CT is the ozone exposure (mg-min/L), TOC is the source water concentration of total organic carbon (mg/L), IOD is the instantaneous ozone demand (mg/L), k is the first order ozone decay rate constant (min^{-1}), and t is time (min) (Gerrity et al, 2014).

2.4 Methodology for EfOM Characterization with UV Absorbance and Fluorescence

When light of a certain wavelength is passed through a sample, some of the molecules in the sample absorb the light. When photons are absorbed, the absorbing molecule is promoted to an electronically excited state, meaning that the outer electrons transition to a higher energy level. Only a fraction of the incident photons are absorbed by molecules in the solution, and the remaining fraction passes through the solution. Using a spectrophotometer, the intensity of the transmitted radiation (I) is compared with that of the incident radiation (I_0), which yields the absorbance or transmittance of the sample (Horiba Scientific, 2012). Wavelength-specific absorbance—typically at 254 nm—is often used as an indicator of water quality. Evaluating

absorbance across the UV spectrum also provides a means of characterizing the organic matter in a sample.

Fluorescence can also be used to assess water quality and characterize organic matter. When the excited electrons eventually relax to their ground state, they release energy in the form of light (i.e., fluorescence). The intensity of the emitted light, which is characterized by a longer wavelength (i.e., less energy) than the incident light, is measured by a spectrofluorometer. These excitation-emission couples can be evaluated across a broad spectrum to generate an excitation emission matrix (EEM), or fluorescence 'fingerprint', for a water sample.

For this study, UV absorbance (or transmittance) and fluorescence were determined with a Horiba Aqualog spectrofluorometer (Edison, NJ). Samples were collected from the pilot reactor, brought to room temperature, and filtered using a 0.7-μm GF/F Whatman syringe filter (GE Healthcare Life Sciences, Piscataway, NJ). The analysis settings that were used are provided in Table 1. Data were processed using Matlab (MathWorks, Natick, MA) to generate contour plots of fluorescence emission and intensity (in arbitrary fluorescence units (AFU)) and identify critical fluorescence peaks and regional intensities. The standard operating procedure used for UV_{254} and fluorescence determination is provided in Appendix 1.

Table 1. **Raman and sample settings for fluorescence analysis.**
The Raman settings are used to calculate the peak Raman area for a blank sample, which is then used to standardize the fluorescence intensities of experimental samples. That allows for direct comparisons between samples analyzed by different analysts, instruments, labs, etc. The sample settings are used to perform the 3D EEM analysis of samples.

Parameter	Raman	Sample
Integration Period	3 s	3 s
Excitation Wavelength Range	350 nm	240-470 nm
Emission Wavelength Range	380-410 nm	280-570 nm

A Matlab code was used for the evaluation of the fluorescence and UV absorbance as a way to characterize the composition of the samples. The EEMs were divided into three regions. Region I is representative of soluble microbial products (SMPs), region II is associated with fulvic-acid-like substances, and region III is indicative of humic-acid-like substances (Gerrity et al., 2011). An EEM showcasing the three regions can be seen below.

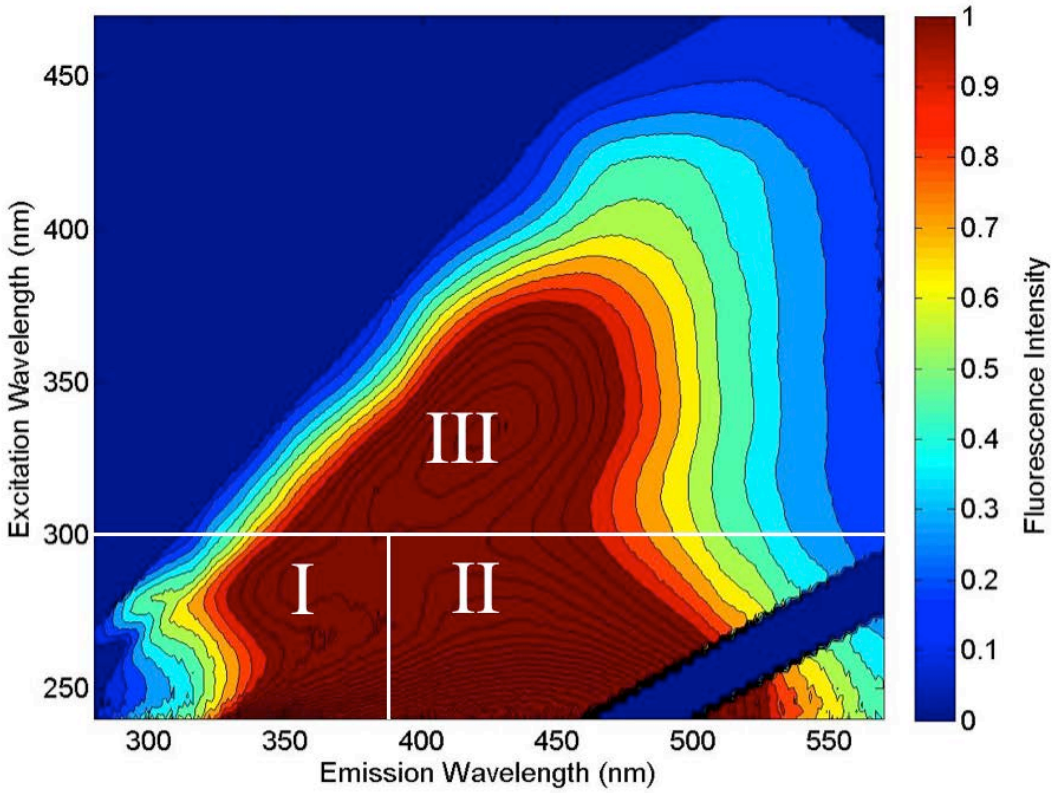


Figure 3. Fluorescence fractioning for the characterization of EfOM.
Region I is representative of soluble microbial products, region II is representative of fulvic acids, and region III represents humic acids.

The fluorescence in each region is computed as the volume under the EEM (Zhou, 2013). Fluorescence and fluorophore concentration are directly related (i.e., the higher the fluorescence, the higher the concentration of fluorophores in that region). The fluorescence index (FI) also gives insight into the source of the organic matter. Higher FI values are generally associated with wastewater-derived organic matter due to the presence of soluble microbial products, biopolymers, and proteins, whereas lower FI values are indicative of terrestrially-derived organic matter (Gerrity et al., 2011). The FI compares the fluorescence at emission wavelengths of 450 nm and 500 nm at a constant excitation wavelength of 370 nm.

2.5 Methodology for EfOM Quantification based on Total Organic Carbon

A Shimadzu TOC V-csn (Kyoto, Japan) was used for TOC analysis. This instrument measures total organic carbon using the non-purgeable organic carbon (NPOC) method. Acid is added to the sample to decrease the pH and convert inorganic carbon (i.e., carbonate species) to CO_2, and then the sample is purged with hydrocarbon-free compressed air to eliminate the CO_2. The sample is then sent to a combustion chamber where the remaining organic carbon is converted to

CO_2 via combustion catalytic oxidation at 680°C. At this point, the CO_2 is sent to a non-dispersive infrared detector and analyzed, and the signals are correlated to TOC concentration. A stock solution using 0.53 g of potassium hydrogen phthalate (KHP) and 250 mL of deionized water was used to produce a 1000 mg/L TOC stock solution. The stock solution was replaced every two months. Standard solutions of 0, 4, 8, 12, 16, and 20 mg/L TOC were prepared using 0, 200, 400, 600, 800, and 1000 µL of stock solution in 50 mL volumetric flasks. These were prepared fresh for each sampling event.

For this study, all glassware were cleaned according to the guidelines provided in Standard Method 5310B. The samples were collected in amber vials (with no headspace), capped with Teflon lined lids, and kept cool prior to analysis. After the samples were acidified using 5N HCl to reduce the pH to less than 2, the samples were covered with parafilm to reduce contamination potential, loaded in the autosampler, and analyzed according to the method parameters provided in Table 2.

Table 2. NPOC analysis parameter settings for both sample analysis and calibration curve determination.

Injection Volume	80 µL
Number of Injections	3/7
Standard Deviation Max	0.100
CV Max	3.00%
Number of washes	2
Auto Dilution	1
Sparge Time	1:30 min
Acid Addition	0

2.6 Methodology for the Evaluation of Biological Activity based on ATP

Adenosine triphosphate (ATP) is a compound used by living organisms to store and transfer energy. When ATP reacts with the Luciferase enzyme, light is produced. This light can be measured with a luminometer to determine the concentration of ATP in the sample. The concentration of ATP can be used as an indicator of the presence of bacteria in a system.

A deposit and surface analysis ATP test kit (Hach, Loveland, CO) was used to quantify the biological activity of the biofilm on the biofilter media according to ASTM D4012. This method measures both the intracellular ATP found inside living bacteria as well as ATP dispersed in the sample from decayed biomass.

For ATP analysis, media samples were extracted from the dedicated sample ports on the biofilter columns using sterile scoopulas and stored in sterile sample containers. Control anthracite that had been stored in the refrigerator upon receipt from the San Jose Creek Water Reclamation Plant was also collected to compare with the media from the pilot-scale reactor. One gram of dry

media was added to individual test tubes containing 5 mL of LuminUltra UltraLyse 7, and the tubes were capped. The tubes were inverted several times for mixing and allowed to sit for five minutes to ensure that the ATP was extracted from the lysed bacteria. A 1-mL volume of the resulting liquid (no solids) was transferred to another tube containing 9 mL of LuminUltra UltraLute (for dilution). Prior to analyzing the samples, an ATP standard calibration was performed by adding 100 uL of LuminUltra Ultracheck1 and Luminase to a test tube and analyzed using a PhotonMaster Luminometer (LuminUltra Technologies Ltd, New Brunswick, CA). This is done to monitor the luciferase enzyme activity in the Luminase. 100 µL of the new solution were transferred to another tube containing 100 µL of Luminase. The final sample tube was placed in the luminometer for analysis within 30 seconds.

2.7 Nutrient Quantification

Nitrate determination was accomplished with method 8039 (Cadmium Reduction Method) using Hach NitraVer 5 powder pillows. This method measures high range nitrate between 0.3 and 30 mg/L NO_3-N. Nitrite was measured using Method 8507 (Diazotization Method) using Hach NitraVer 3 powder pillows for low range nitrite concentrations (0.002-0.3 mg/L NO_2-N). Method 10023 (Salicylate Method) for low range ammonia analysis (0.02-2.5 mg/L NH_3-N) was also used. All of these compounds were measured using a DR5000 spectrophotometer (Hach Corp., USA). For phosphate determination, Method 8048 (Ascorbic Acid) was used with Hach PhosVer 3 powder pillows. Phosphorus was measured using a DR 900 multiparameter handheld colorimeter (Hach Corp., USA).

As will be described later, higher nitrite concentrations were detected in the effluent from the anthracite columns. To determine if any nitrite was originally adsorbed to the media and potentially leaching into the treated water, a leach test was performed on the archived media in the refrigerator. Six samples were evaluated: three crushed samples and three uncrushed samples. A pestle and mortar were used to manually crush the media to very fine particles. A 50 mL test tube was filled with 5.02g of media and filled to the 45 mL mark with distilled water. The samples were allowed to soak for one hour to allow for full saturation of the media. The samples were then placed in a Sorvall Legend RT centrifuge (Thermo Fisher Scientific Inc., USA) for six minutes at 2500 rpm. The supernatant was passed through a 0.7-µm filter and analyzed for nitrite.

2.8 Total Coliform and *E. coli* Quantification

Standard Method 9223, using IDEXX Colilert-18, was used for total coliform and *E. coli* determination in the pilot reactor samples. This method uses defined substrate technology nutrient indicators ortho-nitrophenyl- β -D-galactopyranoside (ONPH) and 4-methylumbelliferyl-β-D-glucuronide (MUG). The β-galactosidase enzyme found in coliform bacteria metabolizes ONPG, producing a yellow color. *E. coli*, on the other hand, uses the β-

glucidonerase enzyme to metabolize MUG, which fluoresces under long-wave ultraviolet light at 366 nm (IDEXX Laboratories, 2015).

For sample analysis, 100 mL of sample was collected in a sterilized, transparent, non-fluorescing IDEXX container containing sodium thiosulfate to quench any residual oxidant present. The samples were collected in triplicate for statistical analysis purposes. The samples were capped and shaken until the sodium thiosulfate dissolved completely. They were then kept cool until the next step could be performed. A Colilert-18 reagent was added to each of the samples and shaken until all nutrients were dissolved. Samples were then transferred to an IDEXX Quanti-Tray/2000 and sealed in an IDEXX Quanti-Tray sealer. The sealed samples were allowed to incubate at 35°C for 18 hours. After incubation, the small and large wells that experienced a color change (yellow) for total coliform or fluorescence for *E. coli* were counted and quantified using the most probable number (MPN) table.

2.9 Trace Organic Contaminants

Due to budget limitations, trace organic contaminants were measured only one time toward the end of the study. Samples of pilot influent, biofiltration effluent (anthracite only), and ozone-biofiltration effluent (ozone-anthracite) were collected in bottles provided by Eurofins Eaton Analytical (Monrovia, CA) and were shipped overnight on ice to the laboratory. Eurofins Eaton Analytical then processed and analyzed the samples for a suite of 94 trace organic contaminants by solid phase extraction, liquid chromatography tandem mass spectrometry (LC-MS/MS), and quantification by isotope dilution. Nine *N*-nitrosamines were also analyzed by the laboratory using EPA Method 521.

3.0 Principal Findings and Significance

3.1 Ozone Demand and Decay

Ozone demand decay curves were generated for O_3/TOC ratios of 0.5, 1.0, and 1.5 (Figure 4). It was not possible to generate a demand decay curve for the O_3/TOC ratio of 0.25 because the instantaneous ozone demand (i.e., the demand at 30 seconds) exceeded the transferred ozone dose.

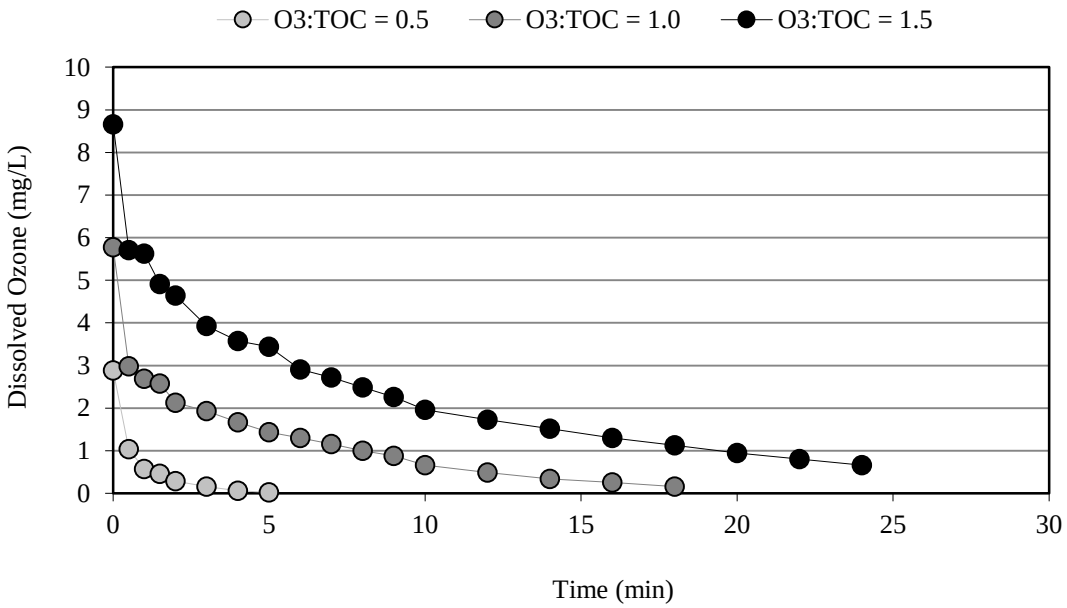


Figure 4. Ozone demand decay curves for the MBR filtrate as a function of O_3/TOC ratio.
The decay witnessed by the ozone is due to a combination of natural ozone decay in pure water and ozone demand from the organic and inorganic compounds in the source water matrix.

The curves indicate the rate at which the ozone decays in this particular water matrix. This gives insight into the composition of the water (i.e., pH, the presence of ozone-reactive organic and inorganic compounds, and the reactivity of those compounds). The extended period of time necessary for complete ozone decay in the presence of 9.5 mg/L of TOC indicates that the bulk organic matter is highly recalcitrant. This is further supported when comparing the pseudo first-order ozone decay rate constants from the study (obtained from performing regressions on the decay curves) versus literature values for similar water matrices (Table 3).

Table 3. Ozone decay regression and rate constants at different ozone dosing conditions.
The ozone decay rate constants observed in the study were much lower than the literature values, indicating the presence of ozone resistant compounds in the source water.

O_3/TOC	Regression	R^2	Study Rate Constant (min^{-1})	Literature Rate Constant[a] (min^{-1})
0.5	$O_3 = 1.89e^{-0.887t}$	0.98	0.89	1.17-3.78
1	$O_3 = 3.56e^{-0.168t}$	0.98	0.17	0.51-0.83
1.5	$O_3 = 5.68e^{-0.093t}$	0.97	0.09	0.15-0.59

[a]Gamage et al. (2013); four secondary treated wastewater effluents

It is important to note that ozone decay naturally occurs in pure water due primarily to its reaction with OH^- ions (Staehelin & Hoigné, 1982). The presence of organic matter should increase the ozone rate of decay because of the additional reaction pathways available. According to Staehelin & Hoigné (1982), the rate of decay of ozone in pure water at a pH of 7 is 1.05×10^{-3} min^{-1}. Compared with the values in Table 3, this value is significantly smaller. This indicates that the decomposition of ozone that occurred during the demand/decay test is

representative of the reaction between ozone and the source water matrix and not due to natural decomposition alone.

3.2 Nutrient Removal

Nutrient removal was monitored over a two-month period for the anthracite columns (C1-C4 and the control) with preozonation and for the BC column without preozonation. Ammonia, nitrate, nitrite, and phosphate were monitored. Nitrate and nitrite concentrations for the anthracite columns were aggregated due to their values, and the averages were plotted alongside the concentrations of the reactor influent and BC effluent (Figures 5 and 6).

Influent concentrations of ammonia, nitrate, and nitrite were low because of the nitrification and denitrification achieved by the full-scale MBR. In fact, ammonia concentrations were negligible in all samples. Nitrate concentrations in the reactor influent (i.e., MBR filtrate) varied from <0.3 to ~10 mg-N/L; the biofiltration effluent typically exhibited similar concentrations. Although the BC contains denitrifying bacteria, the performance of these bacteria is dependent on having low dissolved oxygen levels and a sufficient carbon source to serve as the electron donor. During this phase of testing, the feed to the BC column was non-ozonated effluent, which limited the dissolved oxygen level in the feed water, but there was still 3.3 mg/L of dissolved oxygen present. More importantly, there was an inadequate carbon source to drive the denitrification process. On the other hand, the anthracite columns likely had a sufficient carbon source to drive the process, but the supersaturated dissolved oxygen levels inhibited the denitrification pathway. No phosphate removal was witnessed through the biofilters, and the phosphate concentrations in the reactor influent (i.e., MBR filtrate) were highly variable (data not shown).

One interesting observation was a consistent accumulation of nitrite in the anthracite columns, as shown in Figure 6. This nitrite accumulation did not occur in the BC column, however. The anthracite media was evaluated to determine if nitrite leaching was occurring. The average nitrite concentrations obtained from the uncrushed and crushed media were 0.001±0.004 mg-N/L and 0.007±0.004 mg-N/L, respectively. These concentrations are extremely low and indicate that the higher nitrite concentrations observed were not due to leaching from the media. One possible explanation is the presence of micro-communities within the larger biofilter community. Although high influent dissolved oxygen concentrations were supplied to the anthracite filters, it is possible that small portions of the filter developed anoxic conditions, thereby supporting the conversion of nitrate to nitrite. Although no significant nitrate removal was observed, the low levels of nitrite witnessed would not require substantial nitrate transformation. Once the nitrite was formed, it is possible that an inadequate supply of carbon limited nitrite conversion to nitrogen gas. A study by Liu et al. (2006) documented high levels of denitrification intermediates, such as nitrite, in biofilter effluent due to low concentrations of electron donors. Other sources also cite an inadequate supply of organic matter as the reason for residual nitrite in biofilters (Sison et al., 1995; Sison et al., 1996).

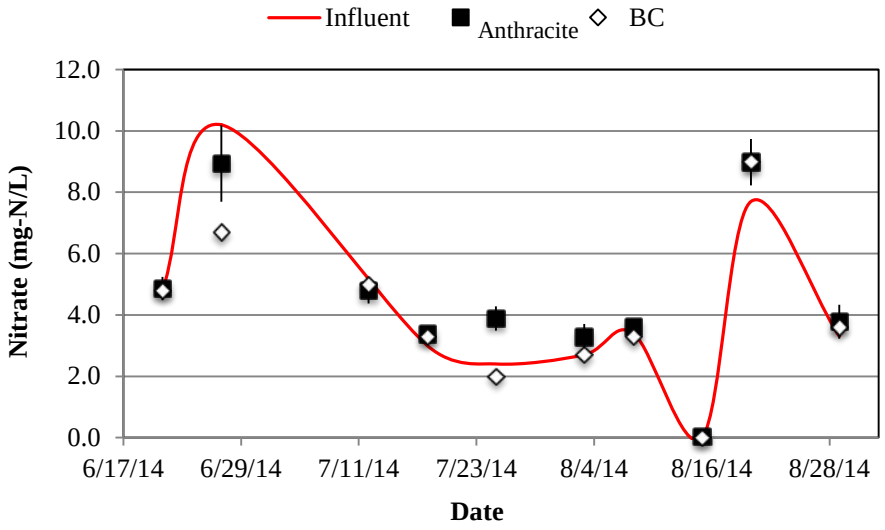


Figure 5. Nitrate concentrations in the influent and biofilter effluents.
The anthracite data represent average concentrations (± 1 standard deviation) based on aggregated data from all of the columns.

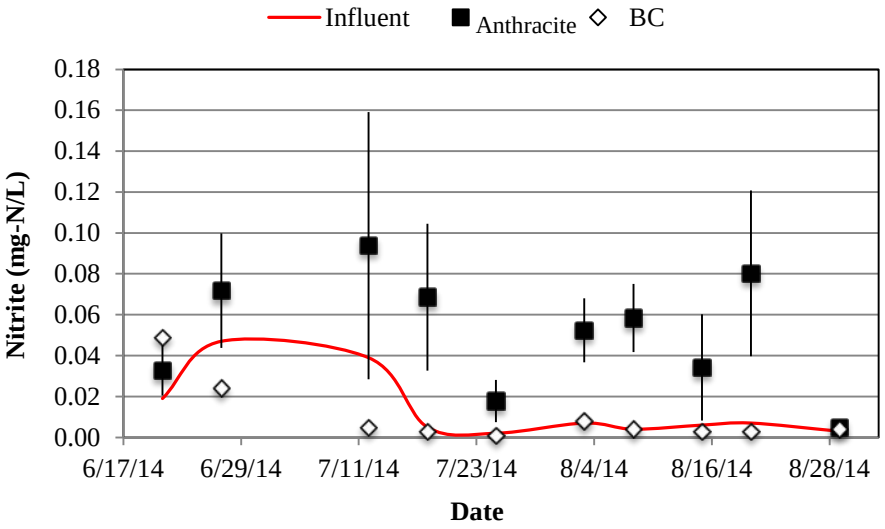


Figure 6. Nitrite concentrations in the influent and biofilter effluents.
The anthracite data represent average concentrations (± 1 standard deviation) based on aggregated data from all of the columns. The anthracite columns consistently exhibited higher nitrite concentrations than the influent or BC effluent.

3.3 TOC Removal with Ozone-Biofiltration

3.3.1 UV_{254} and O_3/TOC Correlation

Based on data obtained from the ozone demand/decay test, a correlation between UV_{254} and ozone dose was developed (Figure 7). The correlation was consistent with similar correlations

presented in the literature (Gerrity et al., 2012). The corresponding regression equation was used to estimate the transferred ozone doses during the kinetics testing (*Section 3.3.3*).

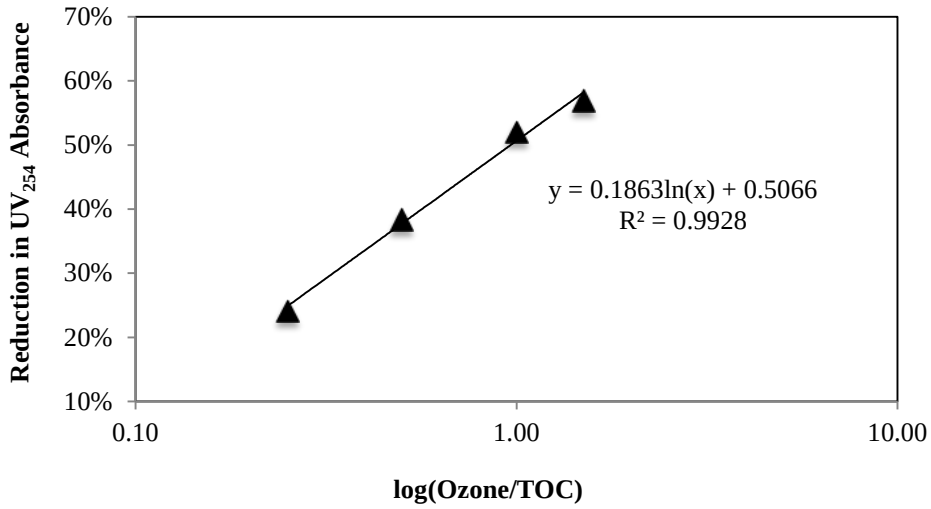


Figure 7. Relationship between O_3/TOC and reductions in UV_{254} absorbance.
Data from the ozone demand/decay testing was used to develop the relationship.

3.3.2 Long-Term Testing of Ozone-Biofiltration

The initial hypothesis of the study was that by providing higher ozone doses and longer empty bed contact times, ozone-biofiltration systems could achieve lower effluent TOC concentrations. In practice, the benefits of longer empty bed contact times (e.g., greater reductions in TOC) have been demonstrated in full-scale ozone-BAC systems in Australia, which employed empty bed contact times ranging from 9 – 45 minutes (Reungoat et al., 2012). However, the feed water and ozone doses differed at each plant so it is difficult to determine whether the extent of TOC removal was primarily impacted by EBCT. Therefore, the current study targeted a more controlled experiment by evaluating the impacts of varying EBCT in parallel columns.

As mentioned earlier, ozonation was required to initiate growth of the microbial community on the filter media. Prior to ozonation, limited TOC removal (~5%) was observed through the biofilters, thereby indicating minimal adsorption and biodegradation, but after initiation of the ozone process, TOC removal in the biofilter effluent increased rapidly (Figure 8). It is important to note that there was an insignificant level of TOC removal due to ozonation alone, thereby indicating that biodegradation in the biofilters was the dominant mechanism responsible for reductions in TOC. Once the TOC removal (and presumably the microbial community) had stabilized, the EBCTs were set at values ranging from 5 – 15 minutes, as described in *Section 2.1.3*, and the ozone dose was maintained at a relative low O_3/TOC ratio of ~0.35.

Based on the performance of the full-scale ozone-BAC systems in Australia, higher EBCTs should result in lower effluent TOC concentration. Despite the range of EBCTs, the TOC removal through the biofilters receiving ozonated water was relatively constant at ~15% (Figure 9), even for the BC. Coupled with the conclusion from the ozone demand/decay testing that the

MBR filtrate was recalcitrant, the revised hypothesis was that the low ozone dose was unable to generate a sufficient concentration of biodegradable organic matter to necessitate EBCTs longer than ~5 minutes. As a result, the experimental approach shifted from long-term operation at a range of EBCTs to short-term kinetics testing at lower EBCTs and with greater time resolution.

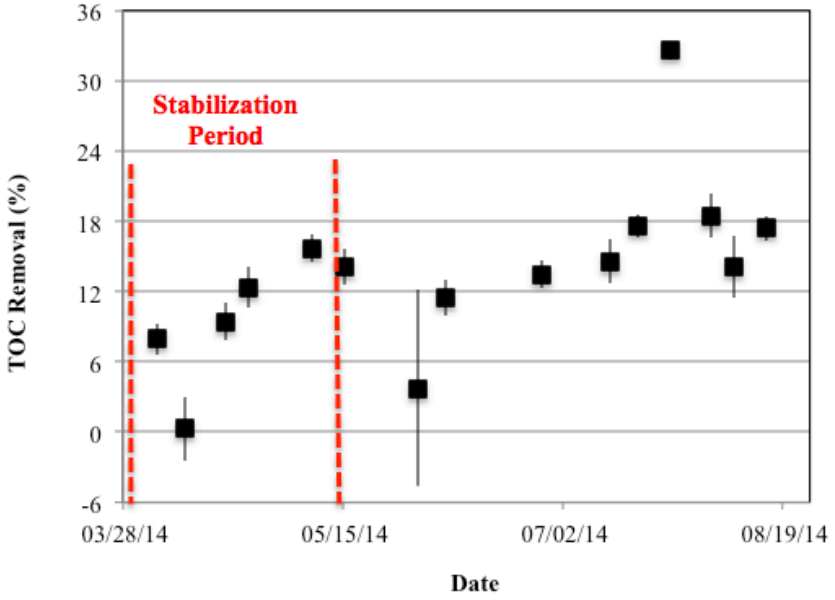


Figure 8. TOC removal in the anthracite and BC biofilters after initiation of the ozone process. The data represent average concentrations (± 1 standard deviation) based on aggregated data from all of the columns receiving ozonated water. The relatively low TOC removal observed in late May 2014 was attributed to an operational upset in the ozone contactors.

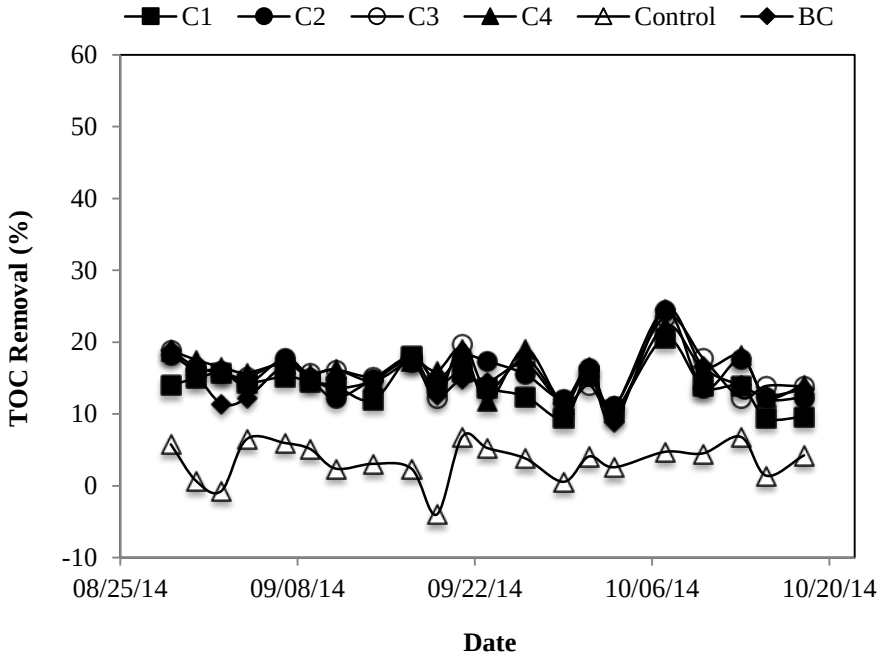


Figure 9. TOC removal in the anthracite and BC biofilters with varying EBCTs. The EBCTs were as follows: C1=5 min, C2=10 min, C3=10 min, C4=15 min, Control=10 min, and BC=10 min. During this phase of testing, all columns except the BC contained anthracite, and all columns except the Control were receiving ozonated feedwater.

3.3.3 Kinetics Tests Results

Three kinetics tests were performed for this study with each test targeting a different O_3/TOC ratio. During the long-term operational testing phase, an average reduction in UV_{254} absorbance of 30% (O_3/TOC of 0.35) was achieved; therefore, this setting was used for the first kinetics test. The second test targeted the highest achievable reduction in UV_{254} absorbance (based on the limitation of the ozone generator), which was about 50% or an estimated O_3/TOC of 1.12. Lastly, kinetics test 3 achieved an average reduction in UV_{254} absorbance of 40% (O_3/TOC of 0.62). It should be noted that the only samples collected during the kinetics tests were influent, C2 (ozone+anthracite), C3 (ozone+exhausted GAC), control (anthracite), and effluent (ozone). Due to the complexity of the operational and sampling procedures, it was not feasible to collect samples from C1 (ozone+anthracite) and C4 (ozone+anthracite). Due to severe compaction of the polyvinyl alcohol biobeads experienced with the BC, the flow rate was extremely low and could not be adjusted to flow rates necessary for the tests. Therefore, the BC column was decommissioned for the duration of the project.

The effect of the changing ozone dose can be seen in Figure 10, which illustrates changes in fluorescence. Humic-like substances (Region III; refer to Figure 3) were considerably reduced with an O_3/TOC of 0.35, biopolymers experienced a significant decrease in fluorescence with an O_3/TOC of 0.62, and fulvic-like substances were significantly transformed at an O_3/TOC of 1.12. As indicated visually in Figure 10 and quantitatively in Table 4, increasing ozone doses improve water quality in all regions and yield EEMs that are more consistent with natural surface waters. Therefore, ozone is effective in eliminating the 'wastewater identity' of the matrix, which is critical for public perception in potable reuse applications This is supported by the reduction in the FI between the influent and ozonated effluent samples (Table 4), which corresponds with reductions in microbially-derived organic matter in region I.

Table 4. EfOM characterization at different ozone doses
Increasing the ozone dose resulted in reductions in fluorophores in all regions and a reduction in FI from the influent to ozonated effluent samples.

Sample	O_3/TOC	Region I	Region II	Region III	Total Fluor.	TF Removal (%)	FI
Inf	0.00	18,305	24,792	12,289	55,386	--	1.91
Eff	0.35	5,214	6,200	2,437	13,851	75	1.37
Inf	0.00	16,827	20,627	9,122	46,577	--	1.70
Eff	0.62	2,517	3,930	1,453	7,900	83	1.41
Inf	0.00	13,965	17,206	8,154	39,325	--	1.76
Eff	1.12	576	1,381	615	2,572	93	1.46

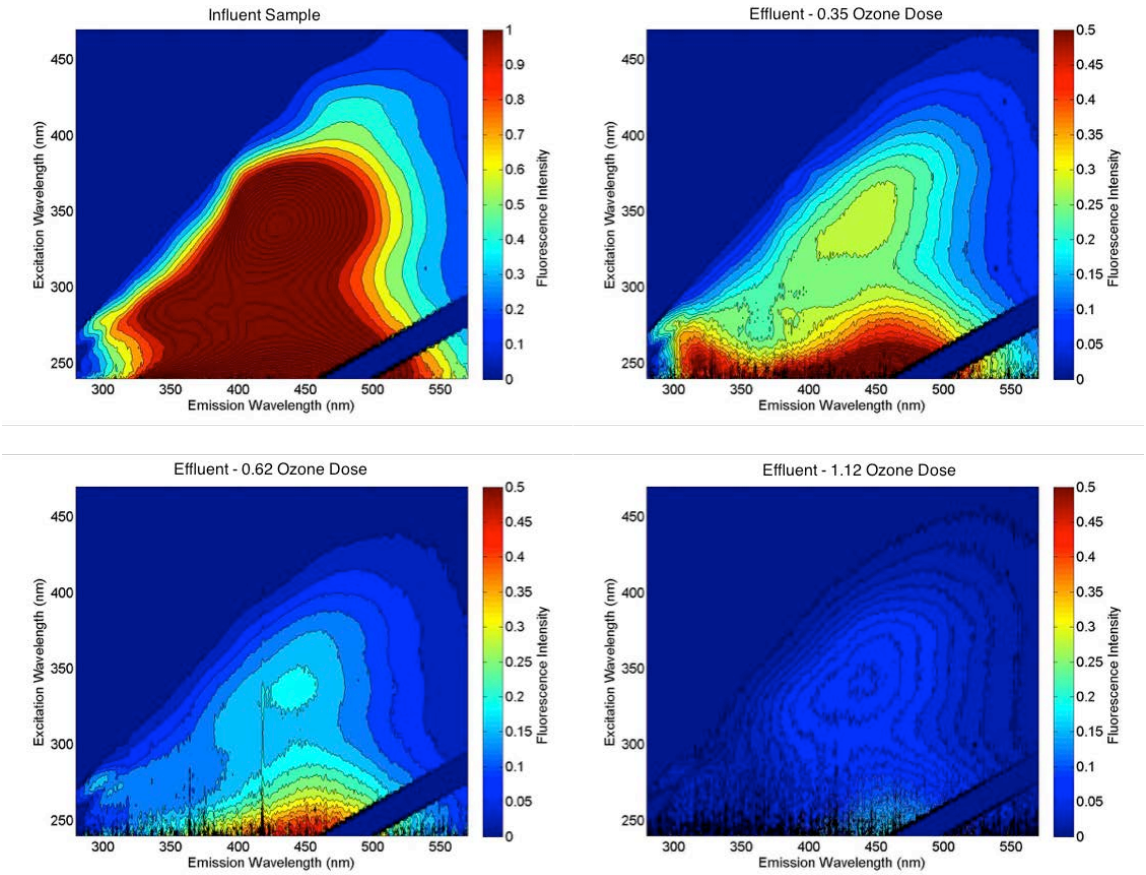


Figure 10. EEM comparison of three ozone doses.
The changes in the fluorescence intensity of the EEM are indicative of the level of organic matter transformation from ozone oxidation. As the ozone dose increases, the more recalcitrant compounds are oxidized, and the number of fluorescing compounds is reduced. *Note the change in scale from the influent to the effluent samples, which increases the resolution of the treated EEMs.*

The results of kinetics test one (O_3/TOC=0.35) are provided in Figure 11. A positive correlation can be seen between TOC removal and EBCT up to around six minutes of contact time. After six minutes, little improvement is observed with increasing contact time. This is consistent with the observed performance of the biofilters during the long-term testing phase with EBCTs ranging from 5 – 15 minutes. It is important to reiterate that C3 contained exhausted GAC (or BAC) during this phase of testing. Therefore, C2 and C3 provide a direct comparison of anthracite and exhausted GAC for ozonated feedwater, and C2 and the control provide a direct comparison of ozonated vs. non-ozonated feedwater with anthracite as the surface for biofilm attachment. Based on the results of this initial test, the anthracite and exhausted GAC performed similarly, while the control column receiving non-ozonated effluent exhibited inferior performance. The performance of the control column was consistent with the start-up period for all columns prior to initiation of the ozone process and the initial long-term testing of varying EBCTs.

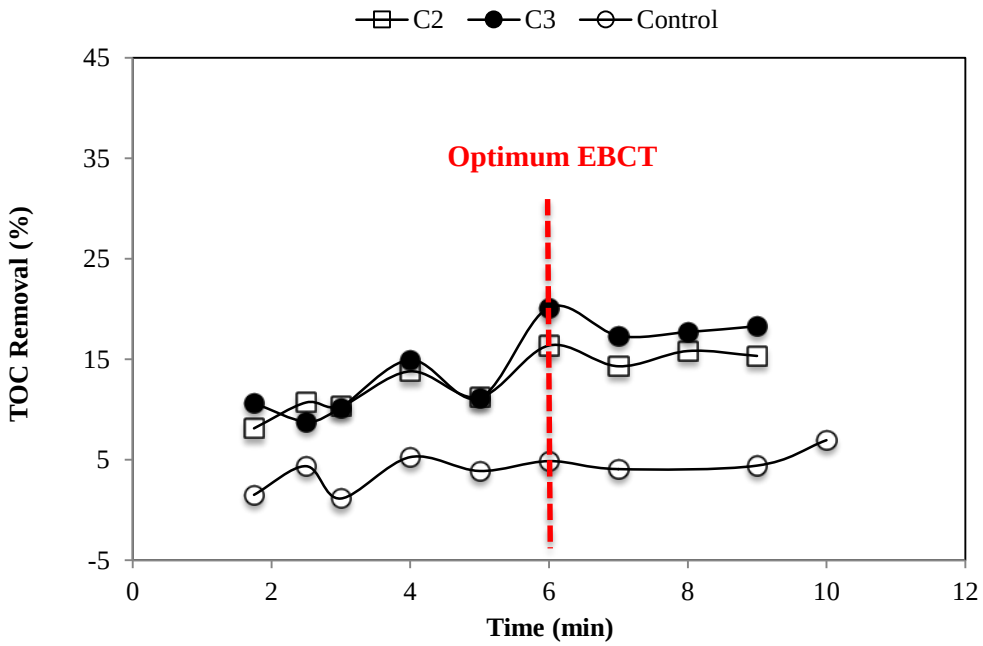


Figure 11. Kinetics test one (O_3/TOC=0.35) results for TOC removal at various EBCTs

Kinetics test two was performed at an O_3/TOC of 1.12. The same trend observed in the first kinetics test was also apparent at this higher ozone dose. However, the additional ozone led to greater TOC removal through the columns, as indicated in Figure 12. Also, the contact time after which the TOC removal stabilized increased from six minutes to ~10 – 12 minutes.

The final kinetics test was performed using an intermediate O_3/TOC of 0.62. Again, the results are similar to the previous kinetics tests, as seen in Figure 13. As expected, based on tests 1 and 2, the TOC removal stabilized at an intermediate contact time of nine minutes, assuming the large fluctuations at higher EBCTs were attributable to experimental error/variability rather than operational performance. The experimental error/variability assumption is supported by the similar trends observed for all three media, but it is unclear what caused this effect.

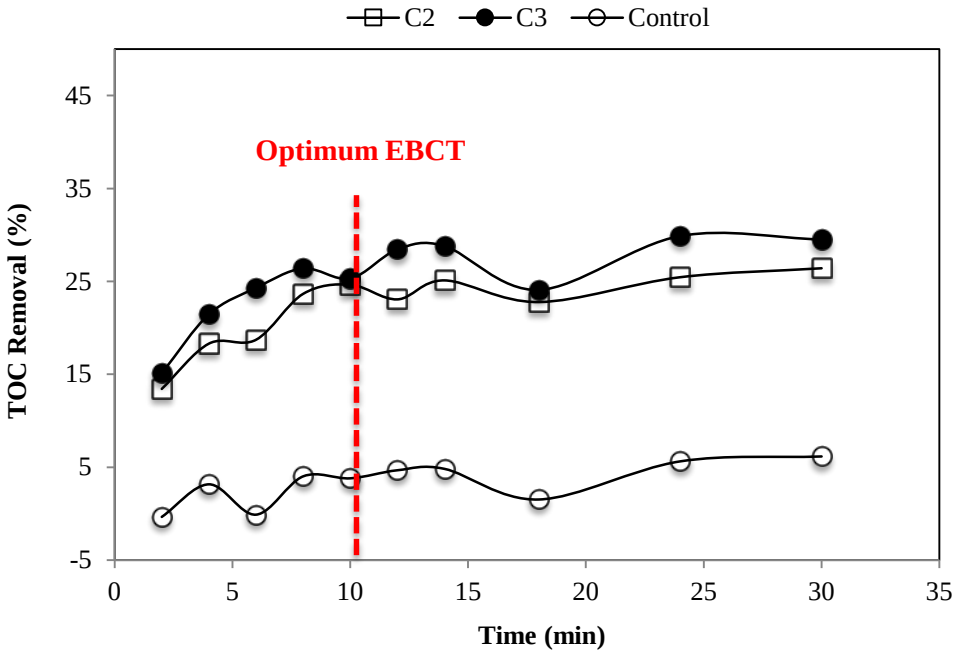


Figure 12. Kinetics test two (O_3/TOC=1.12) results for TOC removal at various EBCTs

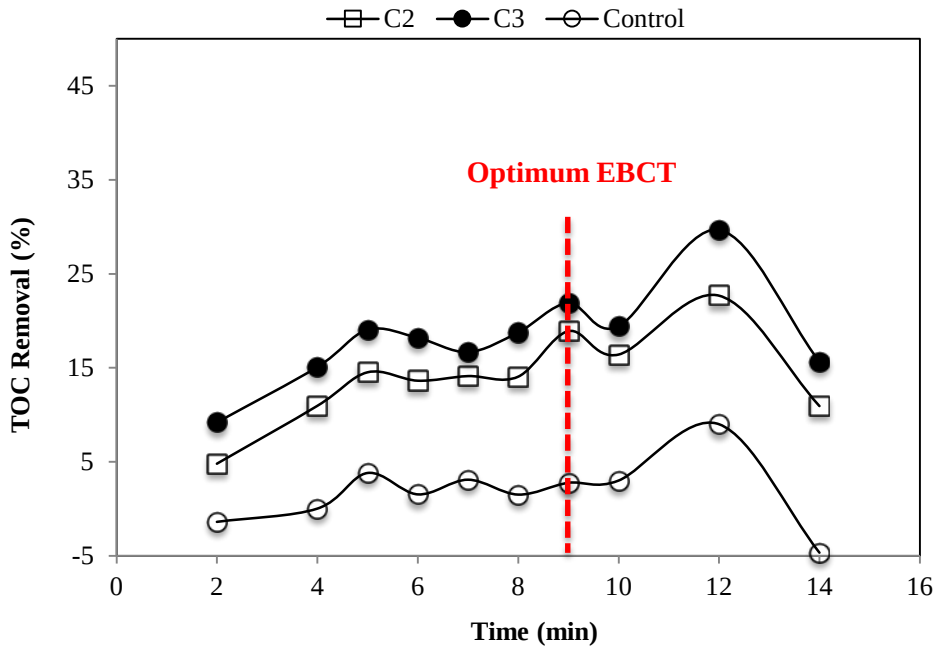


Figure 13. Kinetics test three (O_3/TOC=0.62) results for TOC removal at various EBCTs

It can be seen that higher ozone doses lead to greater reductions in TOC during biofiltration, as would be expected. Kinetics test 2, which had the highest ozone dose (O_3/TOC=1.12), provided the best treatment for both biofilters evaluated. The exhausted GAC generally exhibited better performance than the anthracite, although the differences were small, while the control anthracite column consistently achieved reductions in TOC of less than 5%.

Each kinetics test was examined individually to determine the optimum EBCT at the specific ozone dose. These points were chosen through graphical observation of the point of diminishing return, or the time after which little improvement in treatment efficacy is observed. Providing additional contact time with little treatment enhancement would have negative consequences to a full-scale system; longer retention times equate to lower flow rates or greater structural footprints and higher costs per unit of water treated. These results are tabulated in Table 5.

Table 5. Comparison of optimum conditions and treatment efficacy.

O_3/TOC	Optimum EBCT	TOC Removal (1.2-mm Anthracite)	TOC Removal (0.95-mm Exhausted GAC)	Minimum TOC Achieved
	minutes	%	%	mg/L
0.35	6	16	20	6.4
0.62	9	19	22	5.7
1.12	10 – 12	25	25	5.0

If higher ozone doses were used, it is possible that the minimum TOC values could be reduced even further (i.e., more extensive transformation of bulk organic matter and greater removal of TOC after biodegradation). Unfortunately, due to constraints with the ozone generator, this was the highest achievable ozone dose. Based on Table 5, it appears that a relationship exists between ozone dose and optimum EBCT. To evaluate this potential relationship, the two parameters were graphed against each other and analyzed. The relationship between ozone dose and EBCT is illustrated in Figure 14. A logarithmic function provides some estimation of the relationship, but additional data are clearly warranted to further validate the model. Interestingly, the current data suggest a point of diminishing return for the combination of ozone and EBCT; in other words, there may be a limit to the practical extent of transformation and the generation of biodegradable organics that ultimately serve as food for the microbial community.

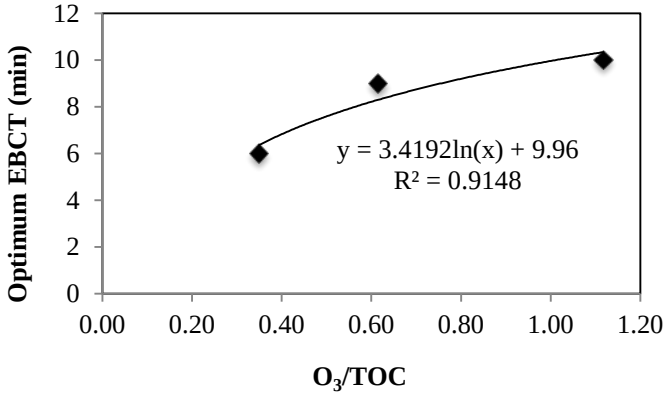


Figure 14. Relationship between ozone dose and optimum EBCT.

3.4 ATP Testing

It is evident from the kinetics tests that C3 (ozone+0.95 mm diameter exhausted GAC) outperformed C2 (ozone+1.2 mm diameter anthracite) at every ozone dose. There are two potential explanations for this observation: (1) the smaller media provides more surface area for biological growth or (2) the smaller media is better suited for the column size and enhances the hydraulic efficiency of the filter. To evaluate these theories, ATP concentrations corresponding to attached growth on the various media were analyzed at three different times during the study (Tables 6 – 8). Table 6 summarizes the ATP concentrations for the initial start-up period while the columns were receiving non-ozonated effluent and minimal TOC removal was observed. Values from the literature are also provided as a basis for comparison. Table 7 summarizes the ATP concentrations for the various columns after they had been receiving ozonated feedwater for six months. At the time of collection, the control column had been receiving non-ozonated feedwater for approximately one month, but it had received ozonated feedwater for five months prior to promote biological growth. Conversely, the BC had been receiving ozonated feedwater for approximately one month, but it had received non-ozonated feedwater for several months prior. Finally, Table 8 provides a comparison of the data from Table 7 and a subsequent sample event approximately 6 months later.

Tables 6 – 8 illustrate the development of the microbial communities over time, particularly following the initiation of the ozone process. The ATP concentrations increased several orders of magnitude following ozone start-up, at which point they were more consistent with values reported in the literature (Table 6). With respect to media depth, Table 8 indicates that both C2 and C3 exhibited significantly higher ATP concentrations at the top of the filter. Furthermore, the ATP concentration at the bottom of the filter exhibited a greater relative decline for the smaller media. This could be linked to more rapid depletion of available organic substrate at the top of C3, which might have hindered biological growth lower in the column.

The higher ATP concentration at the top of C3 versus C2 (Table 8) might be linked to media size (i.e., higher specific surface area of the smaller GAC media) and biofilter performance. Additional biological growth may have contributed to the superior performance of C3, but it cannot be stated absolutely because the potential impact of hydraulic inefficiency was not evaluated. It is also interesting to note that once bacteria colonized the control media when it was originally fed with ozonated water, the high levels of ATP persisted despite the fact that the column was transitioned to non-ozonated effluent at the end of the study. Therefore, the organic matter proved to be too recalcitrant to promote significant reductions in TOC, but it was sufficient to maintain the biomass in the system. Alternatively, the biomass may have become dormant or inactivated, but the ATP test still detected the residual cellular materials present on the media.

It was also observed that the BC had significantly higher ATP concentrations, but there was no appreciable improvement in biofilter performance. The same level of treatment was achieved with C2, which had much less ATP than the BC. Moreover, better treatment was witnessed in C3, which also exhibited a lower ATP concentration. This further proves that higher biomass concentrations do not always equate to more biological activity as measured by TOC removal, which has been stated in previous publications (Pharand et al., 2014). The higher ATP

concentration for the BC may have been attributable to ATP originating from inside the biobeads, whereas the anthracite and exhausted GAC could not support growth within their internal structures. Diffusion limitations may have negatively impacted the ability of the internal biomass to degrade the bulk organic matter in the BC column.

Table 6. Summary of the initial ATP analysis of the anthracite compared against ATP data from the literature.
Prior to ozonation, the ATP concentrations found on the anthracite in the study were significantly lower than typical literature values. This indicates the gross underdevelopment of the biological community in the biofilters prior to start-up of the ozone process. C3 had not been switched to exhausted GAC at this point in the study.

Media Sample	Sample Source	ATP (pg ATP/g media)	Reference
Stored Anthracite	Refrigerator	$0.6x10^2$	Current Study
C1 (anthracite)	Bottom of Contactor	$6.6x10^2$	Current Study
C3 (anthracite)	Bottom of Contactor	$2.3x10^2$	Current Study
Control (anthracite)	Bottom of Contactor	$2.9x10^2$	Current Study
Literature	75-day old GAC	$1.8x10^6$	Velten et al., 2007
Literature	90-day old GAC	$8.0x10^5$-$1.8x10^6$	Velten et al., 2011
Literature	30 GAC filters from 9 WWTPs	$1.4x10^4$-$2.5x10^5$	Magic-Knezev, 2004

Table 7. Summary of ATP concentrations six months after start-up of the ozone process.
Relative to the data in Table 6, the ATP concentrations increased by several orders of magnitude after the feedwater was ozonated. C3 had not been switched to exhausted GAC at this point in the study. At the time of sampling, the control anthracite was receiving non-ozonated feedwater, but it had been receiving ozonated feedwater for 5 months prior.

Media Sample	Sample Location	ATP (pg ATP/g media)
C1 (anthracite)	Bottom	$0.34x10^6$
C2 (anthracite)	Bottom	$0.20x10^6$
C3 (anthracite)	Bottom	$0.30x10^6$
C4 (anthracite)	Bottom	$0.14x10^6$
Control (anthracite)	Bottom	$0.06x10^6$
Biocatalyst	Top	$2.00x10^6$
Stored Biocatalyst	Refrigerator	$0.31x10^6$

Table 8. ATP concentrations at two biofilter depths over time and as a function of media type.
ATP concentrations were higher at the top of the columns. The 0.95 mm exhausted GAC exhibited a higher concentration than the 1.2 mm anthracite, which may be due to the larger specific surface area of the smaller media.

Sample	tATP at Top of Column (pg ATP/g media)		tATP at Bottom of Column (pg ATP/g media)	
	9/10/14	3/24/15	9/10/14	3/24/15
C2 (O_3/anthracite)	--	$0.63x10^6$	$0.20x10^6$	$0.31x10^6$
C3 (O_3/BAC)	--	$0.91x10^6$	--	$0.22x10^6$
Control (No O_3/anthracite)	--	--	$0.06x10^6$	$0.20x10^6$
Biocatalyst (O_3/biocatalyst)	$2.0x10^6$	$2.3x10^6$	--	--

*Dashes represent samples that were not collected

3.5 Total Coliform and *E. coli* Evaluation

One of the concerns associated with biofiltration is the reintroduction of bacteria into the water. Considering that the water might ultimately be used for potable reuse applications, *E. coli* are also relevant in terms of compliance with U.S. EPA drinking water regulations. Samples were collected for the reactor influent (MBR filtrate), effluent (ozone only), C2 (ozone+anthracite), and the control column (anthracite only), and the results are provided in Table 9. All samples tested negative for *E. coli*. There was a small amount of total coliform bacteria present in the influent water. Theoretically, there should be no coliform bacteria present in the influent water because it is MBR filtrate, and the pore size of the membrane is smaller than the size of bacteria. The presence of coliform bacteria may be attributable to growth in the system tubing connecting the MBR sample port to the pilot reactor as well as growth within the reactor components. In fact, noticeable growth was observed in the static mixer located upstream of the influent sample port. Once ozonated, all of the coliform bacteria in the water were inactivated, as indicated by the ozone effluent values. Also, no coliform bacteria were present in the C2 effluent, which indicates that regrowth and release of coliform bacteria did not occur in the ozone-anthracite column (at least during this sample event). Interestingly, the control column had the highest number of coliform bacteria—even higher than the influent. This means that some of the biofilm may have been detaching from the media and entering the water. This suggests that the addition of ozone may not only aid in biofilm growth but also in biofilm attachment to the media or the selection of bacterial species other than coliform bacteria. The issue of microbial, specifically pathogen, regrowth and release into the final product water could potentially be problematic in full-scale operations and would warrant a final disinfection process downstream of the biofilters. Regardless of the regrowth potential, recent regulatory frameworks for potable reuse would likely necessitate a final disinfection process to achieve specified levels of inactivation and/or removal of target pathogens (NWRI, 2013).

Table 9. Total coliform bacteria in the pilot system.
The total coliform concentration increased through the control column, which suggests detachment of the biofilm into the water. Ozone was effective at inactivating coliform bacteria resulting in <1 MPN/100 mL in the ozonated effluent and C2 effluent.

Sample	Total Coliform (MPN/100 mL)			
Replicate	1	2	3	Average
Influent	8.5	3.1	6.3	6.0±2.7
C2	<1	<1	<1	<1
Control	13.4	14.5	8.6	12.2±3.1
Effluent	<1	<1	<1	<1

3.5 Trace Organic Contaminant Mitigation

Only 33 of the 103 trace organic contaminants (TOrCs) and nitrosamines monitored in this study were detected in either the pilot influent, control (anthracite only) effluent, or C2

(ozone+anthracite) effluent (Table 10). Some TOrCs were removed by biofiltration alone (e.g., the antibiotics amoxicillin, sulfamethoxazole, and trimethoprim), but others (e.g., the artificial sweetener acesulfame-K) actually increased during biofiltration. The combination of ozone and biofiltration proved to be effective in reducing the concentrations of many TOrCs, and only 21 of the 103 target compounds were detected in the ozone-biofiltration effluent. The literature suggests that the vast majority of these compounds pose no threat to public health at these low concentrations. One exception is *N*-nitrosodimethylamine (NDMA), which has a 10-ng/L notification level in California. The concentration of NDMA was actually <MRL in the effluent from the control column, but it was 12 ng/L in the ozone-anthracite effluent. Some studies have reported significant formation of NDMA during ozonation of wastewater effluents (Gerrity et al., 2014), and post-ozone biofiltration has been shown to reduce NDMA concentrations (Gerrity et al., 2015). Additional testing would be necessary to determine if ozone-induced NDMA formation would be an issue in this particular matrix and whether the biofiltration process could be used to reduce NDMA concentrations. Regardless, the potential for NDMA exceedances would necessitate further treatment to consistently comply with the notification level.

Table 10. Summary of trace organic contaminant concentrations (ng/L) in the reactor influent, biofiltration (anthracite only) effluent, and the ozone-biofiltration (ozone-anthracite) effluent.

Target Compound	Influent	Biofiltration	Ozone-Biofiltration	Target Compound	Influent	Biofiltration	Ozone-Biofiltration
Acesulfame-K	570	1500	730	Isobutylparaben	<5	8.1	<5
Albuterol	34	<5	<5	Lidocaine	450	410	<5
Amoxicillin	3100	1800	<20	Linuron	10	15	<5
Atenolol	370	68	17	Meprobamate	36	36	44
Bisphenol A	25	270	190	Naproxen	<10	30	16
Butalbital	<5	76	53	NDEA[2]	<2	2	<2
Caffeine	29	<5	<5	NDMA[3]	9.9	<2	12
Carbamazepine	74	70	<5	NMOR[4]	2.8	5.2	3
Cotinine	64	41	46	Primidone	220	200	61
DEET[1]	41	49	12	Sucralose	22000	23000	23000
Dehydronifedipine	7.5	6.4	5.6	Sulfamethoxazole	810	530	13
Diclofenac	<5	160	130	TCEP[5]	96	69	78
Diltiazem	82	35	<5	TCPP[6]	920	800	620
Erythromycin	77	86	<10	TDCPP[7]	240	4900	230
Fluoxetine	20	<10	<10	Triclosan	<10	13	15
Gemfibrozil	<5	36	12	Trimethoprim	160	38	<5
Iohexal	980	2200	730				

[1]*N*,*N*-diethyl-meta-toluamide, [2]*N*-nitrosodiethylamine, [3]*N*-nitrosodimethylamine, [4]*N*-nitrosomorpholine, [5]tris(2-chloroethyl)phosphate, [6]tris(1-chloro-2-propyl)phosphate, [7]tris(1,3-dichloro-2-propyl)phosphate.
*Shading represents compounds that were present at concentrations lower than their method reporting limits (MRLs). 100 additional target compounds were <MRL in all samples so they were omitted from the summary table.

4.0 Conclusions

This study evaluated the performance of an ozone-biofiltration pilot reactor using anthracite, exhausted GAC (or BAC), and a proprietary biocatalyst as the filter media. Biological growth was monitored based on TOC removal and ATP concentrations. Once a stable biological community was established, enhanced TOC removal was evaluated by increasing ozone dose and/or EBCT. Based on the results of the study, several conclusions can be made regarding ozone-biofiltration system performance and recommendations for operational conditions.

4.1 Findings Confirming Previous Work

- Up to 92% of the humic-like substances were transformed through ozonation, with an additional 2% reduction provided through biofiltration. In addition, there was minimal reduction in TOC during ozonation but a substantial reduction in TOC during biofiltration. Therefore, fluorescence and TOC are complementary bulk organic parameters for evaluating the ozone and biofiltration performance, respectively, of ozone-biofiltration treatment trains.
- A logarithmic relationship was identified between UV_{254} removal and O_3/TOC, which allows for estimation of ozone dosing based on changes in UV_{254} absorbance. A similar relationship could be developed for changes in fluorescence, but fluorescence was not monitored during the ozone demand/decay test in this study.
- Higher ozone doses yielded better reductions in TOC, UV_{254} absorbance, and fluorescence when coupled with biofiltration.
- ATP concentrations were higher at the top of the biofilters as compared with the bottom of the biofilters. The small media provided more surface area for biological growth, which potentially explains the higher ATP concentration per gram of small media. This may also explain the better treatment efficacy achieved with the small media, although hydraulic performance may also be a contributing factor.
- The biocatalyst housed the highest concentration of ATP (~50% more ATP) but did not yield additional TOC removal. This suggests that higher biofilm concentrations do not necessarily correlate with better treatment in all applications.

4.2 Significant Findings of this Study

- The anthracite biofilters were unable to remove NO_3-N, NO_2-N, and PO_4-P from the water and actually contributed to higher nitrite concentrations in the treated water. The biocatalyst did not experience the same increase in nitrite, but it was unable to achieve reductions in nitrate, despite the fact that it contained denitrifying bacteria. However, the biocatalyst is intended for suspended growth systems—not packed bed configurations. In addition, the supersaturated dissolved oxygen conditions after ozonation are not conducive to denitrification. Therefore, additional testing of the biocatalyst with more appropriate operational conditions is recommended. In fact, denitrification will be crucial in potable reuse applications because of the 10 mg-N/L maximum contaminant level (MCL) for nitrate in drinking water. The full-scale MBR was able to achieve the

nitrate MCL without additional treatment, but other biological treatment systems may not have such an effective denitrification process. Therefore, the use of a denitrifying biocatalyst might be advantageous if it can be effectively integrated into ozone-biofiltration systems.

- No *E. coli* was found at any point in the system, but total coliform bacteria were discovered in the influent and control effluent. The control exhibited higher total coliform numbers than the influent, which suggests that the biofilm may have detached from the media and entered the water. The ozonated column, however, did not contribute any coliform bacteria despite the higher ATP concentration present. This suggests that ozonation may impact biological attachment to the media or it may select for different species of bacteria due to the change in biodegradable organic matter.
- One of the major objectives of the study was to identify a relationship between ozone dose and empty bed contact time for TOC removal. Based on the kinetics tests performed, optimum EBCTs for various ozone doses were identified. The optimum EBCTs for O_3/TOC of 0.35, 0.62, and 1.12 were found to be 6, 9, and 10 – 12 minutes, respectively. These EBCTs are significantly lower than what is used in some full-scale facilities so it is unclear whether EBCTs of up to 45 minutes are actually warranted. When plotting optimum EBCT versus O_3/TOC, a logarithmic relationship appeared to exist between the two parameters. Based on this relationship, it appears that relatively low EBCTs are necessary for TOC removal. However, additional testing is warranted to validate the relationship and determine whether water quality (e.g., MBR filtrate vs. conventional activated sludge) impacts the optimum EBCT. Based on a comparison with the literature, the MBR filtrate in this study seemed to be highly recalcitrant with respect to oxidation and biodegradation. It is unclear whether this was a site-specific finding or if it is related to the efficacy of the MBR treatment process.
- Another key finding derived from the kinetics tests was the resilience in bulk biological activity to changes in the EBCT based on TOC removal after a stable biological community is developed. A time interval equivalent to three hydraulic retention times was sufficient for biological activity stabilization in the biofilters. However, this study did not fully address the development of micro-communities within the biofilters, which might be capable of specific treatment objectives (e.g., biodegradation of N-nitrosodimethylamine (NDMA), denitrification, bromate reduction). These micro-communities might be sensitive to rapid changes in EBCT.
- The highest ozone dose of 1.12 O_3/TOC achieved the greatest reductions in bulk organic matter due to oxidation and subsequent biofiltration. At this ozone dose, reductions of up to 95% of fluorophores associated with soluble microbial products (region I), 92% of fluorophores associated with fulvic-like acids (region II), and 92% of fluorophores associated with humic-like substances (region III) were achieved. At this ozone dose and the optimum EBCT, the minimum TOC concentration attained was 5.0 mg/L. It is assumed that higher ozone doses would achieve even better water quality, but the data suggest that there is a point of diminishing return for ozone dosing as well.

- Some trace organic contaminants (e.g., the artificial sweetener acesulfame-K) actually increased during biofiltration, and 21 of the 103 target compounds were still detected after ozone-biofiltration. However, the literature suggests that the vast majority of these compounds pose no threat to public health at these low concentrations. The one exception is NDMA (12 ng/L in the ozone-anthracite effluent), which would necessitate further treatment to achieve the 10-ng/L regulatory threshold in California.
- Despite optimization, ozone-biofiltration systems alone appear to be insufficient to meet the stringent TOC target of 0.5 mg/L specified in California's potable reuse regulations. If higher ozone doses are used, this goal may be more attainable, but the current study suggests that TOC removal will plateau far before reaching a 0.5-mg/L effluent TOC concentration. Therefore, additional treatment steps would be necessary to achieve significantly lower concentrations of bulk organic matter. Possible strategies to comply with existing potable reuse regulations include increasing the percentage of diluent water or adding additional treatment steps. The 0.5 mg/L target in California applies to 100% recycled water. If diluent water is added, this target value will increase accordingly. Additional treatment options include a supplementary GAC column (with adsorption capacity) or an ion exchange resin suitable for TOC removal. Humbert et al. (2005) demonstrated 80% DOC removal with a 30-minute contact time using strong anion exchange resins, and GAC columns operated at 13 minute EBCTs achieved 65% TOC removal and over 70% DOC removal (Gibert et al., 2013). However, it is important to note that not all states have such stringent regulations on effluent TOC concentrations, and it is currently unclear whether such requirements have any direct link to public health. It is important to note that ozone-biofiltration is unable to reduce total dissolved solids (TDS) concentrations, which are typically high in wastewater. Therefore, additional treatment for TDS may also be needed in potable reuse applications.

5.0 Future Work

This study demonstrated the potential synergisms of ozone and biofiltration, but additional research must be conducted to validate and/or expand on some of the significant findings. The apparent logarithmic relationship that exists between O_3/TOC and EBCT was not fully established. Higher ozone doses must be explored and larger data sets gathered to better understand the relationship that may exist. Also of interest would be examining this relationship based on various water matrices and media type. If different water qualities yield similar results, correlations could be made and utilized by water utilities at any location. This would save time and money on ozone dosing and biofilter performance experiments.

Another possible avenue for research would be to analyze the effect of ozonated water on biofilm attachment to media and the composition of the microbial community. Conditions within biofiltration systems, particularly related to micro-communities, are largely unknown and must be evaluated to better understand how to optimize this type of water treatment. The

increasing understanding and use of metagenomics and proteomics may aid in this research endeavor.

Based on the performance of the biocatalyst in removing TOC, it would be worth investigating different column configurations and operational conditions to enhance the intended use of this media. For instance, an upflow biofilter (i.e., a fluidized bed) may be more suited for the biocatalyst since it was not designed for packed-bed applications. This would alleviate the issue of compaction and caking if fluidization could be achieved. The presence of dissolved oxygen likely inhibited denitrification; operating the biocatalyst in series with a BAC filter or using nitrogen gas for sparging may help alleviate this problem.

Finally, it is unclear what possible health effects are associated with bulk organic matter in ozone-biofiltration effluent. Therefore, the 0.5-mg/L TOC threshold may not be necessary to maintain public safety in potable reuse applications. Further examination of this bulk parameter is needed.

6.0 Information Transfer Activities

1. Selvy, A., D. Gerrity. 2014. Optimization of Ozone-Biological Activated Carbon Treatment for Potable Reuse Applications. Nevada Water Resources Association 2014 Annual Conference, Las Vegas, NV, Jan. 2014. (2^{nd} Place in Student Poster Contest) (see Appendix 2)
2. Selvy, A., D. Gerrity. 2015. Impacts Of Ozone Dose And Empty Bed Contact Time On Total Organic Carbon Removal Through Ozone-Biological Activated Carbon Treatment. 19^{th} Annual Water Reuse and Desalination Research Conference, Huntington Beach, CA, May 2015. (see Appendix 3)

7.0 Student Support

This grant largely funded the research endeavors (time, instruments, and travel) during completion of Ashley Selvy's M.S.E. degree, which was completed in May 2015. During her studies, Ashley presented a poster at the Nevada Water Resources Association's 2014 Annual Conference and was awarded 2^{nd} Place in the student poster competition. Ashley also presented a poster at the 19^{th} Annual Water Reuse and Desalination Research Conference. She intends to compile the information from this study in a manuscript that will be submitted to a peer-reviewed water/environmental engineering journal.

During the project, Dr. Gerrity also advised a team of senior design students who designed a hypothetical potable reuse treatment facility employing advanced oxidation and biological activated carbon for Searchlight, Nevada.

Dr. Gerrity also leveraged the information gathered during this study and the existing pilot

infrastructure to obtain additional external funding from the U.S. Environmental Protection Agency's STAR program: Early Career Awards – Human and Ecological Health Impacts Associated with Water Reuse and Conservation Practices (EPA-G2014-STAR-F2). The three-year EPA project is expected to start in August 2015 and will support at least two graduate students and several undergraduate students.

8.0 References

1. CDPH. (2014). Regulations related to recycled water. California Department of Public Health. Division of Drinking Water. http://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/documents/lawbook/RWregulations_20140618.pdf. Accessed: May 14, 2015.
2. Gerrity, D., Gamage, S., Holady, J.C., Mawhinney, D.B., Quinones, O., Trenholm, R.A. and Snyder, S.A. (2011). Pilot-scale evaluation of ozone and biological activated carbon for trace organic contaminant mitigation and disinfection. Water Research, 45, 2155-2165.
3. Gerrity, D., Gamage, S., Jones, D., Korshin, G., Lee, Y., Pisarenko, A., . . . Snyder, S. (2012). Development of surrogate correlation models to predict trace organic contaminant oxidation and microbial inactivation during ozonation. Water Research, 46(19), 6257-6272.
4. Gerrity, D., Pecson, B., Trussell, R.S., Trussell, R.R. (2013) Potable reuse treatment trains throughout the world. Journal of Water Supply: Research and Technology, 62, 321-338.
5. Gerrity, D., Owens-Bennett, E., Venezia, T., Stanford, B.D., Plumlee, M.H., Debroux, J., Trussell, R.S. (2014). Applicability of ozone and biological activated carbon for potable reuse. Ozone Sci. Eng., 36, 123-127.
6. Gerrity, D., Pisarenko, A.N., Marti, E., Trenholm, R.A., Gerringer, F., Reungoat, J., Dickenson, E. (2015). Nitrosamines in pilot-scale and full-scale wastewater treatment plants with ozonation. Water Research, 72, 251-261.
7. Gibert, O., Benoit, L., Fernandez, M., Bernat, X., Paraira, M., & and Pons, M. (2013). Fractionation and removal of dissolved organic carbon in a full-scale granular activated carbon filter used for drinking water production. Water Research, 47, 2821.
8. Horiba Scientific. (2012). Aqualog software user's guide for version 3.6 (Version 3.6 rev. B ed.). HORIBA Scientific.
9. Humbert, H., Gallard, H., Suty, H., & Croue, J. P. (2005). Performance of selected anion exchange resins for the treatment of a high DOC content surface water. Water Research, 39(9), 1699-1708.
10. IDEXX Laboratories. (2015). How colilert-18 works. Retrieved from https://www.idexx.com/water/products/colilert-18.html
11. Liu, J., Zhang, X., & Wang, Z. (2006). Nitrification and denitrification in BACF for treating high ammonia source water. Huan Jing Ke Xue, 27(1), 69-73.
12. Magic-Knezev, A., & van der Kooij, D. (2004). Optimisation and significance of ATP analysis for measuring active biomass in granular activated carbon filters used in water treatment. Water Research, 38, 3971-3979.
13. NWRI. (2013). Examining the criteria for direct potable reuse. National Water Research Institute. WateReuse Research Foundation. Alexandria, VA.

14. Pharand, L., Van Dyke, M., Anderson, W., & Huck, P. (2014). Assessment of biomass in drinking water biofilters by adenosine triphosphate. Journal American Water Works Association, 106(10), 63-64.
15. Rakness, K., Wert, E., Elovitz, M., & Mahoney, S. (2010). Operator-friendly technique and quality control considerations for indigo colorimetric measurement of ozone residual. Ozone-Science & Engineering, 32(1), 33-42.
16. Reungoat, J., Escher, B.I., Macova, M., Argaud, F.X., Gernjak, J.K. and Keller, J. (2012). Ozonation and biological activated carbon filtration of wastewater treatment plant effluents. Water Research 46, 863-872.
17. Reungoat, J., Macova, M., Escher, B.I., Carswell, S., Mueller, J.F. and Keller, J. (2010). Removal of micropollutants and reduction of biological activity in a full scale reclamation plant using ozonation and activated carbon filtration. Water Research 44, 625-637.
18. Sison, N., Hanaki, K., & Matsuo, T. (1995). High loading denitrification by biological activated carbon process. Water Research, 29(12), 2776-2779.
19. Sison, N., Hanaki, K., & Matsuo, T. (1996). Denitrification with external carbon source utilizing adsorption and desorption capability of activated carbon. Water Research, 30(1), 217-227.
20. Staehelin, J., & Hoigne, J. (1982). Decomposition of ozone in water: Rate of initiation by hydroxide ions and hydrogen peroxide. Environmental Science and Technology, 16(10), 676.
21. Velten, S. (2011). Development of biomass in a drinking water granular active carbon (GAC) filter. Water Research, *45*, 6347-6354.
22. Velten, S. (2007). Rapid and direct estimation of active biomass on granular activated carbon through adenosine tri-phosphate (ATP) determination. Water Research, *41*, 1973-1983.
23. Zhou, J. (2013). Improved fluorescence excitation-emission matrix regional integration to quantify spectra for fluorescent dissolved organic matter. Journal of Environmental Quality, 42, 925.

Appendix 1. Fluorescence standard operating procedure.

Samples should be at room temperature, filtered (0.7 μm), and analyzed within 48 hours

1. Turn on computer and Aqualog instrument
2. Allow Aqualog to warm up for 15 minutes
3. Open Aqualog software
4. Raman measurement (perform 3 separate times each time the Aqualog is turned on)
    a. Fill sample cell with nanopure water and place into holder
    b. Click **H2O** button to initialize instrument and open 'Aqualog Main Experiment Menu'
    c. Click **Spectra**
    d. Click **Emission 2D**
    e. Load archived experimental settings file or create new protocol
        i. Use a consistent filing system so that you can recall old settings
    f. Verify settings:
        i. Change Data Identifier (used to identify sample in workgroup)
        ii. Integration = 3 s
        iii. Accumulations = 1
        iv. Excitation Wavelength Park = 350 nm
        v. Emission Wavelength Increment = 0.82 nm (2 pixel)
        vi. CCD Gain = Medium
        vii. Blank/Sample Setup = Sample Only
    g. Click **Run**
    h. **(Only on first run of workgroup)** Choose directory to save project file
        i. Use a consistent filing system so that you can recall old files
        ii. Only run 5-10 samples per project file (workgroup) to limit file size
    i. Click **Emission Sample Data** tab
        i. Click **File → Export → ASCII** and save file as a **.txt file** with tab separator
    j. Open Excel and then open the exported file and save as an Excel Workbook
5. Sample Measurement
    a. Fill one sample cell with nanopure water (to be used for blank)
    b. Fill second sample cell with sample to be analyzed
    c. Click **H2O** button to initialize instrument and open 'Aqualog Main Experiment Menu'
    d. Click **3D**
    e. Click **EEM 3D CCD + Absorbance**
    f. Load archived experimental settings file or create new protocol
        i. Use a consistent filing system so that you can recall old settings
    g. Verify settings:
        i. Change Data Identifier (used to identify sample in workgroup)
        ii. Integration = 3 s
        iii. Excitation Range: High = 470 nm, Low = 240, Increment = 1 nm
        iv. Emission Wavelength Increment = 0.82 nm (2 pixel)
        v. CCD Gain = Medium

vi. Blank/Sample Setup = Sample and Blank
    1. Collect blank on first run or load archived blank from that day

h. Click **Run**
i. **(Only on first run of workgroup)** Choose directory to save project file
    i. Use a consistent filing system so that you can recall old files
    ii. Only run 5-10 samples per project file (workgroup) to limit file size
j. Click **Abs Spectrum Sample** tab
    i. Click **File → Export → ASCII** and save file as a **.txt file** with tab separator
k. Click **Sample – Blank Waterfall Plot** tab
    i. Click **Inner Filter Effect** button (next to H_2O)
l. Click **Processed Graph: IFE** tab
    i. Click **Rayleigh Masking** button (next to IFE)
    ii. "Mask 1st Order Rayleigh" should be checked
    iii. "Mask 2nd Order Rayleigh" should be checked
    iv. "SUM of slit widths (in bandpass)" should be 10
m. Click **Processed Data: IFE_RM** tab
    i. Click **File → Export → ASCII** and save file as a **.txt file** with tab separator
n. Save all files in a permanent folder named according to sample description

6. Process the data with MATLAB
    a. Open Excel and then open the **Processed Data: IFE_RM** file and save as an Excel Workbook
    b. Open the **Abs Spectrum Sample** file and save as an Excel Workbook
    c. Move a copy of the following files to your "working" folder for MATLAB analysis
        i. The 3 **Raman** Excel files
        ii. The **Processed Data: IFE_RM** Excel file
        iii. The **Abs Spectrum Sample** Excel file
        iv. Verify that your "working" folder also contains the **ABS and FRI** Excel files
    d. Open the appropriate MATLAB code (Aqualog.m)
    e. Verify that the MATLAB home screen is linked to your working folder.
    f. Verify that all directories in the MATLAB code (purple text) are valid.
    g. Run the program.
    h. Move all processed data to permanent folder. Only move a copy of the **FRI** and **ABS** Excel files to your permanent folder. These files must remain in your working folder for future processing.

Optimization of Ozone-Biological Activated Carbon Treatment for Potable Reuse Applications

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INTRODUCTION

In the face of climate change, pollution, and population growth, water scarcity has become a global threat. Many populations have witnessed their drinking water sources dwindle to an unsustainable level. These severe conditions have sparked interest in potable reuse as an increasingly viable alternative to typical 'pristine' drinking water sources. Although potable reuse has been practiced for decades, the public has become more supportive of the concept over the past few years based on the historical success of several benchmark facilities in the United States and abroad.

There is considerable debate as to the level of treatment needed to ensure protection of public health with regards to potable reuse. Among existing potable reuse guidelines and regulations, the California Department of Public Health (CDPH) provides the most stringent requirements for water quality. Currently, the best way to meet these standards is through the use of full advanced treatment (FAT). Figure 1 provides an example of the typical FAT layout.

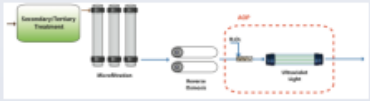

Figure 1: Typical FAT system schematic (e.g., Orange County Water District)

Issues concerning FAT systems:

- High capital and O&M costs
- Energy intensive
- Disposal of concentrated brine stream

Alternative treatment trains composed of ozone and biological activated carbon (BAC) have been employed in several locations throughout the world, but these systems have not yet been optimized and are unable to compete with RO-based treatment trains on the basis of total organic carbon (TOC) removal and reductions in total dissolved solids (TDS). The layout of ozone-BAC treatment is depicted in Figure 2.

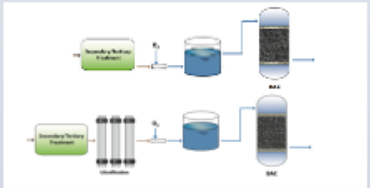

Figure 2: Ozone-BAC system schematic (e.g., El Paso and Gwinnett County)

With the exception of TOC and TDS, which are generally more relevant to aesthetics rather than public health, ozone-BAC is capable of producing a water quality similar to that of RO-based treatment trains on the basis of pathogen reduction, trace organic contaminant (TOrC) mitigation, and a variety of other parameters (Rougeot et al., 2010, 2012; Gerrity et al., 2011, 2014). There are also significant energy and cost savings for the ozone-BAC alternative as there is an incentive to optimize such treatment trains to achieve greater TOC removal (Gerrity et al., 2014; Snyder et al., 2014).

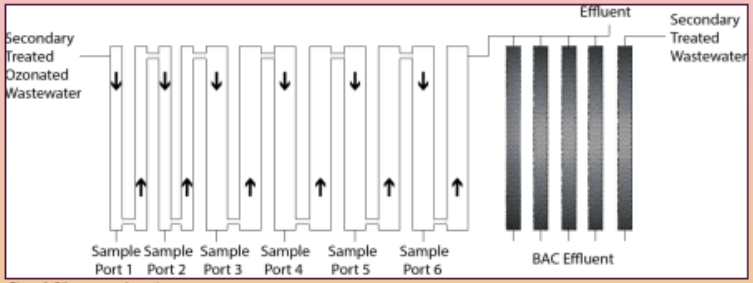

Figure 3: Pilot system schematic

OBJECTIVES

This study aims to identify necessary pre-treatment, post-treatment, and operational conditions needed for ozone-BAC treatment trains to compete with FAT with regards to TOC reduction, pathogen inactivation and removal, and trace organic contaminant mitigation. According to the Groundwater Replenishment Reuse Regulations set by CDPH in 2013, potable reuse supplies should not contain more than 0.5 mg/L of wastewater-derived TOC. Optimization of the process through varying the ozone dose and empty bed contact time (EBCT) will be explored based on this 0.5-mg/L threshold.

SYSTEM DESCRIPTION

- Membrane bioreactor filtrate feeds the 1 liter per minute pilot system.
- A mass flow controller and venturi injector are used for ozone dosing, which is based on O_3:TOC ratio
- The ozone contactors range from 1-2 inches in diameter and provide a total of approximately 17 minutes of contact time
- Exhausted Calgon carbon from the Upper Occoquan Service Authority is loaded into parallel 1-inch columns with a range of EBCTs
- The BAC has been seeded with secondary effluent from a local wastewater treatment facility, and the biofilm is developing.
- A peristaltic pump is used for backwashing the BAC columns and for tracer and challenge studies

OZONE DEMAND DECAY

An ozone demand decay study was performed on the MBR filtrate using the indigo trisulfonate colorimetric method for dissolved ozone (Figure 4). The O_3:TOC of 0.25 was not graphed because the instantaneous ozone demand (IOD) exceeded the applied ozone dose (i.e., ozone CT – 0 mg-min/L).

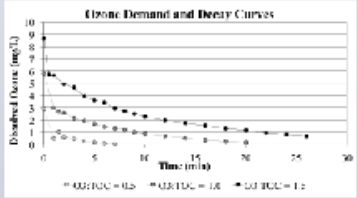

Figure 4: Ozone demand and decay curves for various ozone doses

HYDRAULIC DETERMINATION

A sodium chloride step input tracer study was performed on the ozone contactors to characterize the hydraulics of the system. The tracer was quantified based on electrical conductivity. This will aid in the determination of ozone CT through each contactor.

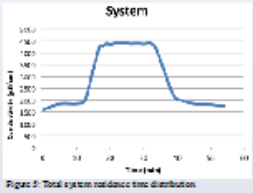

Figure 5: Total system residence time distribution

Table 1: Actual (t) and theoretical (t) hydraulic retention times for each sample port

Location	t (min) Q = 2 lpm	t (min) Q = 2 lpm	t (min) Q = 1 lpm
Port 1	0.45	0.50	0.50
Port 2	1.19	0.90	1.40
Port 3	2.19	2.40	3.80
Port 4	4.01	4.80	7.70
Port 5	6.66	7.10	11.5
Port 6	9.67	9.50	15.5
System	10.19	10.60	17.20

Economic Justification (10-MGD Comparison)

Table 2: Cost comparison between FAT and ozone-BAC treatment systems

Process	Capital ($M)	Annual O&M ($M)	Process	Capital ($M)	Annual O&M ($M)
MF	$11.4	$1.5	MF	$11.4	$1.5
RO	$22.5	$5.5	Ozone	$5.4	$0.1
UV/H_2O_2	$2.2	$0.5	BAC	$7.4	$0.6
--	--	--	UV	$2.2	$0.5
Total	$36.4	$8.1	Total	$26.4	$2.5
Savings	--	--	Savings	$10.0	$2.5

Based on data from Snyder et al. (2014) and Gerrity et al. (2014)

Ozone Contactors

BAC Columns

FUTURE WORK

- Evaluation of biological activity in the BAC columns based on adenosine triphosphate (ATP) and TOC removal
- Investigation of O_3:TOC ratios of 0, 0.25, 0.50, 1.00, and 1.50 based on bulk organic matter transformation and TOrC oxidation
- Evaluation of EBCTs of 0, 5, 10, 15, and 30 minutes based on TOC and TOrC removal
- Evaluation of proprietary biocatalyst in addition to BAC
- Characterization of UV absorbance and fluorescence using a Horiba Aqualog spectrofluorometer to assess bulk organic matter transformation during ozonation and removal through BAC
- Optimization of ozone-BAC with regards to the 0.5 ppm CDPH threshold for potable reuse water
- Quantification of total and fecal coliforms using the Colilert method and Quantiray 2000 quantification protocol

REFERENCES

1. Gerrity, D., Gamage, S., Holady, J.C., Mawhinney, D.B., Quinones, O., Trenholm, R.A. and Snyder, S.A. (2011) Pilot-scale evaluation of ozone and biological activated carbon for trace organic contaminant mitigation and disinfection. Water Research 45, 2155-2165.
2. Gerrity, D., Owens-Bennett, E., Venczel, T., Stanford, B.D., Plumlee, M.H., Debroux, J., Trussell, R.S. (2014) Applicability of ozone and biological activated carbon for potable reuse. Ozone Sci. Eng., in press.
3. Rougeot, J., Escher, B.I., Macova, M., Argaud, F.X., Gernjak, J.K. and Keller, J. (2012) Ozonation and biological activated carbon filtration of wastewater treatment plant effluents. Water Research 46, 863-872.
4. Rougeot, J., Macova, M., Escher, B.I., Carswell, S., Mueller, J.F. and Keller, J. (2010) Removal of micropollutants and reduction of biological activity in a full scale reclamation plant using ozonation and activated carbon filtration. Water Research 44, 625-637.
5. Snyder, S.A., von Gunten, U., Amy, G. and Debroux, J. (2014) Use of ozone in water reclamation for contaminant oxidation. WRF-08-05. WaterReuse Research Foundation.

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I would like to thank Dr. Daniel Gerrity for providing me with the opportunity to pursue this research. I would also like to thank the City of Henderson for providing not only a continuous supply of water but also the space to perform the research. The BAC was provided by the Upper Occoquan Service Authority through their support of WateReuse Research Foundation Project 11-02. Finally, I would like to thank the Nevada Water Resources Research Institute (NWRRI) for providing the primary funding source for this research.

Appendix 2. Poster from the Nevada Water Resources Association's 2014 Annual Conference.

Impacts of Ozone Dose and Empty Bed Contact Time on Total Organic Carbon Removal Through Ozone-Biological Activated Carbon Treatment

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Department of Civil and Environmental Engineering and Construction, University of Nevada, Las Vegas

Funded by:
Nevada Water Resources Research Institute

INTRODUCTION

In the face of climate change, pollution, and population growth, water scarcity has become a global threat. These severe conditions have sparked interest in potable reuse as an increasingly viable alternative to typical 'pristine' drinking water sources.

There is considerable debate as to the level of treatment needed to ensure protection of public health with regards to potable reuse. Among existing potable reuse guidelines and regulations, the California Division of Drinking Water (DDW) provides the most stringent requirements for water quality. For example, wastewater-derived total organic carbon (TOC) is limited to 0.5 mg/L for direct injection of non-diluted reuse water into any California aquifer. Currently, the only way to meet these standards is through the use of full advanced treatment (FAT), which consists of reverse osmosis (RO) and an advanced oxidation process (AOP). While RO is highly effective at treating water, it is also energy intensive, expensive, and produces a concentrated brine stream that is difficult to manage.

Alternative treatment trains composed of ozone and biological activated carbon (BAC) have been employed in several locations throughout the world, but these systems have not yet been optimized and are unable to compete with RO-based treatment trains on the basis of TOC removal.

With the exception of TOC and total dissolved solids (TDS), which are generally more relevant to aesthetics rather than public health, ozone-BAC is capable of producing a water quality similar to that of RO-based treatment trains on the basis of pathogen reduction, trace organic contaminant (TOrC) mitigation, and a variety of other parameters (Reungoat et al., 2010, 2012; Gerrity et al., 2011, 2014). There are also significant energy and cost savings for the ozone-BAC alternative so there is an incentive to investigate various operational configurations for ozone-BAC treatment trains to achieve greater TOC removal (Gerrity et al., 2014; Snyder et al., 2014).

OBJECTIVES

The objective of this study is to identify the necessary operational conditions needed for ozone-BAC treatment trains to compete with FAT on the basis of TOC reduction. The hypothesis is that by coupling greater effluent organic matter transformation via increased ozone doses with longer empty bed contact times, ozone biological activated carbon systems can achieve better TOC removal and possibly approach the 0.5-mg/L threshold for TOC set by the DDW .

SYSTEM DESCRIPTION

Membrane bioreactor (MBR) filtrate fed the 0.6 lpm pilot system (Figure 1). An oxygen concentrator, ozone generator, and venturi injector were used for ozone generation and injection into the feed water. The ozone contactors ranged from 1-2 inches in diameter and provided a total of approximately 25 minutes of contact time. Exhausted 1.2 mm Calgon carbon was loaded into parallel 1-inch columns (C1, C2, C4) receiving ozonated MBR filtrate and a control column that received non-ozonated MBR filtrate (Figures 1B and 1C). C3 contained exhausted 0.95 mm Cabot carbon, and BC held a proprietary biocatalyst (polyvinyl alcohol bead containing denitrifying microorganisms).

BIOFILTER EVALUATION

The ozone-BAC pilot was operated long term for biofilm stabilization and to evaluate the effect of EBCT on TOC removal. After observing no significant difference in TOC removal with varying EBCT during long-term operation, the system was re-evaluated to determine the cause of the unexpected results. Two theories were tested:

1. If the media to column diameter ratio is too large, hydraulic inefficiencies due to wall effects may occur. To test this theory, C3 was replaced with a smaller media of 0.95 mm 11 months after startup.
2. It was theorized that the bioavailable TOC was being consumed quicker than expected and that once the biofilm was stabilized, changing the EBCT would have little impact on bulk biological activity. To study these theories, kinetics tests with shorter EBCTs were performed.

Three kinetics tests were performed for this study. Each one was targeting a different O_3/TOC ratio. It should be noted that the only samples collected during the kinetics tests were influent, C2, C3, control, and effluent. C1 and C4 were redundant given that they contained the same media as C2. Due to severe compaction of the polyvinyl alcohol biobeads, the flow rate in the BC column was extremely low and could not be adjusted to flow rates necessary for the tests.

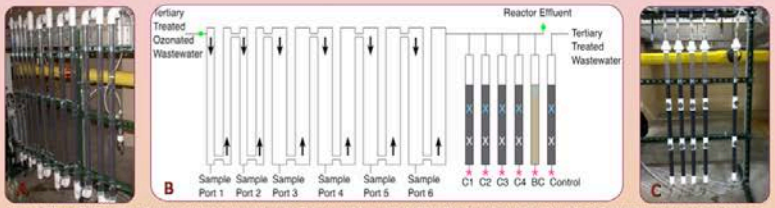

Figure 1. Photo of the (A) ozone contactors and (B) pilot system schematic. The pink asterisks mark the biofilter effluent sampling locations, the white "X's" represent the lower media sampling locations at a 17.5-inch filter media depth, and the blue "X's" represent the upper media sampling locations at a depth of 5.5 inches. The green circles denote the non-filtered samples, the influent and effluent sample locations. The influent sample port was located prior to ozone injection into the water stream, and the effluent sample location was located after the ozone contactors. The column containing the biocatalyst is denoted as BC. Photo of the (C) BAC columns (column BC is not shown).

OZONE DEMAND/DECAY

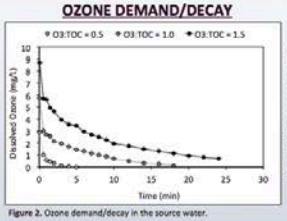

Figure 2. Ozone demand/decay in the source water.

An ozone demand/decay test was performed, and the data are plotted in Figure 2. The UV_{254} data collected from this test was used to develop a correlation between differential UV_{254} absorbance and ozone dose (Figure 3), which is consistent with similar correlations presented in the literature (Gerrity et al., 2012). This function was used to estimate the ozone dose for the kinetics testing.

OZONE DOSING

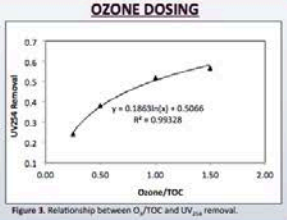

Figure 3. Relationship between O_3/TOC and UV_{254} removal.

KINETICS TESTS

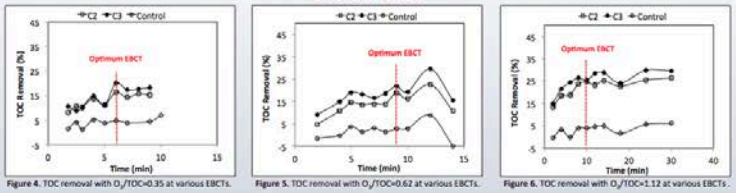

Figure 4. TOC removal with O_3/TOC=0.35 at various EBCTs.

Figure 5. TOC removal with O_3/TOC=0.62 at various EBCTs.

Figure 6. TOC removal with O_3/TOC=1.12 at various EBCTs .

KINETICS TEST RESULTS SUMMARY

Table 1. Comparison of optimum conditions and treatment efficacy

O_3/TOC	Optimum EBCT	TOC Removal (1.2-mm Media)	TOC Removal (0.95-mm Media)	Minimum TOC Achieved
	minutes	%	%	mg/L
0.35	6	16	20	6.4
0.62	9	19	22	5.7
1.12	10	23	25	5.0

BIOFILM CHARACTERIZATION

Table 2. ATP concentration at various points in the filters

Sample	tATP at Top of Column	tATP at Bottom of Column
C2	$6.3x10^5$ pg ATP/g media	$3.1x10^5$ pg ATP/g media
C3	$9.1x10^5$ pg ATP/g media	$2.2x10^5$ pg ATP/g media
Control	N/A	$2.0x10^5$ pg ATP/g media
BC	$2.3x10^5$ pg ATP/g media	N/A

- The data from Table 1 was used to develop a relationship between optimum EBCT and ozone dose. A logarithmic function appears to capture the relationship well, but additional data are warranted to further validate the relationship.
- The concentration of ATP can be used as an indicator of the presence of bacteria and the degree of biological growth in a system.

EBCT AND OZONE DOSE RELATIONSHIP

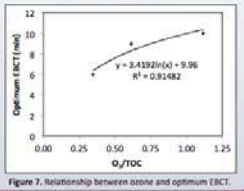

Figure 7. Relationship between ozone and optimum EBCT.

CONCLUSIONS

- The optimum EBCT for O_3/TOC of 0.35, 0.62, and 1.12 were found to be 6, 9, and 10 minutes, respectively. When plotting optimum EBCT versus O_3/TOC, a logarithmic relationship seems to exist between the two parameters. Based on this relationship, it appears that relatively low EBCTs are necessary for TOC removal in some matrices; however, additional testing is warranted to validate the relationship.
- The small media provided more surface area for biological growth, which explains the higher ATP concentration found on the small media. This may also explain the better treatment efficacy achieved with the small media, although improved hydraulic performance may also contribute.
- At the highest O_3/TOC ratio of 1.12 and EBCT of 10 minutes, the minimum TOC concentration attained was 5.0 mg/L. It is assumed that higher ozone doses would achieve even better water quality, but the data suggest that there is a point of diminishing return for ozone dosing as well.
- Despite optimization, O_3/BAC systems alone are insufficient to meet the stringent DDW TOC requirement of 0.5 mg/L. If higher ozone doses were used, this goal may have been more attainable, but the data suggests the TOC removal would plateau far before reaching a 0.5 mg/L effluent TOC concentration.
- Possible strategies to comply with existing potable reuse regulations include increasing the percentage of diluent water or adding additional treatment steps, such as ion exchange or activated carbon with adsorptive capacity.

FUTURE WORK

- The apparent logarithmic relationship that exists between O_3/TOC and EBCT was not fully established. Higher ozone doses must be explored and larger data sets gathered to better understand the relationship that may exist.
- Analyze the effect of ozonated water on biofilm attachment to media and biological community makeup.
- It is unclear what possible health effects are associated with TOC; therefore, the 0.5 mg/L threshold may not be necessary to maintain public safety in potable reuse applications. Further examination of this bulk parameter is needed.

REFERENCES

1. Gerrity, D., Gamage, S., Holady, J.C., Mawhinney, D.B., Quinones, O., Trenholm, R.A. and Snyder, S.A. (2011) Pilot-scale evaluation of ozone and biological activated carbon for trace organic contaminant mitigation and disinfection. Water Research 45, 2155-2165.
2. Gerrity, D., Owens-Bennett, E., Venezia, T., Stanford, B.D., Plumlee, M.H., Debroux, J., Trussell, R.S. (2014) Applicability of ozone and biological activated carbon for potable reuse. Ozone Sci. Eng. 36, 123-137.
3. Reungoat, J., Escher, B.I., Macova, M., Argaud, F.X., Gernjak, J.K. and Keller, J. (2012) Ozonation and biological activated carbon filtration of wastewater treatment plant effluents. Water Research 46, 863-872.
4. Reungoat, J., Macova, M., Escher, B.I., Carswell, S., Mueller, J.F. and Keller, J. (2010) Removal of micropollutants and reduction of biological activity in a full scale reclamation plant using ozonation and activated carbon filtration. Water Research 44, 625-637.
5. Snyder, S.A., von Gunten, U., Amy, G. and Debroux, J. (2014) Use of ozone in water reclamation for contaminant oxidation. WRF-08-05. WaterReuse Research Foundation.

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I would like to thank Dr. Daniel Gerrity for providing me with the opportunity to pursue this research. I would also like to thank staff at the study site for providing not only a continuous supply of water but also the space to perform the research. The project described in this publication was supported by Grant/Cooperative Agreement Number G11AP20092 from the United States Geological Survey. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the USGS.

Appendix 3. Poster from the 19th Annual Water Reuse and Desalination Research Conference.

Estimation of Atmospheric Wet and Dry Deposition of Nutrients to Lake Tahoe Snowpack

Basic Information

Title:	Estimation of Atmospheric Wet and Dry Deposition of Nutrients to Lake Tahoe Snowpack
Project Number:	2013NV195B
Start Date:	3/1/2013
End Date:	2/28/2016
Funding Source:	104B
Congressional District:	NV002
Research Category:	Water Quality
Focus Category:	Nutrients, Hydrology, Water Quality
Descriptors:	None
Principal Investigators:	Rina Schumer

Publications

1. Obrist, D., Moore, C. W., Pearson, C., Pierce, A. M., Schumer, R., Helmig, D., Van Dam, B., Fain, X., Steffen, A., Staebler, R., Nghiem, S., Douglas, T., 2013: Mercury in alpine and Arctic snow: atmospheric deposition and fate processes, Seminar, Graduate Program of Hydrologic Sciences, University of Nevada: Reno, NV, April 1, 2013
2. Pearson, C., Obrist, D., Schumer, R., 2013: Nutrient and Mercury Concentrations and Loads in Tahoe Basin Snowpack, AGU Fall Meeting: San Francisco, CA, December 9, 2013
3. Pearson, C., Obrist, D., Schumer, R., 2013: Nitrogen and Phosphorus Concentrations and Loads within Lake Tahoe Snowpack, University Council on Water Resources 2013 Annual Conference: Lake Tahoe, CA, June 1, 2013, Published
4. Obrist, D., Moore, C. W., Douglas, T. A., Steffen, A., Staebler, R. M., Pearson, C., 2012: Concentrations of total and dissolved Hg in snow and vapor deposition collected during Atmospheric Mercury Depletion Events (AMDEs) in Barrow, Alaska during the BROMEX campaign. Abstract A31D-0059, AGU Fall Meeting: San Francisco, CA
5. Pearson, C., Obrist, D., Schumer, R., 2012: Quantifying Nutrient and Mercury Concentrations and Loads in Tahoe Snowpack, Water Summit 2012: Milwaukee, WI.
6. Pearson, C., Obrist, D., Schumer, R., 2012: Quantifying Nutrient and Mercury Concentrations and Loads in Lake Tahoe Snowpack, Abstract B23H-0540, Presentation at AGU 2012 Fall Meeting: San Francisco, CA, December 3, 2012.
7. Trustman, B. D., Obrist, D., Schumer, R., Pearson, C., Moore, C. W., 2014: Nutrient and Mercury Dynamics in the Lake Tahoe basin Snowpack, Mountain Observatories: Reno, NV, July 18, 2014
8. Pearson, C., Schumer, R., Trustman, B. D., Rittger, K., Johnson, D. W., Obrist, D. (2015). Nutrient and mercury deposition and storage in an alpine snowpack of the Sierra Nevada, USA, Biogeosciences Discuss., 12, 593-636

Estimation of atmospheric wet and dry deposition of nutrients to Lake Tahoe Snowpack

Rina Schumer, Division of Hydrologic Sciences, Desert Research Institute
Daniel Obrist, Division of Atmospheric Sciences, Desert Research Institute

Problem and Research Objectives

This study aims to fill gaps in nitrogen, phosphorous, and mercury deposition loads contained in snowfall and snowpack throughout the Tahoe basin using experimental measurements and spatial modeling. Developing atmospheric deposition constraints is needed to understand mobility and transport pathways of N and P from the watersheds to the lake, which account for a significant fraction of nutrient inputs to the lake. Comprehensive management practices to reduce N and P loads to Lake Tahoe will benefit from improved estimates of nutrient deposition to terrestrial basin areas, particularly since these provide a long-term source for potential N and P inputs to Lake Tahoe. Measurement and modelling of mercury deposition was added to this project in an effort to extend expertise in Mercury cycling in high alpine watersheds.

We will fill the gap in Tahoe Basin terrestrial atmospheric deposition estimates using an integrated approach that includes experimental measurements of wet deposition loads and snowpack accumulation in the Lake Tahoe watershed, combined with spatial modeling to extrapolate snowpack loads of N, P, and Hg to the entire watershed area. The role of snowpack deposition of nutrients is considered key in the Lake Tahoe basin given that seventy percent of annual precipitation occurs during winter and spring as snow and results from a National Atmospheric Deposition Program (NADP) stations in Sagehen Creek indicating that up to two-thirds of annual wet deposition of nutrients is associated with winter and spring snowfall.

Methods

Wet deposition loads in the basin are measured using both wet deposition samplers and snowpack core sampling. A number of wet deposition samplers are deployed in the Lake Tahoe watershed to continuously collect wet deposition samples, one located at a high elevation, remote site on top of Homewood ski area and a second location in Incline Village, NV. Since the Lake Tahoe Basin straddles the boundary of Nevada and California, field work will occur in both states. Wet deposition samples are collected every two weeks. Along with bi-weekly wet deposition samples, snowpack core samples will be collected at seven sites in the basin, starting with the first measureable snowpack accumulation until spring melting has ended. These sites are distributed across elevations and along eastern and western transects, with three sites located in the western part of the watershed in California and four sites in Nevada

Basin-wide loads and distribution are assessed using chemical concentrations and loads measured throughout the snow seasons as well as basin-wide mean peak SWE estimates

from SWE reconstruction for the Sierra Nevada from 2000 to 2011. Sierra SWE reconstruction employs accurate estimates of snow depletion rates based on MODIS Snow Covered Area and Grain size (MODSCAG) in order to estimate peak SWE. MODSCAG calculates fractional snow cover area and grain size from Moderate Resolution Imaging Spectroradiometer (MODIS) data.

Principal findings and significance

Bi-weekly snowpack core samples were collected at seven sites along two elevation gradients in the Tahoe Basin during two consecutive snow years to evaluate total wintertime snowpack accumulation of nutrients and pollutants in a high elevation watershed of the Sierra Nevada. Additional sampling of wet deposition and detailed snow pit profiles was conducted the following year to compare wet deposition to snowpack storage and assess the vertical dynamics of snowpack chemicals. Results show that on average organic N comprised 48% of all snowpack N, while nitrate (NO3--N) and TAN (total ammonia nitrogen) made up 25 and 27%, respectively. Snowpack NO3--N concentrations were relatively uniform across sampling sites over the sampling seasons and showed little difference between seasonal wet deposition and integrated snow pit concentrations in agreement with previous studies that identify wet deposition as the dominant source of wintertime NO3--N deposition. However, vertical snow pit profiles showed highly variable concentrations of NO3--N within the snowpack indicative of additional deposition and in snowpack dynamics. Unlike NO3--N, snowpack TAN doubled towards the end of winter and in addition to wet deposition, had a strong dry deposition component. Organic N concentrations in snowpack were highly variable (from 35 to 70%) and showed no clear temporal or spatial dependence throughout the season. Integrated snowpack organic N concentrations were up to 2.5 times higher than seasonal wet deposition, likely due to microbial immobilization of inorganic N as evident by coinciding increases of organic N and decreases of inorganic N, in deeper, aged snowpack. Spatial and temporal deposition patterns of snowpack P were consistent with particulate-bound dry deposition inputs and strong impacts from in-basin sources causing up to 6 times enrichment at urban locations compared to remote sites. Snowpack Hg showed little temporal variability and was dominated by particulate-bound forms (78% on average). Dissolved Hg concentrations were consistently lower in snowpack than in wet deposition which we attribute to photochemical-driven gaseous remission. In agreement with this pattern is a significant positive relationship between snowpack Hg and elevation, attributed to a combination of increased snow accumulation at higher elevations causing limited light penetration and lower photochemical re-emission losses in deeper, higher elevation snowpack. Finally, estimates of basin-wide loading based on spatially extrapolated concentrations and a satellite-based snow water equivalent reconstruction model identify snowpack chemical loading from atmospheric deposition as a substantial

source of nutrients and pollutants to the Lake Tahoe basin, accounting for 113 t of N, 9.3 t of P, and 1.2 kg of Hg each year.

Information Transfer Activities

Presentations

Trustman, B. D., Obrist, D., Schumer, R., Pearson, C., Moore, C. W., 2014: Nutrient and Mercury Dynamics in the Lake Tahoe basin Snowpack, Mountain Observatories: Reno, NV, July 18, 2014

Obrist, D., Moore, C. W., Pearson, C., Pierce, A. M., Schumer, R., Helmig, D., Van Dam, B., Fain, X., Steffen, A., Staebler, R., Nghiem, S., Douglas, T., 2013: Mercury in alpine and Arctic snow: atmospheric deposition and fate processes, Seminar, Graduate Program of Hydrologic Sciences, University of Nevada: Reno, NV, April 1, 2013

Pearson, C., Obrist, D., Schumer, R., 2013: Nutrient and Mercury Concentrations and Loads in Tahoe Basin Snowpack, AGU Fall Meeting: San Francisco, CA, December 9, 2013

Pearson, C., Obrist, D., Schumer, R., 2013: Nitrogen and Phosphorus Concentrations and Loads within Lake Tahoe Snowpack, University Council on Water Resources 2013 Annual Conference: Lake Tahoe, CA, June 1, 2013, Published

Obrist, D., Moore, C. W., Douglas, T. A., Steffen, A., Staebler, R. M., Pearson, C., 2012: Concentrations of total and dissolved Hg in snow and vapor deposition collected during Atmospheric Mercury Depletion Events (AMDEs) in Barrow, Alaska during the BROMEX campaign. Abstract A31D-0059, AGU Fall Meeting: San Francisco, CA

Pearson, C., Obrist, D., Schumer, R., 2012: Quantifying Nutrient and Mercury Concentrations and Loads in Tahoe Snowpack, Water Summit 2012: Milwaukee, WI,

Pearson, C., Obrist, D., Schumer, R., 2012: Quantifying Nutrient and Mercury Concentrations and Loads in Lake Tahoe Snowpack, Abstract B23H-0540, Presentation at AGU 2012 Fall Meeting: San Francisco, CA, December 3, 2012,

Publications

Pearson, C., Schumer, R., Trustman, B. D., Rittger, K., Johnson, D. W., Obrist, D. (2015). Nutrient and mercury deposition and storage in an alpine snowpack of the Sierra Nevada, USA, *Biogeosciences Discuss.*, 12, 593-636

Student Support

This project supported the Master's work for Chris Pearson, who graduated in December 2013 and partially supported Master's student Benjamin Trustman. The project produced numerous conference presentations (above) and a published manuscript with both students as coauthors in *Biogeosciences.*

Quantifying Surface Runoff and Water Infiltration in Arid and Semi-arid Areas

Basic Information

Title:	Quantifying Surface Runoff and Water Infiltration in Arid and Semi-arid Areas
Project Number:	2013NV196B
Start Date:	3/1/2013
End Date:	2/28/2016
Funding Source:	104B
Congressional District:	NV001
Research Category:	Climate and Hydrologic Processes
Focus Category:	Surface Water, Models, Water Quantity
Descriptors:	None
Principal Investigators:	Yong Zhang, Li Chen, Donald M Reeves

Publications

1. Zhang, Y., L. Chen, D. M. Reeves, and H.G. Sun, Fractional-derivative models for surface runoff along heterogeneous ground surface. Abstract will be published in the 2014 International Conference on Fractional Differentiation and its Applications, Catania, 23-25 June, 2014.
2. Sun, H.G., M. M. Meerschaert, Y. Zhang, J.T. Zhu, and W. Chen, A fractal Richards' equation to capture the non-Boltzmann scaling of water transport in unsaturated media, Advances in Water Resources, 52, 292-295, 2013.
3. Sun, H.G., Y. Zhang, W. Chen, and D. M. Reeves, Use of a variable-index fractional-derivative model to capture transient dispersion in heterogeneous media, Journal of Contaminant Hydrology, 157, 47-58, 2014.
4. Chen, L., 2014: Modeling Rainfall Runoff Process and Scaling Effects in Complex Arid Environments, 1st Congress of China Geodesy and Geophysics: Beijing, October 25, 2014-October 26, 2014.
5. Chen, L., Sela, S., Svoray, T., Assouline, S., 2014: Hydrological responses and scaling effects in vegetated semi-arid areas with surface sealing, (Invited talk), AGU Fall Meeting: San Francisco, December 15, 2014-December 19, 2014.
6. Zhang, Y., L. Chen, D. M. Reeves, and H. G. Sun, A fractional-order tempered-stable continuity model to capture surface water runoff, Journal of Vibration and Control, 2014. doi: 10.1177/1077546314557554.

Quantifying surface runoff and water infiltration in arid and semi-arid areas

Li Chen

Problem and Research Objectives

Surface runoff is a fundamental hydrologic component that can affect many processes in hydrology, morphology, biology, and ecology. In arid and semi-arid areas, surface runoff in the form of Horton overland flow is often observed, due to the typical short-duration and high-intensity rainfall that easily exceeds the soil infiltration capacity. It can cause environmental effects (i.e., nonpoint source pollution and water sustainability), agriculture issues (i.e., reduction of crop productivity due to soil conservation), and natural hazards (i.e., flooding). Surface runoff is also the main driving force for soil erosion and may trigger debris flow hazards. It also produces horizontal redistribution of water resources that may affect habitats and ecosystem in watersheds, and rapidly transport of nutrients or contaminates along land surfaces which affects multiple biological and ecological processes. Accurate quantification of surface runoff, however, has remained a challenge in hydrology for many decades. This project proposes the use of novel mathematical models to quantify the complex dynamics of surface runoff and infiltration in order to protect water resources and the environment in Nevada.

Methodology

In this study, we applied state-of-the-art physical theories and novel mathematical tools to build and solve the physical models for the surface runoff process. Random walk theories combined with the subordination technique can account for the nonlocal movement of water packages along a ground surface exhibiting fractal complexity. The local variations of flow behavior can also be captured by conditioning on local soil and topography properties. The resultant model explains the scale evolution of surface runoff within and across sub-basins through the use of the new mathematical concept of tempered stable laws. We have also generalized the Richards' equation using the physical concept of fractal time, which accounts for both the sub-diffusive and super-diffusive anomalous motion of moisture observed in heterogeneous, unsaturated soils. Meanwhile, we developed a high resolution, multidimensional conceptual modeling approach to simulate infiltration and runoff at various scales to explore the scale dependence runoff generation processes.

Principal Findings and Significance

To date, the following findings are obtained through this study:

1. The infiltration process can be efficiently captured by a fractal Richards' equation (FRE). The traditional Richards' equation corresponds to the Boltzmann scaling of wetting front, with travel distance growing as the square root of time. In laboratory experiments and field measurements, the evolution of a horizontal wetting front can deviate significantly from Boltzmann scaling. The proposed fractal Richards' equation is able to capture the non-Boltzmann scaling of water transport in unsaturated soils, which replaces the integer-order time derivative of water content with a fractal derivative. Applications show that the FRE fits well water content curves from various previous literature.

2. The multi-scale heterogeneity nature of soils, especially the soil fractal dimension, may result in a full range of anomalous dynamics in water infiltration. This includes the sub-diffusive regime, when regions of flow permeability can retard flow, and super-diffusion, where the wetting front is accelerated along preferential flow paths. The fractal time index in the FRE model may be related to soil texture parameters, especially the fractal dimension.

3. Surface runoff and water infiltration in arid and semi-arid areas may not exhibit constant scaling; instead transition between diffusive states (i.e., super-diffusion, sub-diffusion, and Fickian diffusion) may occur at various transport scales. These transitions are likely attributed to physical properties of the medium, such as spatial variations in heterogeneity in soil and topography. This "transient dispersion" can be modeled with a variable-index fractional-derivative law (FDL) which is not limited to stationary heterogeneous media as the standard constant-index FDL limited. Applications show that the variable-index theory can efficiently quantify the observed scale transitions, with the scale index varying linearly in time or space.

5. A fractional-order continuity equation can be used to quantify the behavior of surface runoff, where the influence of heterogeneity on flow dynamics can be characterized using spatiotemporally nonlocal terms built upon fractional derivatives. The model is found capable to describe the general patterns of hillslope runoff hydrograph. Numerical analysis show that the space-fractional diffusive term in the flow model does not lead to apparent early arrivals in the rising limb of a hydrograph. Meanwhile, the time-fractional term in the model can account for the strong time-nonlocal influence of net recharge on the receding limb of a hydrograph, and a wide range of late-time behavior of flow.

6. The high resolution modeling study shows that surface runoff has a decline trend with the increase of the spatial scale in complex arid environment comprising a number of impacting environmental factors such as soil, topography and vegetation. This trend can be described by a power-law runoff-scale relationship. Such a relationship, however, can be affected by soil hydraulic properties, rainfall pattern and intensity. Large spatial

heterogeneity may increase the scale effect, while higher rainfall intensity may reduce the effect. This trend can be potentially used to guide the upscaling strategies in larger scale hydrologic modeling practice.

Information Transfer Activities:

Papers:

- Chen, L. Sela S. Assouline, S., Zhang, Y. Scaling of rainfall-runoff in complex semi-arid environments. Water Resources Research (submitted).
- Zhang, Y., L. Chen, D. M. Reeves, and H. G. Sun, A fractional-order tempered-stable continuity model to capture surface water runoff, Journal of Vibration and Control, 2014. doi: 10.1177/1077546314557554.
- Sun, H.G., M. M. Meerschaert, Y. Zhang, J.T. Zhu, and W. Chen, A fractal Richards' equation to capture the non-Boltzmann scaling of water transport in unsaturated media, Advances in Water Resources, 52, 292-295, 2013.
- Sun, H.G., Y. Zhang, W. Chen, and D. M. Reeves, Use of a variable-index fractional-derivative model to capture transient dispersion in heterogeneous media, Journal of Contaminant Hydrology, 157, 47-58, 2014.

Presentations:

- Chen, L., Sela, S., Svoray, T., Assouline, S., 2014: Hydrological responses and scaling effects in vegetated semi-arid areas with surface sealing, (Invited talk), AGU Fall Meeting: San Francisco, December 15, 2014-December 19, 2014.
- Chen, L., 2014: Modeling Rainfall Runoff Process and Scaling Effects in Complex Arid Environments, 1st Congress of China Geodesy and Geophysics: Beijing, October 25, 2014-October 26, 2014.
- Zhang, Y., L. Chen, D. M. Reeves, and H.G. Sun, Fractional-derivative models for surface runoff along heterogeneous ground surface. International Conference on Fractional Differentiation and its Applications, Catania, 23-25 June, 2014.

Student support

This project provided partial support for the Ph.D. graduate student Nudthawud Homtong and postdoctoral fellow Peng Jiang.

Impact of climate on mercury transport in the Carson River-Lahontan Reservoir system and Management Alternatives to Mitigate Response

Basic Information

Title:	Impact of climate on mercury transport in the Carson River-Lahontan Reservoir system and Management Alternatives to Mitigate Response
Project Number:	2013NV197B
Start Date:	3/1/2013
End Date:	2/28/2016
Funding Source:	104B
Congressional District:	NV002
Research Category:	Water Quality
Focus Category:	Non Point Pollution, Surface Water, Models
Descriptors:	None
Principal Investigators:	Rosemary Woods-Hart Carroll

Publications

1. Flickinger, A. K., Carroll, R. W., Warwick, J. J., Schumer, R. (2015). Impact of Climate Change on Mercury Transport along the Carson River-Lahontan Reservoir System, Nevada Water Resources Association: Reno, NV, January 26, 2015.
2. Flickinger, A. K., Carroll, R. W., Warwick, J. J., Schumer, R. (2014). Impact of climate change on mercury transport along the Carson River-Lahontan Reservoir system, American Geophysical Union: San Francisco, CA, December 15, 2014-December 19, 2014, H31H-0734.

Impact of climate on mercury transport in the Carson River-Lahontan Reservoir system

Problem and Research Objective

The United States Environmental Protection Agency (US EPA) designated the Carson River and Lahontan Reservoir (CRLR) as a superfund site in 1991 for its contamination by mercury (Hg) as a result of historic mining practices. Fish populations in Lahontan Reservoir exceed the Federal Action limit for consumption (1 μg/g). The rate of Hg transported through the CRLR system and the resulting bioaccumulation in the reservoir is non-linearly related to in-stream flow (Carroll et al., 2000; Carroll et al., 2001; Carroll et al., 2004; Warwick and Carroll, 2008; Carroll, 2010). It is therefore vitally important to relate changes to in-stream flow regimes caused by climate change with the fate of Hg in this important freshwater system. Studies (IPCC, 2007; USGCRP, 2009) suggest that the future envelopes of climate variability may differ from historical data, with all regions in the southwestern US predicted to have increased temperatures and most regions predicted to experience a change in precipitation. The US Bureau of Reclamation (USBR, 2011) developed hydrologic responses associated with 112 down-scaled climate projections from the World Climate Research Programme Coupled Model Inter-comparison Project 3. Hydrologic projections are based on the Variable Infiltration Capacity (VIC) macro-scale hydrology model. The projected flows at Fort Churchill on the Carson River show that while uncertainty in projected flows increases significantly by 2070s, there is also significant increase in the median seasonal flow volume in the winter and a decrease in flows during the spring-summer runoff period. This change in precipitation will lead to changes in stream flow, which could affect the Hg transport in the CRLR system. This project aims to establish the impact of projected climate on Hg transport through the CRLR system and the significance of change in terms of timing and total mass of each Hg species modeled (total Hg, total dissolved Hg, total methylmercury (MeHg) and total dissolved MeHg).

Methodology

Data Preparation

1. Observed flows at the Woodfords gage (10310000) on West Carson, Gardnerville gage (10309000) on the East Carson were correlated to predict historic flows at the Carson City Gage (gage number 10311000) on the main Carson River, from 1990 to present. This transfer is necessary since the CRLR Hg transport model uses the Carson City gage as its upstream flow boundary, not the Woodsville or Gardnerville gages predicted by VIC. Data were separated by month and log-log regressions were performed to correlate the data. September and October were split in half to improve correlation and was likely required based on late season irrigation practices.
2. The bias correction process from "West-Wide Climate Risk Assessments: Bias-Corrected and Spatially Downscaled Surface Water Projections" was followed to reduce the existing bias between the observed and VIC predicted flows in the historical period. To do this, an empirical cumulative distribution function (ECDF) was found for both the observed and the projected data from 1950-1999. The VIC predicted output was then transformed by finding the percentile of the flow according to the ECDF of the projected data, and then using that percentile to find the corresponding flow in the ECDF of the observed data, which was used as the bias corrected flow and performed for Woodfords, Gardnerville and Fort Churchill Gages (10312000). Error in VIC bias-corrected flows reduced error (nrmse) from greater than 14% at all gage locations to 3.4% at Woodfords, 3.6% at Gardnerville and 4.1% at Fort Churchill for the historic record 1950-1999.
3. Bias correction factors calibrated for the historic record were applied to future VIC predictions for years 2000 to 2099. Future Woodsville and Gardnerville flows were then translated into

Carson City Gage flows based on regression statistics described in (1). Carson City gage and Fort Churchill gage daily flows were used to develop RIVMOD input.

4. The Hg transport model requires the stage of the Lahontan Reservoir and the discharge from the Lahontan Dam as inputs. A spreadsheet model was created to model these parameters from the available data. The inputs to the spreadsheet are the average monthly flow from the Fort Churchill gage, representing the input from the Carson River, and the Truckee Canal (which enters the Lahontan Reservoir near the dam on the northern end of the reservoir). The initial storage, found from USGS gage data (10312100), is also set. Stage is then tracked as a function of inputs as well as the required release due to agricultural demands downstream and maximum reservoir stage.
5. The calibrated reservoir model was run over future VIC scenarios from 2000 to 2099 using average monthly Truckee Canal inflows to establish future reservoir stages and discharged based on historic reservoir operations. Reservoir discharge and stage, along with Truckee Canal inflows were input to RIVMOD at the daily stress period for future WASP/RIVMOD simulations.

Model Modifications

Several modifications were made to the Hg Transport model in order to ensure that the runs could accurately run for a century. The original model had not been run for longer than a decade, and early attempts to continue the model past a decade found that the time variables needed to have more significant digits in order to prevent round off error. There was also a numerical instability that was solved by limiting the maximum change in Hg concentration between time steps, and the accuracy of the results was improved by raising the maximum number of iterations per time step from 10 to 20.

Analysis:

Analysis was done for all 112 climate projections with respect to each modeled Hg species and total mass transported at the FCH as well as each of the three reservoir basins. Decadal daily-averages were determined as well as the 80% confidence interval based on ranking. Changes in decadal means were tested using the two-sample Kolmogorov-Smirnov test. Output related to the dissolved DMeHg in the reservoir for the decadal averages and confidence intervals were then used in the bioaccumulation and mercury mass balance model (BioHg) to determine possible changes in Hg bioaccumulation in Sacramento Blackfish.

Principal Findings and Significance

- High flow events in the beginning of the century may contribute large amounts of Hg to enter the river system (as expressed at Fort Churchill) but these high flow events cause the channel to widen over time to cause the magnitude of flow depth and associated bank erosion to decrease over time. This, along with reduced spring and summer stream flows, can lead to significant decreases in THg and TMeHg loading in the spring and summer.
- Fort Churchill DHg concentrations increase noticeably during the summer/fall low-flow periods due to larger stream bottom area from channel widening and associated diffusion. However, the decrease in flow, however, causes DHg loads to decrease significantly during these seasons. In contrast, DHg loading increases significantly during the winter and spring months.
- The highest DMeHg concentrations at Fort Churchill occur during the lowest flows while the lowest concentrations occur during the highest flows. Subsequently, DMeHg loads decrease across all seasons, with largest and most significant decreases occurring in the spring and summer.

- The three sections of the reservoir differ in the response of their DMeHg concentrations, with a modest increase during the summer and fall in the South and Middle Basin and an overall decrease in the North Basin. This is due to the North Basin receiving inflow from the Truckee Canal, effectively diluting the concentrations of DMeHg. As flows decrease throughout the century, this inflow has a stronger dilution effect on the North Basin.
- Overall, the results from each of the three scenarios show the fish exceeding the federal consumption advisory level of 1 μg of Hg per g of mass by the end of their first year of life, and this is true for both the first and last decade of the century. These results suggest that the Sacramento blackfish will continue to be unsafe to consume throughout the century unless some sort of mitigation strategy is attempted

Information Transfer Activities

Research results were presented one regional and one international conference.

- Flickinger, A. K., Carroll, R. W., Warwick, J. J., Schumer, R. (2015). Impact of Climate Change on Mercury Transport along the Carson River-Lahontan Reservoir System, Nevada Water Resources Association: Reno, NV, January 26, 2015.
- Flickinger, A. K., Carroll, R. W., Warwick, J. J., Schumer, R. (2014). Impact of climate change on mercury transport along the Carson River-Lahontan Reservoir system, American Geophysical Union: San Francisco, CA, December 15, 2014-December 19, 2014, H31H-0734.

A Master thesis has been submitted to the University of Nevada, Reno and is currently being converted into a paper for peer review. Likely submittal to Journal of Ecologic Modeling.

In addition the following paper has been submitted for review.

- Carroll, R. W., Warwick, J. J. (2015). Modeling the Highly Dynamic Loading of Mercury Species in the Carson River and Lahontan Reservoir System, Nevada, *Science of the Total Environment*, Submitted

Student Support

Allison Flickenger is supported at the Masters level within the Graduate Program of Hydrologic Sciences at the University of Nevada, Reno.

References Cited

Carroll, R.W.H. 2010. Modeling mercury transport and bioaccumulation in the Carson River and Lahontan Reservoir System, Nevada. Dissertation. University of Nevada, Reno. 170 pp.

Intergovernmental Panel on Climate Change (IGPCC). 2007. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Avery, M. Tignor, and H.L. Miller (eds.). Cambridge University Press, Cambridge, United Kingdom and New York, New York, United States, 996 pp. Available at http://www.ipcc.ch/ipccreports/ar4-wg1.htm.

United States Bureau of Reclamation (USBR). 2011. West-wide climate risk assessments: bias-corrected and spatially downscaled surface water projections. Technical Memorandum no. 86-68210-2011-01. 138 pp.

United States. Global Change Research Program (USGCRP). 2009. Global Climate Change Impacts in the United States, T.R. Karl, J.M. Melillo, and T.C. Peterson, (eds.). Cambridge University Press, 196 pp.

Impact of Climate Change on Low-probability, High-risk Flooding Events in the Southwestern United States

Basic Information

Title:	Impact of Climate Change on Low-probability, High-risk Flooding Events in the Southwestern United States
Project Number:	2015NV204B
Start Date:	3/1/2015
End Date:	2/28/2016
Funding Source:	104B
Congressional District:	NV-003
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	None
Principal Investigators:	Li Chen

Publications

1. Crystal DuBose, Li Chen, Peng Jiang, Zhongbo Yu. 2016. Estimating Future Flood Frequency in the Southwest. 2016 NWRA Annual Conference, March 1-2, 2016, Las Vegas, NV.
2. Crystal DuBose, Li Chen, Peng Jiang, Zhongbo Yu. 2016. A Statistical Analysis and Future Estimation of Flood Frequency in Relation to Extreme Precipitation Events for Regions in the Southwest. 11th Annual GeoSymposium, Department of Geoscience, University of Nevada Las Vegas, April 29, 2016, Las Vegas, NV.
3. Jiang, P., Yu, Z., Chen, L., Acharya, K. (2015). Changing storm properties and their impacts on the flood frequency in southern Nevada, UCOWR/NIWR/CUAHSI 2015 Annual Conference: Las Vegas, NV, June 15, 2015-June 18, 2015
4. Jiang, P., Acharya, K., Chen, L., Yu, Z. (2016). The impacts of changing storm properties on water fluxes and solute transport in Las Vegas Valley, Lower Colorado River Science Symposium, Las Vegas, NV, February 8-9, 2016
5. Chen, L., Sela, S., Svaroy, T., Assouline, S. Influences of vegetation and rainfall patterns on scaling in Hortonian rainfall-runoff processes. AGU Fall Meeting, December 15-19, 2015, San Francisco, CA.

Annual Report for
Impact of Climate Change on Low-probability, High-risk Flooding Events in the Southwestern United States

PI: Li Chen
May 23, 2016

Problem and Research Objectives

Climate change is expected to be a contributor to extreme precipitation events, events in the 95th percentile of intensity. According to the National Climate Assessment, the most extreme precipitation events, daily events in the 99th percentile, show an overall increasing trend regionally across the mainland of the United States from 1958 to 2012. Assuming emissions continue to increase, the Southwest is expected to experience extreme precipitation events nearly twice to three times as often by 2081. Concerns of property damage, environmental alterations, and even loss of life due to extreme flooding events are continuous issues to regions within the southwestern United States. Sudden downpours, high intensity storms of short duration, often lead to flash flooding due to lack of vegetation, high runoff rates, and low absorption rates from dry soils in this region. While many precipitation models in the Southwest have projected these extreme variations in rainfall due to climate change, a statistical relationship between extreme precipitation events and future flood frequency has yet to be developed. Furthermore, precipitation characteristics used to develop a statistical relationship currently do not incorporate nonstationary data regarding climate change. Thus, storm events may underestimate the increasing intensity of precipitation and cause unreliability in future hazard assessments. Our goal is to develop statistical and hydrologic models that incorporate nonstationary precipitation characteristics to project future flood frequency of extreme flooding events for locations in the Southwest. The models will quantify future precipitation conditions and be used to determine whether statistical parameters alone can accurately derive future flood frequency.

Methodology

In order to estimate the future flood frequency based on precipitation characteristics, statistical and hydrologic analyses of the Virgin River watershed has been conducted. The following tasks show the major components of the methodology.

Create IDF Curves through Statistical Analysis

To address extreme flooding through statistical analysis, a correlation between precipitation characteristics and flood frequency will be derived. In order to derive this correlation, we must identify any varying precipitation characteristics. In order to identify these characteristics, changes in intensity, duration, frequency (IDF) relationships need to be accounted for.

In order to create the necessary IDF curves, hourly precipitation gage data was collected from the National Centers for Environmental Information (NCEI). Gridded data was retrieved from the North American Regional Climate Change Assessment Program (NARCCAP). A combination of eleven different global and regional climate models each with a thirty year stimulated historical (1971-2000) and future (2041-2070) record was then used to produce a total of twenty-two different IDF curves for each location. Thus, each location had a total of twenty- three IDF curves – eleven historical IDF curves, eleven future IDF curves, and one observation-based IDF curve. A Matlab program was developed to facilitate the calculation of IDF curves.

Synthesizing data based on Bayesian Model Averaging

Since no one climate model best represented the observed data, we used Bayesian Model Averaging to compare stimulated historical conditions with the observed data. In using Bayesian Model Averaging, each historical nonstationary curve will have a weighted average based on the projected likelihood of a particular curve appearing over time. Thus, no curve is represented as best, rather the eleven historical nonstationary curves will be weighted and used to create only one historical nonstationary curve. The weighted results will then be used to calibrate one futurist nonstationary IDF curve for each location.

Evaluate changes in IDF relationships

The historical IDF curve were compared with the future IDF curve to determine the relative difference of average rainfall intensity. The difference in average rainfall intensity will provide us with one way to evaluate the potential changes in future precipitation characteristics and be applied in flood frequency prediction.

Create Flood Frequency Curve

Stream gauge data of a thirty-year period has been collected from the United States Geological Survey (USGS). Two locations, Littlefield and Virgin, were chosen in correspondence to St. George and Zion National Park. Two flood frequency curves were then created by the software PeakFQ using the similar statistical analysis to that of IDF curves. Furthermore, then same distribution, Log Pearson Type III, was used for both IDF curves and flood frequency curves.

Correlate precipitation characteristics with flood frequency

Observed precipitation characteristics such as IDF curves are currently being correlated with flood frequency curves using multiple linear regressions. We are using a weighted least squares linear regression to calibrate an average return period for the chosen future IDF curves and observation-based IDF curves. Since IDF and flood frequency curves are developed in an exponential matter, log transformations will allow us to convert back and forth between

exponential and linear models. Once one a correlation is developed for the observed data, it can be applied to the projected precipitation data to derive future flood frequency.

Hydrologic Analysis

To address extreme flooding through a hydrologic analysis, the hydrologic model, HEC-HMS (Hydrologic Engineering Center of the U.S. Army Corps of Engineers), was used to simulate the floods in the study watershed and determine future flood frequency. Three climate scenarios were selected for future flood predictions, including: (1) Natural Climate Change- No changes in emission scenarios and the frequency of ocean oscillations; (2) Anthropogenic Climate Change 1- Changes in emission scenarios from emissions path A1b and B1; (3) Anthropogenic Climate Change 2 - Changes in both emission scenarios and the frequency of ocean oscillations due to human-induced global warming. The hydrologic model has been parameterized to run for the Virgin River watershed and predict the floods for future flood frequency analysis and the results compared with the statistical analysis.

Principal Findings and Significance

The major findings so far can be summarized as follows:

- Precipitation frequency for future conditions shows more extreme storm events could occur in the future at the same frequency as present, or the same magnitude storm events could occur more frequently in the future;
- Using multiple climate models is likely to reduce the uncertainty in the precipitation frequency prediction;
- Flood frequency can be related to precipitation frequency. The relation of flood frequency versus precipitation IDF can be described by an exponential function;
- Flood frequency seems more related to gridded data based precipitation frequency than point data based precipitation frequency.

These findings can provide guidance is developing local precipitation and flood frequencies for future climate conditions to cope with the impact of climate change on future extreme storm and flooding events.

Information Transfer Activities

Research results of this project have been presented to hydrologists and colleagues from CUAHSI (Consortium of Universities for the Advancement of Hydrologic Science, Inc.) during CUAHSI's watershed master class in University of Arizona's Biosphere 2, and several local and national academic conferences. The publications are listed below:

Crystal DuBose, Li Chen, Peng Jiang, Zhongbo Yu. 2016. Estimating Future Flood Frequency in the Southwest. 2016 NWRA Annual Conference, March 1-2, 2016, Las Vegas, NV.

Crystal DuBose, Li Chen, Peng Jiang, Zhongbo Yu. 2016. A Statistical Analysis and Future Estimation of Flood Frequency in Relation to Extreme Precipitation Events for Regions in the Southwest. 11th Annual GeoSymposium, Department of Geoscience, University of Nevada Las Vegas, April 29, 2016, Las Vegas, NV.

Jiang, P., Yu, Z., Chen, L., Acharya, K. (2015). Changing storm properties and their impacts on the flood frequency in southern Nevada, UCOWR/NIWR/CUAHSI 2015 Annual Conference: Las Vegas, NV, June 15, 2015-June 18, 2015

Jiang, P., Acharya, K., Chen, L., Yu, Z. (2016). The impacts of changing storm properties on water fluxes and solute transport in Las Vegas Valley, Lower Colorado River Science Symposium, Las Vegas, NV, February 8-9, 2016

Chen, L., Sela, S., Svaroy, T., Assouline, S. Influences of vegetation and rainfall patterns on scaling in Hortonian rainfall-runoff processes. AGU Fall Meeting, December 15-19, 2015, San Francisco, CA.

Student Support

Funding of this project has provided support to a Masters graduate student Crystal DuBose in Department of Geoscience, University of Nevada Las Vegas, to work on this project as research towards her degree. A postdoctoral fellow, Dr. Peng Jiang with DRI, was partially supported by this project in his research.

Testing the Mortality and Settlement of Quagga Mussel Veliger Under Various Chemical Treatments

Basic Information

Title:	Testing the Mortality and Settlement of Quagga Mussel Veliger Under Various Chemical Treatments
Project Number:	2015NV205B
Start Date:	3/1/2015
End Date:	2/28/2016
Funding Source:	104B
Congressional District:	NV-003
Research Category:	Biological Sciences
Focus Category:	Invasive Species, Ecology, Water Quality
Descriptors:	None
Principal Investigators:	Kumud Acharya

Publications

There are no publications.

Final Report

Title: **Testing the Mortality and Settlement of Quagga Mussel Veliger under Various Chemical Treatments**

PI: Kumud Acharya, DRI

Problem and Research Objectives

The quagga mussel (*Dreissena bugensis*) is an aquatic invasive species that is spreading throughout Lake Mead and other western waterways. Lake Mead exhibits year round warm temperatures, high calcium levels and a lack of natural predators, all of which are very favorable conditions for their growth and spread. *Dreissena bugensis* reproduce and colonize hard surfaces and filter large amounts of water removing algae and other food sources for zooplankton. They disrupt the aquatic food chain and interfere with underwater infrastructure such as dam penstocks, intake pipes etc. Not only does Lake Mead present opportunities for *Dreissena bugensis* to cause ecological damage, but economic damage to infrastructure is also a major concern.

More than 80% of the water used in the Las Vegas Valley is obtained from Lake Mead. The Southern Nevada Water Authority (SNWA) pumping plant draws up to 300 million gallons per day. Based on 2007 estimate more than 700 million veligers per day are likely being pumped through the plant in the summer months. The *Dreissena bugensis* has become the most serious non-indigenous biofouling pest introduced into North American freshwater systems. It has the ability to tolerate a wide range of environmental conditions, is extremely adaptable and has very high growth and reproductive rates. It has the potential to significantly alter and degrade water delivery systems. It has been agreed by the researchers and water managers that the invasion of the lower Colorado River is a big challenge as these are the first large reservoir systems invaded by quagga mussels. The quagga mussels can be expected to result in the replacement of good forms of algae/phytoplankton by less desirable and harmful forms. Simultaneously, there will be an accumulation of large quantities of quagga mussel pseudofeces at the sediment surface adversely affecting water chemistry, transferring nutrients from pelagic to benthic water creating inhospitable environment for organisms and threaten the quality of the reservoir as a drinking water source. As the population continues to grow, it can transform the shoreline into numerous dead shells and requiring increased maintenance and costs.

The majority of research conducted on mussel infestations and their impacts has been specific to zebra mussels with much less emphasis on quagga mussels until they were discovered in Lake Mead. While the two species have many similar characteristics, existing research does not provide reliable information to predict the potential impacts of the current infestation in the Colorado River system or on the water suppliers that draw from this system. What is apparent, even at this early date, is that the quagga invasion is proceeding at a more rapid pace than was

experienced in the eastern United States. As a result, water managers have had little advanced notice prior to experiencing serious system impacts. The research conducted on quagga mussels in the last 6-7 years at Acharya Lab (DRI), SNWA, University of Nevada Las Vegas (UNLV), and University of Nevada Reno (UNR) have provided some valuable information on the life history characteristics such as growth rate (Link, 2010) and spawning (Schwaebe, 2012) and containment (Ultra Violet, Zequanox), however, the scientific community is nowhere near a breakthrough to stop these mussels either at the source or from spreading to other water bodies. On top of that the issue of quagga mussels has been even more urgent in the Southwestern U.S. recently especially in the wake of record drought in Southern California and precipitous decline of Lake Mead level and disappearing discharge in the Colorado River. Therefore the primary goal of this research is determination of quagga mussel veliger (larval stage) mortality under various chemical treatment conditions that can be deployed at the water treatment pumping stations.

The current practice of using chlorination has its own problem, such as excessive Trihalomethane (THM) formation. To avoid THM formation, we propose to test potassium permanganate and copper sulfate solutions to kill and prevent settlement of quagga mussel veligers. Potassium permanganate is a potent oxidizing agent that has been extensively used for the disinfection of bacteria, fungi, plants, and parasites. Primarily it inactivates pathogens via direct oxidation of cell material or destruction of specific enzymes by the permanganate ion (MnO_4^-) (Weber, 1972). Its application also leads to the precipitation of manganese dioxide, which is an additional method for removing microorganisms from drinking water (Cleasby et al. 1964). Though not a primary disinfectant, potassium permanganate has served as an alternative to pre-chlorination or other oxidants at stages of treatment where chemical oxidation is desired to control taste and odors as well as remove color, iron, manganese, and undesirable algae from the water (Chen and Yeh, 2005). In a secondary role, potassium permanganate has been useful in preventing formation of THMs and other disinfection byproducts (DBPs) via oxidation of precursors, reducing demand for other disinfectants (Bryant et al., 1992). Past studies have suggested that potassium permanganate may be effective against adult zebra mussels at continuous dosages ranging from 0.5 to 2.5 mg/L (Klerks and Fraleigh, 1991) as well as adult quagga mussels (Boelman et al. 1996), but its effect on veligers of either species at smaller dosages is not known, beyond an experiment studying its effectiveness against zebra mussel veligers (Matisoff et al., 1990).

Copper sulfate in dilute solutions has often been used in treating aquarium fish infested with parasites and as an aquatic herbicide. In pellet form, copper sulfate has served as an agent for eradicating large mollusk species in aquatic environments (Murray-Gulde et al., 2002). Most nuisance species have been controlled with relatively low concentrations (around 2 mg/L). Previous studies have also suggested that copper sulfate is effective at low concentrations against zebra mussel veligers and at high concentrations over long periods against adults (Kennedy et al., 2006). Some research has suggested that copper sulfate may work well with quagga mussels but that may depend on other water factors like temperature and calcium levels (Claudi et al., 2014).

Therefore, effective dosage of potassium permanganate and copper sulfate solutions to control quagga mussel veligers are studied.

Methodology

Water for all the assays was collected from Lake Mead on a regular basis. Lake Mead water was collected from a site on the lake shore near Lake Mead Marina and transported in 10 and 25 L plastic carboys. Carboys were triple rinsed with lake water prior to collection. Once in the laboratory, all water was filtered through a 35 micron mesh filter to remove veligers, competing zooplankton, and any other foreign bodies from the water. The filtered Lake Mead water (FLMW) was stored in covered 20 L plastic, opaque buckets with aeration and kept at ambient laboratory temperature (~20 ^{o}C) during the course of the experiment.

All veligers used in this study were collected from Lake Mead in bulk using a 44 micron plankton net. The plankton net was sunk to approximately 12 meters below the water surface after being thrown out into the lake and towed horizontally. The site of collection was at Lake Mead Marina for summer months. Samples were transferred into plastic sampling bottles and sent to the laboratory in chilled coolers. Once in the laboratory, samples were combined into Imhoff cones and allowed to settle for at least one hour at ambient laboratory temperature before identification and selection of specimens.

Specimens selected for the experiment were active quagga mussel veligers of the umbonal stage of growth with a shell area of ~0.040 mm^2. A transfer pipette was used to transfer water samples from the bottom of the Imhoff cones onto watch glass for observation. Organisms were identified as quagga mussel veligers using polarized light microscopy, which identifies them by the characteristic “iron cross” patterns on their shells (Johnson, 1995). Large and active veligers of the umbonal stage were hand selected and transferred individually from the watch glass to petri dishes via pipette. The umbonal stage is easily identified as the hinge line grows from straight (in the previous life stage) to curved outwards as the umbo become distinct (Nichols and Black, 1994). The shell of the veliger remained transparent at this stage, allowing for visual observations of digestion and organ health for the sake of determining mortality rates.

Specimens were transferred to quadruple replicate petri dishes (28 veligers per treatment) filled with 50 mL of FLMW with the gradient of concentrations of the experimental treatment chemicals, potassium permanganate ($KMnO_4$) and copper sulfate ($CuSO_4$). Three set of five different concentrations for each treatment chemical were tested. Concentrations used in the experiments are listed in Table 1. The amended treatment waters were made in separate batches for each treatment dosage prior to the start of the experiment. The batches were made through serial dilution from the solution with the highest concentration for any given set of the experiments. Due to the small amount of chemicals used, slight differences in actual concentrations may exist. Amended treatment waters were stored in the incubator for the duration of the mortality experiments.

During the assays, ammonia levels and pH were tested to ensure that a stable environment is maintained in each water sample. Ammonia was tested for levels above 1 mg/L and pH was tested for anything outside the normal range for Lake Mead water.

Table 1. Concentrations used in the experiments

Chemical	Set Number	Concentrations of Treatment (mg/L)				
		1	2	3	4	5
$KMnO_4$	1	0.1	0.5	1.0	2.0	4.0
	2	1.0	2.0	4.0	6.0	8.0
	3	8.0	12.0	16.0	24.0	32.0
$CuSO_4$	1	0.01	0.1	0.5	1.0	2.0
	2	0.5	1.0	2.0	4.0	8.0
	3	8.0	10.0	12.0	16.0	20.0

Exposure times for all individual experimental groups were 1.0, 4.0, 8.0, 24, 48, and 72 hours, with control groups handled in the same way as the other groups but only with FLMW. For exposure periods after the initial 8.0 hours, all experimental and control groups were kept in an incubator at a constant temperature of 20 ^{o}C and subjected to 12 hour day/night cycles with LED lighting in order to duplicate their natural environment until the maximum 72 hour exposure time had been reached. After every exposure period, each group was examined for mortality rates. A veliger was counted as deceased when it had a lack of internal movement for at least two minutes even with external stimulation and had a shell that was empty or had visible signs of decomposition. Results were analyzed as a comparison across all six water treatments (five chemical treatments and the control).

Principal Findings and Significance

Figures 1 and 2 show average veliger mortalities of different concentrations of potassium permanganate and copper sulfate, respectively. The mortality of the control treatment was less than 10% throughout the experiments indicating that quagga mussels have successfully established in the experimental condition. Mortality of veligers in the potassium permanganate assays shows apparent dependence on chemical dosages, as all treatment groups observed at the same times displayed increasing mortality with increasing chemical concentration (Figure 1). Notable exceptions were the 1.0 and 2.0 mg/L treatments of experiment set 1, which both had slightly higher mortality than the 4.0 mg/L treatment at the end of the experiment period (Figure 1a). The mortalities in any dosage were high during the first eight hours. This trend was more obvious with medium and high concentrations (Figures 1b and 1c). The average final mortality rates for veligers in the potassium permanganate treatments was 25% for concentrations below 8.0 mg/L and 55% for those above 8.0 mg/L. The highest mortality rate was approximately 78% after 72 hours of exposure to 32.0 mg/L potassium permanganate.

The copper sulfate treatments followed similar trends to potassium permanganate with even fewer individuals surviving and average final mortality rates of 35% for concentrations below 8.0 mg/L and 60% for those above 8.0 mg/L. Similar to potassium permanganate, mortality was high at the beginning and reduced as exposure time increased. However, the effect of potassium permanganate after eight hours of exposure time was slightly higher than that of potassium permanganate. It is also worth noting that the copper sulfate consistently had higher rates of mortality compared to potassium permanganate across all treatments.

Both potassium permanganate and copper sulfate treatments failed to reach 50% mortality at or below 8.0 mg/L concentration. Only when the experiments were expanded to include higher concentrations, treatment levels begin showing mortality for the majority of veligers exposed to the chemicals. The lethal 50% concentration (LC_{50}) after 72 hours for copper sulfate was substantially lower than for potassium permanganate. The final LC_{50} for copper sulfate ended up being around 9.5 mg/L, while the final LC_{50} for potassium permanganate was 14.6 mg/L.

Both pH and ammonia in the control treatment water remained constant throughout the assays. The pH ranged from 7.5 to 8.0 for the Lake Mead treatments. Ammonia remained below 1 mg/L throughout the entire course of the assays.

Statistical analysis was conducted to compare the means for the mortality using Wilcoxon rank score since the data did not represent a normal distribution. Results of low concentrations of potassium permanganate (set 1) were not statistically different. Statistically significant differences were observed for results of medium concentrations of potassium permanganate (set 2) after eight hours of exposure time and high concentrations of potassium permanganate (set 3) throughout the exposure. The results indicated that mortality was affected by potassium permanganate above certain level (≈ 0.5 mg/L) of dosages. On the other hand, results of low (set 1) and medium (set 2) concentrations of copper sulfate showed statistically significant differences after 24 hours of exposure time. Results of high concentrations of copper sulfate (set 3) showed statistically significance throughout the exposure time. The result implies that copper sulfate treatments have more effect on mortality of veligers compared with potassium permanganate treatments since low dosages with longer exposure periods also showed statistically significant differences.

The results of the mortality assay with the amended chemical treatments at the prescribed concentrations partially supported the hypothesis concerning the existence of a direct relationship between mortality and chemical dosages. As chemical concentrations increased, whether it was potassium permanganate or copper sulfate, so did the mortality rate. In addition, we saw that the relative mortality rates for copper sulfate treatments were higher than those for potassium permanganate, as suggested by some studies into the toxicity of copper sulfate on freshwater benthic invertebrates at concentrations of 0.1 to 0.8 mg/L (Montz et al., 2010). One noticeable observation was that veligers seemed to be discolored but still alive when they were exposed to potassium permanganate. Therefore, low chemical dosage of potassium permanganate may not be lethal but cause some effects on veligers.

Our results for the potassium permanganate assays was similar to the zebra mussel veligers results reported by Matisoff et al. (1990), which showed about 27% of mortality rates with concentrations ranging from 0.5 to 2.5 mg/L. Furthermore, mortality of quagga veligers with up to 8.0 mg/L potassium permanganate concentrations throughout the first 72 hours of exposure time was reported as approximately 40% by Coyle et al. (2014), which was similar to our results (about 45% mortality with 8.0 mg/L potassium permanganate for 72 hours exposure).

Similarly, Claudi et al. (2014) reported low effectiveness of copper sulfate treatments required to kill quagga mussel adult within 72 hours of exposure time. However, they were also pointing out that the use of copper sulfate products such as Natrix™ and EarthTec® work better on killing quagga mussel adult. Similar results were also reported by Watters et al. (2013). They reported that near total mortality of quagga mussel veligers was observed with 5.0 mg/L EarthTec® product, which is a mixture of copper sulfate pentahydrate and base acid, after 72 hours of exposure. Furthermore, use of different copper compounds also affect mortality of mussels differently as reported by Kennedy et al. (2006). They used low concentration of chelated copper and achieved high mortality of newborn zebra mussels within hours.

Overall, the results from both the potassium permanganate and copper sulfate assays suggest that achieving total mortality with these two chemical treatments may be impractical. Our study limited exposure times to 72 hours since it is a reasonable residency time for chemicals in drinking water after application. Both assays only achieved mortality above 50% after 72 hours with dosages above 8.0 mg/L, and even then only 75-80% mortality was achieved at the maximum concentrations (20.0 mg/L for copper sulfate, 32.0 mg/L for potassium permanganate). Such concentrations would be expensive, and may cause threaten non-target species, outweighing the benefits of quagga mussel eradication.

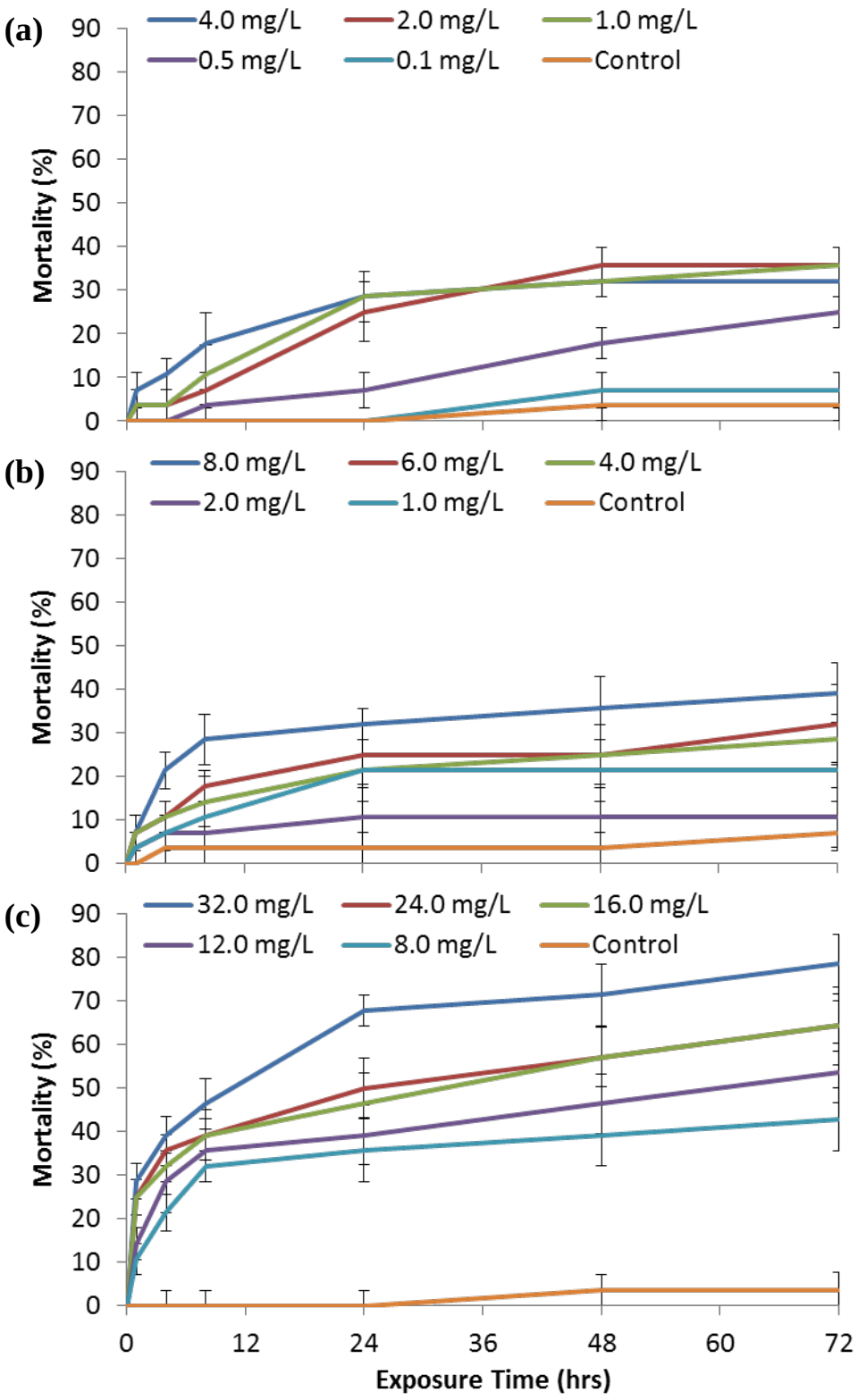

Figure 1. Average mortality rate of potassium permanganate treatments with (a) low, (b) medium, and (c) high concentrations. Error bars represent the standard error with four sample size.

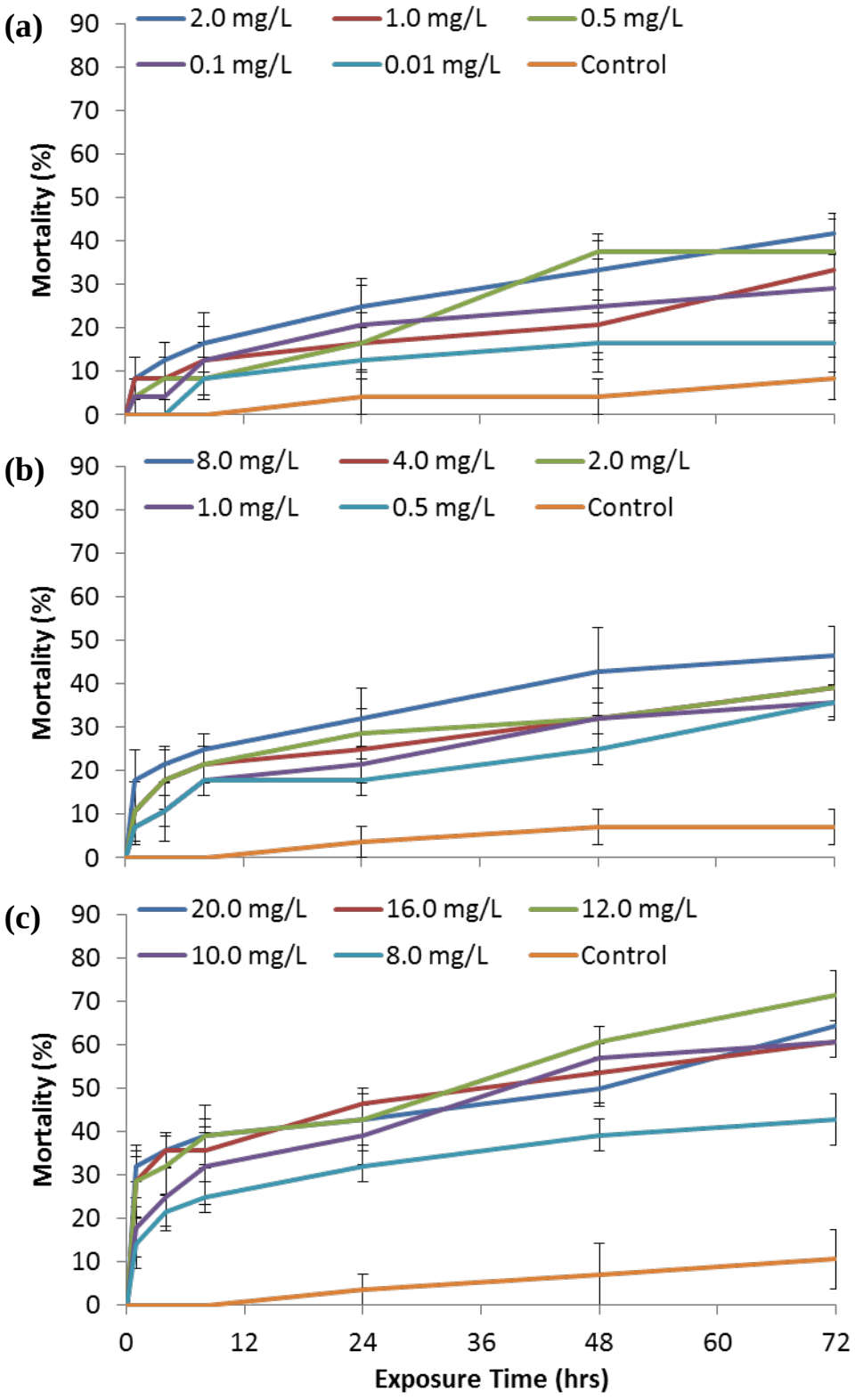

Figure 2. Average mortality rate of copper sulfate treatments with (a) low, (b) medium, and (c) high concentrations. Error bars represent the standard error with four sample size.

Summary and Further Work

The study showed that effectiveness of potassium permanganate and copper sulfate on mortality of quagga mussel veligers is low and it may not be practical to use for achieving total mortality. However, further studies are suggested, since the effects of the two chemicals on quagga mussel veligers are still relatively unknown. Studies into alternative treatments such as alternative chemical compounds may also be warranted to have a better understanding of possible control methods for quagga mussels in Lake Mead and the western United States.

Information Transfer Activities

Results will be presented in an appropriate conference available in near future.

Student/other Support

The project supported an undergraduate student's summer internship at DRI (Michael Zhou, Princeton University). The project also supported two graduate students, Sachiko Sueki and YuZhen Feng (University of Nevada, Las Vegas) partially.

References

Boelman, S.F., Neilson, F.M., Dardeau, E.A., and Cross, T. 1996. Zebra mussel (*Dreissena polymorpha*) control handbook for facility operators, first edition. Miscellaneous Paper EL-97-1, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.0.

Chen, J. J., and Yeh, H. H. 2005. The mechanisms of potassium permanganate on algae removal. *Water Research 39*(18): 4420-4428.

Claudi, R., Prescott, T. H., Mastisky, S., and Coffey, H. 2014. Efficacy of copper based algaecides for control of Quagga and Zebra Mussels. *Report for California department of water resources, aquatic nuisance species program*, 1-58.

Cleasby, J.L., Baumann, E.R., and Black, C.D. 1964. "Effectiveness of Potassium Permanganate for Disinfection." *J. AWWA.* 56: 466-474.

Coyle, B. P., Lord, P. H., Wong, W. H., and Albright, M. F. 2014. Potassium permanganates effect on zebra mussel adults and veligers. Retrieved from http://www.oneonta.edu/academics/biofld/publications.asp.

Bryant, E.A., Fulton, G.P. and Budd, G.C. 1992. *Disinfection Alternatives for Safe Drinking Water*. Van Nostrand Reinhold, New York, NY.

Johnson, L.E. 1995. Enhanced early detection and enumeration of zebra mussel (Dreissena spp.) veligers using cross-polarized light microscopy. *Hydrobiologia, 312*(2): 139-146.

Kennedy, A.J., Millward, R.N., Stevens, J.A., Lynn J.W., and Perry, K.D. 2006. Relative sensitivity of zebra mussel (Dreissena polymorpha) life-stages to two copper sources. *J. Great Lakes Res.* 32: 596-606

Klerks, P.L., and Fraleigh, P.C. 1991. Controlling Adult Zebra Mussels with Oxidants. *J.AWWA.* 83(12): 92-100.

Link, C. L. 2010. Filtration and growth rate of Lake Mead quagga mussels (Dreissena bugensis) in laboratory studies and analyses of bioaccumulation (M.S. thesis). ProQuest, UMI Dissertation Publishing. UMI number: 1479079.

Matisoff, G., Fraleigh, P., Greenberg, A.B., Gubanich, G., Hoffman, G.L., Klerks, P.L., ... & Wenning, M.E. 1990. Controlling zebra mussels at water treatment plant intakes—Part II. Veliger Dose/Response Static tests, Abstract, International Macrofouling Symposium. *Electric Power Research Institute, Orlando, FL.*

Montz, G.R., Hirsch, J., Rezanka, R., and Staples, D.F. 2010. Impacts of copper on a lotic benthic invertebrate community: response and recovery. *Jour. Fresh. Ecol.* 25(4): 575-587.

Murray-Gulde, C.L., Heatley, J.E., Schwartzman, A.L., and Rodgers Jr, J.H. 2002. Algicidal effectiveness of clearigate, cutrine-plus, and copper sulfate and margins of safety associated with their use. *Archives of Environmental Contamination and Toxicology 43*(1): 19-27.

Nichols, S.J., and Black, M.G. 1994. Identification of larvae: the zebra mussel (*Dreissena polymorpha*), quagga mussel (*Dreissena rosteriformis bugensis*), and Asian clam (Corbicula fluminea). *Canadian Journal of Zoology 72*(3): 406-417.

Schwaebe, L.M.S. 2012. *Spawning, veliger growth and desiccation of Dreissena bugensis* (M.S. thesis). ProQuest, UMI Dissertation Publishing. UMI number: 1517008.

Watters, A., Gerstenberger, S.L., and Wong, W.H. 2013. Effectiveness of EarthTec® for killing invasive quagga mussels (*Dreissena rostriformis bugensis*) and preventing their colonization in the Western United States. *Biofouling*, *29*(1), 21-28.

Weber Jr., W.J., 1972. Physicochemical Processes in Water Quality Control. John Wiley & Sons, New York, NY.

Geologic and Seismic Effects of Large Scale Groundwater Withdrawal from Northeastern Nevada Basins

Basic Information

Title:	Geologic and Seismic Effects of Large Scale Groundwater Withdrawal from Northeastern Nevada Basins
Project Number:	2015NV206B
Start Date:	3/1/2015
End Date:	2/28/2016
Funding Source:	104B
Congressional District:	NV-002
Research Category:	Climate and Hydrologic Processes
Focus Category:	Groundwater, Water Use, Water Quantity
Descriptors:	None
Principal Investigators:	Rina Schumer

Publications

1. Geologic and seismic effects of large-scale groundwater withdrawal from northeastern Nevada basins B. Anderson R. Schumer, S. McCoy Nevada Water Resources Association Annual Conference, March 1-3, 2016, Las Vegas, NV
2. Geologic and seismic effects of large-scale groundwater withdrawal from northeastern Nevada basins B. Anderson R. Schumer, S. McCoy UCOWR/NIWR Conference on June 21-13, 2016, Pensacola Beach, FL.

Geologic and Seismic Effects of Large Scale Groundwater Withdrawal from Northeastern Nevada Basins

Rina Schumer
Division of Hydrologic Sciences
Desert Research Institute
Project dates: March 1, 2015 – February 28, 2016

Problem and research objectives

In 2012, the Nevada state engineer approved annual withdrawals of 61,127 acre feet of groundwater from Spring Valley, 5,235 acre feet from Cave Valley, 11,584 acre feet from Dry Lake Valley and 6,042 acre feet from Delamar Valley in Lincoln and White Pine Counties for transfer to the Las Vegas water system.

What magnitude of groundwater withdrawal and reduction of pore pressure within large capture zones in eastern Nevada basins (specifically, Spring Valley basin) is sufficient to modify the crustal stress field, affect basin faults, and possibly induce seismicity? How does this compare with proposed withdrawal rates?

The balance between pore water pressure and aquifer matrix stress in balancing the force imposed by an overburden is a fundamental concept in hydrogeology. In particular, it explains why excessive groundwater withdrawal has led to surface deformation and subsidence in many areas. This phenomenon has been observed in Nevada, where groundwater from alluvial aquifers has been an important water source throughout Nevada, and in the Las Vegas Valley groundwater withdrawal has led to cracks near preexisting faults and also ground subsidence of more than six feet. While local subsidence issues resulting from pumping have long been well known, more recently it has been determined that changes in surface mass (glaciers, surface water bodies) and groundwater withdrawal can affect regional fault motion and seismicity [*Gonzalez et al.*, 2012; *Hampel and Hetzel*, 2006]This can been explained by unloading of mass from the crust affecting the vertical stress state of the lithosphere as induced flexure and rebound alters the horizontal stress [*Hetzel and Hampel*, 2005].While the effects of altering eastern Nevada surface hydrology and ecology have been a focus of environmental impact statements, the possibility of affecting fault slip rates and possibility of inducing seismicity has not. The technology to evaluate this possibility has been developed in the last decade and will provide a cutting-edge research project for a graduate student who will be trained with cross-disciplinary expertise in structural geology, rheology, and hydrogeology.

The project objectives were to

1. Use publically available hydrologic data to determine the order of magnitude of overburden mass loss in Spring Valley under future pumping scenarios.

2. Use existing analytical equations parameterized with values from regional studies to relate groundwater withdrawal to unloading of the crust and its effect on vertical slip rates.

3. Compare estimates of mass-unloading necessary to modulate seismicity in the Spring Valley with mass reduction estimated from proposed pumping.

Methods

Hydrologic Data

An existing USGS MODFLOW model of the Spring Valley area [*Halford and Plume*] was used to evaluate aquifer mass loss response to long-term pumping. 3D grid data used to calculate mass loss and the change in force from MODFLOW include grid size, drawdown, and specific yield. The equivalent force N_i generated by changing mass of each grid cell i can be calculated using

$$N_i = (area_i) \times (drawdown_i) \times (specific\ yield_i) \times \rho \times g$$

Where ρ is water density and g is gravity (1,000 kg/m^3 and 9.81 m/s^2, respectively).

Calculation of normal and shear stress acting on the fault

A point load exerts a stress on points directly beneath itself, but the influence from a single point load is felt by surrounding nodes as well. Thus, to calculate the change in stress at cells across a fault, the effect of the change in load acting at every cell with mass removal must be considered. By the principle of superposition, the effect of the force exerted by all other cells can be summed to resolve the cumulative state of stress at any point [*Jaeger et al.*, 2007]. We assume that the force at each cell can be approximated as a point when calculating stress at a large distance $r=x^2+y^2+z^2$. The components of the three-dimensional symmetric stress tensor

$$\begin{bmatrix} \tau_{xx} & \tau_{xy} & \tau_{xz} \\ \tau_{yx} & \tau_{yy} & \tau_{yz} \\ \tau_{zx} & \tau_{zy} & \tau_{zz} \end{bmatrix} = \begin{bmatrix} \tau_{xx} & \tau_{xy} & \tau_{xz} \\ \tau_{xy} & \tau_{yy} & \tau_{yz} \\ \tau_{xz} & \tau_{yz} & \tau_{zz} \end{bmatrix}$$

at a point in a homogenous, isotropic, elastic, half-space resulting from a point-load at the surface of the half-space can be computed as

$$\tau_{xx} = \frac{N}{2\pi}\left[\frac{3x^2z}{r^5} + \frac{(1-2v)(y^2+z^2)}{r^3(z+r)} - \frac{(1-2v)z}{r^3} - \frac{(1-2v)x^2}{r^2(z+r)^2}\right]$$

$$\tau_{yy} = \frac{N}{2\pi}\left[\frac{3y^2z}{r^5} + \frac{(1-2v)(x^2+z^2)}{r^3(z+r)} - \frac{(1-2v)z}{r^3} - \frac{(1-2v)y^2}{r^2(z+r)^2}\right]$$

$$\tau_{zz} = \frac{N}{2\pi}\left[\frac{3z^3}{r^5}\right]$$

$$\tau_{xy} = \frac{N}{2\pi}\left[\frac{3xyz}{r^5} - \frac{(1-2v)xy(z+2r)}{r^3(z+r)^2}\right]$$

$$\tau_{yz} = \frac{N}{2\pi}\left[\frac{3yz^2}{r^5}\right]$$

$$\tau_{zz} = \frac{N}{2\pi}\left[\frac{3xz^2}{r^5}\right],$$

where ν is the Poisson's ratio of the material.

If the orientation of the force/stress grid is not aligned with the strike and dip of the fault, the stress tensor must undergo coordinate transformation to properly distinguish normal from shear stress acting on the fault plane. Coordinate rotation for a three dimensional tensor is computed using

$$T' = L\,T\,L^T,$$

where *T'* is the transformed matrix, *T* is the original matrix, *L* is the transformation matrix, and L^T is the transpose of the transformation matrix [*Jaeger et al.*, 2007].

Mechanics of stress transfer/stress change

The first-order controls on the stick-slip pattern of motion along a fault is governed by two opposing forces. First, shear stress, which is often due to very slow tectonic motion, drives motion along the fault. This is opposed by the strength of the fault, which is a function of the normal stress acting on the fault multiplied by a coefficient of friction of the fault [*Jaeger et al.*, 2007; *Twiss and Moores*, 1992]. When the strength of the fault is greater than the shear stress, the fault is locked and strain accumulates over time. A decrease in normal stress unclamps the fault, which allows for failure at lower levels of shear stress. The normal stress is typically a function of the lithostatic stress acting on the fault, and it varies inversely with the soil-water pore-pressure: the presence of pore-water pressure will decrease the effective normal stress acting on the fault [*Twiss and Moores*, 1992]. The resulting governing equation for the state of stress on a fault is given by the coulomb failure function (CFF):

$$\sigma_c = \tau_s - \mu_f(\sigma_n - p) + s$$

Where σ_c is the coulomb stress, τ_s is the shear stress on the fault, μ_f is the coefficient of friction (material property), τ_n is the normal stress on the fault, *p* is pore pressure, and *s* is material cohesion. When the shear stress overcomes the strength of the fault, the fault ruptures and typically results in an earthquake.

The subsurface state of stress on faults at seismogenic depth is close to the strength of the fault, even in stable intraplate areas. This means that only a small CFF perturbation in the right direction has the potential to bring a fault to failure. Earthquakes themselves have given clues to

how much change in CFF this may be. Large earthquakes release stress and can in turn increase stress on surrounding faults [*Stein*, 1999]. Correlation of spatial patterns of static stress changes in the crust with locations of aftershock occurrence have revealed 'triggering thresholds', or static stress changes in the subsurface above which there is a higher frequency of earthquake occurrence [*Anderson and Johnson*, 1999a; *Hardebeck et al.*, 1998; *King et al.*, 1994; *Lockner and Beeler*, 1999; *Reasenberg and Simpson*, 1992]. A static stress change as low as 0.01 MPa (10 kPa or 0.1 bar) marks the boundary between a low to high degree of correlation with aftershock occurrence [*Anderson and Johnson*, 1999a], thereby stress changes on a fault above 0.01 MPa value represent a likely chance of failure.

Principal findings and significance

A synthesis of related studies on unloading and fault slip is summarized in Table 1. The static stress changes that caused rupture (Table 1) are similar to those thresholds discussed in the aftershock studies. Due to the prominent similarity, the threshold of 0.01 MPa ΔCFF static stress change that was statistically determined [*Anderson and Johnson*, 1999a; b] can be applied as a useful threshold to mark the lower end of the ΔCFF range for potential danger of fault failure in future large-scale groundwater pumping scenarios. While this threshold does not guarantee a fault rupture, this value is used as a "danger zone" threshold, where a ΔCFF of this magnitude puts the fault in a category of a potential failure.

Table 1. Comparison of studies implicating groundwater pumping as cause of earthquake rupture

Study	Fault type	Unloading Source	Unloading Amount	Unloading Area	Unloading Time Period	ΔCFF on applicable fault(s)
Gonzalez et al.	Strike Slip - rake = 36°	Agricultural GW pumping in Alto-Guadelentin basin	20 km^3	80 km^2	50 yrs	5-10 kPa
Trugman et al.	Strike Slip	Cerro Prieto Geothermal Field	0.36 km^3	16 km^2	~37 yrs	10-15 kPa/yr
Kundu et al.	Thrust	Agricultural GW pumping from Indo-Gangetic Plains	1240 km^3	1.5e5 km^2	~55 yrs	3-8 Kpa
Amos et al.	Thrust / Strike Slip	California Central Valley (San Joaquin Basin)	160 km^3	27,000 km^2	~150 yrs	10-15 kPa

Using the idea of a "danger zone" above, preliminary results suggest that proposed groundwater pumping in Spring Valley puts local faults within this danger zone after 50-100 years. Please refer to the Master's Thesis of Brian Anderson for final results, expected in August 2016.

Information Transfer Activities

A Master's thesis and one manuscript based on this research will be produced from this work.

Conference Proceedings:

Geologic and seismic effects of large-scale groundwater withdrawal from northeastern Nevada basins – B. Anderson R. Schumer, S. McCoy Nevada Water Resources Association Annual Conference, March 1-3, 2016, Las Vegas, NV

Geologic and seismic effects of large-scale groundwater withdrawal from northeastern Nevada basins – B. Anderson R. Schumer, S. McCoy UCOWR/NIWR Conference on June 21-13, 2016, Pensacola Beach, FL.

Student Support

This project supported Brian Anderson, a Master's student in the Graduate Program of Hydrologic Sciences at the University of Nevada, Reno. His thesis defense is scheduled for August, 2016. This year Brian attended and presented material at the Nevada Water Resources Association Annual Conference and will be presenting at the 2016 UCOWR/NIWR Conference. We expect a manuscript based on his thesis to be submitted to the Journal of Geophysical research in the fall.

References

Anderson, G., and H. Johnson (1999a), A new statistical test for static stress triggering: Application to the 1987 Superstition Hills earthquake sequence, *Journal of Geophysical Research-Solid Earth*, *104*(B9), 20153-20168, doi: 10.1029/1999jb900200.

Anderson, G., and H. Johnson (1999b), A new statistical test for static stress triggering: Application to the 1987 Superstition Hills earthquake sequence, *Journal of Geophysical Research-Solid Earth*, *104*, 20153-20168.

Gonzalez, P. J., K. F. Tiampo, M. Palano, F. Cannavo, and J. Fernandez (2012), The 2011 Lorca earthquake slip distribution controlled by groundwater crustal unloading, *Nature Geoscience*, *5*(11), 821-825, doi: 10.1038/ngeo1610.

Halford, K. J., and R. W. Plume Potential Effects of Groundwater Pumping on Water Levels, Phreatophytes, and Spring Discharges in Spring and Snake Valleys, White Pine County, Nevada, and Adjacent Areas in Nevada and Utah 52 pp.

Hampel, A., and R. Hetzel (2006), Response of normal faults to glacial-interglacial fluctuations of ice and water masses on Earth's surface, *Journal of Geophysical Research-Solid Earth*, *111*(B6), doi: 10.1029/2005jb004124.

Hardebeck, J. L., J. J. Nazareth, and E. Hauksson (1998), The static stress change triggering model: Constraints from two southern California aftershock sequences, *Journal of Geophysical Research-Solid Earth*, *103*(B10), 24427-24437, doi: 10.1029/98jb00573.

Hetzel, R., and A. Hampel (2005), Slip rate variations on normal faults during glacial-interglacial changes in surface loads, *Nature*, *435*(7038), 81-84, doi: 10.1038/nature03562.

Jaeger, J. C., N. G. W. Cook, and R. Zimmerman (2007), *Fundamentals of Rock Mechanics, 4th edition*, 488 pp., Wiley-Blackwell.

King, G. C. P., R. S. Stein, and J. Lin (1994), STATIC STRESS CHANGES AND THE TRIGGERING OF EARTHQUAKES, *Bulletin of the Seismological Society of America*, *84*(3), 935-953.

Lockner, D. A., and N. M. Beeler (1999), Premonitory slip and tidal triggering of earthquakes, *Journal of Geophysical Research-Solid Earth*, *104*(B9), 20133-20151, doi: 10.1029/1999jb900205.

Reasenberg, P. A., and R. W. Simpson (1992), RESPONSE OF REGIONAL SEISMICITY TO THE STATIC STRESS CHANGE PRODUCED BY THE LOMA-PRIETA EARTHQUAKE, *Science*, *255*(5052), 1687-1690, doi: 10.1126/science.255.5052.1687.

Stein, R. S. (1999), The role of stress transfer in earthquake occurrence, *Nature*, *402*(6762), 605-609, doi: 10.1038/45144.

Twiss, R. J., and E. M. Moores (1992), *Structural Geology*, 500 pp., W.H. Freeman and Company, New York.

Information Transfer Program Introduction

GreenPower Mission Statement: To support Nevada s preK-12 educators in science-based, environmental education by providing the tools, resources, and knowledge they need.

What is a Green box? Developed for K-12 educators, Green Boxes introduce environmentally focused topics that emphasize sustainable practices and natural resource conservation. Each Green Box is centered around a green topic written for either a particular grade or range of grade levels. Every box contains the material and curriculum needed to engage students in hands-on activities with real-life applications that are both informative and fun! Best of all, our program is completely free for educators to use. We have a large list of titles available and more coming online all the time.

Who Creates Green boxes? Green Boxes are made by educators for educators. Once created, each box is then vetted through our Advisory Green Box Committee, which is comprised of education and environmental experts. Upon committee approval, the Green Boxes then make their way to GreenPower schools.

What Does a Greenbox Contain? Curriculum aligned to Nevada State, Common Core, and Next Generation Science Standards. Enough content for 1 2 weeks of instruction, complete with hands-on-activities and projects. Most materials needed for activities (both consumables and non-consumables). A flash drive with curriculum and supplemental materials.

Greenpower-Empowering Nevada

Basic Information

Title:	Greenpower-Empowering Nevada
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DRI GreenPower Green Box

Watersheds

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Watersheds

Created by:

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&
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Learning Cycle 5E Lesson

Based upon and modified from Roger Bybee* (1990)

**Bybee, R & Landes, N. (1990). Science for life and living: An elementary school science program from Biological Sciences Curriculum Study (BSCS). American Biology Teacher. 52 (2). 92-98.*

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Introduction to the Green Box

Welcome to the Watersheds Green Box!

Since its inception in 2000, GreenPower has been the outreach program for the Desert Research Institute (greenpower.dri.edu). We aim to increase scientific knowledge and understanding of the earth's environment, promoting preservation of diverse ecosystems, advancing responsible resource management, and improving human health and welfare. In addition to our Green Boxes, we offer workshops and trainings throughout the year and various school support by providing speakers, grants, and field trip opportunities as well.

The Watersheds unit is composed of lessons that align with the Next Generation Science Standards (NGSS).

Upon completion of this unit, students will be able to:

- Understand the concept of mimicry.
- Recognize how the external structure of plants and animals help the organism survive and grow.
- Explain how the external structure of a plant or animal determines its function.
- Design a solution to a human problem using their knowledge of mimicry, structure, and function.

This Green Box was created Aarone Dehne and Brian Fitzgerald. Aaron is a chemistry teacher at Clark High School in Las Vegas, NV. He has a B.S. in both Chemistry and Teaching Broadfield Science and a M.A. in Curriculum and Instruction. Brian's interests include surface water quality sampling, data acquisition, analysis, work-up, and report writing for a multitude of studies. He has a B.S. in Mechanical Engineering and a M.S. in Environmental Sciences and Health.

We hope you and your students enjoy using this Green Box!

Lesson 1

What Causes Eutrophication?

Next Generation Science Standards

- HS-LS1-5. Use a model to illustrate how photosynthesis transforms light energy into stored chemical energy. [Clarification Statement: Emphasis is on illustrating inputs and outputs of matter and the transfer and transformation of energy in photosynthesis by plants and other photosynthesizing organisms. Examples of models could include diagrams, chemical equations, and conceptual models.]

- HS-LS2-4. Use mathematical representations to support claims for the cycling of matter and flow of energy among organisms in an ecosystem. [Clarification Statement: Emphasis is on using a mathematical model of stored energy in biomass to describe the transfer of energy from one trophic level to another and that matter and energy are conserved as matter cycles and energy flows through ecosystems. Emphasis is on atoms and molecules such as carbon, oxygen, hydrogen and nitrogen being conserved as they move through an ecosystem.]

- HS-LS2-6. Evaluate the claims, evidence, and reasoning that the complex interactions in ecosystems maintain relatively consistent numbers and types of organisms in stable conditions, but changing conditions may result in a new ecosystem. [Clarification Statement: Examples of changes in ecosystem conditions could include modest biological or physical changes, such as moderate hunting or a seasonal flood; and extreme changes, such as volcanic eruption or sea level rise.].

- HS-LS4-5. Evaluate the evidence supporting claims that changes in environmental conditions may result in: (1) increases in the number of individuals of some species, (2) the emergence of new species over time, and (3) the extinction of other species. [Clarification Statement: Emphasis is on determining cause and effect relationships for how changes to the environment such as deforestation, fishing, application of fertilizers, drought, flood, and the rate of change of the environment affect distribution or disappearance of traits in species.].

- HS-ESS2-2. Analyze geoscience data to make the claim that one change to Earth's surface can create feedbacks that cause changes to other Earth systems. [Clarification Statement: Examples should include climate feedbacks, such as how an increase in greenhouse gases causes a rise in global temperatures that melts glacial ice, which reduces the amount of sunlight reflected from Earth's surface, increasing surface temperatures and further reducing the amount of ice. Examples could also be taken from other system interactions, such as how the loss of ground vegetation causes an increase in water runoff and soil erosion; how dammed rivers increase groundwater recharge, decrease sediment transport, and increase coastal erosion; or how the loss of wetlands causes a decrease in local humidity that further reduces the wetland extent.]

Background Knowledge

Teacher:

Lakes and other waterways can be classified into three types: oligotrophic, mesotrophic, and eutrophic. Oligotrophic refers to waterbodies with no excess nutrients and, therefore, not excessive plant growth and a wide diversity of plant and animal life. Oligotrophic waterways typically are deep and clear, with colder temperatures; Lake Tahoe is a good example of an oligotrophic lake. The opposite of oligotrophic is eutrophic. A eutrophic waterway has an excess of nutrients, which results in excessive plant growth and low biodiversity. Famous examples of eutrophic waterways include the Chesapeake Bay, the dead zone at the mouth of the Mississippi River in the Gulf of Mexico, and the Sacramento-San Joaquin delta. Eutrophic lakes typically are shallow, have warmer temperatures, have mucky bottoms, and experience algal blooms. In the middle of these two extremes are mesotrophic waterways. Mesotrophic lakes contain some excess nutrients and plant growth.

Some areas of the world are so rich in nutrients as a result of human disturbance that they are classified as hypereutrophic. To give some scale, a typical eutrophic lake registers phosphates at >50 µg/L (parts per billion [ppb]); a hypereutrophic lake can have phosphate concentrations > 100 µg/L. Eutrophication at these levels causes widespread algal blooms because of the excess of nutrients. These algal blooms significantly reduce the light that penetrates the water, killing many aquatic plant species. When the algae eventually die, they sink to the bottom of the lake and decompose. The decomposition process uses up dissolved oxygen from the water and turns it into gaseous carbon dioxide, effectively removing dissolved oxygen from the lake bottom. The lower oxygen levels can lead to regular fish kills.

Eutrophication is a natural process, though this process is usually accelerated by human interactions with the environment. It is estimated that 53% of lakes in the

United States are eutrophic (International Lake Environment Committee (ILEC)/ Lake Biwa Research Institute n.d.).

Eutrophication is usually due to an input of excess nutrients like nitrate and phosphate that are used to enhance agricultural output or to make residential lawns greener. It is usually phosphate that limits algae production in many lakes, but in some conditions nitrogen is the limiting nutrient. The limiting nutrient is a nutrient whose concentration in the environment of an organism determines the growth and productivity of that organism.

The main source of phosphates going into lakes is usually nonpoint pollution. Unlike point source pollution, which enters the water from specific locations (such as a regulated discharge pipe into the watershed), nonpoint source pollution comes from surface runoff from farms, lawns, septic systems, and anything dumped directly into the water or on the land in a surrounding watershed. One major source of nonpoint pollution in a rural watershed is past and present agricultural practices. Point source pollution in watersheds comes from companies that discharge water directly into the watershed, but the U.S. Environmental Protection Agency (EPA) regulates the amount of phosphate that can be discharged; therefore, companies and utilities usually do not make a big contribution of phosphate into watersheds today.

Suggested Class Preparation and Format

Although the investigation explores relatively mild contaminants, it will demonstrate that it does matter what you put in the water, even in a small amount. This lesson was chosen as the first in our Watersheds Green Box because it engages the students on the question of water quality and how human activities often influence outcomes. It also takes a period of days for the experiment to mature correctly. There is an important lesson to be demonstrated on the principle of "limiting reagents" in a chemical reaction (i.e., the reactants in a chemical reaction that limit the amount of product that can be formed).

For aquatic plant life, there are three primary things that are required for algae to grow: sunlight, nitrogen nutrients, and phosphorus nutrients. Having ample access to all three will cause an algal explosion or bloom in the water. By restricting any one of these ingredients, algal growth will be limited. This investigation can provide a visual demonstration of this concept, which is central to all chemical reactions.

Preparing the phosphate and nitrate solutions along with setup for students will take a minimal amount of time. You can provide the surface water sample, ask the students to bring one, or start with deionized water (DI) water to which algae is added. If collecting a sample, it should be from stagnant water, such as a pond,

within the top 0.5-1.0 m of water; plenty of algal cells should be found in the sample. If starting with DI water, algae can be purchased from a science supply company at minimal cost.

To give students the option of having a "low" and "high" concentration of each of the pollutant solutions, just add 9 drops of pollutant to 100 ml of water to make a low concentration and 18 drops of pollutant to 100 ml of water to make a high concentration. The pollutants should be added to the water samples every day after data have been collected or whenever the students check the beakers. Use 100 ml water samples.

If labware is limited, the investigation can be divided among larger groups of students. One group could test only the effects of nitrate, while another could test only the effects of phosphate, and so on. In addition, plastic cups could be used in place of beakers. Student pairs could be assigned just one trial instead of two, and then class data could be combined.

Beaker	Option 1: Amount of Nutrient (liquid)	Option 2: Amount of Nutrient (tablet)
Control 1	None	
Control 2	None	
Low P0 1	9 drops of KPO4	
Low P0 2	9 drops of KPO4	
High P0 1	18 drops of KP04	
High P0 2	18 drops of KP04	
Low N0 1	9 drops of KN03	
Low N0 2	9 drops of KN03	
High N0 1	18 drops of KN03	
High N0 2	18 drops of KN03	
Low both 1	9 drops of KP04 , 9 drops of KN03	
Low both 2	9 drops of KP04 , 9 drops of KN03	
High both 1	18 drops of KP04 , 18 drops of KN03	
High both 2	18 drops of KP04 , 18 drops of KN03	

Student:

A. *Prior Standards:* Look to the standards. What should the students have as prior knowledge before beginning this lesson? What standards should have been covered in previous grades? If this is an introductory lesson, indicate as such.

B. *Life Experience:* Has the student seen algae in a river, pond, or lake?

Time

If the investigation is conducted in a traditional way (see Student Handout A), it will take one 50-minute class period for lab setup. The investigation will need to run for at least 10-14 days, so a few minutes will need to be set aside every other day or every three days for students to perform the water quality tests and to make observations. One 50-minute class period will be needed for data analysis. If the inquiry approach is used, an additional 50-minute period will be needed for students to conduct background research and design the investigation.

Materials List

- surface water sample (can be collected in advanced by one person, typically need 2-3 L per test) OPTIONAL
- sunny window or plant grow lights
- deionized (DI) water (or any type of purified water)
- 14—200 ml beakers or plastic cups (Note: Since most commercial detergents contain phosphorus, try to thoroughly rinse glassware cleaned with soap before conducting any part of the experiment.)
- 7 plastic pipettes or droppers
- M potassium phosphate, dibasic solution (KH POJ4); this will be the phosphate pollutant
- M potassium nitrate solution (KN03); this will be the nitrate pollutant
- plastic wrap
- compound microscope (optional) slides and coverslips (optional)
- algae (optional)-any species will work
- Vernier sensors or water quality tests for turbidity and dissolved oxygen (optional)
- nitrate pollutant : 0. 10 g of potassium nitrate to 100 ml of DI water
- phosphate and nitrate pollutant : 0.14 g of potassium phosphate (dibasic) and 0. 10 g of potassium nitrate to 100 ml of DI water

Engagement

Objectives: Students will be able to graph data collected from scientific research in order to determine how the crab population has declined and how environmental factors influence Hematodinium outbreaks.

Lesson Materials:

- Anticipation Guide
- Data for the graphs
- Graph paper or Computer with MS Excel

Procedures:

1. Students will complete the anticipation guide on the Maryland Blue Crab and Hematodinium individually by reading each statement and writing true or false in the "before" column.
2. The teacher will hold a discussion with the class to determine the validity of each statement. The teacher can use a "thumbs-up, thumbs-down" method for quick student feedback. All statements on the anticipation guide are true.
3. Students will be given 1 of 4 sets of data: (1 & 2) date, prevalence, water temperature, and water salinity, (3) year, prevalence, average temperature, and total precipitation, (4) year, crab harvests. The data was compiled from a series of published research articles.
4. Display all graphs for students to see.
5. Discuss with students the relationship between water temperature and Hematodinium, salinity and Hematodinium, salinity and total precipitation, average temperature and water temperature, and finally crab harvests and all environmental factors.
6. Students will develop a hypothesis to answer the question "What is killing the Maryland Blue Crab?"

Exploration

Objectives: students will learn about how eutrophication impacts waterbodies. Through small group brainstorming and class discussions, students will begin thinking about why it should matter to them.

Collection of Surface Water

1. Rinse the collecting bottle out three times with sample water. Collect water from the top 0.5-1.0 m. Select a stagnant pond to ensure there will be algae present. Label the bottle with the location and refrigerate until used.

Testing the Effects of Nutrients on Eutrophication

2. Obtain 3 L of pond water from the teacher. [Teacher Note: As an alternative, students can collect their own samples.]

3. Measure the turbidity and dissolved oxygen content of the sample. Record the data on the table on your answer sheet.

4. Write down visual observations that include water color, clarity, and odor.

5. Pour 100 ml of pond water into a beaker. Repeat this step 13 more times, for a total of 14 beakers. Label the beakers as follows: control 1, control 2, low PO 1, low PO 2, high PO 1, high PO 2 low NO 1, low NO 2, high NO 1, high NO 2, low both 1, low both 2, high both 1, high both 2. [Teacher Note: This will give two trials for the investigation.]

6. Pollute the pond water samples with the appropriate amount of nutrients. Use Table 1 as a guide.

Sample	Color	Clarity	Odor
Control 1	Clear	Good	None
Control 2	Clear	Good	None
Low $P0_4$ 1	Green growth on bottom	Good	None
Low $P0_4$ 2	Green growth on bottom	Good	None
High $P0_4$ 1	Green growth on bottom	Good	None
High $P0_4$ 2	Green growth on bottom	Good	None
Low $N0_3$ 1	Green growth on bottom more than $P0_4$	Fair	None
Low $N0_3$ 2	Green growth on bottom more than $P0_4$	Fair	None
High $N0_3$ 1	Green on bottom, thicker with clumps of algae	Fair	None
High $N0_3$ 2	Green on bottom, thicker with clumps of algae	Fair	None
Low both 1	Green growth on bottom more than $P0_4$	Fair	None
Low both 2	Green growth on bottom more than $P0_4$	Fair	None
High both 1	Green growth on bottom more than $P0_4$	Fair	None
High both 2	Green growth on bottom more than $P0_4$	Fair	

7. Cover the beakers with plastic wrap and set in a sunny window or under grow lights.

8. Check the beakers every 1-3 days. Perform steps 2-6 every time the beakers are checked. The only water quality tests that need to be performed regularly from now on are dissolved oxygen and turbidity.

9. Run the lab for 10-14 days. Beakers do not have to be checked on weekends.

Optional Extensions

• If you do not have access to a turbidity meter, you can measure the amount of algae relative to the control by comparing the mass with an analytical balance. To find the mass of the algae, wait until the last day of the experiment and then filter the samples through pre-weighed filters and weigh the algae. Compare the mass of the experimental group with that of the control group to obtain a quantitative measure of growth.

• Sometimes in algal blooms there are a lot of blue-green algae because they can fix their own nitrogen. Other types of algae, which cannot fix their own

nitrogen, have limited growth because of the lack of this nutrient. If you have diagrams available for students to identify the different types of algae, this would be an additional parameter to measure—to determine if types of algae concentrations change depending on availability of nutrients. Have the students examine samples of their pond water under the microscope and identify the algae present every time they check their beakers.

Explanation

For this section, write at least five questions that you would ask the students in debriefing in order to see if they learned the content that they were supposed to learn. Be sure this section includes scientific vocabulary and definitions (Tier 3 vocabulary) that are introduced (E.g. Word Wall).

In this section of the lesson, the students will display their results to the class. The class will then agree or disagree if Hematodinium is the culprit using their data from the lab. The teacher will explain that there are additional methods such as qualitative PCR that provide quantitative, conclusive data for DNA fingerprinting.

Objectives:

Students will be able to analyze their gel in order to determine if Hematodinium DNA is present in crab hemolymph. Students will also be able to investigate various biotech techniques available to scientists in order to select the best methods for positively identifying a suspect.

Answers to Questions in Student Handouts:

1. P04

2. nonpoint sources, surface runoff

3. Answers will vary depending on starting water. Students should support their answer with data.

4. algal blooms on lake, mucky lake bottom, low oxygen levels on bottom of lake, fish kills

5. Homeowners can use low-phosphorus or no-phosphorus fertilizers on lawns. People living next to streams and waterways can leave a buffer strip. Farmers can use different farming methods to prevent soil erosion and buffer strips.

Elaboration

Students will investigate the environmental benefits of the Maryland Blue crab, detrimental Lake Mead quagga mussels, or detrimental Lake Tahoe Asian clams by performing Internet research. Students will then create an informational brochure or commercial to notify the public about the ecological role of the crab, the population decline since the 1990's, reasons for that decline including Hematodinium, and management techniques to control Hematodinium and other environmental pressures. For mussels and clams, the potential negative impacts of these invasive species should be explained.

The students should realize that the tidal range is not the same at the two locations and is greater at the location closer to the ocean. The students will be given the opportunity to compare a tidal freshwater marsh to a wetland that is not affected by tides—a peatland. The students will see that although peatlands are not affected by tides, they still have plant community stratification due to inundation levels. In peatlands, this stratification is due only to elevation differences. A slight elevation change can completely alter the plant communities in the area.

Objectives: Using tidal data, students will compare the tidal range of two geographic locations. Students will compare a tidal freshwater marsh and a peatland in regard to how hydrology affects the distribution of plant communities in the two wetlands.

Evaluation

Students will be able to demonstrate their understanding of importance of the blue crab to the health of the Chesapeake Bay and the dangers of the parasite Hematodinium spp., or the health of Lake Mead or Lake Tahoe by writing a formal lab report, creating an information brochure to increase public awareness, and implementing a blue crab population and/or Hematodinium prevalence management technique.

Formative: Qualitative Data

Includes on-going teacher observation, questioning, what you will be looking for and/or asking questions about, etc.

As an additional part of the evaluation, display the students' answers to the discussion questions presented during the Engagement phase.

Allow the students to again discuss the questions in their small groups and modify or change any answers with which they no longer agree.

Summative: Quantitative Data

Students will write a formal lab report to answer the question "Is Hematodinium the culprit?" that includes purpose of the lab, amendments to the materials list and procedure, picture of gel (if available), data analysis and conclusions.

Students will follow the rubric for the "Why save the blue crab?" or “Invasives in Lake Mead and Lake Tahoe” informational brochure/commercial.

Students can implement one of the management techniques identified in their brochures/commercials and journal their experiences and evaluate the technique's effectiveness.

Clean-up

Students should assist with proper disposal of algal solutions (pour down the drain, not outside) and cleaning up any spills that may have occurred.

Lesson 2

Identifying Plants

Next Generation Science Standards

- HS-LS1-5. Use a model to illustrate how photosynthesis transforms light energy into stored chemical energy. [Clarification Statement: Emphasis is on illustrating inputs and outputs of matter and the transfer and transformation of energy in photosynthesis by plants and other photosynthesizing organisms. Examples of models could include diagrams, chemical equations, and conceptual models.]

Background Knowledge

Teacher:

This investigation gives students the opportunity to view the outside world and bring science into the field. Teaching students how to identify plants gives them a greater appreciation for nature, just as learning to read words lets young students begin to appreciate literature. As older students learn to identify plants, they become educated about what lives in the watershed and they develop a greater desire to preserve it. They also become familiar with invasive species that may be invading their watershed.

Many students do not like the memorization that comes with plant taxonomy, but with modest effort everybody can successfully identify by name most of the plants in their community. By the end of this field exercise, students will feel pride in their identification abilities.

Preparation time for this investigation will depend on your familiarity with using dichotomous keys and existing plant knowledge. A visit ahead of time to the location that the students will explore is highly recommended. Identifying all the plants could take a relatively short time or an extended time period, depending on the degree of prior knowledge of plants.

Some counties offer plant identification programs through the parks and recreation department. It is likely that a local park near your school has at least some of the trees already identified along the trails.

Teacher will need to select a key or book to teach plant identification for your specific area. There is a plethora of dichotomous keys on the internet (e.g., wwwfor.msu.edulextension/ExtDocs/ Ident- keylopening.htm), but because books are still the most portable of field equipment, using a book with a dichotomous key is the best choice. The Peterson Field Guide Series is one option for tree identification; this series is useful because the investigation is targeted toward tree identification. Once the primary knowledge is gained, herbaceous plants could be included. A nice guide that uses a dichotomous-type key for flowers is Newcomb's Wildflower Guide (Newcomb 1977), which covers flowers in northeaster n and north-central America.

If you need to brush up on plant terminology, refer to the plant identification book(s) selected for the investigation. These books contain definitions, explanations, and pictures. The Student Handout included in this investigation contains some definitions and illustrations of very basic terms.

Use the first class period (indoors) of this identification lesson to teach students what a dichotomous key is and how to use it, as well as basic plant terminology and leaf morphology. The best way to teach the terminology is to first show students a picture from the identification book and then show them several fresh specimens in the classroom (five different specimens would suffice). Additional class periods indoors may be necessary to teach the basics before taking students into the field. For a wrap-up exercise indoors, have students complete their worksheets on basic plant terminology; alternatively, you can ask them to fill out the worksheets as the material is introduced. Additional plant specimens will be needed to complete the worksheet. You should use the same specimens that students were shown in class, along with some specimens they have not seen before.

Once the students have a good grasp on how to use the dichotomous key, take them outside and have them work in pairs to "key out" the plants. To keep students who finish early engaged, assign them additional species to identify. Have students keep a field notebook of all plants identified, listing habitat and major characteristics. Give the students helpful hints along the way to aid them in their memorization; for example, the peeling bark of a sycamore tree can make it look "sick"; black cherry bark looks like burnt potato chips; and the mnemonic MAD HORSES, which stands for maples, ashes, dogwoods, and horse chestnuts, can be used to identify trees that have opposite leaves. Most other tree families commonly encountered will have alternate leaves.

Plant identification can be done anywhere. City areas may have a great number and variety of tree species, but if the opportunity exists, take a field trip to a natural area near you.

Materials List

- dichotomous key (teacher's choice)
- plant specimens (for possible species, see Table 3.1 at the end of the section "Answers to Questions in Student Handout")

Engagement

Procedures:

The Engage portion of this laboratory will consist of a "K-W-L" activity, or "Know—Want to know—Learned". This may be very familiar to your classroom, or completely unfamiliar. In this activity, you will introduce the lesson topic, "Plant Identification", and the reasons why it is important. Being a junior naturalist is a wonderful hobby, but many of our students may have never been exposed to this subject matter. The import of this topic is more than their own edification. Scientists study plants to improve and secure the food supply for an increasing world population, identify new sources of bioactive compounds and medicines, improve fiber production and identify sources of biofuels and biorenewable resources. Many careers require knowledge of plants: farmer, forester, fire fighter, chef, etc. After introducing the topic, ask the students to write down on a StickyNote one thing that they already "Know" about plant identification and/or photosynthesis and stick it on the whiteboard, under the "Know" heading. Next, ask the students to write down on a StickyNote one thing that they "Want to know" about plant identification and stick it on the whiteboard, under the "Want to know" heading. The "Learned" part of the K-W-L is good for formative post-assessment after the lesson.

Exploration

Objectives: Teaching plant identification in the field can feel overwhelming, but it does not have to be if the students are prepared for the activity. It is up to you to determine how much time to spend on plant identification; as familiarity grows, this exercise can become more extensive each year. Identification can be taught alone or with other topics. For example, plant identification most likely would be included in a biology unit on plants. Plant identification would also be useful in an ecology unit with plant population counts or in a unit on invasive species.

Tips for Identifying Plants

1. Examine the plant and determine if the leaves are opposite or alternate.
2. Look at an individual leaf and follow the leaf stalk back until you see the bud. Is the leaf compound or simple?
3. Look at the leaf shape and margins.
4. Examine the bark.
5. Use these observations with a dichotomous key to identify the plant.

Preserving Plant Specimens

Collecting Specimens

Select specimens for the collection that are free from any type of blemish or damage. Use a knife or scissors to cut branches. For herbaceous plants (plants that are fleshy), cut the stem off all the way to the ground. Make sure when cutting trees or herbaceous plants that the specimen has enough leaves to determine leaf arrangement. When cutting flowers, one is sufficient. Include seeds with the specimen whenever possible. When collecting, identify the plant first before collecting it. Write the specimen's name on a piece of masking tape and wrap the tape around the stem or branch of the plant. Place the specimen in a plastic bag (such as a plastic grocery bag) with some wet pa- per towels. Keep the bag closed at all times. The wet paper towels keep the humidity high inside the bag to keep the plants from wilting. Plants should be pressed as soon as possible. If unable to press plants the same day, place the closed bag inside of a refrigerator. Most plants will keep for a few days.

Plant Press

Lay out the specimen on one side of a piece of a newspaper that is folded in half. Make sure the leaves are not overlapping and one leaf is turned over. Sometimes this can be tricky. Masking tape can be used to tape down branches. If using masking tape for the branches, put it on your fingers several times to take off most of the stickiness so it will not damage the specimen. Do not use masking tape on the leaves, because the tape will rip them when they are dry.

Fold the remaining half of the newspaper over the specimen. Write the species name on margin of the newspaper for later use.

Put several newspapers and, if available, a piece of corrugated cardboard in be- tween specimens. Set heavy objects such as books on top of the stack of specimens, and wait three to five days for the specimens to dry. Try to place them in a location with little humidity, and use a fan (if available) to speed up the evaporation of the water from the plants.

Mounting Specimens

Never tape or staple specimens to the paper. Use your finger or a paintbrush to spread a thin layer of glue over the entire leaf sur- face. After covering all of the leaves on the specimen and its stem, affix the specimen to the paper. When gluing down, make sure to keep the one leaf that was turned over in the drying process so the back can be seen. Some species of plants, such as poplars or aspens, naturally turn a blackish color when dried; others tend to bubble up. These types of things are to be expected and should not cause any deduction of points on the collection. Do not cover the specimens with any type of plastic or contact paper. It is important that the viewer be able to touch the specimens for identification.

Labeling

Each specimen needs a label in the lower left corner of the herbarium paper. As shown in the sample label format below, the label should include the following information: Latin name of the plant; family name of the plant; common name of the plant; your name; date collected; city or township, county, and state where collected; specific location of collection; and habitat. The label may be typed or written in black ink.

Turning in the Collection

The collection must be in alphabetical order according to family. Prepare a table of contents for the collection that consists of the family name, Latin name, common name, and page number for each specimen. Place specimens with the table of contents and the rubric on top in a brown paper bag, and turn the collection in to the teacher.

Species Name	Opposite, Alternate or Whorled	Blade Type: Simple or Compound	Margin of Blade: entire, serrated, undulated, lobed
Any species of Maple (except Ashleaf aka Boxelder)	Opposite	Simple	Lobed (With the different species notice the shape of the sinus. Some are "V" shaped and some are "U" shaped indicating its species.
Red, Black, White, Bur Oak	Alternate (oak leaves tend to cluster)	Simple	Lobed (With the different species notice the lobes tip differ, each difference indicating its species.)

Bitternut Hickory	Alternate	Pinnately Compound	Serrate
Any species of Ash	Opposite	Pinnately Compound	Entire to serrate depending upon species
Redbud	Alternate	Simple	Entire with leaf having a heart shape
Ohio Buckeye or Horsechesnut	Opposite	Palmately Compound	Serrate

Optional Extensions

• Have your students teach basic plant identification to elementary students. This provides a great assessment for the high school student and gives the elementary student a safe and supervised encounter with nature.

• Have your students make a guided walking trail by making labels for trees and plants they have identified. Index cards with information can be laminated inexpensively at a copy center.

• Have your students make a herbarium collection, which is a collection of dried plant specimens preserved on herbarium-quality paper. Instructions for preserving plant specimens and a herbarium collection rubric are included at the end of the Teacher Information section. The collection should be limited to 25 specimens; a larger collection will require too much time and effort.

Explanation

For this section, write at least five questions that you would ask the students in debriefing in order to see if they learned the content that they were supposed to learn. Be sure this section includes scientific vocabulary and definitions (Tier 3 vocabulary) that are introduced (E.g. Word Wall).

In this section of the lesson, the students will display their results to the class. The class will then agree or disagree if Hematodinium is the culprit using their data from the lab. The teacher will explain that there are additional methods such as qualitative PCR that provide quantitative, conclusive data for DNA fingerprinting.

Objectives:

Students will be able to analyze their gel in order to determine if Hematodinium DNA is present in crab hemolymph. Students will also be able to investigate

various biotech techniques available to scientists in order to select the best methods for positively identifying a suspect.

Answers to Questions in Student Handouts

1. P04

2. non-point sources, surface runoff

3. Answers will vary depending on starting water. Students should support their answer with data.

4. algal blooms on lake, mucky lake bottom, low oxygen levels on bottom of lake, fish kills

5. Homeowners can use low-phosphorus or no-phosphorus fertilizers on lawns. People living next to streams and waterways can leave a buffer strip. Farmers can use different farming methods to prevent soil erosion and buffer strips.

Evaluation

Students will be able to demonstrate their understanding of importance of

Formative: Qualitative Data

Includes on-going teacher observation, questioning, what you will be looking for and/or asking questions about, etc.

As an additional part of the evaluation, display the students' answers to the discussion questions presented during the Engagement phase.

Allow the students to again discuss the questions in their small groups and modify or change any answers with which they no longer agree.

Revisit the K-W-L activity. Ask the students to write down on a StickyNote one thing that they "Learned" about plant identification and stick it on the whiteboard, under the "Learned" heading. Compare answers to the Know and Want-to-know answers received before the lab.

Summative: Quantitative Data

Students will submit their worksheets.

Lesson 3

Create a Watershed

Next Generation Science Standards

- **HS-ESS2-5.** Plan and conduct an investigation of the properties of water and its effects on Earth materials and surface processes. [Clarification Statement: Emphasis is on mechanical and chemical investigations with water and a variety of solid materials to provide the evidence for connections between the hydrologic cycle and system interactions commonly known as the rock cycle. Examples of mechanical investigations include stream transportation and deposition using a stream table, erosion using variations in soil moisture content, or frost wedging by the expansion of water as it freezes. Examples of chemical investigations include chemical weathering and recrystallization (by testing the solubility of different materials) or melt generation (by examining how water lowers the melting temperature of most solids).

Background Knowledge

Teacher:

The land we live on is divided into watersheds. A watershed is a land area whose runoff drains into any river, stream, lake, or ocean. Small watersheds, such as the watershed for the creek behind your house, or the watershed for the pond down the road, drain into small bodies of water, and cover small land areas. The runoff from small watersheds join together, and their combined areas become a new, larger watershed. Large watersheds, such as the Mississippi Basin and the Chesapeake Bay watershed, drain into large bodies of water, and cover immense land areas. Despite their differences in sizes, all watersheds share common properties. They all perform the same function of transporting water over the Earth's surface. The watersheds encompass suburban lawns, parking lots and city streets. Water seeps down through the soil to aquifers, which are underground formations in rock and soil that contain enough ground water to supply wells and springs.

Many human activities have an effect on watersheds. Construction projects like dams can limit the flow of water; construction of roads and buildings can divert and even increase the flow of water. Agricultural fertilizers can run off of crop fields and inadvertently fertilize harmful microorganisms in rivers and lakes, having an adverse effect on water quality and marine life. The irresponsible

disposal of household and industrial chemicals can be harmful because these chemicals travel through the watershed, poisoning life and damaging natural ecosystems.

Watersheds can also have an effect on humans. Many communities use rivers, streams, and aquifers as their source of drinking water. Water treatment prepares this water for human consumption, but if the water is laden with chemicals and microorganisms, it can be difficult to treat effectively. Floods are one of the major events in a watershed. Homes built on flood plains, low lying areas adjacent to rivers, are susceptible to flooding conditions when heavy precipitation exceeds the watershed's capacity to absorb water. Rivers, streams, and lakes overflow, threaten human lives, and damage or destroy roads, buildings, and flood control measures. Watersheds can also become dry, causing water shortages for those who depend on their lakes and rivers for drinking water.

It is clear that humans have a close relationship with watersheds. The responsible planning of watershed use and development is important to ensure that the ecosystems sustained by the watersheds are not destroyed, and to protect the health and safety of our communities.

Extension Note: Prior to the demonstration, the teacher should engage the students in activities involving identification of a local watershed. Maps can be used to facilitate this activity, and a field trip to a local river or pond can serve to demonstrate the concept of a watershed. Ask students to identify where the water is coming from. How far does the watershed extend? For a small stream, the answer may be several hundred feet; but for a lake or river, the watershed may be much larger. Visit EPA's "Surf Your Watershed" for local watershed information (http://www.epa.gov/surf/iwi).

Student:

A. *Prior Standards:*

   HS-LS2-1. Use mathematical and/or computational representations to support explanations of factors that affect carrying capacity of ecosystems at different scales.

   HS-ESS2-1. Develop a model to illustrate how Earth's internal and surface processes operate at different spatial and temporal scales to form continental and ocean-floor features.

B. *Life Experience:* Have the students experienced rain? Visited a river? Drunk water from a faucet?

Materials List

- 1 large Tupperware or container (about 1.5'W x 3'L x 1'H)
- 2 lbs. of modeling clay
- 3 lbs. of sand (any type of sand will do)
- 2 lbs. of aquarium gravel
- 1 roll of wax paper (or any other impervious, water repellant surface, tin foil, plastic wrap, etc.)
- 1/4 cup of cocoa mix, iced tea mix, or other flavored drink mix (to represent chemicals)
- 1 spray bottle or bucket full of water

Engagement

Objectives: By relating this "Water Teaser", students will learn about what areas constitute a watershed. Through small group brainstorming and class discussions, students will begin thinking about how water moves around our planet and their watershed.

Procedures:

Relate this "joke" about water to your students in any fashion that seems reasonable in your classroom:

> "This is no joke but a call to *BAN* dihydrogen monoxide, otherwise known as the invisible, killer substance. For your information, dihydrogen monoxide (DHMO) is colorless, odorless, tasteless, and kills thousands of people every year. Most of these deaths are caused by accidental inhalation of DHMO in its liquid form, but the dangers of dihydrogen monoxide do not end there. Prolonged exposure to its solid form causes tissue damage and contact with its gaseous form causes burns. DHMO use is widespread. For those who have become dependent on it, DHMO withdrawal means death. DHMO can be an environmental hazard: it is a major component of acid rain, contributes to the "greenhouse effect", leads to the erosion of natural landscapes and hastens the corrosion of most metals. Being so prevalent (quantities are found in every stream, lake and reservoir), DHMO contamination is at epidemic proportions. Despite the dangers, DHMO is often used as an industrial solvent, as a fire retardant, in nuclear power plants and (can you believe this) in certain food products. Companies dump

waste dihydrogen monoxide into rivers and the ocean, and nothing can be done to stop them because this practice is still legal. Stop the horror now! The American government and the United Nations have refused to ban the production, distribution or use of this chemical due to its "economic importance." The navy and certain other military organizations are highly dependent on DHMO for various purposes. Military facilities receive tons of it through a sophisticated underground distribution network. It is also stored in large quantities for military emergencies. But, it's not too late! You can help. Act *NOW* to prevent further contamination. Write your representatives. Send e-mails. Inform your friends about the dangers. What you don't know *CAN* hurt you and every individual throughout the world."

Exploration

Objectives: This experiment illustrates the basic properties of a watershed: how water flows from higher elevations to lower elevations, and how watersheds are interconnected. The students will understand how the placement of buildings, roads, and parking lots can be important to watershed runoff, and how careless use and disposal of harmful contaminants can have a serious effect on downstream watershed denizens.

1. Wash the aquarium gravel carefully to remove any powdery residue that may add cloudiness to the water. Fill the container to about 2 inches from the bottom with the gravel. Slope the gravel slightly so, that at one end (downslope), the gravel is only about ½ inch deep and, at the other end (upslope), the gravel is about 3 inches deep. This gravel layer will represent the aquifer.

2. Mix the clay and the sand. The consistency of this mix should be gritty, with slightly more clay than sand. This mixture should allow water to run freely over it, but if left standing, the water should slowly permeate the surface. Add this mixture to the container carefully, so as not to disturb the slope of the aquifer already placed. The slopes should be similar, with about 2 inches of sand/clay mix overlying the gravel already placed, and on the downhill end there should be about 3" of gravel left exposed.

3. Carve a channel in the middle of the clay/sand layer, about ½ inch deep and about 1 inch wide. This channel will represent the main river of the watershed. Near the top of the slope, split the channel into two or three separate channels to represent tributaries. You may wish to add other tributaries along the main branch of the "river" to further illustrate other watersheds.

4. With some extra clay/sand mix, build little hills between the tributaries. These hills separate the smaller watersheds, but when looked at as a whole, the entire "river" system is one watershed. You may also wish to add some small model trees or green felt to represent forests or fields. Buildings can be represented with small blocks of wood.

5. Along the main river, flatten out an area that is about 8 inches by 3 inches. Cut out a piece of wax paper to be about 4 inches by 3 inches in size. Stick this down onto the clay sand mix, sloping it slightly towards the river. If necessary, use some clay to hold the edges down. Explain to students that this wax paper represents the impervious surface of a parking lot.

6. Fill the bottom of the aquarium up to about 2 inches from the bottom with water. The water should fill all of the aquarium gravel "aquifer" area, and should just reach up to the lowest extent of the clay/sand mixture. Explain to students that the aquifer captures and transports water that seeps down through the soil.

7. Using the spray bottle, simulate rain over the flattened soil area and the parking lot. Ask the students to note that the "rain" soaks through the soil, but runs off the parking lot to the river. Ask them what the effect would be if the entire watershed was "paved".

8. Sprinkle some cocoa mix over the sides of one of the smaller watersheds. Tell the students that the cocoa represents pollution. Over one of the unpolluted "watersheds," cause some rain with the spray bottle (*it may be necessary to cause more rain by pouring water). Note that the runoff from the rain is clean. Now, make it rain over the polluted area. Ask the students to note how the pollution travels down through the watershed, contaminating all downstream areas. Discuss with the students why the pollution is a problem, and what can be done to fix the problem.

Explanation

Objectives: Students will clearly communicate through question/answer what they have learned about the concepts of water moving down through ever-increasing sizes of creek to river. Through a slide show or presentation, students will see actual photographs of the water cycle. The instructor should allow students to offer information and then correct or elaborate on student input.

Discussion questions:

- Where does water come from?
- How does it get in to your river?

- How does water get to your kitchen or bathroom?
- What is chemical weathering?
- What is mechanical weathering?

Vocabulary

- A watershed is any area that drains to a common waterway
- Without water, the other nutrient cycles would not exist in their present forms, and life on earth could not exist.
- Water keeps us alive, moderates climate, sculpts the land, removes and dilutes wastes and pollutants, and moves continuously through the hydrologic cycle.
- Only about 0.02% of the earth's water supply is available to us as liquid water.

Elaboration

The students will now take the knowledge they acquired during the Exploration and Explanation phases of the lesson and apply them to their geographic locations. During the first part of the Extension, the students will form new groups of 2-4 children, or retain their prior groups, to develop a skit whereby they represent some aspect of the hydrologic process that they just investigated. In the second part of the Extension, these groups will perform their skit in front of the class. The rest of the class will attempt to guess what aspect they are trying to represent. Think charades.

Objectives: Using observation of the flow of water in their "watershed", students will compare discoveries between groups. Discussion can also be linked to the watershed in which they live.

Evaluation

Formative: Qualitative Data

For discussion, at individual, group, or class level:

1. What are some possible sources of watershed pollution in your community?
2. Where are the boundaries of our (where we live) watershed?
3. What other impervious surfaces besides parking lots can cause excessive runoff in a watershed?
4. What can be done to reduce our impact on watersheds and their environment?

5. Using a map of the area around your house and EPA's "Surf Your Watershed," identify where the runoff from your driveway will end up. Can you track the path of potential pollution to a large body of water (i.e., ocean or bay)?

Summative: Quantitative Data

All activities completed by the students throughout the lesson (topographic profiles, explanation paragraphs, questions/answer) will be assessed using a rubric to determine the students' understanding and to assign grades.

Clean-up

Students should help put away clay, gravel, baking tins (or Tupperware), etc.

Closure

Objectives of this laboratory were to describe and investigate the hydrologic cycle with a model. Students should grasp the mechanical and chemical weathering patterns in a river and its entire watershed.

Lesson 4

Stream Channel Morphology

Next Generation Science Standards

- **HS-ESS2-2**. Analyze geoscience data to make the claim that one change to Earth's surface can create feedbacks that cause changes to other Earth systems. [Clarification Statement: Examples should include climate feedbacks, such as how an increase in greenhouse gases causes a rise in global temperatures that melts glacial ice, which reduces the amount of sunlight reflected from Earth's surface, increasing surface temperatures and further reducing the amount of ice. Examples could also be taken from other system interactions, such as how the loss of ground vegetation causes an increase in water runoff and soil erosion; how dammed rivers increase groundwater recharge, decrease sediment transport, and increase coastal erosion; or how the loss of wetlands causes a decrease in local humidity that further reduces the wetland extent.]

- **HS-ESS2-5**. Plan and conduct an investigation of the properties of water and its effects on Earth materials and surface processes. [Clarification Statement: Emphasis is on mechanical and chemical investigations with water and a variety of solid materials to provide the evidence for connections between the hydrologic cycle and system interactions commonly known as the rock cycle. Examples of mechanical investigations include stream transportation and deposition using a stream table, erosion using variations in soil moisture content, or frost wedging by the expansion of water as it freezes. Examples of chemical investigations include chemical weathering and recrystallization (by testing the solubility of different materials) or melt generation (by examining how water lowers the melting temperature of most solids).]

- **HS-LS2-1**. Use mathematical and/or computational representations to support explanations of factors that affect carrying capacity of ecosystems at different scales. [Clarification Statement: Emphasis is on quantitative analysis and comparison of the relationships among interdependent factors including boundaries, resources, climate, and competition. Examples of mathematical comparisons could include graphs, charts, histograms, and population changes gathered from simulations or historical data sets.] [Assessment Boundary:

Assessment does not include deriving mathematical equations to make comparisons.]

Background Knowledge

Teacher:

Historically, streams and rivers were the major transportation routes for people and goods in this country. Many large cities, such as Chicago, Detroit, and Grand Rapids (Michigan), were built next to waterways because the easiest and most efficient means of transporting goods was via the river to the Great Lakes.

With farming and urban development of watersheds, our streams and waterways began to change. For example, in Holland, Michigan, the Ottawa Indians, who used the area for summer hunting grounds long before the Europeans arrived in 1849, noted that streams were being dammed up and polluted when they left the area in 1850. As part of this early development, the land was cleared of trees, which resulted in streams being warmed as the shade canopy was removed. To use this new source of lumber, sawmills became commonplace. The sawdust from sawmills and erosion due to loss of vegetation filled in the streams, making them more shallow, warmer, and more turbid. In addition to erosion, the clearing of the land also increased runoff, which in time lowered the base flow of the waterways (Van Koevering 1960).

As development continued, people manipulated streams by changing their channel morphology (shape). Straight stream channels transport water and sediment faster, whereas meandering streams slow the flow of water. When land was being settled in this country, it was a common practice to straighten stream channels and make new ones to drain the land more efficiently.

When humans change the natural topography, certain effects are likely to be seen in a watershed. The transportation of water and sediment increases when streams are straightened and, with the increased flow rate of water, erosion also increases. All of these factors increase turbidity and can have a negative effect on the waterways and most lakes. A degraded visual appearance will be the most noticeable effect, but animal and plant life will also begin to change as streams are changed.

Student:

A. *Prior Standards:*

HS-LS2-1.Use mathematical and/or computational representations to support explanations of factors that affect carrying capacity of ecosystems at different scales.

HS-ESS2-1. Develop a model to illustrate how Earth's internal and surface processes operate at different spatial and temporal scales to form continental and ocean-floor features.

B. *Life Experience:* Have the students experienced rain? Visited a river? Drunk water from a faucet?

Materials List

- 100 g of sand
- waste container to catch effluent, with an opening 30 cm wide electronic balance (students may share at each lab table)
- 50 ml graduated cylinder stopwatch
- Styrofoam stream table (You can buy Styrofoam at any home improvement store. See instructions below for making the stream table.)
- aluminum foil

Engagement

Objectives: Students will learn how and why rivers form where they do.

Procedures:

1. Ask students to read the article "Wetland Plants" taken from the Smithsonian Environmental Research Center's website, "It's all in the Watershed," http://serc.si.edu/education/resources/watershed/, or another article related to wetland plants.

2. Divide the students into small groups (no more than four students) to discuss the following questions. Students should only use their prior knowledge and experiences to answer the questions.

3. Stress to the students that they will not be penalized for wrong answers. This activity is to simply see what they already know about the topic.

4. Discussion Questions:

   a. Have you ever been to a wetland such as a swamp, marsh, or peatland? If so, what characteristics did you observe?

   b. What factors might cause the water level near the surface of the soil in a wetland to change?

c. Do you think that all wetlands experience the same patterns of fluctuation in water level near the surface of the soil? Why or why not?

d. Would you expect to find the same types of plants growing in every area of a wetland? Explain your answer.

5. After the students have been given ample time to discuss the questions in their small groups, have each group share their answers with the rest of the class.

6. Hang large sheets of paper or poster board on the wall. Ask each group to write on the paper a summary of what they discussed or their one best answer for each question.

7. Review the responses on the large sheets of paper.

8. Do not tell the students if what they have discussed is correct or incorrect. Save the large sheets of paper to refer back to at a later time.

Exploration

Part I: Determine Placement of Deposition in Stream Channels

l. Place the Styrofoam stream board at a 30-degree angle on the lab table.

2. Position a waste container under the stream channel outlet to catch any effluent.

3. Weigh 10 g of sand and pour it into the top quarter of the straight channel.

4. Measure 25 ml of water and pour it into the straight channel at a steady rate.

5. Draw a diagram of where the sand was deposited in the channel.

6. Repeat steps 2-5 with the meandering channel.

7. You may need to redo this until you see a pattern of sedimentation. Be sure to rinse out any residual sand between measurements.

Part II: Determine the Rate of Flow in Stream Channels

1. Place the Styrofoam stream board at a SO-degree angle (low gradient) on the lab table.

2. Position a waste container under the stream channel outlet to catch any effluent.

3. Measure 25 ml of water and pour it into the straight channel at a steady rate.

4. As pouring begins, start the stopwatch; stop it when the water first reaches the bottom of the stream. This may have to be practiced a few times to get an accurate result.

5. Record all data, enter the data on a spreadsheet, and calculate the average time for the class.

Part III: Determine the Amount of Sediment Transported in Stream Channels

1. Using the Styrofoam stream board with the depression side up, position the board at a 30-degree angle.

2. Position a waster container below the outlet to catch any effluent.

3. Get a piece of aluminum foil that is about the size of a sheet of paper and cut out 2 cm x 7 cm rectangles. Fold the rectangles in half and place them within the depressions in both stream channels. [Teacher Note: The students will need 12 rectangles total, if they will be performing this twice using the two different gradients as suggested in the optional extension.]

4. Using your finger, mold each aluminum foil rectangle to fit each depression; cut off any excess.

5. Using a Sharpie marker, label the depressions of the straight channel on the back of each aluminum foil cup, as follows: ST (top), SM (middle), and SB (bottom); then mark the depressions of the meandering channel as MT, MM, and MB.

6. Using the electronic balance, weigh each aluminum cup and record the mass on a spreadsheet.

7. Weigh 10 g of sand.

8. Measure 25 ml of water.

9. Pour the 10 g of sand in the straight channel between the top and the first depression.

10. Place the aluminum cups in their proper depressions.

11. Pour 25 ml of water down the straight channel, again making sure all the water flows into the channel. Pour at a steady rate. Note any sediment coming out of the bottom of the stream.

12. Very carefully remove the aluminum cups and set them on a piece of paper towel on the lab counter. The paper towel should be labeled in advance with the same abbreviations listed in step 5.

13. Repeat this procedure (steps 6-12) for the meandering channel.

14. Let the aluminum foil cups dry overnight. [Teacher Note: As a quicker alternative, the cups could be set on a hot plate for about 20 minutes to dry.]

15. Weigh the aluminum cups with the sand, and subtract the initial mass of the aluminum foil to find the mass of the sand deposited in each location. Record the data on a spreadsheet.

Optional Extensions

- All three procedures can be repeated using a gradient of 60 degrees to demonstrate the role of topography in flow rate and sediment deposition.
- For Part II of the investigation, velocity could be calculated and graphed instead of only time being recorded as indicated in the procedure.

Explanation

The goal of this investigation is to understand the river system. Bring the class back together and discuss the following essential questions:

1. What things about a river would be important to measure?
2. What river characteristics and conditions affect flow?
3. What river conditions affect the amount of erosion?

Make a list of student responses on the board and go over the key words in the lesson.

Scientific Vocabulary

- Erosion = “the entrainment or picking up of sediment to be moved by water”
- Deposition = “the laying down or dropping of sediment from the water”

- Entrainment = “picking up of sediment by the water”
- Morphology = “the shape of the stream”
- Ephemeral = “a stream that does not flow all the time”
- Perennial = “a stream that does flow all the time, never goes dry”
- Flood = “when streamflow is larger than the normal amount, or when the stream overflows its channel”
- Gravity = “the force that moves objects towards the earth”
- Slope = Rise/Run “the slope of the land”
- Velocity = Distance / Time “the speed of the water moving through the stream”
- Discharge = Area x Velocity “amount of water moving through the stream”

Make it a point to say that velocity and discharge were not be measured, but only observed, in this investigation because it is difficult to make measurements accurately on such a small model.

Next let them write their predictions on their worksheet, tell them it’s okay if they don’t know and to write how they think the two variables in each question relate to one another. If they are having a lot of trouble help them by reminding them how water moves through water slides, washed near their houses, hoses, etc. Introduce the stream tables and their components to the class. Make sure to set any rules that you have in your classroom, for example, “Do not put more than the prescribed amount of water in the table, you will ruin your experiment” or “Do not add more sediment unless you check with a teacher first”. These are important for the experiment, as well as the first step towards minimizing the mess after the class is finished!

Did they find actual values or just observing relative similarities and differences?

What did they notice about the stream movement at this velocity, slope, etc.?

Are there any areas along the rivers that are eroded faster? Any areas that deposition occurs more? Is it even throughout the river?

Elaboration

1. Now that we understand how a river works, let's think about how that helps us as humans.

    a. If you were building a house by a river what would you keep in mind to make sure your house lasted?

    b. If you wanted to go on a river rafting/kayaking trip and wanted lots of rapids and fast water and beaches to camp on, where would you need to be on the river?

    c. If you wanted to fish, where could you find fish that like the swift water? Where could you find the fish that liked the calm and still water?

2. This is a great time to bring out the transparencies and squirt bottles to talk about how pavement and urban areas create large areas of high overland flow and can cause large amounts of water to go into the nearby stream. There are lots of things that can be talked about here; it really depends on your own goals for your classroom. Other ideas and insightful observations to include:

    - House and infrastructure placement near streams and flooding problems.

3. Have students place their house where they think it is safe, flood the stream and see if their house survives. Have them relate this to what locations they would zone as residential versus farmland only uses.

    - Runoff from urban areas and water harvesting

4. Use transparencies to represent pavement and spray water over the top and watch the runoff concentrate and erode a small channel to the larger river. Have them design a system to move water to the stream or disperse water effectively.

    - Dam placement and breaking

5. Have them design embankment or earth fill dams (or other dams with play dough) to see how they block up the water or create a large drop for electricity. Let the students destroy the dam with a flood at the end, have them write down observations about how it failed and how they could build the dam better next time.

    - Diversion and flood hazard minimization

6. Have student engineer channels to "save" a neighborhood from floods. You could set up a scene before class for each group. Have them explain the setup, draw to scale, etc. and then test it with a design flood.

    - Delta formation and sedimentation

7. At the end of the stream table (non-filled in area) the sediment will create a delta feature.

8. Talk about features of a delta and relate the ones they make (have them draw them) to real ones on Google Earth or other pictures. Talk about how these systems occur at the mouth of all rivers and can influence trade routes (think Mississippi River).

    - Fossil preservation

9. Use plastic dinosaurs to show how river (or lake) sedimentation covers and preserves dinosaurs after death (or during death like La Brea tar pits).

    - Placer deposits and grain size and density

10. Use different materials in the stream table to show how the different densities group into deposits in specific locations in the river, these are called placer deposits and are mined by people. Also different grain sizes will move to different locations of the stream, have them draw and observe where these occur.

Evaluation

The Evaluation phase will be used to determine what additional information about wetlands—especially related to tides, elevation, and plant communities—the students have learned throughout the lesson. Most of the Evaluation is accomplished by assessing the activities completed by the students during the other phases of the lesson. However, the Evaluation also includes revisiting the discussion questions presented early in the lesson and giving the students an opportunity to modify or change their responses.

Formative: Qualitative Data

Includes on-going teacher observation, questioning, what you will be looking for and/or asking questions about, etc.

As an additional part of the evaluation, display the students' answers to the discussion questions presented during the Engagement phase.

Allow the students to again discuss the questions in their small groups and modify or change any answers with which they no longer agree.

Summative: Quantitative Data

Describe final projects with grading rubrics.

All activities completed by the students throughout the lesson (topographic profiles, explanation paragraphs, tidal graphs and questions, Venn diagrams) will be assessed using a rubric to determine the students' understanding and to assign grades.

Closure

Some things that can be seen clearly in the model may not be able to be seen as easily in real-world examples, such as the Truckee River or Las Vegas Wash. Have them keep this in mind while looking at this model and make sure the kids understand that a model is used for observation and assumptions here may not be correct for all other models.

Rivers are extremely important in understanding natural systems of an area. Remember that rivers are studied for many different reasons and any or all of these embraced by the students is a step in the right direction.

Lesson

5

Aquatic Macroinvertebrate Identification

Next Generation Science Standards

- **HS-LS2-4.** Use mathematical representations to support claims for the cycling of matter and flow of energy among organisms in an ecosystem. [Clarification Statement: Emphasis is on using a mathematical model of stored energy in biomass to describe the transfer of energy from one trophic level to another and that matter and energy are conserved as matter cycles and energy flows through ecosystems. Emphasis is on atoms and molecules such as carbon, oxygen, hydrogen and nitrogen being conserved as they move through an ecosystem.]

Background Knowledge

Teacher:

Everyone is familiar with terrestrial insects such as dragonflies, houseflies, mosquitoes, and beetles, but many of us are unaware that many insects live in the water during their larval stages. These insects are part of a larger group of organisms called aquatic macroinvertebrates, which also includes crustaceans, worms, and mollusks. They are called macroinvertebrates because they can be seen with the naked eye and lack vertebrae. The information that follows focuses only on aquatic insects.

Aquatic insects can undergo a complete or an incomplete metamorphosis. In complete metamorphosis (Figure 10.1), the egg hatches and the larva emerges. During the larval stage the insect grows into a pupa, which in tum grows into the adult. The larva and pupa do not look like the adult. Aquatic insects that go through complete metamorphosis include beetles, caddisflies, dobsonfiies, and true flies.

Aquatic insect eggs that go through incomplete metamorphosis hatch into nymphs and then the nymphs mature into the adult insects (Figure 10.2). The nymphs look similar to the adults. Nymphs have exoskeletons that they molt several times before emerging as adults. Aquatic insects that go through incomplete

metamorphosis include mayflies, dragonflies, damselflies, stoneflies, and true bugs.

The gills on mayflies and stoneflies are easy to see. Some species of mayflies have gills that run along their abdomens on the outside of their bodies, and these gills can be seen "beating" in the water. Dragonflies have their gills inside their abdomen, and water is continually pumped through the abdomen out its posterior end. The dragon- fly can use this as method of jet propulsion to make a quick getaway if needed. Most of the true bugs spend their entire life cycle in the aquatic environment and obtain oxygen by surfacing and carrying bubbles of oxygen under their wings or on hairs. All true bugs have piercing, sucking mouth parts, which the bug uses to liquefy the inside of its prey (Singletary, McGinley, and Bledzki 2008).

Aquatic macroinvertebrates make up the basis of the food web of streams and wetlands and are great indicators of water quality. Each species has a different level of pollution tolerance, with some species being very sensitive to and others extremely tolerant of pollution. The website www.roaringfork.org/imageslotherlaquaticinverte-bratesheet.pdf groups the aquatic macroinvertebrates according to pollution tolerance.

Students of all ages love collecting insects. This investigation could be done with any class for exposure to outdoor learning. Most likely it would be used in a biology class when discussing insects. It could also be used in an environmental science class studying water quality, because macroinvertebrates are great indicators of the health of a stream.

Very little setup is required for this investigation if you are experienced with collecting and identifying aquatic macroinvertebrates. If you have not had prior training, be sure to visit the site ahead of time to collect and identify some of the organ- isms first.

One class period indoors should be used to teach the students about the organ- isms they will be collecting by setting up samples of the aquatic invertebrates with dissecting scopes and note cards containing identifying characteristics about each. If you are just starting out and do not have a collection of organisms, pictures of the aquatic macroinvertebrates could be used instead

At least one more class period will be needed for collecting the insects. Students could collect them in one day or over several days. Students could collect and identify the organisms in the field or preserve them in alcohol and return to the classroom to identify them at a later date. If you plan on conducting sampling routinely, we suggest that the organisms be sampled and then released. To help motivate students when they are collecting, you can give the class goals (e.g., they

need to collect 10 different species) and then give student points based on the number collected. Once students can identify the macroinvertebrates, you can give them a list of species they need to find. Students could keep the organisms in a jar until the end of the hour and then bring them up to you to confirm identification. If both a stream and a pond habitat are available, you can have students sample in both locations and compare and contrast the species collected. There are many different methods that can be used to collect macroinvertebrates, as well as many different ways to sample their population. A dichotomous key should be used to identify the organisms. There is a plethora of dichotomous keys; for high school students the best option is a simple picture key because of the easy terminology. One for rivers can be accessed at http://clean-water.uwex.edulpubslpdjlwav.riverkey.pdj and one for ponds at http://clean-water.uwex.edulpubslpdflwav.pondkey.pdj. Both of these keys were developed by the University of Wisconsin-Extension in cooperation with the Wisconsin Department of Natural Resources; the keys may be reproduced for educational purposes as long as proper credit is given.

Student:

A. *Prior Standards:*

- **HS-LS2-1**.Use mathematical and/or computational representations to support explanations of factors that affect carrying capacity of ecosystems at different scales.
- **HS-ESS2-1**. Develop a model to illustrate how Earth's internal and surface processes operate at different spatial and temporal scales to form continental and ocean-floor features.

B. *Life Experience:* Have the students experienced rain? Visited a river? Drunk water from a faucet?

Materials List

- access to stream and/ or pond
- dip net (D-frame dip nets work best) for streams or wetlands or kick net for streams
- tweezers
- shallow pan
- dichotomous key for macroinvertebrates in streams and ponds
- dissecting scope

- informational note cards about insects (make your own or order a set from a science catalog)

Engagement

Objectives: Students will learn how and why invertebrates are essential to *living* rivers and streams. They will also see how gradients of abiotic factors create multiple niche habitats that are inhabited by specialist populations and learn about the metabolic diversity of prokaryotes that live as chemotrophs, phototrophs, autotrophs and deterotrophs. The will observe the cycling of sulfur and other nutrients in a natural system.

Procedures:

Tell the students they are going to learn more about macroinvertebrates and water quality. Ask them the following questions:

1. Describe what your mind sees (what colors, what motions, etc.) when you hear the words "water pollution."

2. Is pollution something you can always see, smell or touch?

3. What is pollution?

4. How does pollution affect our ecosystems?

Exploration

Optional Extension:

If working in a stream, students could also conduct an index of biological integrity (IBI) study to determine the water quality of the stream. Visit the Save Our Streams Program website (www.people.virginia.edu/sos-iwla/Stream-Study/Methods/FormIntro.HTML) to learn more about conducting an IBI and to obtain a data form. There are many different types of IBIs available online, but the Save Our Streams Program link is one of the easiest to use.

Once students are familiar with the aquatic macroinvertebrates, you could have the high school students team up with elementary school students to collect samples. Elementary students love working with older students, and it gives the older students a sense of accomplishment and pride to share what they know with younger students. Additionally, as every teacher knows, the best learning comes through teaching the material. At first, some high school students may be turned off by working with younger students, but we have found over several years that

almost all of our high school students enjoy this experience. It is one item on the end-of-the-year class sur- vey that the students say to keep doing.

Collecting Insects in a Stream

1. Put the dip net onto the stream bottom, facing the net upstream.

2. For each pair of students, one person should stand in front of the net and gently kick the material on the streambed. Any insects present will flow downstream into the net.

3. Pick up any large rocks and gently brush any insects off into the net.

4. Dump the contents of the net into a shallow pan with some water.

5. Sort through the insects and use the key to identify them.

6. Record the data, including names and quantity of insects found, on a sheet of paper.

Collecting Insects in a Pond

1. Most of the insects in the pond will be on the edges near the vegetation. This is where the collecting should take place.

2. Insert the net into the water and sweep it just above the bottom, to avoid the mud, and then along the vegetation. Do a few sweeps.

3. Dump the contents of the net into a shallow pan with some water.

4. Sort through the insects and use the key to identify them.

5. Record the data, including names and quantity of insects found, on a sheet of paper

Explanation

The goal of this investigation is to understand the river system. Bring the class back together and discuss the following essential questions:

- What things about a river would be important to measure?
- What river characteristics and conditions affect flow?
- What river conditions affect the amount of erosion?

Make a list of what they say on the board and go over the key words in the lesson:

Scientific Vocabulary

- Aquatic insect = An insect that spends part or all of its life cycle in the water.
- Benthic macroinvertebrate = Bottom dwelling aquatic animals, without a backbone, that can be seen with the naked eye.
- Deposition = The laying down or dropping of sediment from the water.
- Discharge = Area x Velocity. Amount of water moving through the stream.
- Diversity = Number of different types of organisms that can live together in a certain habitat.
- Entrainment = Picking up of sediment by the water.
- Ephemeral = A stream that does not flow all the time.
- Erosion = the entrainment or picking up of sediment to be moved by water.
- Flood = When streamflow is larger than the normal amount, or when the stream overflows its channel.
- Gills = Structures that organisms that live in water use to get oxygen.
- Gravity = The force that moves objects towards the earth.
- Habitat = The place where animals and plants live. In a stream it includes pools, riffles, deep water, undercut banks, vegetation, gravel and rocks or sand.
- Larva = The immature form of an insect that transforms through complete metamorphosis.
- Macroinvertebrate = Animals that lack a backbone and can be seen with the naked eye.
- Moderately tolerant organism = Organism that can survive with some pollution.
- Morphology = The shape of the stream.
- Nymph = The immature form of an insect that transforms through incomplete metamorphosis.

- Perennial = a stream that does flow all the time, never goes dry.
- Pollution = Any substance that changes our environment in a harmful way and stresses living things. The quality of the environment is impaired.
- Pupa = In insects with complete metamorphosis, a stage where the immature insect is enclosed in a tissue-like cocoon, where reorganization occurs and an adult emerges (usually an immobile stage).
- Sensitive organism = An organism that dies with exposure to a low level of pollution.
- Slope = Rise/Run "the slope of the land"
- Somewhat sensitive organisms = Organisms that can survive with some pollution.
- Tolerant organism = Organisms that can survive in polluted conditions.
- Velocity = Distance / Time. The speed of the water moving through the stream.

Make it a point to say that velocity and discharge were not be measured, but only observed, in this investigation because it is difficult to make measurements accurately on such a small model.

Next let them write their predictions on their worksheet, tell them it's okay if they don't know and to write how they think the two variables in each question relate to one another. If they are having a lot of trouble help them by reminding them how water moves through water slides, washed near their houses, hoses, etc. Introduce the stream tables and their components to the class. Make sure to set any rules that you have in your classroom, for example, "Do not put more than the prescribed amount of water in the table, you will ruin your experiment" or "Do not add more sediment unless you check with a teacher first". These are important for the experiment, as well as the first step towards minimizing the mess after the class is finished!

Did they find actual values or just observing relative similarities and differences?

What did they notice about the stream movement at this velocity, slope, etc.?

Are there any areas along the rivers that are eroded faster? Any areas that deposition occurs more? Is it even throughout the river?

Evaluation

The Evaluation phase will be used to determine what additional information about wetlands—especially related to tides, elevation, and plant communities—the students have learned throughout the lesson. Most of the Evaluation is accomplished by assessing the activities completed by the students during the other phases of the lesson. However, the Evaluation also includes revisiting the discussion questions presented early in the lesson and giving the students an opportunity to modify or change their responses.

Formative: Qualitative Data
Includes on-going teacher observation, questioning, what you will be looking for and/or asking questions about, etc.

As an additional part of the evaluation, display the students' answers to the discussion questions presented during the Engagement phase.

Allow the students to again discuss the questions in their small groups and modify or change any answers with which they no longer agree.

Summative: Quantitative Data
Describe final projects with grading rubrics.

All activities completed by the students throughout the lesson (topographic profiles, explanation paragraphs, tidal graphs and questions, Venn diagrams) will be assessed using a rubric to determine the students' understanding and to assign grades.

Closure

Some things that can be seen clearly in the model may not be able to be seen as easily in real-world examples, such as the Truckee River or Las Vegas Wash. Have them keep this in mind while looking at this model and make sure the kids understand that a model is used for observation and assumptions here may not be correct for all other models.

Rivers are extremely important in understanding natural systems of an area. Remember that rivers are studied for many different reasons and any or all of these embraced by the students is a step in the right direction.

USGS Summer Intern Program

None.

Student Support					
Category	**Section 104 Base Grant**	**Section 104 NCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	1	0	0	0	1
Masters	5	0	0	0	5
Ph.D.	3	0	0	0	3
Post-Doc.	1	0	0	0	1
Total	10	0	0	0	10

Notable Awards and Achievements