University of Kentucky
UKnowledge

Theses and Dissertations--Public Health (M.P.H. & Dr.P.H.)

College of Public Health

2016

Reduction of Fecal Coliform Bacteria Through the Elimination of Sewage Discharges in West Virginia Streams

Kady Rogers
University of Kentucky

Follow this and additional works at: https://uknowledge.uky.edu/cph_etds

Part of the Public Health Commons

Right click to open a feedback form in a new tab to let us know how this document benefits you.

Recommended Citation

Rogers, Kady, "Reduction of Fecal Coliform Bacteria Through the Elimination of Sewage Discharges in West Virginia Streams" (2016). *Theses and Dissertations--Public Health (M.P.H. & Dr.P.H.)*. 79.
https://uknowledge.uky.edu/cph_etds/79

This Dissertation/Thesis is brought to you for free and open access by the College of Public Health at UKnowledge. It has been accepted for inclusion in Theses and Dissertations--Public Health (M.P.H. & Dr.P.H.) by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.

STUDENT AGREEMENT:

I represent that my capstone and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student's advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student's capstone including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Kady Rogers, Student

Wayne Sanderson, PhD, MS, Committee Chair

Corrine Williams, ScD, MS, Director of Graduate Studies

REDUCTION OF FECAL COLIFORM BACTERIA THROUGH THE ELIMINATION OF SEWAGE DISCHARGES IN WEST VIRGINIA STREAMS

CAPSTONE PROJECT PAPER

This paper is submitted in partial fulfillment of the requirements for the Master of Public Health, with a concentration in Environmental Health, from the University of Kentucky

By

Kady Rogers

Lexington, KY

April 2016

Committee Members

Dr. Wayne Sanderson PhD, MS Committee Chair

Dr. David Mannino, MD

Dr. Nancy Johnson, DrPH, MSPH

TABLE OF CONTENTS

ABSTRACT

Background and Objectives

Early environmental regulations such as the Federal Water Pollution Control Act of 1948, and the 1972 amendments—which would later become known as the Clean Water Act—have improved the quality of the drinking and recreational waters here in the United States. This has been achieved as a result of strict regulations on discharges, proper management strategies, as well as thorough sampling and testing for contaminants. One such contaminant is fecal waste; these materials are a public health concern because of the potential risk of gastrointestinal diseases. Scientists have used fecal coliforms as an indicator of the presence of these potential pathogens since the early 1900's. It is especially important to sample for these in areas where straight pipes, faulty septic tanks, and inadequate management facilities are in use, as they are potential sources of contamination. The purpose of this study is to assess the fecal coliform levels prior to sewer construction improvements, and again after construction has been completed to determine if there has been a significant reduction in fecal coliforms in four streams located in West Virginia.

Methods

Fecal coliform data from four streams in West Virginia where sewage management upgrades were obtained: Boggs Run, Dunloup Creek, Soak Creek, and Warm Spring Run. Samples were collected upstream and downstream, before and after the upgrades were complete. The data were analyzed using log transformation, F-test, Student's T-test, and Fisher's exact test to determine which sites had significant reductions in fecal readings.

Results

Two downstream sites, Dunloup MP 11.9 and Warm Spring Run 5.8, had significant decreases in the geometric mean fecal coliform readings. All sites showed a reduction in the median, arithmetic, and geometric mean fecal readings after the sewage management projects were completed, though two of these findings were not significant.

Conclusion

The results of this study suggest updates to, or the replacement of, inadequate sewage management facilities, as well as the elimination of discharges are an effective way to reduce the amount of fecal contamination in streams and rivers. It is also important to consider the source of fecal contamination, environmental impacts, public health implications, when determining the best management practices for dealing with fecal impacts to surface waters.

INTRODUCTION

In 1972 the United States Congress passed the Clean Water Act with the following goals: eliminating pollutants from being discharged into water systems as well as prohibiting toxic pollutants from entering water systems, and improving water quality for wildlife and recreational purposes (Adler, 2011). These goals were to be met by 1985, yet there are still pollutants and toxins being discharged into streams and rivers today. Many of these pollutants are unregulated, and considered to be nonpoint source discharges because they are not associated with a National Pollutant Discharge Elimination System or NPDES permit. One common contaminant is fecal waste which can enter the water through the discharge of raw sewage through straight pipes, failing septic systems, agricultural runoff, and natural land uses (EPA, 2002).

Concern surrounding this particular contaminant is due to the potential pathogenic fecal bacteria in the waste. These pathogens pose a threat to public health and water quality in many areas of the country; however, some regions are especially vulnerable. The Appalachian region stretches from New York to Mississippi and is made up of some of the most disadvantaged areas in the nation. Approximately 22 percent of the counties in this region are considered to be “distressed”—poverty and unemployment rates are 150 percent above the national average. (O'DELL, 2005)

West Virginia is home to many of those distressed counties; though there is funding through the federal government, many people throughout the state still have straight pipes discharging raw sewage, containing high concentrations of fecal bacteria, into streams (O'DELL, 2005). These discharges can lead to water quality impairments. The West Virginia Department of Environmental Protection (WVDEP) uses fecal coliform bacteria as an indicator for fecal pathogenicity in public water sources. This study focuses on fecal coliform samples taken upstream and downstream, before and after construction improvements to determine if these improvements have significantly reduced the amount of fecal material in the four streams of interest.

LITERATURE REVIEW

Historic Laws and Regulations

The first national law to address water pollution was the Federal Water Pollution Control Act of 1948. Since that time many federal and state laws have been passed to ensure the citizens of the United States have access to clean drinking water, as well as adequate recreational waters. The Act was expanded and reorganized in the 1972 amendments—which would later become known as the Clean Water Act (CWA). The CWA addressed unlawful industrial and residential discharges, provided funding for the construction of wastewater treatment facilities, maintained existing regulations and quality standards, and granted authority to implement these provisions to the U.S. Environmental Protection Agency. Today the EPA, along with other federal and state agencies, works to ensure that the goals and regulations set forth by the CWA are being met for all surface waters in the U.S. (EPA, 2014)

The EPA published the National Primary Drinking Water Regulations (NPDWRs) which include legally enforceable drinking water standards. For the purpose of this paper, the contaminant of interest is fecal coliform bacteria. The NPDWRs list total coliform as the contaminant pertaining to fecal pollution where a maximum contamination level goal (MCLG) is 0 mg/L, or 5% maximum contaminant level (MCL) meaning no more than 5% of samples taken in any given month can be total coliform positive (EPA, 2016). The agency in charge of regulating and monitoring water quality conditions in West Virginia is the Department of Environmental Protection. This agency conducts assessments on all watersheds in the state, issues and enforces all waste permits, and works to ensure water quality standards are being met throughout the state (WVDEP, 2016). One of these regulations pertains to the allowable level of fecal coliform in recreation waters which is not to exceed 400/100ml (WVDEP, 2016). It is very important to test for these fecal coliforms as they are valuable indicators of the presence of pathogenic microbes in the water.

Use of Indicator Species

The use of indicators to detect the presence of certain bacteria in different mediums has been in use since the early 1900’s. An indicator species is not necessarily a pathogen, but it could indicate the presence other pathogenic organisms (Griffin, Lipp, McLaughlin, & Rose, 2001). The detection of fecal contamination in water samples became possible in 1904 with the development of the fecal coliform assay (Doyle & Erickson, 2006). An indicator species should meet the following criteria: present in high numbers in human intestine and feces, inability to grow outside the intestinal tract, resistant to environmental conditions, strong association with the presence of pathogenic microorganisms (Cabral, 2010; Savichtcheva & Okabe, 2006). The three most common indicators for fecal contamination are total coliforms, fecal coliforms, and *E. coli* (Noble, Moore, Leecaster, McGee, & Weisberg, 2003).

Total coliforms are a group of related bacteria that are not harmful to humans (Cabral, 2010). These are "facultative anaerobic, gram-negative, non-spore forming, oxidase negative, rod-shaped bacteria that ferment lactose to acid and gas within 48 hours at 35°C" (APHA, Clesceri, & Greenberg, 1998). Total coliforms include *Escherichia* spp., *Klebsiella* spp., *Shigella* spp., *Salmonella* spp., and *Yersinia* spp. (Griffin et al., 2001). They are abundant in the intestinal flora of humans and other warm blooded animals and so were used as indicators for fecal contamination (Winfrey, Strosnider, Nairn, & Strevett, 2010). Sources of total coliforms include fecal material, soils, and water; as a result these are considered to be less reliable indicators of fecal contamination (Tallon, Magajna, Lofranco, & Leung, 2005).

Fecal coliforms are currently the most widely used indicator for fecal contamination. They are gram negative bacilli, nonspore formers, oxidase-negative, optional aerobic or anaerobic, and are able to multiple in the presence of bile salts or other surface agents with equivalent properties (Doyle & Erickson, 2006). These differ from total coliforms as they are able to grow in elevated temperatures and ferment lactose in 48 hours at 44.5°C in mediums with bile salts (Cabral, 2010; Griffin et al., 2001). The range of detectable species with this indicator assay is much lower than those detected by total coliforms (Cabral, 2010). The genera include *Escherichia* spp., *Enterococci* spp., and *Clostridium perfringens*. These bacteria are considered to be more reliable as indicators as they are present specifically in the intestinal tract and feces of humans and warm blooded animals.

E. coli has been increasingly used in the past decade as many researchers prefer it to other indicators. It is being recommended as a replacement to the current fecal coliform assay for two key reasons: first, it is apparent that some fecal coliforms are not fecal in origin, and second testing methods for *E. coli* have improved significantly (Tallon et al., 2005). *E. coli* is the most common fecal coliform, and although most strains are not considered to be pathogenic, *E. coli* O157:H7 does pose a serious risk to human health (Cabral, 2010; Jamieson, Gordon, Sharples, Stratton, & Madani, 2002).

Environment

Fecal coliform bacteria are generally non-disease causing organisms; they are distributed throughout the gastrointestinal tract, with the majority in the large intestine (Cabral, 2010). Human and animal waste contains large amounts of these bacteria, and their presence or absence in the environment allows scientists to determine the type of impacts affecting water systems. Once in the environment they may exist in wastewater storage systems, soils, groundwater, and surface waters. The sources of fecal material in streams include straight pipes, failing septic systems, agricultural runoff and urban runoff. (Cabral, 2010)

Though the CWA of 1977 made it illegal to dispose of any waste directly into water systems without a permit, many straight pipes are still in use. This is especially true of the Appalachia region due to lack of funding for proper sewage disposal or geology that prevents the use of septic tanks. These open pipes transport raw, or partially settled sewage into close by streams or ditches. It is often impractical for rural residences to have underground septic tanks due to limited lot sizes or bedrock geology. Federal funding has been made available to many states where straight pipes are still in use as a way to improve infrastructure and reduce sewage impairments. (O'DELL, 2005)

Urban areas also contribute to the impairment of water systems through runoff. Urban wet-weather sources of fecal contamination include storm water, combined sewer overflows, and sanitary sewer overflows. This type of pollution is difficult to monitor and control as it is dependent upon the weather, magnitude of flow, and concentration of contamination. The concentration of bacteria in storm water mainly comes from domestic animals, wildlife, human waste, and growth of microorganisms in standing waters. These wet-weather sources can be very detrimental to receiving waters, especially if they are used for recreation or drinking water as they are subject to strict pollution guidelines. Dry-weather sources of bacteria include ground water infiltration and sanitary sewer cross-connections. (Marsalek & Rochfort, 2004)

Livestock agriculture is also a source of bacterial contamination to surface and ground waters. A major pathway for contamination is the application of manure as fertilizer to tile drained land. In a rain event, the bacteria present in the manure could be collected in runoff that will eventually end up in streams. Depending on the distance from the stream channel it is also possible for the manure to enter the stream in a flood. Another route of contamination from livestock is through the direct access to streams. Many farmers rely on streams and creeks to provide water for their animals, and the livestock sometimes use these sources to cool off during warmer seasons. The survival of these bacteria in the soil is dependent on many factors such as soil type, moisture content, temperature, and pH. (Jamieson et al., 2002)

Though fecal bacteria enter the environment through a number of ways their survival is not assured; it is dependent upon many environmental factors including moisture, soil type, temperature, and pH. Several research studies have found enteric bacteria survive best in high moisture, even flooded, soils. (Hagedorn et al., 1978) observed an increase in E. coli populations just after major rain events led to a rise in the water table. It has been proposed that environments with limited moisture availability are not ideal for enteric bacteria. The type of soil also determines the survivability of enteric bacteria; because the moisture content is important, soils that can retain water tend to promote bacterial growth. The temperature of soil or water can promote or reduce the survival of bacteria; Filip et al. (1988) found that E. coli could survive in mixtures of soil and water for over 100 days at 10°C, though warmer temperatures would have been optimal for growth. Finally, a water or soil pH of 6 or 7 was shown to be optimal for enteric bacterial growth. (Jamieson et al., 2002)

Detection Methods

Water is usually tested for these bacteria using simple metabolic reactions. Traditional sampling methods for total and fecal coliforms include multiple-tube fermentation (MTF) and membrane filtration (MF) (Tallon et al., 2005). MTF provides a most-probable number, and is generally used in highly contaminated samples (Cabral, 2010). MTF is performed after growth of total coliforms in a liquid medium. MF is used for low concentrations of contamination; it is plated on agar and used to detect CFU/100mL count (Edberg, Allen, & Smith, 1988). Neither method can isolate and identify bacteria to species or differentiate total coliform from fecal coliform (Edberg et al., 1988).

Management

The influence of fecal contaminants on water systems is a great concern to public health; and as such, strategies have been developed over time to manage and mitigate their influence. Currently, wastewater is being treated with a multiple-barrier approach; this is a combination of processes set up to prevent or reduce the contamination of water so it can be returned to the environment with an acceptable purity level. This approach includes three major components: source water protection, drinking water treatment, and drinking water distribution. (Spellman, 2013)

Within this management approach are septic systems which keep contaminants such as fecal materials out of streams and other surface water systems. These onsite systems process household and commercial sewage and include wastewater treatment plants, package plants, and individual septic tanks systems (EPA, 2004). If planned and designed properly, these systems offer health benefits by reducing the exposure to pathogens and as a result decreasing the risk of disease. However, if the design was poorly executed or the acceptable flows are exceeded septic systems could overflow or leach and cause other problems (NESC, 2015).

According to the WV Department of Environmental Protection (WVDEP), approximately 7,000 septic tanks are installed each year in the state of WV. These are typically underground holding systems with two main parts: a septic tank and a drainfield. Wastewater flows from the residence through and inlet pipe, and then into the watertight tank. The wastewater is effectively treated while in the tank as the solids and liquids separate forming three layers: scum, partially clarified water, and sludge. Sludge is semi-liquid waste produced from sewage; it is a major product of septic tank and activated sludge systems. Bacteria in the tank break down the solids while the liquids flow from the tank, through and outlet pipe, and into the drainfield. The drainfield is a usually made up of a network of trenches or deep layer of fine gravel buried under the surface that acts as a biological filter. Perforated pipes run along the drainfield so the liquids are dispersed evenly. (NESC, 2015)

A package plant is an alternative to in-ground wastewater treatment options. Package plants are extended aeration processes used primarily in small communities, suburban subdivisions, rest areas, or trailer parks where flow rates are below 0.5 MGD; though they can be designed to treat flows as low as 0.002 MGD (Spellman, 2013). These types of facilities are different from larger wastewater treatment plants because they are pre-fabricated, delivered to the site, and generally require little day-to-day maintenance (EPA, 2000). The most common type of package plant is the extended aeration model. This system utilizes biological treatment of biodegradable waste in aerobic conditions (EPA, 2000). Simplified, wastewater enters the system and the large particles are screened out immediately, then the waste is passed through a grinder, it is aerated, clarified, and the material left over is either returned as activated sludge (RAS) or removed for disposal as waste activated sludge (WAS) (EPA, 2000). The advantages of package plants include: easy installation and operation, better equipped to handle flow fluctuations, odor free with small footprints, and low sludge yields (EPA, 2000). The disadvantages include; limited flexibility if regulation changes, longer aeration requires additional energy to run processes, and without additional units these processes do not achieve denitrification or phosphorus removal (EPA, 2000).

These facilities are not inexpensive; and many times small communities are unable to update these elements without funding through the state and federal governments. West Virginia has a program called the Clean Water State Revolving Fund to address water quality issues through the wastewater facility construction, upgrades, or expansions (WVDEP, 2016). The money for these programs is loaned to communities with low interest rates, and the payments returned to the state are used to fund more project loans and grants. Communities are recommended for funding through the WV Infrastructure and Jobs Development Council, but they must meet certain requirements prior to receiving the loan. Other assistance programs are available through different agencies.

Public Health Implications

Prior to using public sewer systems and treatment facilities much of the biological waste generated by humans was disposed of in privies and cesspools (EPA, 2004). These primitive conditions left people vulnerable to disease transmission and other health risks. Advancements in the field of public health have greatly improved living conditions, and reduced the spread of diseases from pathogenic fecal coliforms; however it is estimated that approximately 560,000 people suffer from waterborne diseases each year in the United States (Cabral, 2010).

The greatest risk of microbial infection is the ingestion of contaminated water (Cabral, 2010). The three most common water transmitted bacterial gastrointestinal diseases are cholera, salmonellosis, and shigellosis. Their level in the water is generally very low and occurs sporadically or erratically (Cabral, 2010). Public health concerns for streams similar to the ones in this study include recreational uses such as swimming, fishing, and wading.

It can be difficult for public health practitioners to determine the source of outbreaks of gastrointestinal related to water systems such as streams and rivers. Many factors influence the level of fecal coliforms in surface water; and so tracking the outbreak to its source is a challenge. These pathogenic bacteria survive differently in varying environmental conditions, and the detection of these organisms is time sensitive and relatively costly. Understanding environmental systems and how they influence the distribution and survival of pathogenic bacteria is a primary concern for many public health officials. (Barb Peichel, 2009)

Research objectives

1. Determine whether sites showed a significant reduction in fecal coliform colonies after construction upgrades and improvements were made.
2. Determine which upgrades or improvements made during construction had the greatest contribution to the reduction of fecal coliform.

METHODS

Fecal coliform data were obtained for four locations from the West Virginia Department of Environmental Protection. Boggs Run, Dunloup Creek, Soak Creek, and Warm Spring Run were the sites where construction upgrades were made to local sewage outflows and treatment facilities. Water samples were collected just upstream and immediately downstream from the construction areas, at low flow, in 100mL sterile containers and quickly packed on ice for membrane filtration analysis. Membrane filtration analysis was reported in colonies/100mL. Water samples with readings above 400 colonies/100mL are above the DEP's maximum daily criterion and considered contaminated.

Sample Sites

Boggs Run

Boggs Run is located in the community of Moundsville in Marshall County, West Virginia. Four areas in the community were approved for construction upgrades: Pin Oak Subdivision, Fort Clark Estates, Rustic Hills, and East 4th Street. Pin Oak Subdivision construction included upgrading the existing wastewater collection system and adding an internment sand filter to the treatment plant. Fort Clark Estates construction included upgrades to the existing wastewater collection system, replacement of an existing septic tank treatment facility with a package plant. Rustic Hills construction included upgrading the existing wastewater collection system to reduce the inflow levels, and upgrades to the package plant by installing a flow equalizer. East 4th Street construction upgrades included extended sewer service through two gravity sewers, two sewage pump stations, a grinder pump station, and a sewage force main. Water samples were collected at Boggs Run MP 3.1 from July 2005 to June 2006 prior to construction. Once construction was completed water samples were taken again from MP 3.1 from August to October 2009.

Dunloup Creek

Dunloup Creek is located in Fayette and Raleigh County in West Virginia. Construction for the communities of Kilsyth and Price Hill was approved in order to eliminate raw sewage discharges through straight pipes and failing septic systems. The construction project included upgrading the sludge maintenance and installing a micro-strainer screen at the main lift station of the WWTP. Water samples on Dunloup Creek were collected upstream of construction at MP 13.6 from August 2006 to August 2007, and again after construction from September to October 2009. Water samples were also collected downstream, prior to construction, at MP 11.9 from May 2002 to August 2007, and again after construction from September to October 2009.

Soak Creek

Soak Creek is located in Raleigh County, West Virginia. This project was located in the town of Sophia and included the construction of a new sewer system to eliminate septic tanks and direct discharges. Samples were taken upstream of construction at MP 5.1 from July to August in

2008, and again after construction from September to October 2009. Water samples were also collected downstream, prior to construction from June to August 2008, and again after construction from September to October 2009.

Warm Spring Run

Warm Spring Run is located in Morgan County, West Virginia. The sewage improvement project included extending sewer services to approximately 135 customers, and the construction of a new, activated sludge WWTP. Three locations on Warm Spring Run were sampled: MP 8.2, 5.8, and 4.9. Water samples collected at MP 8.2 were upstream of construction and were taken from June to October 2007, and again after construction from August to October 2009. Sites downstream from construction included MP 5.8 and 4.9, these were sampled between June and October 2007, and again after construction was completed from August to October 2009, and from August 2009 to June 2014.

STATISTICAL ANALYSIS

Data were received from the WVDEP and downloaded for analysis into Microsoft Excel. Descriptive statistics were performed for fecal coliform colonies. Each site had a skewed distribution of coliform samples, so the data were log transformed, and normal distribution statistics were applied. F-tests were used to determine variance equality or inequality. Student’s T-test was used to determine differences in the geometric sample means at each location, before and after construction. The Fisher’s exact test evaluated the proportion of samples above and below the 400 colonies/100mL criterion, before versus after construction. The percentage of samples above and below 400 colonies/100mL, the DEP’s maximum daily criterion, were also determined. ArcMap 10.3 was utilized to develop maps depicting sampling points on each stream and through the state of West Virginia (Figures 1-5).

RESULTS

Site locations, environmental impacts, and proposed improvement upgrades are detailed in Table 1. All sites were surrounded by or in close proximity to roads and residential areas. Dunloup Creek and Soak Creek both had impacts from old mine sites and gas wells. Impacts from residential areas present the greatest risk for the introduction of fecal material into nearby streams.

The descriptive statistics concerning the fecal coliform samples are reported in Table 2. All of the locations showed a decrease in the median fecal coliform colonies after the upgrades to sanitation were installed (Table 2). The largest decrease in median colonies occurred at Dunloup Creek MP 11.9; samples taken before the work was completed had a median value of 6600 colonies/100mL, and 720 colonies/100mL after the work was complete. All of the downstream locations had reduced arithmetic mean fecal coliform readings after the upgrades were completed. The highest reduction was reported for Dunloup Creek MP 11.9 with 9176.0 colonies/100mL, followed by the second highest decrease of 1138.4 colonies/100mL at Warm Spring Run MP 5.8 (Table 2). The geometric mean for each of the eight sites also indicated a reduction in fecal coliform colonies post construction (Table 3). The greatest decrease in geometric mean was 7203.12 and occurred at Dunloup Creek MP 11.9.

Two sites, both located downstream of construction, showed a significant reduction in the geometric mean fecal coliform count: Dunloup Creek MP 11.9, and Warm Spring Run MP 5.8, with respective p values of 0.01 and 0.02 (Table 3); This is visually supported by scatter plots showing a decrease in the sample values once upgrades were complete (Figures 2 and 7).

Table 4 represents the percentage of samples above and below the criterion of 400 colonies/100mL, before and after the upgrades were completed. The percentage of samples at or above 400 colonies/100mL was reduced by at least 20% in all but two sample sites after the upgrades were completed. The two sites with no reduction in percent of samples above the criterion, Dunloup Creek MP 13.6 and Soak Creek MP 3.9, were both located upstream of the construction upgrade sites (Table 3). The site with the highest decrease in the percent of samples above the maximum criterion was Warm Spring Run MP 5.8 with a 51.7% reduction. The Fisher's exact test showed no significant difference in the proportion of samples above or below the criterion before and after construction work was completed (Table 4).

DISCUSSION

The results of this study do indicate that construction upgrades did help to improve fecal coliform levels in these four streams. Though not significant for all sites, each of the downstream locations indicated a reduction in the median, arithmetic, and geometric mean fecal coliform readings. Two sites showed a significant reduction in the fecal coliform contamination: Dunloup Creek and Warm Spring Run.

Dunloup Creek was the most fecal impaired stream in this study prior to the sewage improvements; the downstream sample location had the highest maximum reading, 22,790 colonies/100mL. Once the improvements were made there was a significant reducing in the level of fecal contamination, though it is still above the maximum daily criterion. The upstream location, MP 13.6, had an initial maximum reading of 32; so between MP 13.6 and MP 11.9 there are sources of fecal contamination contributing to the high volume. The elimination of direct discharges, extension of sewer services, and the implementation of a new sludge handling system in the WWTP did greatly reduce the fecal readings at MP 11.9.

Warm Spring Run MP 5.8 also showed a significant reduction in fecal coliform readings. The maximum reading at this downstream location was 2430 colonies/100mL prior to construction, and 410 colonies/100mL once the work had been completed. Only one sample taken after the upgrades was over the maximum criterion for fecal coliform; it was only over by 10 colonies/100mL. Though not statistically significant, the second downstream location, MP 4.9, revealed reductions to the median, maximum, mean, and geometric mean fecal coliform readings. The reduction of fecal coliform at this site is most likely due to the construction of a new WWTP. The previous WWTP was only able to manage and treat 400,000 gallons of wastewater per day; whereas the new activated sludge WWTP was able to process over 1.74 million gallons per day. According to public service district officials in the town Bath, this allowed for greater flow management, and better effluent levels.

CONCLUSION

The results of this study suggest these fecal projects did help to reduce the fecal coliform levels in the four streams of interest. As expected, the sewage improvement projects had a greater impact on the reduction of fecal coliform in the downstream site due to flow direction.

These findings suggest the most effective way to reduce fecal contamination is to upgrade or replace the WWTP to adequately support and treat the level of wastewater being delivered to these sites. Employees at the new WWTP on Warm Spring Run saw significant improvements in their effluent readings, and the results of this study also indicate a significant reduction in fecal coliform in the downstream sampling sites. Warm Spring Run MP 4.9 readings indicate the positive impacts from these upgrades may decrease as distance from the project location and fecal sources increase.

The levels of fecal coliform vary greatly in surface waters due to factors such as environmental influences, interaction between multiple sources, and bacteria survival variation (Peichel et al., 2009). In each of the sites where improvements were made there was an evident decrease in fecal coliforms, though none brought the readings below the maximum daily criterion of 400 colonies/100mL. Further studies could address the source of bacteria—perhaps there are wildlife or agricultural influences that are not being addressed as contributors to contamination. It is also possible that some of the readings are not indicative of enteric bacteria, but rather naturally occurring fecal coliform bacteria.

This study could be used in support of sewage improvement projects in areas where containment systems are not meeting the needs of the community. The dilemma in many of these situations is the need for upgrades to treatment facilities, but a lack of funding for the construction of these projects. Several of the communities included in these sewage upgrades were in violation of WVDEP water quality standards and were pressed into action by environmental enforcement officials. It is important for public health officials in small communities to understand the connection between funding opportunities, infrastructure needs, and the potential disease risks of contact with contaminated water sources.

LIMITATIONS

This study does have several limitations; first, the number of samples taken at each site is small. The highest number of samples taken at a location was twelve, and the lowest number of samples taken was three. As fecal coliform levels vary greatly in the water it is important to sample frequently to capture natural fluctuations. Soak Creek for example, was only sampled three times after construction was complete—though these samples indicate a reduction, a higher amount of samples could have shown a significant decrease in geometric fecal coliform mean.

It is possible that some of the fecal coliform bacteria is not enteric in nature, and therefore not associated with fecal contamination. One way to differentiate between naturally occurring levels of fecal coliform, and fecal coliforms from fecal waste would be to establish a reference stream. This stream should be free of residential impacts and regular recreational activities; it would be sampled regularly to determine a baseline for naturally occurring coliforms. Another way to determine if the readings were truly enteric in nature would be to change indicator species from fecal coliforms to E. coli. This indicator is supported by the EPA as it is more selective in identifying enteric bacteria.

Another potential limitation is no consideration of the time of year in which the samples were collected. Most of the samples taken before the projects were started were collected in June, July, and August when the water temperatures are the highest in West Virginia. The samples collected after the projects were completed were mostly collected in September and October when the water temperature starts to drop. This could have had an effect on the levels of fecal coliform present in the stream, as the bacteria survive better in warmer temperatures. Sampling at each site should have taken place in the same months to reduce bias from bacterial survival at different water temperature ranges.

High flow conditions also impact the amount of fecal coliform in the streams. Some of the samples were collected when waters were slightly turbid—this could indicate a high flow event, resulting in an influx of bacteria from the soil and even the water table. Also there is no record of how many residences located along or near the streams were using straight pipes to discharge household waste, and how many of those were included in the sewage upgrades.

REFERENCES

Adler, R. W. (2011). Returning to the Goals of the Clean Water Act. *Journal of Contemporary Water Research and Education, 88*(1), 5.

APHA, L., Clesceri, A., & Greenberg, A. (1998). Eaton. *Standard Methods for the Examination of Water and Wastewater (20th ed.), American Public Health Association, Washington, DC*.

Barb Peichel, P. V., Art Persons, Pat Conrad, Andrea Plevan, and Toben Lafrancois. (2009). *Literature Summary of Bacteria-Environmental Associations Developed for the Upper Mississippi River Bacteria TMDL Project*. Retrieved from https://www.pca.state.mn.us/sites/default/files/wq-iw8-08l.pdf.

Cabral, J. P. (2010). Water microbiology. Bacterial pathogens and water. *International Journal of Environmental Research and Public Health, 7*(10), 3657-3703.

Doyle, M. P., & Erickson, M. C. (2006). The fecal coliform assay, the results of which have led to numerous misinterpretations over the years, may have outlived its usefulness. *Microbe, 4*, 162-163.

Edberg, S. C., Allen, M. J., & Smith, D. B. (1988). National field evaluation of a defined substrate method for the simultaneous enumeration of total coliforms and Escherichia coli from drinking water: comparison with the standard multiple tube fermentation method. *Applied and Environmental Microbiology, 54*(6), 1595-1601. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC202702/

EPA. (2000). *Wastewater Technology Fact Sheet*. Retrieved from https://www3.epa.gov/npdes/pubs/package_plant.pdf.

EPA. (2002). *Metals, pH and Fecal Coliform Bacteria Total Maximum Daily Loads (TMDLs) for the Dunloup Creek Watershed, West Virginia*. Philadelphia, PA.

EPA. (2004). *Primer for Municipal Wastewater Treatment Systems*. Washington, DC Retrieved from https://www3.epa.gov/npdes/pubs/primer.pdf.

EPA. (2014). *Requirements Governing Water Quality Standards*. Retrieved from https://www.epa.gov/sites/production/files/2014-12/documents/wv-series2.pdf.

EPA. (2016). *Table of Regulated Drinking Water Contaminants* Retrieved from https://www.epa.gov/your-drinking-water/table-regulated-drinking-water-contaminants#four.

Griffin, D. W., Lipp, E. K., McLaughlin, M. R., & Rose, J. B. (2001). Marine Recreation and Public Health Microbiology: Quest for the Ideal Indicator This article addresses the historic, recent, and future directions in microbiological water quality indicator research. *Bioscience, 51*(10), 817-825.

Jamieson, R., Gordon, R., Sharples, K., Stratton, G., & Madani, A. (2002). Movement and persistence of fecal bacteria in agricultural soils and subsurface drainage water: A review. *Canadian Biosystems Engineering, 44*(1), 1-9.

Marsalek, J., & Rochfort, Q. (2004). Urban wet-weather flows: sources of fecal contamination impacting on recreational waters and threatening drinking-water sources. *Journal of Toxicology and Environmental Health, Part A, 67*(20-22), 1765-1777.

NESC. (2015). National Environmental Services Center, WVU. Retrieved from http://www.nesc.wvu.edu/subpages/septic_defined.cfm

Noble, R. T., Moore, D. F., Leecaster, M. K., McGee, C. D., & Weisberg, S. B. (2003). Comparison of total coliform, fecal coliform, and enterococcus bacterial indicator response for ocean recreational water quality testing. *Water research, 37*(7), 1637-1643.

O'DELL, G. A. (2005). Community Self-Help Activism in Water/Sewer Development: Case Studies from McDowell County, West Virginia, and Letcher County, Kentucky. *Appalachian Journal, 33*(1), 54-76.

Savichtcheva, O., & Okabe, S. (2006). Alternative indicators of fecal pollution: relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. *Water research, 40*(13), 2463-2476.

Spellman, F. R. (2013). *Handbook of water and wastewater treatment plant operations*: CRC Press.

Tallon, P., Magajna, B., Lofranco, C., & Leung, K. T. (2005). Microbial indicators of faecal contamination in water: a current perspective. *Water, Air, and Soil Pollution, 166*(1-4), 139-166.

Winfrey, B., Strosnider, W., Nairn, R., & Strevett, K. (2010). Highly effective reduction of fecal indicator bacteria counts in an ecologically engineered municipal wastewater and acid mine drainage passive co-treatment system. *Ecological Engineering, 36*(12), 1620-1626.

WVDEP, W. V. D. o. E. P. (2016). *Water and Waste Management*. West Virginia Retrieved from http://www.dep.wv.gov/WWE/Pages/default.aspx.

ACKNOWLEDGEMENTS

I would like to thank the West Virginia Department of Environmental Protection for providing me with the fecal coliform data used in this study. I would also like to thank the members of my committee for their assistance and guidance in the completion of this study: Capstone Chair Dr. Wayne Sanderson, Committee Member Dr. David Mannino, and Committee Member Dr. Nancy Johnson. In addition, I would like to thank Ryan Pack for his assistance in creating the maps used in this paper.

BIOGRAPHICAL SKETCH

Kady Rogers is originally from Ripley, West Virginia. She earned her Bachelor of Science degree in Biology from West Virginia Wesleyan College. Currently, she is a Master of Public Health candidate in Environmental Health at the University of Kentucky. During her time at the University of Kentucky she was a teaching assistant in Academic Enhancement, as well as a Central Appalachian Regional Education and Research (CARERC) funded fellow.

APPENDIX 1. TABLES AND FIGURES

Table 1. Site descriptions, land use, and construction data.

Stream Name	County	ANCODE	Land Use	Construction Upgrades
Boggs Run	Marshall	WVO-86	residential, roads, powerlines	Extended sewer service, new package plant, updated package plants
Dunloup Creek	Fayette/ Raleigh	WVKN-22	residential, old mines, gas wells, roads	Extended sewer services, eliminate straight pipes and direct discharges, new sludge handing at WWTP
Soak Creek	Raleigh	WVKN-26-K	residential, mining, gas wells, roads	Extended sewer services in two locations
Warm Spring Run	Morgan	WVP-10	residential, roads, powerlines	Eliminate septic tanks for 135 customers, extension of sewer services, new wastewater treatment plant

Table 2. Descriptive statistics and log transformations.

Stream	# of Samples	Maximum	Median	Arithmetic Mean (SD)	Geometric Mean (SD)
Boggs Run					
MP 3.1					
Before	5	2100	1550	1406 (848.63)	1184.26 (1.87)
After	5	2170	587	871.4 (858.02)	573.28 (4.64)
Dunloup Creek					
MP 11.9					
Before	7	22790	6600	11044.3 (9670.2)	8048.57 (4.23)
After	7	6880	720	1868.3 (2524.2)	845.45 (10.01)
MP 13.6					
Before	3	32	18	19.3 (16.04)	13.74 (2.52)
After	5	678	9.5	120.3 (274.3)	13.38 (22.01)
Soak Creek					
MP 3.9					
Before	6	926	171	292.7 (345.52)	187.01 (4.88)
After	3	109	7	39.3(61.273)	9.17 (11.16)
MP 5.1					
Before	6	2286	670	869.0 (794.27)	540.67 (8.08)
After	4	1089	268	411.8 (510.97)	121.48 (14.11)
Warm Spring Run					
MP 4.9					
Before	5	428	350	378.4 (191.74)	337.37 (1.21)
After	6	490	350	300 (186.55)	232.30 (3.89)
MP 5.8					
Before	5	2430	1750	1394 (1702.58)	943.16 (4.89)
After	12	410	302	255.6 (143.66)	180.70 (14.27)
MP 8.2					
Before	5	4170	440	1340 (1765.09)	732.94 (5.53)
After	5	451	375.8	375.8 (175.67)	331.37 (1.54)

Table 3. Change in geometric mean, before and after construction

Stream	Δ Geometric Mean	T-Test (p-value)
Boggs Run		
MP 3.1	610.98	0.22
Dunloup Creek		
MP 11.9	7203.12	0.01
MP 13.6	0.36	0.98
Soak Creek		
MP 3.9	177.84	0.17
MP 5.1	419.19	0.28
Warm Spring Run		
MP 4.9	105.07	0.11
MP 5.8	762.46	0.02
MP 8.2	401.57	0.22

Table 4. Percentage of samples exceeding 400 colonies/100mL maximum daily criterion.

Site	Percentage of Samples above Max Limit (400 colonies/100mL)		Fishers Exact
	Before	After	(p-value)
Boggs Run			
MP 3.1	100.0	60.0	0.44
Dunloup Creek			
MP 11.9	100.0	66.7	0.19
MP 13.6	0	16.7	1.0
Soak Creek			
MP 3.9	16.7	0	1.0
MP 5.1	83.3	50.0	0.50
Warm Spring Run			
MP 4.9	40.0	16.7	0.55
MP 5.8	60.0	8.3	0.053
MP 8.2	60.0	40.0	1.0

Figure 1. Map of the fecal coliform sampling site locations in West Virginia.

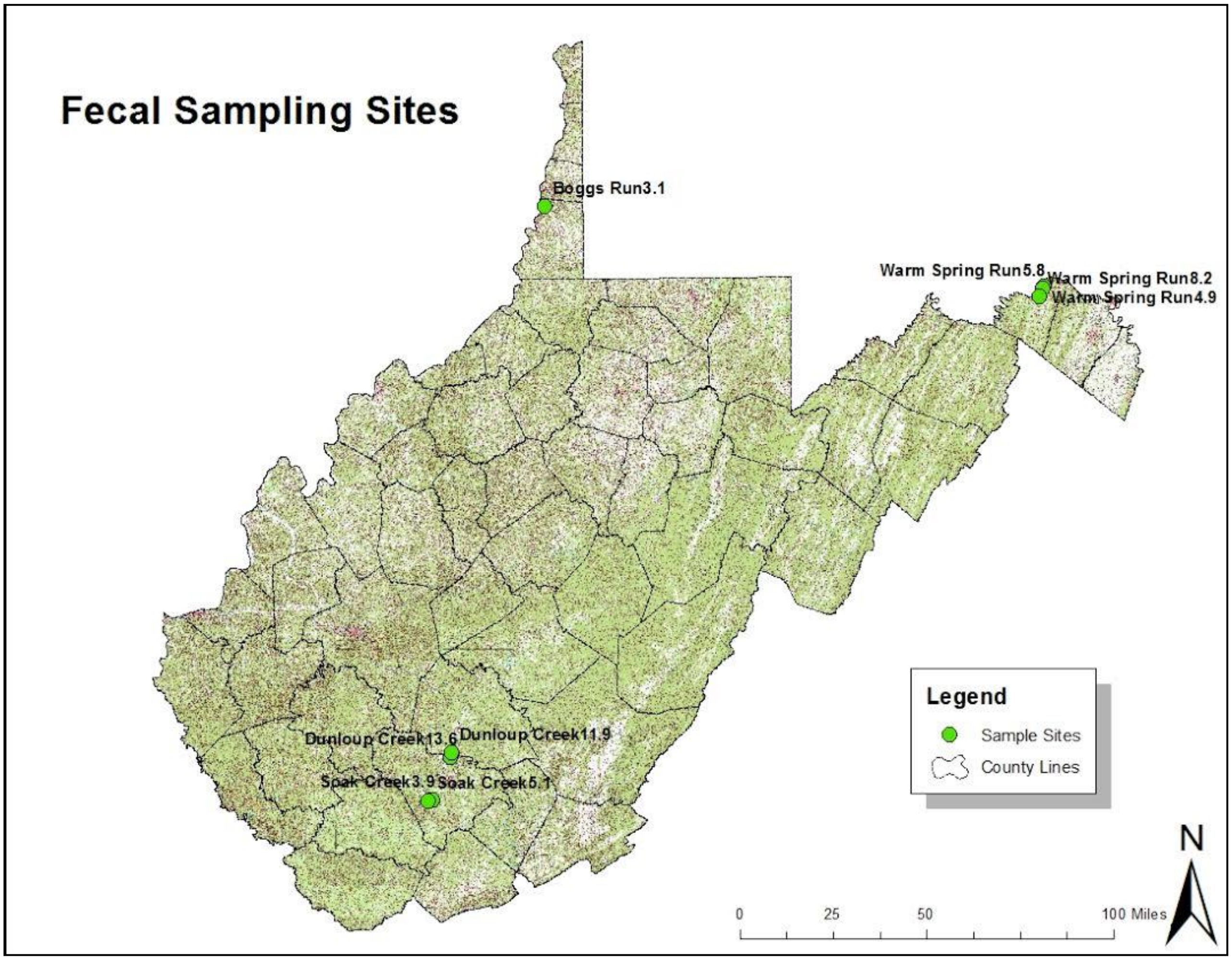


Figure 2. Downstream sampling location at Boggs Run.

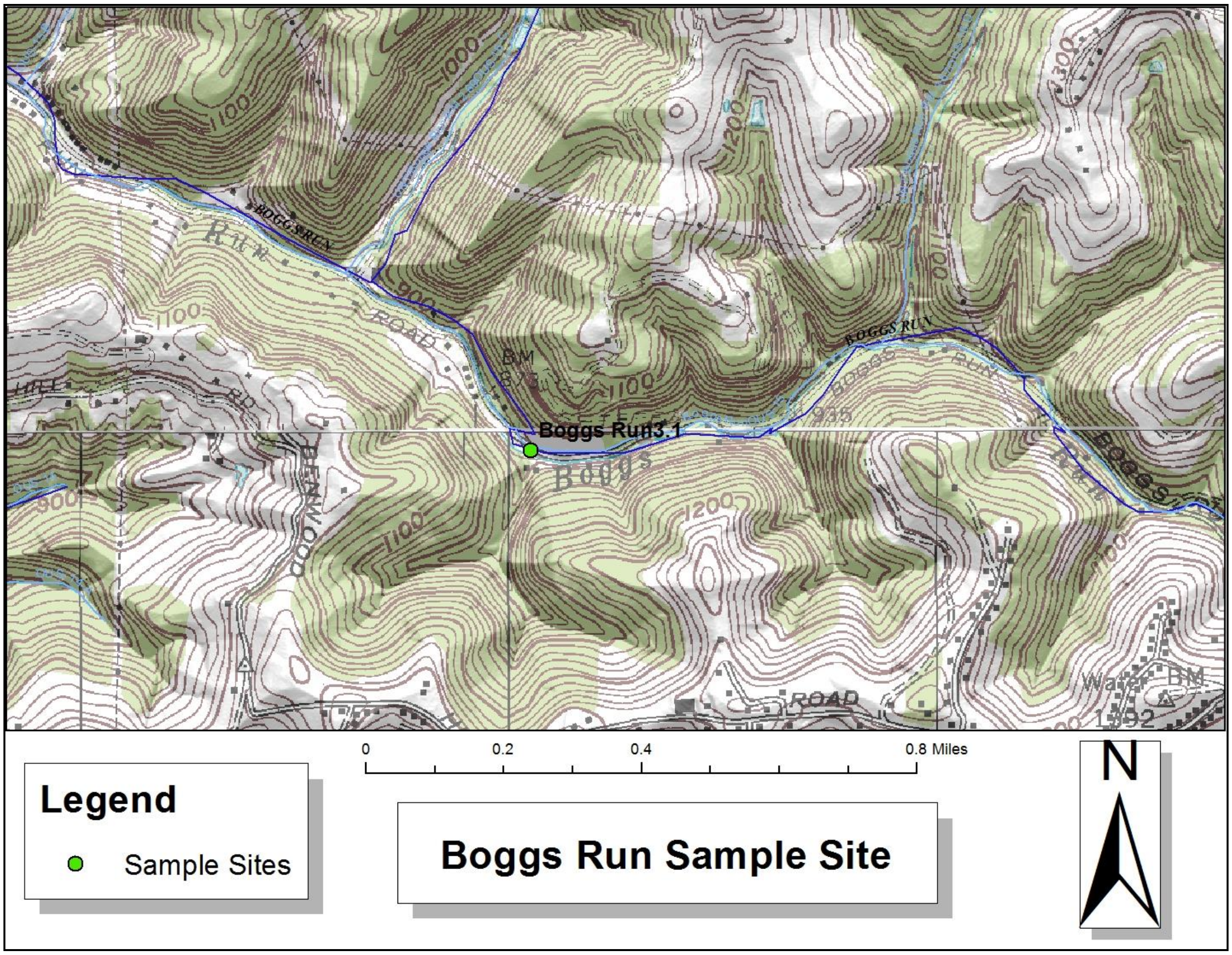


Figure 3. Upstream and Downstream sampling sites on Dunloup Creek.

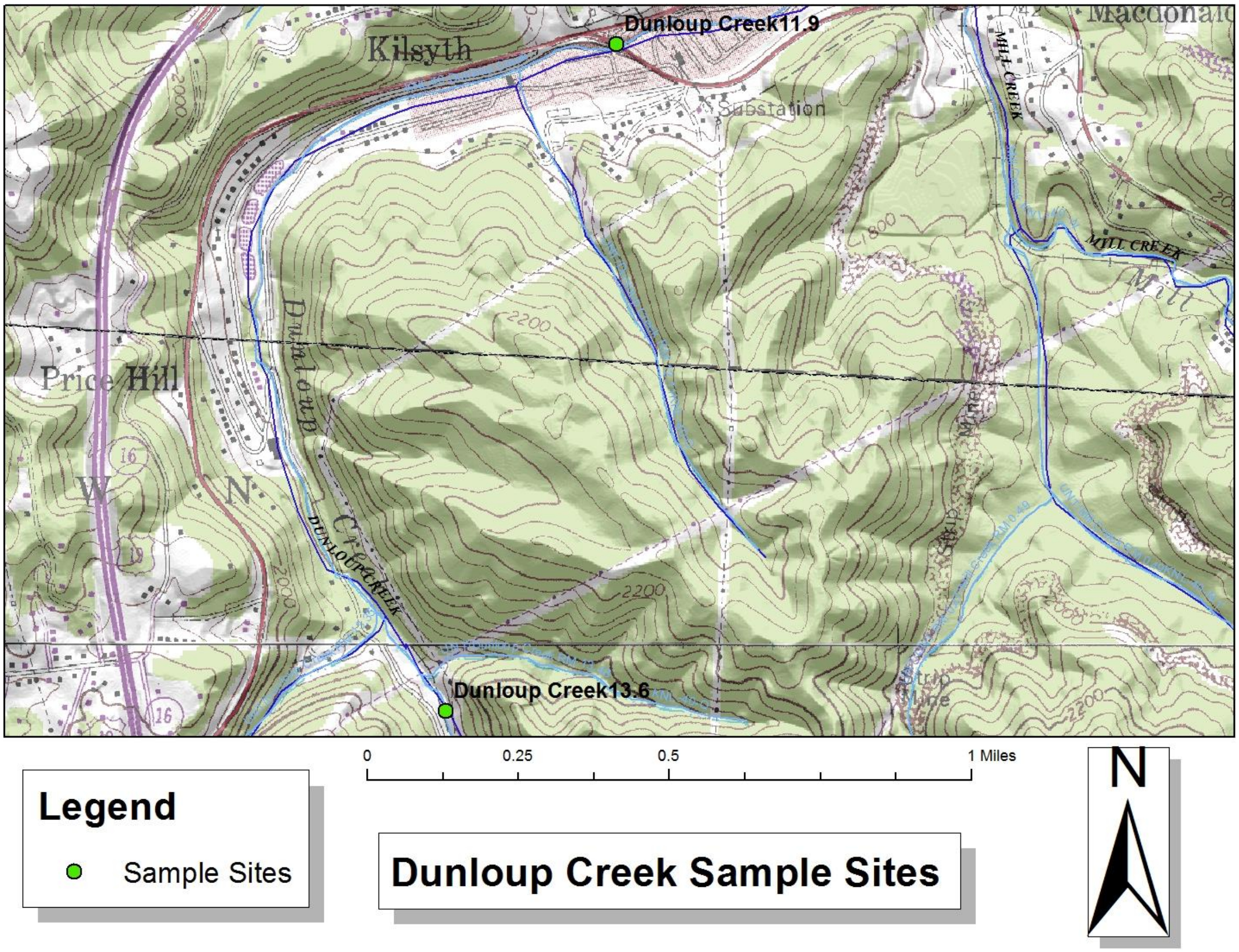


Figure 4. Upstream and Downstream sampling sites on Soak Creek.

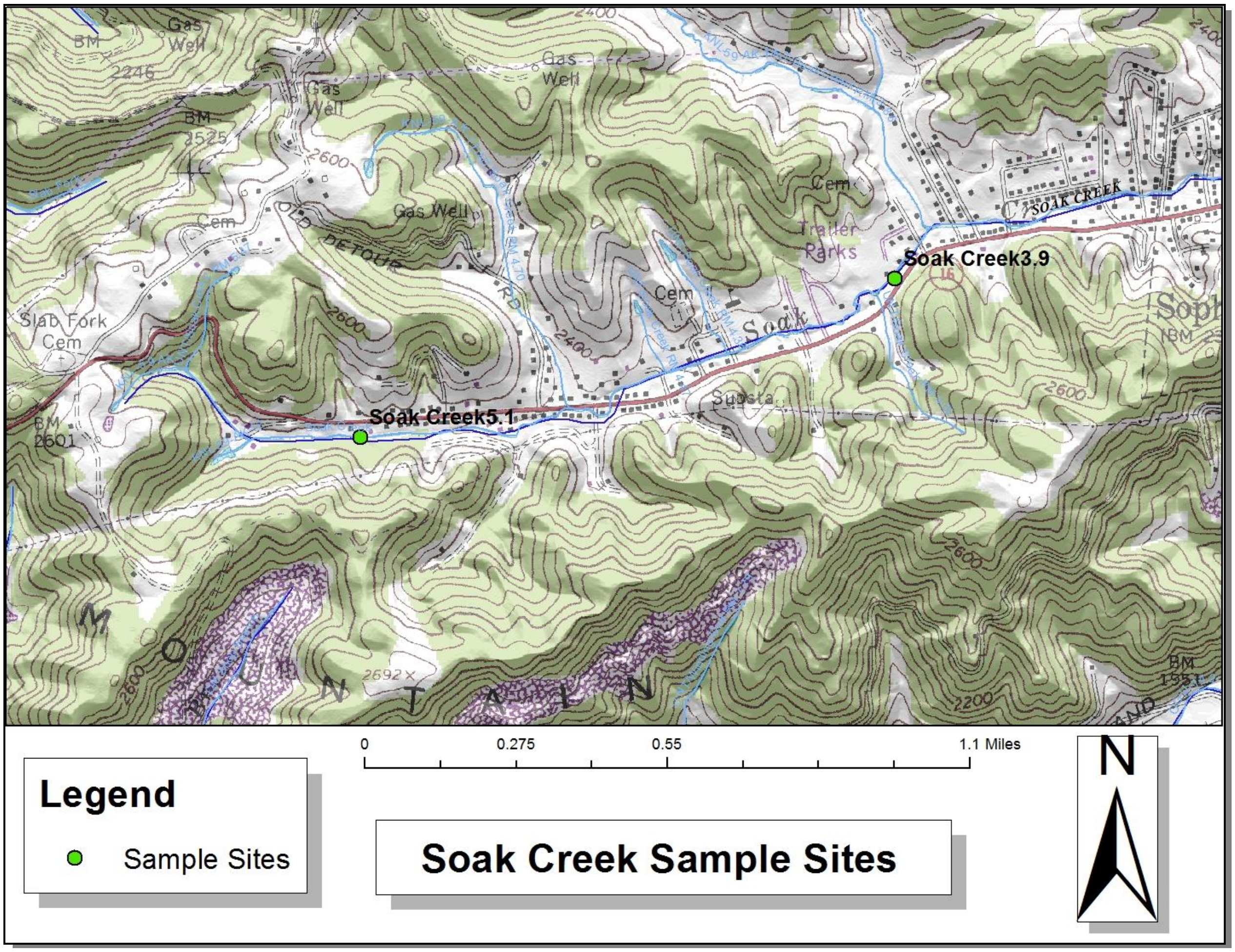
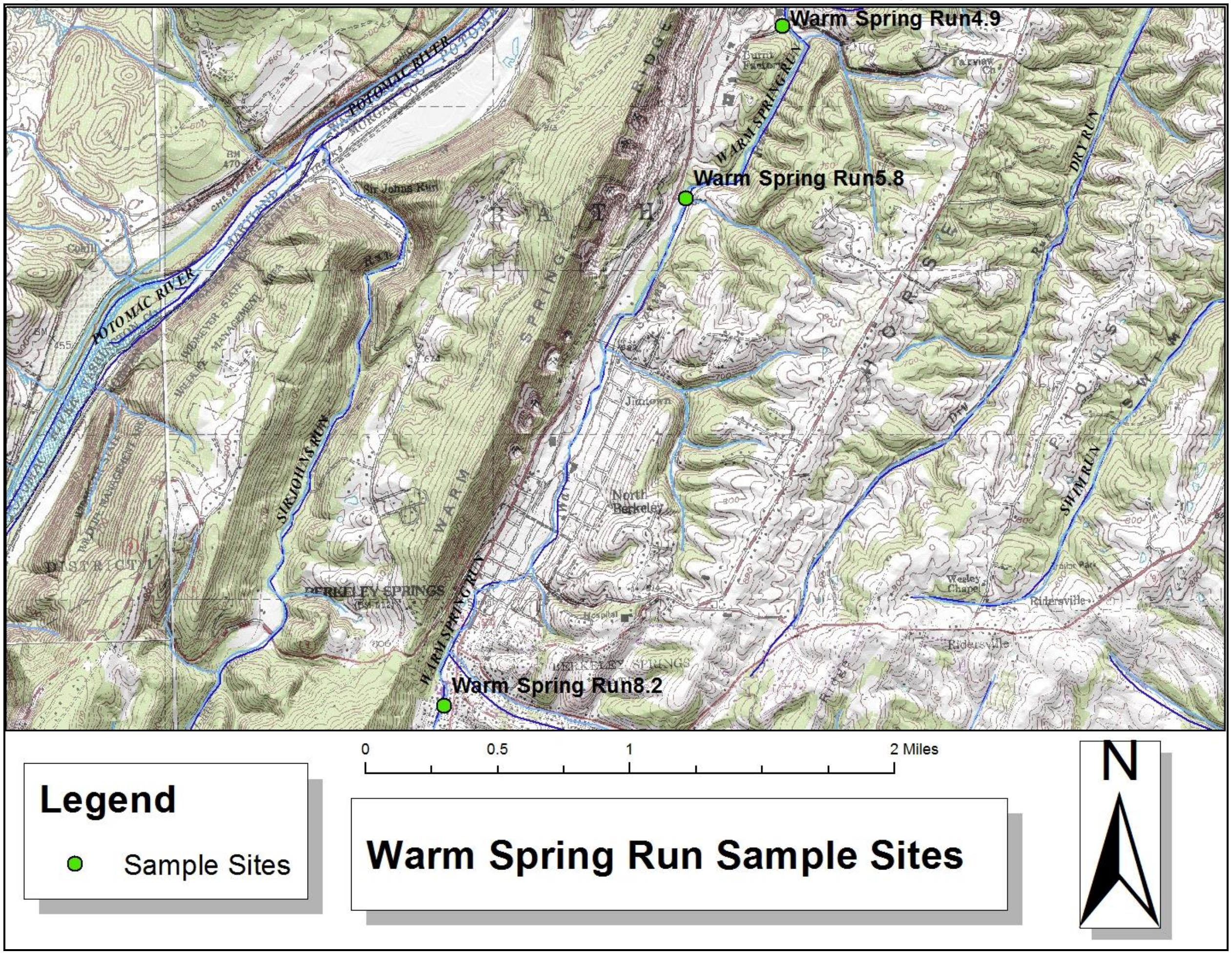


Figure 5. Upstream and downstream sampling locations at Warm Spring Run.



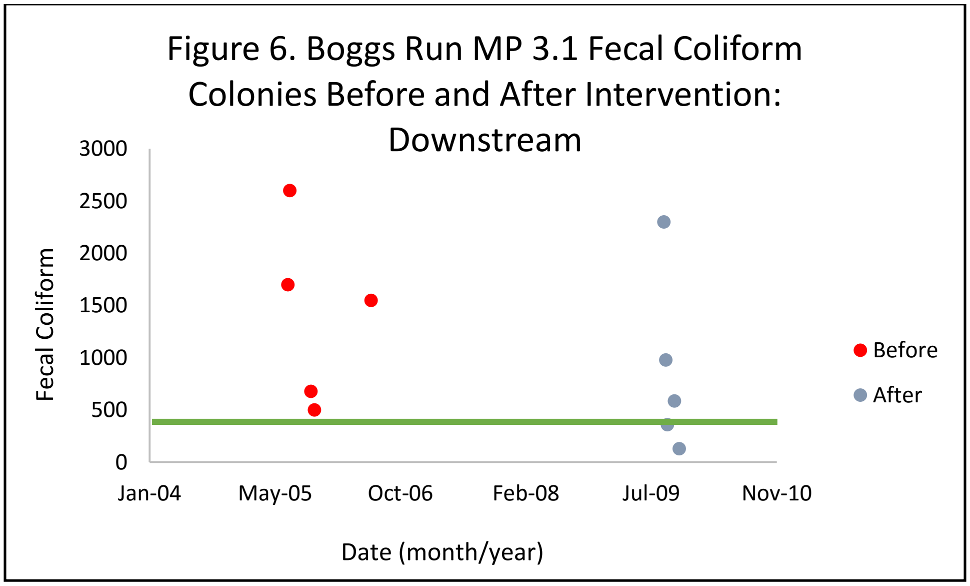


Figure 6. Boggs Run MP 3.1 Fecal Coliform Colonies Before and After Intervention: Downstream

Figure 7. Dunloup Creek MP 11.9 Fecal Coliform Colonies Before and After Intervention: Downstream

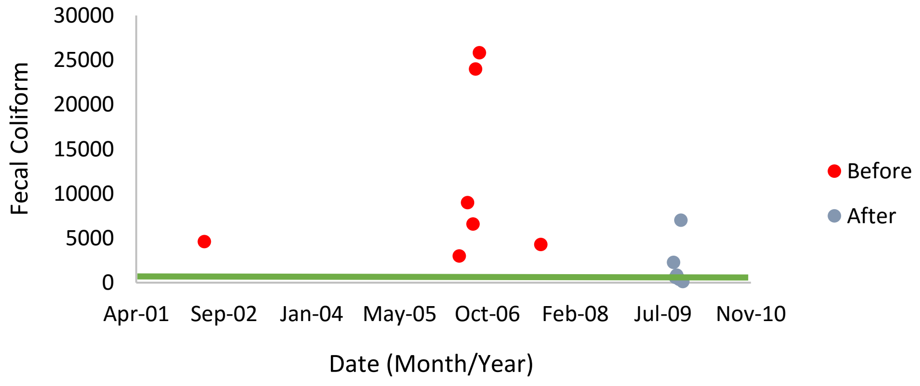


Figure 8. Dunloup Creek MP 13.6 Fecal Coliform Colonies Before and After Intervention: Upstream

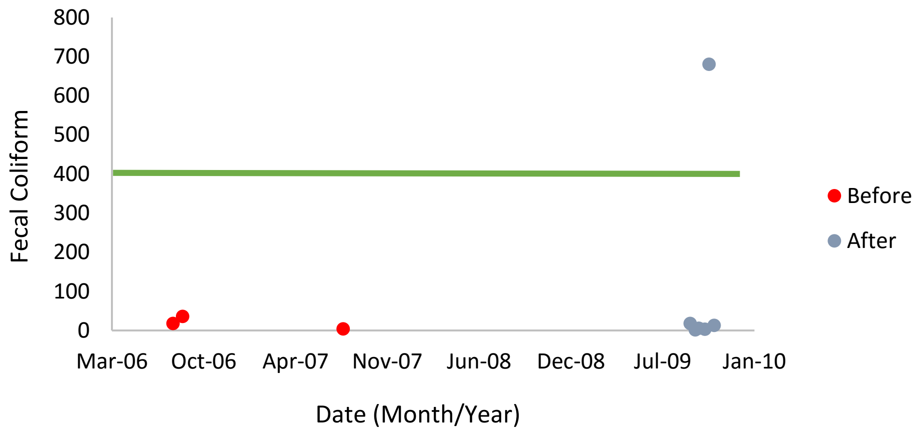


Figure 9. Soak Creek MP 3.9 Fecal Coliform Colonies Before and After Intervention: Downstream

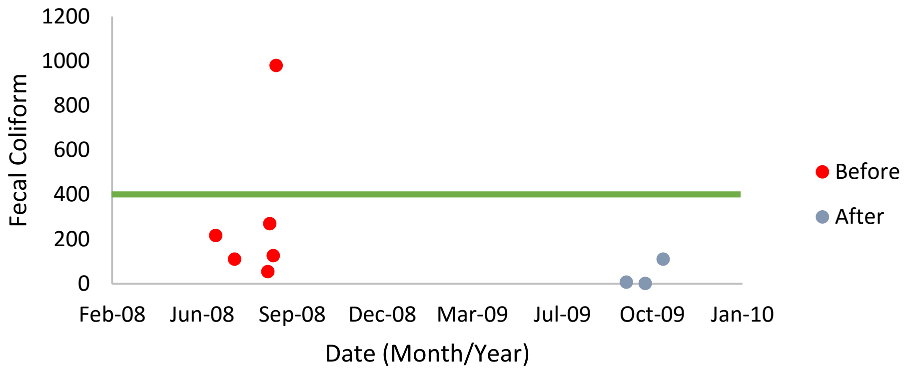


Figure 10. Soak Creek MP 5.1 Fecal Coliform Colonies Before and After Intervention: Upstream

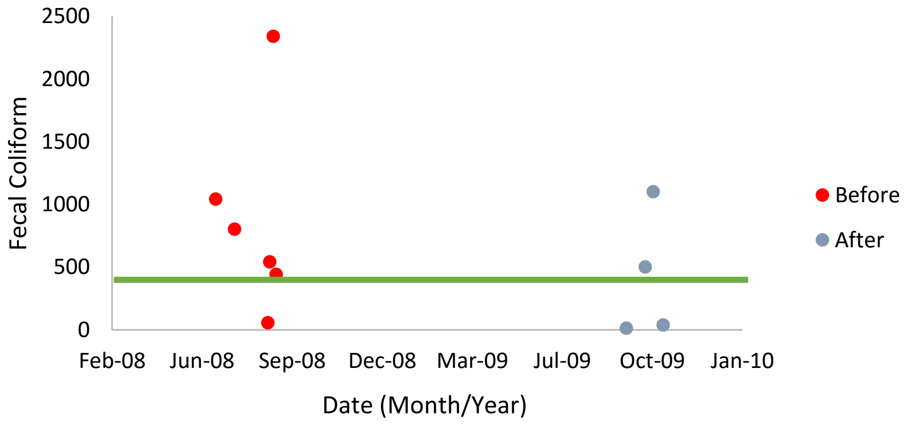


Figure 11. Warm Spring Run MP 4.9 Fecal Coliform Colonies Before and After Intervention: Downstream

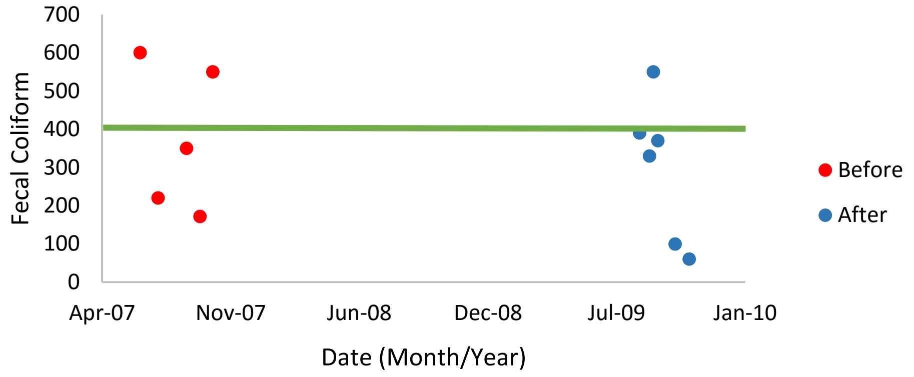


Figure 12. Warm Spring Run MP 5.8 Fecal Coliform Colonies Before and After Intervention: Downstream

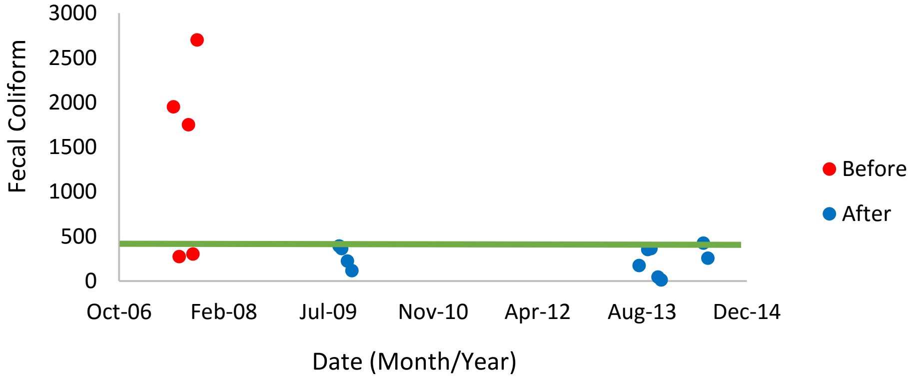


Figure 13. Warm Spring Run MP 8.2 Fecal Coliform Colonies Before and After Intervention: Upstream