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Kaleb Bassett

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INVESTIGATING THE SOURCES AND DYNAMICS OF ESCHERICHIA COLI IN A MISSOURI OZARKS WATERSHED

by

KALEB COLT BASSETT

A THESIS

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Approved by

Dev K. Niyogi, Advisor
David J. Westenberg
Mark W. Fitch

ABSTRACT

Escherichia coli, an intestinal bacterium, can serve as an indication of the presence of pathogenic microorganisms in water systems used by humans for recreation, agriculture, or drinking water. Many aquatic systems in the United States exceed the *E. coli* standard, set by the Environmental Protection Agency, for safe drinking water and recreational use. During 2016, a water sampling program was established in the Mill Creek watershed, a rural watershed located near the city of Newburg in Phelps County, Missouri. Water samples were collected before, during, and after storms throughout the year to examine the relationship between *E. coli* concentrations and measures of surface water runoff, such as turbidity and discharge. Results indicated that *E. coli* was primarily entering the stream (possibly bound to solid particles) via surface runoff during storm events. Sediment samples were also collected and revealed that it was possible for *E. coli* to become stored in the sediment bottom, where it could persist for 60 to 90 days. Disturbance of sediment reservoirs resulted in elevated *E. coli* concentrations in the stream, indicating that sediment reservoirs can prolong the potential for waterborne disease outbreak. Thus, a series of lab and field experiments were designed to investigate potential factors that may influence the survival and longevity of *E. coli* in the water column and sediment of streams. A better understanding of the sources, distributions, and controls of *E. coli* in aquatic systems will help guide management of fecal pollution in watersheds to minimize the threat to public health.

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1. INTRODUCTION

Nonpoint sources of pollution present the greatest challenge for maintaining high water quality conditions in rivers, streams, lakes, reservoirs, and estuaries across the United States (U.S. EPA, National Water Quality Inventory: 2004 Report to Congress, 2009). Nonpoint source pollutants are defined as any pollutants, natural or anthropogenic, which are deposited into water resources by surface runoff. Nutrients, sediments, and fecal pathogens are three regularly monitored nonpoint source pollutants that negatively impact the quality of water resources. The delivery of nonpoint source pollutants to streams and downstream reservoirs can pose a threat to the health of the organisms living in and near these aquatic ecosystems. In some cases, a serious concern for human health can exist.

Pathogenic microorganisms (microbes) pose the greatest water quality concern to humans. Contamination of water resources by waterborne pathogens leads to millions of deaths each year from diseases, such as cholera, cryptosporidiosis, and giardiasis (WHO/UNICEF, Global Water Supply and Sanitation Assessment Report, 2000). The presence of pathogenic microbes in aquatic systems is an indication of fecal pollution and is classified as being attributed to either point (i.e., direct) or nonpoint (i.e., indirect) source pollution (Buck et al. 2004, Eyles et al. 2003). Most sources of fecal pollution are related to domestic (includes agriculture) and wild animal wastes that are deposited directly into aquatic systems or enter indirectly with storm runoff (Wilson et al. 2014).

Additionally, human recreation and leaking septic systems from nearby residences are also known to commonly increase pathogenic microbes in streams and lakes (Pandey et al. 2014).

Escherichia coli (E. coli) is a normal inhabitant of the intestinal tract of humans and other warm-blooded animals, and is excreted in feces. Thus, *E. coli* is commonly used as an indicator of fecal pollution in aquatic systems because it is abundant and easily detectable by modern water sampling methods. Since the establishment of the Clean Water Act in 1972, the quality of the water resources of the United States has improved drastically. However, monitoring by the U.S. EPA, especially in the late 1990s and early 2000s, revealed that water quality is still a major concern. Of the rivers and streams assessed by the U.S. EPA, 44% were reported as impaired or not clean enough to support their designated uses, such as fishing and swimming. Also, 64% of assessed lakes and reservoirs were reported as impaired (U.S. EPA, National Water Quality Inventory: 2004 Report to Congress, 2009). Pollution was often cited as the leading cause of impairment in the water sources assessed. Top sources of pollution included agricultural activities and unknown/unspecified sources (i.e., nonpoint sources of pollution). While point sources of pollution are heavily regulated and lead to improvements in water quality, nonpoint sources of pollution are not regulated. It is very difficult, if not impossible, to identify and regulate nonpoint sources of pollution. For this reason, its contribution to surface water pollution remains largely unaddressed.

OBJECTIVES

In the present study, a relatively undeveloped and rural watershed in the Missouri Ozarks was investigated for the presence of fecal pollution. The research site for this study was the Mill Creek watershed, which is located a short distance from the town of Newburg in Phelps County, Missouri. From September 2015 to May 2017, the research team collected 48 sets of water samples from 20 different site locations in Mill Creek and its major tributaries as part of a hydrological survey of the watershed.

The first two objectives of this study were focused on utilizing the results of the hydrological survey to answer the following two questions about nonpoint sources of fecal pollution: (1) what are the major pathways these pathogens use to enter and travel through watersheds and (2) do stream sediments serve as reservoirs for fecal pathogens in streams? The final objective of this study was to compare the fecal pollution levels of various surface water sources.

The first objective of this study was to gain a better understanding of the sources and dynamics of fecal pathogens (using *E. coli* as an indicator organism) in the Mill Creek watershed. This karst watershed includes several springs and a variety of potential sources of fecal pollutants, including human residences, livestock agriculture, recreational activities such as horseback riding and dispersed camping, and an abundance of wildlife. *E. coli* concentrations in Mill Creek, as well as most other streams, are usually lower during baseflow than high discharge conditions. Storm events result in higher stream discharge conditions because the heavy rainfall amounts lead to additional streamflow from surface runoff. Thus, as part of this study, the research team performed sampling before, during, and after storms and used a simple linear regression to relate *E. coli* concentrations to rainfall characteristics (e.g., rainfall amount and discharge) and turbidity (a measure of surface runoff). The end goal was to determine the point in time on a storm hydrograph where *E. coli* is most abundant in the water column of the stream. Establishing this relationship will provide insight into when fecal contamination of water resources is of greatest concern to human health.

The second objective of this study was to analyze the persistence of fecal pollution in the stream and sediment. Due to the long survival time of *E. coli* in stream sediment, the research team investigated the likelihood of stream sediments serving as reservoirs for fecal pathogens. Previous studies have found evidence to suggest that internal loading of pollutants occurs in streams via suspension of the sediment during disturbance events (e.g., storm events or human recreation). Thus, an important goal of this study was to determine if sediment reservoirs were a major source of high fecal pollution in the Mill Creek watershed. Additionally, a review of the literature was used to identify the main environmental factors that control the survival and longevity of fecal pathogens that enter the stream and sediment. After these main factors were determined, the research team designed lab and field experiments to find the maximum survival times and decay rates of *E. coli* under various environmental conditions (e.g., various levels of solar radiation, water temperatures, and substrate compositions).

The final objective of this study was to compare the fecal pollution levels of various surface water sources and focused on answering the following two questions: how do fecal pollution levels differ between (1) springs, streams, and ponds and (2) urban and rural watersheds? Sampling sites in the Mill Creek watershed included springs, ponds, the stream channel and its tributaries; therefore, the differences in fecal pollution levels of these various surface water sources could be established and compared. Urban watersheds are regularly monitored for fecal pollution by state agencies, but less information is known about the sources, dynamics, and fate of fecal pollutants in less developed or rural watersheds (Missouri DNR, Missouri Integrated Water Quality Report, 2016). Additional water sampling was conducted in the urban setting of Rolla, Missouri. Samples were collected from the Deible Branch near the ACORN trail, off Highway O, to compare fecal pollution levels with Mill Creek. The findings on fecal pollution levels in these urban and rural sites can be applied to other watersheds in Missouri and the United States.

Like the rest of the United States, numerous water resources in Missouri (8,860 stream miles and 287,800 acres of lakes) are categorized as impaired by pollution. Coliform bacteria are listed as the most commonly identified pollutants in Missouri water resources, and nonpoint source runoff is reported as the most common source of pollution (Missouri DNR, Missouri Integrated Water Quality Report, 2016). This study will assist in guiding and improving the management effort of watersheds in the Missouri Ozarks and the United States to protect the aquatic ecosystems and natural resources they provide from nonpoint sources of fecal pollution. The U.S. Forest Service, Missouri Department of Conservation, and Mill Creek Watershed Coalition (local nonprofit group) will also benefit from this study as they plan to restore parts of Mill Creek's watershed in the coming years. The results of this study will provide a better understanding of the sources and dynamics of fecal pathogens, which will result in the establishment of better guidelines for water resource usage that informs the public of the possible risk of fecal contamination and reduces the threat of waterborne diseases to people and animals. Overall, several users, from local citizen groups to government agencies, will benefit from the information that will be gathered and published by this research project.

2. REVIEW OF LITERATURE

2.1. NONPOINT SOURCE POLLUTION

Nonpoint sources of pollution have been identified as the primary threat to many aquatic ecosystems (Carpenter et al. 1998). However, the pathways by which these pollutants travel from watersheds to streams and ponds are not well understood, nor are the processes governing the delivery of the pollutants downstream versus their retention within streams. The three main nonpoint source pollutants of interest in this research include: nutrients, sediments, and fecal pathogens. Nutrients, including nitrogen and phosphorus, are the primary limiting factors for primary production in many ecosystems, especially downstream aquatic systems such as lakes and estuaries. Sediments can include both inorganic and organic particles entering streams via erosion from riparian areas, stream banks, nearby roads, and agricultural fields. Finally, the existence of fecal pathogens in water sources used for recreation, irrigation, or drinking water pose a serious threat to human health. Probable contamination of water by fecal pathogens can be indicated by the presence of indicator organisms, such as *E. coli* (Wilson et al. 2014). Common sources of these three pollutants include fertilizers, detergents, fossil fuel combustion products, domestic and wild animal wastes, and industrial and agricultural wastewaters that enter the watershed via surface runoff (Carpenter et al. 1998).

2.1.1. Nutrients. Excess nitrogen and phosphorus in natural ecosystems is a major environmental problem (Vitousek et al. 1997, Carpenter et al. 1998).

The addition of nutrients from a variety of anthropogenic sources can have profound effects on terrestrial, freshwater, estuarine, and marine ecosystems. One problem of concern is algal blooms that lead to oxygen depletion in aquatic systems, resulting in fish kills. Also, some cyanobacteria produce toxins, which lead to toxic algal blooms that can contaminate drinking water reservoirs (Vitousek et al. 1997). Aquatic ecosystems, especially lakes and estuaries, are of special concern given the effects of eutrophication on the services that these systems provide to humanity, from declines in lake clarity to the imperilment of estuarine fisheries. The Mill Creek watershed is a Blue-Ribbon trout stream and home to a locally threatened species, the plains topminnow (*Fundulus sciadicus*), both of which could suffer from the negative effects of eutrophication. Streams mediate the delivery of nutrients from human-dominated watersheds to lakes and estuaries. Streams have often been viewed as simple pipe-like conduits that passively transport pollutants, but that view is changing because of new research findings. The new view no longer considers streams as simple pipelines, but instead emphasizes that as nutrients are transported in streams, they may be retained or transformed by stream algae and microbes (i.e., stream uptake), thereby lessening the flux of nutrients to downstream ecosystems (Peterson et al. 2001, Mullholland et al. 2008, Niyogi et al. 2010). For example, previous work by Niyogi et al. (2010) at Mill Creek found that dissolved inorganic nitrogen increased as discharge increased, but as flow decreased, stream uptake increased, and less nitrogen was available to travel downstream.

2.1.2. Sediments. The erosion of stream banks, roads, and agricultural fields during stormflows are common sources of additional sediments in aquatic ecosystems (Davies-Colley et al. 2008). Increasing sediment can act as a stressor in streams, negatively affecting aquatic organisms and ecological processes. Suspended sediment can reduce clarity, leading to light limitation of primary producers (Ryan 1991), and mobile sediments scour stream algae, thus further reducing primary production (Biggs et al. 1999, Schofield et al. 2004). Sedimentation also negatively affects many animals through the loss of physical habitat, a decrease in food quality, and possible damage to taxa with delicate gills and mouthparts (Rabeni and Smale 1995, Angradi 1999). In conclusion, sedimentation can affect animals in streams via direct (e.g., physical injury) and indirect pathways (e.g., reduced food from primary production).

2.1.3. Fecal Pollution. Fecal pollution is a public health concern because it can lead to the contamination of water resources used by humans for agriculture, recreation, and drinking water. These pathogens are responsible for the outbreak of deadly diseases, such as cholera and giardiasis, which is one reason why ensuring clean water and sanitation is a global goal for future sustainable development (United Nations, Sustainable Development Goals, 2015). Even in undeveloped or rural watersheds, such as Mill Creek, pathogenic microbes can pose a threat to human health. Fecal pollution can come from a variety of sources in watersheds (Buck et al. 2004, Eyles et al. 2005). Most nonpoint sources of fecal pollution are related to domestic and wild animal wastes that indirectly enter aquatic ecosystems with surface runoff (Wilson et al.

2014). However, direct inputs, also known as point sources, of fecal pollution can also exist. Two common point sources of fecal pollution in aquatic systems are direct pumping and leaking septic systems (Pandey et al. 2014).

E. coli bacteria are commonly used as indicator bacteria to detect and estimate the level of fecal contamination of water because they are more abundant than other fecal bacteria and can be easily enumerated using current methods (U.S. EPA, Water: Monitoring and Assessment, 2012). A great deal of research has been performed on *E. coli* in aquatic ecosystems to determine the sources and understand the movement of pathogenic microorganisms within watersheds. A study by Knierim et al. (2015) found *E. coli* concentrations and stream turbidity to be closely related, with both increasing during high flow events. Other studies have used microbial source tracking techniques to show that animal feces are the most common sources of *E. coli* in aquatic ecosystems (Esseili et al. 2008, Wilson et al. 2014). Thus, unless a point source of fecal pollution is known to exist, it is accepted that surface runoff from storms is loading the waterway with *E. coli* from animal feces in the watershed.

Although knowing the major sources of fecal pollution is important for prevention, understanding more about the final fate of *E. coli* once it reaches the waterway is of equal importance. For this reason, the transport and survival of *E. coli* in streams has become an area of research interest. Davis et al. (2005) looked at the survival of *E. coli* in stream sediments and discovered that during the winter *E. coli* could survive for at least four months in the stream sediment. Research results, including this study, led some researchers to propose the existence of sediment reservoirs, which store *E. coli* and other fecal pathogens in the stream sediment. During disturbance events, such as floods, these sediment stores of *E. coli* can become suspended and lead to dangerously high levels of *E. coli* in the water column (Muirhead et al. 2004, Cho et al. 2010). Further research is needed to investigate various environmental factors, such as solar radiation, water temperature, sedimentation, adsorption, predation, stream vegetation, and nutrient availability, which could affect the survival of *E. coli* in streams. The difference in the geology and hydrology of watersheds also plays a role in the survival and transport of *E. coli* in aquatic systems. Given the karst nature and predominance of springs in the Mill Creek watershed, the pollutant-discharge relationship and dynamics (i.e., survival and transport) of *E. coli* in Mill Creek will likely be unique to its hydrology.

2.2. MILL CREEK WATERSHED HYDROLOGICAL SURVEY

The hydrological survey of the Mill Creek watershed consisted of two major parts: studying water quality and stream flow. *E. coli* concentration and other water quality characteristics are commonly monitored to ensure water sources are safe for public use. Stream flow (discharge) has been studied before, during, and after storm events to determine if it is a strong predictor of *E. coli* concentration in streams.

2.2.1. Water Quality. Water quality is a measure of the suitability of a water source for a designated use based on its physical, chemical, and biological characteristics. Some commonly monitored characteristics of water quality include water temperature, conductivity, dissolved oxygen (DO), pH, salinity, turbidity, and a number of contaminants (e.g., nutrients, heavy metals, toxins, and bacteria). Numeric standards have been established for each monitored water quality parameter. The standards serve as guidelines for determining if a water source is suitable for one or more designated uses such as drinking, recreation, agricultural irrigation, or protection and maintenance of aquatic life (USGS, Water-Quality Information, 2014).

Water quality parameters are important to monitor because they reveal how water sources are being affected, either by natural or anthropogenic causes. For example, changes in the season and sunlight intensity are major factors that affect the water temperature of a stream or pond. Dissolved gases, such as oxygen, are common in natural waters. Adequate oxygen levels are required for fish and most other aquatic life to survive. Dissolved oxygen levels can reveal harmful stream conditions like eutrophication, which can lead to fish kills (Vitousek et al. 1997, Carpenter et al. 1998). Other commonly monitored water quality characteristics include conductivity, turbidity, and fecal pathogens.

Conductivity is a measure of how well water can conduct an electrical current and is determined by the number of dissolved ions in the water. The more ions in the water, the greater the conductivity. Ions are dissolved and released from the soil and rocks as water flows through or over them. Thus, the geology of a watershed directly influences the conductivity (i.e., amount and type of ions) of a waterbody. Spring water typically has a high conductivity due to the abundance of ions collected as the water passes through the ground. Two other factors that affect the conductivity of water include evaporation and rain. Evaporation results in the loss of freshwater, which causes the conductivity of a waterbody to increase as it becomes more concentrated with ions. Rainwater has a very low conductivity (near zero); thus, rain that enters a waterbody will decrease the conductivity (USGS, Water-Quality Information, 2014).

Turbidity is a measure of water clarity. It is an expression of the amount of light that is scattered by the material in the water when a light is shined through it. The more the light is scattered, the higher the turbidity. As water from rain moves over the land and through the ground, it carries plant debris, algae, sand, silt, clay, organic and inorganic matter, and microscopic organisms to rivers and streams, making the water appear muddy or turbid (USGS, Water-Quality Information, 2014). Turbidity can be used as a quantitative measure of surface runoff, which is the main cause of nonpoint source pollution. Water quality characteristics, especially conductivity and turbidity, could provide some evidence to support that the main sources of fecal pathogens in Mill Creek are nonpoint sources.

Fecal pathogens are also a major water quality concern in water sources. Monitoring *E. coli*, a common fecal indicator bacteria, will be the major focus of this study. Some water quality characteristics can be determined directly from a stream or well using a water quality monitoring meter (e.g., water temperature, conductivity, DO, salinity, pH, and turbidity), while others need to be analyzed at a laboratory (e.g., chemical or biological contaminant concentrations). This presents a problem with the current methods for evaluating the threat of fecal pollution on water sources, especially those used for recreation. Enumeration of fecal bacteria requires 24 hours to process in a laboratory after a sample is taken. Thus, agencies responsible for parks and recreation cannot quickly determine if a water source is polluted by fecal bacteria and should be closed to the public. If a relationship can be established between fecal pollution and a water quality characteristic that is directly measured from the stream, then this issue would no longer exist. In this study, water temperature, dissolved oxygen, specific conductivity, and turbidity were directly monitored at sites in the Mill Creek watershed to determine if these characteristics could be related to the concentration of *E. coli*.

2.2.2. Stream Flow. Stream flow or discharge is defined as the volume of water that moves through a specific point in a stream during a given period of time. The additional runoff from storm events results in higher discharge readings and presumably higher concentrations of fecal indicator bacteria as well. Prior studies have shown that the concentrations of fecal indicator bacteria, such as *E. coli*, can vary by several orders of magnitude in streams depending on the amount of storm discharge and the hydrology of the recharge area. Thus, it is important to examine the change in fecal indicator bacteria concentration throughout a storm hydrograph to gain a better understanding of how discharge and fecal pollution from nonpoint sources are related. Davis et al. (2005) determined that *E. coli* concentrations increase rapidly during the rising limb of a storm hydrograph, peak prior to or coincide with the peak of the storm pulse, and decline rapidly, well before the recession of the storm hydrograph. They proposed that this pattern indicated sediment-associated *E. coli*, which form sediment stores in the stream and can be disturbed during storms.

Knierim et al. (2015) monitored *E. coli*, nitrates, and chlorides in a recreational spring (Blowing Spring) and stream (Little Sugar Creek) near the city of Bella Vista, Arkansas. From January 2007 to August 2013, the researchers characterized the water quality of these sites during baseflow and storm events. They found that the concentration of *E. coli* was significantly greater during storm events (median was 649 cfu per 100 mL) than baseflow periods (median was 41 cfu per 100 mL). At Blowing Spring, the researchers also determined that a significant and positive linear relationship existed between *E. coli* concentration and discharge. Due to the increase in *E. coli* concentration with discharge, Knierim et al. (2015) proposed the following pathway for fecal indicator bacteria in karst watersheds. Initially bacteria are sourced from the surface; they are then accumulated at the soil-rock interface (i.e., epikarst), and subsequently flushed into the fractures in the carbonate bedrock during storm events, which leads to the observation of higher bacterial concentrations at springs. Hence, the hydrology of the watershed plays a key role in determining pollutant dynamics.

Davis et al. (2005) examined the survival of *E. coli* in the sediment of springs and streams within the Savoy Experimental Watershed (SEW) in northwest Arkansas. The researchers developed sampling chambers, which they inoculated with *E. coli* and deployed throughout the SEW to assess the viability of *E. coli* in these karst environments over extended periods. The study was performed during the winter and the authors concluded that *E. coli* could survive for at least four months in the stream sediment. The researchers proposed that the cooler stream temperatures led to slowed metabolism in the organisms, which resulted in prolonged existence. It is likely that these results are strongly influenced by seasonal variation and changes in water temperatures. Nevertheless, Davis et al. (2005) revealed the health hazard associated with bacterial persistence in stream sediment. Also, as it pertains to stream flow, they highlighted an additional source of fecal indicator bacteria from sediments, which can be used to further explain the positive relationship between *E. coli* concentration and discharge in streams.

These two studies in the Ozarks of northwestern Arkansas investigated the effect of storms on discharge and *E. coli* concentration. The springs and streams used as study sites by Knierim et al. (2015) and Davis et al. (2005) are representative of mantled karst aquifers found throughout most of the Ozarks of southern Missouri and northern Arkansas. The results of these studies are expected to be similar to Mill Creek, which is sure to have its own unique hydrology, but will still share many characteristics of karst hydrology seen in the rest of the Ozarks.

2.3. SEDIMENT RESERVOIRS

Many studies have examined stream sediments for their ability to store enteric bacteria. One such study, “Bottom Sediment: a Reservoir of *Escherichia coli* in Rangeland Streams” by Stephenson and Rychert (1982) observed the survival of *E. coli* in stream sediments. The results of their research showed that bottom sediments contained 2 to 760 times greater concentrations of *E. coli* than the overlying water. These sediment stores could be resuspended following disturbance simulations and storm events, both of which contributed to the pollution of the overlying water. Stephenson and Rychert (1982) established the importance of considering bottom sediments as stores for *E. coli* and other indicators of fecal contamination.

Since 1982, the majority of research results have supported the findings of Stephenson and Rychert that benthic sediments are able to harbor significantly higher concentrations of enteric bacteria than the overlying water. Sherer et al. (1988) used a rake to disrupt the stream bottom of Bear Creek in Central Oregon and found from 1.8 to 760 million fecal coliforms per square meter could be resuspended and immediately measured downstream. In a later study, Sherer et al. (1992) designed an experiment to test the survival of fecal coliforms and fecal streptococci organisms in the stream sediment. The researchers loaded 4-L plastic containers with 500 grams of sediment (collected from Bear Creek), 2-75 grams of cow manure, and 100 grams of water. These 4-L plastic containers were incubated at 8°C for 25 days and bacterial analysis was performed. Fecal coliform and fecal streptococci bacteria revealed half-lives from 11 to 30 days and 9 to 17 days, respectively, when incubated with sediment (Sherer et al. 1992). When the bacteria were incubated without sediment the half-lives were much shorter (2.8 days). The survival of enteric bacteria was demonstrated to be significantly longer in sediment-laden waters than in those without sediment, which could explain the extreme number of fecal coliforms they were able to resuspend from the stream sediment in their 1988 study.

Davis et al. (2005) also supported these findings when they made the surprising discovery that *E. coli* could survive for at least four months in the stream sediment. Sediment storage of *E. coli* appears to now be an accepted idea; however, the process by which *E. coli* enters the sediment is still relatively unknown. More research is also needed to determine if the fecal pathogens stored in the sediment can be resuspended during disturbance events. The resuspension of fecal pathogens from sediment reservoirs has been termed “internal loading” and is being monitored in streams by some researchers.

Muirhead et al. (2004) created artificial floods to study sediment disturbance and the internal loading of fecal contamination. In the absence of overland flow from the catchment, the only source of fecal bacteria is in-channel stores, which could be assessed during dry weather conditions. Artificial floods were produced by releasing water from a supply reservoir and resulted in two orders of magnitude increase in *E. coli* concentration in the water column (from a background level of 10^2 cfu per 100 mL to over 10^4 cfu per 100 mL). They also found that bacterial peak concentrations and yields declined systematically during a series of artificial flood events and the sum of the bacterial yields could be used to approximate in-channel stores of fecal pollution. More recently, Wilson et al. (2014) examined *E. coli* concentrations at two public swimming beaches at the Lake of the Ozarks State Park in Camden County, Missouri. Using open bottom buckets and paint mixers, the researchers disturbed sediments near the beach edge and found significantly greater concentrations of *E. coli* after resuspending the sediment. This provides evidence that bathers at beaches can resuspend *E. coli*-contaminated sediments, which can be an important source of *E. coli* in the water column. The results of Muirhead et al. (2004) and Wilson et al. (2014) support the existence of sediment reservoirs for *E. coli* and have shown that these in-channel stores can be disturbed by floods or human activity, thereby leading to higher levels of fecal contamination in streams and lakes.

2.4. ENVIRONMENTAL FACTORS AFFECTING E. COLI SURVIVAL

The natural environment is a dynamic system influenced by an array of variables. The survival of pathogenic microbes in the natural environment is influenced by the conditions of its surroundings. Scientific literature indicates that a variety of environmental factors (e.g., physical, chemical, and biological) impact the survival of *E. coli* and other pathogenic microbes in streams and sediments. The main in-stream factors include: solar radiation, water temperature, sedimentation, adsorption, predation, stream vegetation, and nutrient availability. Also, other factors can affect *E. coli* and microbe survival outside of streams, such as the local hydrology, geology, soil characteristics, and the presence (or lack) of riparian vegetation. There is a wide assortment of reported survival times for pathogenic microbes outside the gastrointestinal tract, which reflects the impact of various factors in the natural environment. However, there are also differences in research methodologies and bacterial strains used during experimentation that must be considered to explain some of the reported differences in survival time. For these reasons, the importance of some environmental factors to pathogenic microbe survival in aquatic systems is still debated by researchers.

2.4.1. Solar Radiation. Sunlight is a portion of the electromagnetic radiation emitted by the sun and experienced on earth, which includes visible light, infrared (IR) radiation, and ultraviolet (UV) radiation. UV radiation causes damage to the cells and DNA of living organisms and is commonly used for sterilization in the fields of microbiology and medicine. Of the environmental factors influencing *E. coli* survival in aquatic systems, solar radiation has been reported as the single most important parameter affecting the die-off of *E. coli* (Whitman et al. 2008, Gutiérrez-Cacciabue et al. 2016).

Whitman et al. (2008) performed a study on Dunes Creek, a small coastal stream of southern Lake Michigan, where they impounded an upper portion of the creek to form an artificial pond. They examined the effect of sunlight and season on *E. coli* inactivation in the pond for 30 months from pre- to post-pond construction. The main goal of the study was to determine the effectiveness of artificially ponding streams to reduce fecal contamination in downstream reaches. Results from the study suggested that sunlight exposure was the most important factor reducing *E. coli* at the pond outflow. The researchers estimated that 26% of *E. coli* reduction in the pond was due solely to solar inactivation (Whitman et al. 2008). Similar results have been seen in laboratory studies on *E. coli* and sunlight inactivation. A study by Gutiérrez-Cacciabue et al. (2016) found

E. coli exposed to sunlight suffered an immediate inactivation, a 3-log (99.9%) reduction in less than four hours. For comparison purposes, the researchers found that it took 219 hours (about 9 days) for 99.9% reduction of *E. coli* that was kept in the dark. The inactivation of *E. coli* via solar radiation appears to be an obvious solution for reducing fecal indicator bacteria in aquatic systems. However, aquatic systems are dynamic and other environmental factors must be considered such as sediment and vegetation, which can shield *E. coli* and other pathogenic microbes from the harmful effects of solar radiation.

2.4.2. Water Temperature. As the water temperature increases, so does the rate of *E. coli* inactivation. Flint (1987) found that *E. coli* could survive for up to 260 days at temperatures from 4°C to 15°C in autoclaved filtered river water. Survival of *E. coli* was greatest at 4°C and was lowest at 37°C (Flint 1987). Whitman et al. (2008) found similar results in the artificial pond they created on Dunes Creek. Reduction of *E. coli* concentration between pond inflow and outflow was only 17% during the winter months, which is much lower than the 98% reduction of *E. coli* seen during the summer months (Whitman et al. 2008). The literature presents two main reasons for the increase in *E. coli* survival at colder water temperatures. First, during the winter there is less direct sunlight, thus lowering the temperature and reducing the amount of inactivation due to solar radiation. This implies that the effects of water temperature on *E. coli* survival in aquatic systems are directly proportional to the effects of solar radiation. Some researchers even suggest that water temperature plays little to no role in *E. coli* persistence. However, the second reason for why *E. coli* survives longer at colder water temperatures does not support this suggestion. The second reason is *E. coli* enters an inactive state at lower temperatures, which allows it to survive for longer periods (Flint 1987).

2.4.3. Sedimentation and Adsorption. A study by Byappanahalli et al. (2003) investigated the sources of *E. coli* in Dunes Creek, a small Lake Michigan coastal stream, which has chronically elevated *E. coli* levels near its outfall next to a bathing beach. The researchers found that *E. coli* was most common within submerged sediment and wetted bank sediments, and numbers rapidly decreased landward beyond the banks. Their results indicate that sediments and soils in the Dunes Creek watershed harbored *E. coli*, and the persistently elevated counts in the stream are perhaps due to the washing of the sediment-borne organisms into the water. Research by Jamieson et al. (2005) further explained the transport and deposition of sediment-associated *E. coli* in natural streams using tracer-bacteria. The main goal of the study was to unravel the mechanism behind the storage of *E. coli* in stream sediments. In the discussion, the authors proposed a method by which *E. coli* and other bacteria adhere to fine sediment particles in aquatic environments. Bacteria are initially drawn to the surfaces of fine solids by London-van der Waals forces, and then once the bacteria are positioned close to the solid's surface, they use extracellular polymers to form strong, permanent attachments. Jamieson et al. (2005) concluded that the majority of negatively charged bacteria in the water column are attached to the surfaces of fine solids. It is this attachment to fine sediments that influences and ultimately determines the transport characteristics of *E. coli* and other fecal pathogens in streams. Eventually, over the length of the stream, the fine sediments will settle out of the water column and contribute to the sediment reservoirs observed by Stephenson and Rychert (1982).

Boutilier et al. (2009) performed a similar study on the process of adhesion and sedimentation of *E. coli* within wastewater treatment wetlands. Adsorption is defined as the adhesion of atoms, ions, or molecules from a gas, liquid, or dissolved solid to the surfaces of solid bodies with which they are in contact. Sedimentation is the tendency for particles in suspension to settle out due to the forces of gravity. Adsorption and sedimentation are related and both mechanisms participate in controlling the deposition of *E. coli* in streams. If *E. coli* remains free-floating or adsorbs to small and lightweight organic particles, then sedimentation will be negligible. However, if *E. coli* adsorbs to larger and denser inorganic particles, then sedimentation will likely have significance in the removal of *E. coli*. The results of the study showed that in dairy wastewater (the natural environment in this study), approximately 90% of *E. coli* was found to be free-floating or associated with organic particles less than 5 μm in size (Boutilier et al. 2009). This would suggest that sedimentation is negligible in this case and would contradict the theory that bottom sediments serve as sinks for *E. coli* and other fecal indicator bacteria. This is just one example of the complexity and conflicting results that have kept researchers from making decisive conclusions about the transport and deposition of sediment-associated *E. coli*.

The results of research by Gutiérrez-Cacciabue et al. (2016) suggest that adsorption to sediments (i.e., solid particles) in streams protects *E. coli* and leads to longer survival times. Gutiérrez-Cacciabue et al. (2016) studied the inactivation of indicator bacteria by sunlight in freshwater, with and without solid particles. In the absence of solid particles, *E. coli* bacteria that were exposed to sunlight were immediately inactivated (i.e., 3-log reduction occurred in less than four hours). However, in the presence of solid particles, *E. coli* did not see a 3-log reduction until 70 hours (about 3 days) had passed. The protective role of sediments was made even clearer when the indicator bacteria were kept in the dark (i.e., removing solar radiation, which is a known sterilizer). For a 3-log reduction of the *E. coli* bacteria in the samples to occur it took 219 hours (about 9 days) without solid particles and with solid particles it took 1,354 hours (about 56 days). Gutiérrez-Cacciabue et al. (2016) concluded that solid particles serve as protective shields, which could increase survival and persistence of fecal indicator bacteria in freshwater sources. If adhesion to stream sediments does in fact protect and result in longer persistence of fecal bacteria in streams, then it could allow more time for sedimentation to occur. Thus, *E. coli* and other fecal pathogens could become stored in the sediment forming sediment reservoirs.

2.4.4. Predation. Hall et al. (1996) performed a field study on the consumption of bacteria by invertebrates in streams. In the study, fluorescently labeled bacteria (FLB) were used to look at the uptake length of bacteria particles in the stream and to study the bacterial removal rates by invertebrate consumption. They performed two releases of FLB, one in July and the other in August. They took water samples at 5-meter intervals (from 5 to 45 meters) at 20 minutes and 40 minutes after bacterial release. This was then used to calculate the uptake length of the bacteria particles in the stream stretch. For the second part of the experiment, they collected seven insect taxa from 5 to 12 meters below the release site and used gut analyses to calculate the rate of FLB consumption by each insect group.

From the results, Hall et al. (1996) determined the uptake length of FLB in the stream to be 78 and 83 meters for the two releases. Also, they found that *Simulium*, a filter-feeding blackfly larva, had the highest uptake rate. Two net-spinning caddisflies (*Diplectrona* and *Parapsyche*) also had high FLB uptake rates, but not as high as the *Simulium*. Invertebrate ingestion per square meter of stream bottom was only 7% of total stream uptake, and 91% of the invertebrate uptake was performed by *Simulium* (Hall et al. 1996). Thus, invertebrate consumption did not play a major role in stream uptake; instead adhesion of FLB to the substrate seemed to be more important in the uptake.

In the discussion, Hall et al. (1996) states that the abundance of *Simulium* was low at the test site and the calculated uptake length of FLB only due to ingestion by *Simulium* was 1.4 km. If the abundance of *Simulium* in the stream was ten times the amount observed in this study (which it has been in other stream studies), then *Simulium* could be capable of removing 60% of FLB in the water column of the stream, shortening the uptake length to 142 meters. The stream conditions also play a major role in the bacterial uptake by invertebrates. Invertebrates can take up more bacterium when the stream’s depth is low and discharge is high. A low depth allows the invertebrates to filter more of the water column and a higher discharge means that more particles can be filtered, leading to higher consumption. Thus, in aquatic environments where there are high densities of blackflies or caddisflies (i.e., filter-feeding invertebrates) and the right stream conditions (i.e., shallow, high flow), there is the potential for invertebrates to regulate bacterial survival and transport.

2.4.5. Aquatic Vegetation and Nutrient Availability. The shared occurrence of bacteria and aquatic vegetation has been consistently observed in aquatic environments (Sherer et al. 1992, Byappanahalli et al. 2003, Moreira et al. 2012). However, the ecological significance of these associations is not fully understood. Bacterial survival and growth in aquatic habitats increases when they are attached to particles or other solid surfaces, such as aquatic vegetation. The roots and surfaces of aquatic vegetation, especially algae, provide bacteria with attachment sites, which in turn provides protection against harmful solar radiation and predation leading to increased bacterial survival (Byappanahalli et al. 2003). The surfaces of stream sediments, soil particles, and algae also provide higher concentrations of organic matter and nutrients, which can prolong the survival and in some cases, lead to the growth of bacteria in aquatic environments (Sherer et al. 1992).

A study by Moreira et al. (2012) investigated the biofilm-forming capability of *E. coli* in three temperate freshwater lakes. They found that periphytic *E. coli* populations (*E. coli* that are attached to the surfaces of plants or rocks above the bottom sediments) were continuously present in the three lakes they studied. Using a crystal violet assay, they determined that isolates from this periphytic *E. coli* population are superior biofilm formers, which can form 2.5 times as much biofilm as human *E. coli* isolates and 7.5 times as much as bovine *E. coli* isolates. The results of this study may have revealed the existence of selective pressures in freshwater environments that may favor *E. coli* capable of forming biofilms. It also indicates that forming attachments to surfaces, such as aquatic vegetation, can lead to improved survival and persistence, maybe even growth, of bacteria in aquatic environments.

The potential ability of *E. coli* to grow in aquatic environments has led some researchers to question its feasibility as a fecal indicator bacteria. However, even less is known about the role of aquatic vegetation and nutrient availability on the survival and potential growth of other fecal indicator bacteria or the more harmful waterborne pathogens they are used to indicate (i.e., *Giardia* or *Cryptosporidium*). It may be possible for plants or other aquatic vegetation to control the abundance of fecal bacteria in aquatic environments. Plant uptake is an effective bioremediation tool for removing heavy metals and other toxic pollutants from contaminated soils and waters (Salt et al. 1995). However, little to no research has been published on using phytoremediation to control fecal pollution levels in surface water and groundwater sources.

2.5. LEVELS OF FECAL POLLUTION IN SURFACE WATERS

About three quarters of the Earth's surface is covered by water. Most of that water exists in the oceans as saline or salt water (about 97%). The other three percent is considered fresh water, which is either frozen in glaciers and ice caps, stored in aquifers (i.e., groundwater), or readily available as surface water.

Groundwater and surface water sources, such as springs, streams, rivers, ponds, and lakes, are continually under threat from fecal pollution. Each state in the U.S. has established water monitoring programs to ensure water quality is kept within safe standards for the designated uses of groundwater and surface water sources in rural and urban areas.

2.5.1. Springs, Ponds, and Streams. Springs, ponds, and streams all differ in their physical and chemical properties, which can lead to varied levels and responses to fecal pollution between these surface water sources. Springs are natural wells that bring water from underground aquifers to the earth's surface. The unique hydrological characteristics of groundwater, such as filtration and recharge, strongly influence the fecal contamination levels observed at springs. As water travels through the pores in the ground, contaminants can be filtered out and removed. Thus, springs tend to have lower concentrations of fecal bacteria than streams or ponds (Byappanahalli et al. 2003). However, a serious water quality problem can still exist if sources of fecal contamination are present in a spring's recharge area.

Streams (or rivers) come in lots of different shapes and sizes. They cover the earth's surface like the branching veins and arteries of the human body, where they serve as the main conduits of surface water. Streams carry water from a variety of different sources, such as springs, snowmelt, and high-altitude lakes, until they empty into the ocean or a large inland body of water. The vast length of streams and rivers exposes them to numerous sources of fecal pollution. For this reason, streams are expected to have higher levels of fecal pollution than springs or ponds.

Ponds (or lakes) are inland bodies of standing or slowly moving water. This lack of stream flow is the key feature that distinguishes ponds from springs and streams. Ponds typically have warmer water temperatures due to a combination of more direct solar radiation (because of the pond’s shape and size) and the lack of flowing water. The increased solar radiation and warmer temperatures lead to greater bacterial inactivation (i.e., fecal pathogen removal) in ponds and lakes than in springs or streams (Whitman et al. 2008). Byappanahalli et al. (2003) monitored *E. coli* contamination in different surface water sources within the Dunes Creek watershed. They concluded that median *E. coli* counts were highest in stream sediments and bank sediments, followed by, in order of decreasing magnitude, running water, standing water, and spring water.

2.5.2. Rural and Urban Streams. A common source of *E. coli* in streams is runoff from rural (i.e., agricultural) and urban landscapes. Watersheds, in both rural and urban areas, are unique and can differ in land use, size, and management practices. These differences can impact the sources, transport, and amount of fecal contamination in nearby streams. The primary sources of bacterial contamination in urban watersheds include domestic animals and sewage from failing infrastructure and aging sewer lines. In less urbanized or rural watersheds, bacterial contamination can come from a variety of sources such as septic systems, livestock, wildlife, and the use of manure or compost fertilizer on agricultural lands (Harmel et al. 2010). Urbanization can increase the transport of bacterial contaminants to nearby surface waters. Impervious surfaces such as roads, parking lots, and roofs, allow bacterial contaminants to quickly reach receiving waters, which results in significantly greater amounts of fecal contamination in urban streams during runoff periods (Bushon et al. 2017). In rural watersheds, bacterial contaminants can be removed from runoff, via filtration by the ground and riparian vegetation, before reaching the stream. Thus, urban streams are expected to have higher concentrations of fecal indicator bacteria after storm events than rural streams. However, few studies have compared fecal pollution levels in rural and urban streams. An improved scientific understanding of the sources and dynamics of *E. coli* will assist in the establishment of best practices for assessing, managing, and regulating fecal contamination in rural and urban watersheds.

3. MATERIALS AND METHODS

3.1. RESEARCH SITE DESCRIPTION

This research project focused on monitoring and assessing *E. coli* concentrations in surface water sources near the Missouri S&T campus in Rolla, Missouri. The majority of sampling sites were in the Mill Creek watershed near the town of Newburg in Phelps County, Missouri (Figure 3.1). Water samples were collected throughout the length of Mill Creek's main branch and within its two major tributaries. The watershed contains several springs and ponds, which were also examined for the presence of *E. coli* bacteria.

The Mill Creek watershed drains a catchment of 12,064 hectares in the Missouri Ozarks (Niyogi et al. 2010). Land use within the catchment includes: mostly forest (83.1%) and grassland (15.9%), with a very small amount of cropland and pasture (0.1%). About 60 percent of the catchment consists of U.S. national forest land (Mark Twain National Forest). The human population density of the watershed is low, with only about 370 people (3.1 people per km^2) living in the catchment (Niyogi et al. 2010). A few small farms near Hardester Hollow have livestock (cows and goats) and numerous horseback riding trails are present within Kaintuck Hollow. Fecal matter at these sites was expected to be a major source of *E. coli* entering Mill Creek, primarily via storm runoff, which could negatively impact the water quality at a recreational picnic area located downstream.

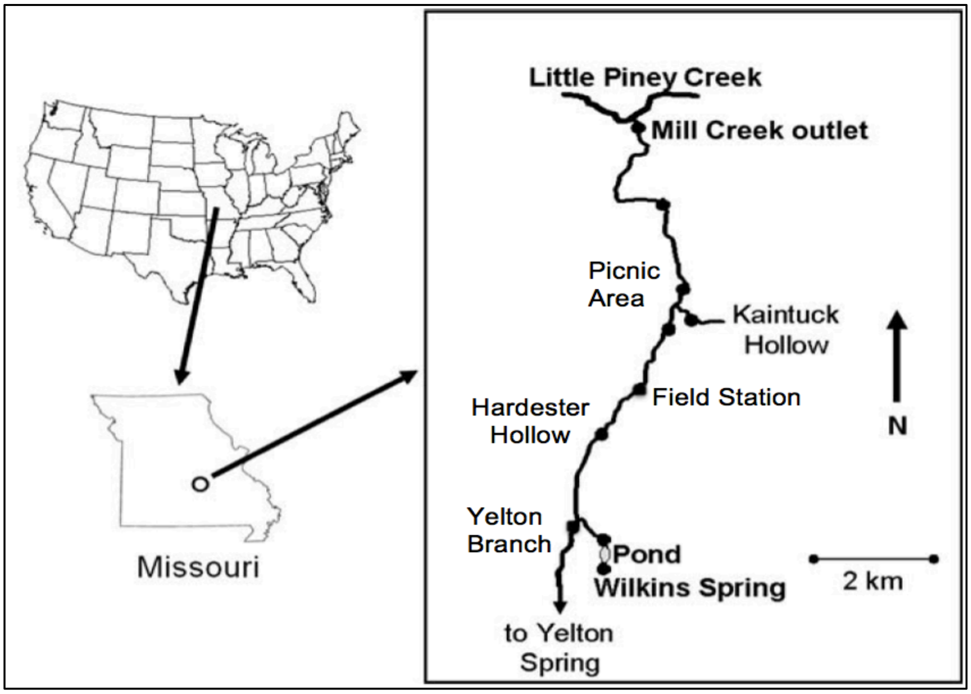


Figure 3.1. Site map of Mill Creek, Phelps County, Missouri. Circles indicate sampling sites.

Mill Creek and the surrounding area receive an annual average of 1,073 mm of precipitation, mostly in the form of rain (Niyogi et al. 2010). Rainfall amounts are, for the most part, evenly distributed throughout the year; although, spring storms lead to the highest monthly average for May (about 125 mm). The Missouri Ozarks are well known for their caves, sinkholes, and springs, given the karst geology, so topographic divides often do not always correspond to water sources. Mill Creek is fed by several springs; the four main springs that usually contribute water are Yelton Spring, Wilkins Spring, Hudgens Spring, and Elm Spring. Yelton Spring is commonly dry during drought conditions. Therefore, most water to Mill Creek during baseflow originates from Wilkins Spring. Two perennial tributaries to Mill Creek are streams draining Kaintuck Hollow and Hardester Hollow. Given the minimal flow of these two major tributaries, the size of Mill Creek does not vary greatly downstream. Mill Creek flows for 10 km from Wilkins Spring to its confluence with Little Piney Creek, which is a tributary of the Gasconade River. Throughout its length, it is well lit (about 50% canopy cover during summer) because of its width (average channel width of about 20 m). Stream temperature is fairly constant (about 13°C) at the spring source, and varies from 4°C in winter to 25°C during low flows in summer at the outlet to Little Piney Creek (Niyogi et al. 2010).

Bacterial analysis was performed on several ponds in the city of Rolla to expand the dataset to include more urban sampling sites. Sampled ponds included: Frisco Pond in Schuman Park near the Missouri S&T campus, Rolla Lodge Pond located at Ber Juan Park, and the Lion’s Club Park pond. The Deible Branch, a perennial stream that drains a catchment of 583 hectares near the ACORN trail and Highway O in Rolla, Missouri, was also sampled and assessed for contamination by *E. coli*. A comparison was made between the average *E. coli* concentration observed in the Deible Branch, which represented an urban watershed, and the more rural watershed of Mill Creek. The data collected from the springs, ponds, and streams in the Mill Creek watershed and in the city of Rolla were used to determine if a difference exists between the average *E. coli* concentration in springs, ponds, and streams.

3.2. MILL CREEK WATERSHED HYDROLOGICAL SURVEY

The hydrological survey of the Mill Creek watershed consisted of two major parts: studying water quality and stream flow. *E. coli* concentration and other water quality characteristics are commonly monitored to ensure water sources are safe for public use. Stream flow (discharge) has been studied before, during, and after storm events to determine if it is a strong predictor of *E. coli* concentration in streams.

3.2.1. Water Quality. The hydrological survey of the Mill Creek watershed began in September of 2015 and water samples were collected through May of 2017. Three to four grab samples were collected in the field each month at chosen sites (between 15 and 20 depending on if flow was present) along Mill Creek and its tributaries. Samples were collected more frequently during storm events. The water samples were left unfiltered and kept on ice during collection and transport to the lab, where bacterial analysis was performed within six hours of collection.

In the lab, the turbidity and *E. coli* concentration of each water sample was analyzed by standard methods. A nephelometric turbidity meter (Hach 2100P Portable Turbidimeter) was used to measure turbidity (in nephelometric turbidity units or NTU). *E. coli* concentrations were determined using a membrane filtration method (EPA Method 1603) and reported as colony forming units per 100 milliliters (cfu/100 mL). In EPA Method 1603, water samples are filtered through 0.45 µm membrane filters, which retains the *E. coli* and allows them to grow on a selective agar medium, such as modified mTEC (membrane-

Thermotolerant *Escherichia coli*) agar. After incubation (2 hours at 35°C, then at 44.5°C for 22 hours), *E. coli* form distinct purple colonies on the modified mTEC agar plates and can be counted (Figure 3.2). The direct counts are then used to calculate the *E. coli* concentration of a site at the time of sampling (U.S. EPA, Method 1603, 2002). Dilutions may be necessary to achieve countable plates when fecal pollution levels are high, such as during storm events, after sediment disturbance, or when point sources are present. Other water quality characteristics, specifically water temperature (degree Celsius, °C), dissolved oxygen (milligrams per liter, mg/L), and specific conductivity (microSiemens per centimeter, µS/cm), were recorded directly from the stream at each site using a YSI ProDO water quality meter.

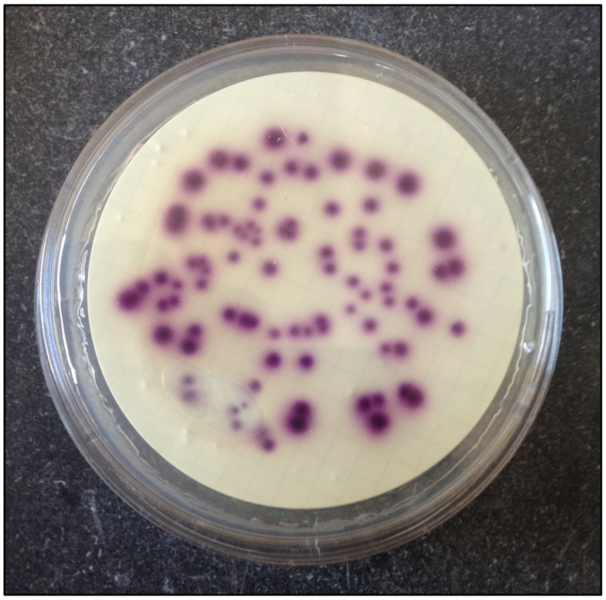


Figure 3.2. Positive result for E. coli on modified mTEC agar. E. coli form distinct purple colonies.

3.2.2. Stream Flow. Stream discharge is commonly quantified using the velocity-area method, in which discharge is calculated by finding the cross-sectional area of the stream and multiplying it by the mean stream velocity at this cross-sectional point (Gore 2006). However, determining the cross-sectional area and mean velocity of the entire stream can be very difficult. Thus, measurements of width, depth, and velocity are taken at equal intervals across the stream and the discharge of each interval is calculated. The overall stream discharge can then be determined as the sum of the discharges from each interval within the stream cross-section.

A cross-section of Mill Creek, near the picnic area site, was selected for measuring stream discharge. This section of the stream had a uniform streambed and flow, with few boulders and little to no dead water near banks, which is most optimal for accurately measuring stream discharge. The overall width (in feet) of the stream was determined with a measuring tape (the overall width is divided by the number of interval measurements to determine the width of each interval). A top setting wading rod and Marsh-McBirney Flo-Mate 2000 were used to measure depth (in feet) and mean velocity (in feet per second) at each interval across the stream (Gore 2006). Stream velocity varies along the vertical plane in a stream due to friction, from zero at the stream bottom to a maximum near the water’s surface. To correct for this variation, the mean velocity was measured at six-tenths of the total depth below the surface, which was determined empirically to be a close approximation to the mean velocity at a vertical line in the stream (Gore 2006). The stream discharge of Mill Creek, at the picnic area site, was calculated in cubic feet per second (cfs) using the velocity-area method.

Stream discharge was sampled at the picnic area site during each sampling trip at Mill Creek to determine if a relationship exists between discharge and *E. coli* concentration. Additional samples were collected during storm events when possible, given limitations for safety, to analyze how *E. coli* concentrations are associated with stormflows from heavy precipitation and surface runoff. Multiple water samples and discharge measurements were collected at the picnic area site before, during, and after major storm events. During these storm sampling events, a sample was collected about an hour before the storm began and then additional samples were taken every hour during the storm. In some cases, more samples had to be collected the day following the storm because the discharge had not receded on the day of the storm. The continuous discharge data collected from these storm sampling events was used to construct storm hydrographs for Mill Creek. The water samples were analyzed for turbidity and *E. coli* concentration, which were recorded and added to the storm hydrograph to show how these water quality characteristics are affected throughout a storm event.

3.3. SEDIMENT RESERVOIRS

It was predicted that *E. coli* would be present and more abundant in the stream sediment of Mill Creek. To test this hypothesis, sediment disturbance samples were collected at the picnic area site during each sampling trip to measure the concentration of *E. coli* stored in the sediment. A comparison could then be made between the *E. coli* concentration in the sediment and surface water at the picnic area, which could provide further insight into the existence of sediment reservoirs of fecal pathogens in streams. Also, a comparison of the suspendable concentrations of *E. coli* from the sediment to *E. coli* concentrations in the water column after storm events may reveal the importance of the sediment reservoir to stormflow concentrations.

The site chosen to perform sediment disturbance samples had a stream bottom that was representative of Mill Creek. The Mill Creek substrate can be described as a mixture of primarily fine silt and sand particles and small gravel (i.e., pebbles), but a few larger chunks of rock (i.e., cobbles or boulders) also are present. Sediment disturbance samples were collected using enclosed cylinders (i.e., open-bottom buckets) and a cordless power drill with a spiral paint mixer (Wilson et al. 2014). The sediment disturbance samples were collected about one meter from the stream bank in approximately eight to ten inches of water. The open-bottom bucket was pushed several inches into the stream sediment and the drill was used to disturb the sediment for 45 seconds. A grab sample was collected, from within the bucket, 30 seconds after disturbing the stream sediment. This sample was kept on ice and the *E. coli* concentration was determined in the lab using the membrane filtration method.

To use the membrane filtration method, the water samples from the sediment disturbance had to be diluted with autoclaved stream water because otherwise too many fine sediment particles would block the small pores on the membrane filter. Thus, 10 mL of water from the sediment disturbance sample was added to 90 mL of autoclaved stream water from Mill Creek. Then, all 100 mL of this new sample was used for membrane filtration to determine the *E. coli* concentration present in the stream bottom. It was also determined through experimentation that waiting 30 seconds before collecting the disturbed water sample from the bucket yielded best results. Samples collected immediately after to within 30 seconds of disturbing the sediment were too turbid to filter, even after performing a dilution with autoclaved stream water.

3.4. ENVIRONMENTAL FACTORS AFFECTING E. COLI SURVIVAL

To further investigate the dynamics and fate of *E. coli* in Mill Creek, several lab experiments were designed to test survival. A simple mesocosm approach was used, in which the natural environment of the stream was simulated and conditions were controlled to determine the effect of various environmental factors on *E. coli* survival. Sunlight, temperature, sedimentation, and adsorption were the primary environmental factors examined. One-meter plastic gutters with aquarium pumps and tubing were used to simulate the stream conditions of a small order stream with low flow, similar to the tributaries of Mill Creek. Experiments assessing the effect of sunlight, sedimentation, and adsorption on *E. coli* survival were performed in these “gutter mesocosms.” However, the effect of temperature on *E. coli* survival was done in 500 mL Erlenmeyer flasks instead of the one-meter gutters because the gutters were too large to store and maintain at the desired temperature conditions.

Due to the complexity of stream systems in the natural environment, it is practically impossible to isolate and study each independent factor affecting the survival of *E. coli* in the field. Thus, controlled lab experimentation is required. Many researchers have performed lab studies (and some field studies have been conducted) on *E. coli* survival, but none have utilized one-meter plastic gutters and aquarium pumps for mesocosms. Thus, a major goal of these survival experiments was to determine the effectiveness of the “gutter mesocosms” by comparing the results (i.e., decay rates) with the findings from other research reports. Additionally, the survival experiment results will provide more information and a better understanding of how fecal pathogens exist in aquatic environments, which could be used to better manage watersheds and protect people and animals from fecal contamination and waterborne disease.

3.4.1. Solar Radiation. An experiment was designed using two one-meter gutter mesocosms to test the rate of sunlight inactivation on *E. coli* concentration at the Hardester Hollow tributary of Mill Creek. Water was collected from Hardester Hollow in ten-liter carboys, generally a few days after a rainstorm to ensure the concentration of *E. coli* was elevated, and three liters were added to each mesocosm (water was mixed thoroughly inside the carboys before being added). The mesocosms were sterilized with ethanol prior to adding the contaminated water from Hardester Hollow. The two treatment conditions for the experiment were: sunlight and no sunlight. Thus, one mesocosm was placed in the shade, to serve as a control, and the other mesocosm was placed in direct sunlight. Experiments were conducted for two hours in the field at the Missouri

S&T field station or outside at the Missouri S&T campus, on sunny days during the summer of 2016, specifically during the middle of the day when the sun was directly overhead. Samples of 25 mL were taken in sterile conical tubes from each mesocosm at the start of the experiment (i.e., initial sample used to determine initial concentration) and then again after one hour and two hours of incubation time. The samples were kept on ice until they were processed after the completion of the experiment. The turbidity of each sample was recorded using a Hach meter and the *E. coli* concentration was determined using the membrane filtration method. The percent survival after two hours and decay rate of *E. coli* concentration in the two mesocosms were calculated and used to quantify the role sunlight has on the survival, more specifically the inactivation and removal, of fecal pathogens in aquatic systems.

3.4.2. Water Temperature. Two three-month-long temperature experiments were conducted as part of this research project (from Oct. 2016 to Jan. 2017 and June 2017 to Sept. 2017). The general procedure used to perform both experiments is as follows. In the temperature experiments, nine 500 mL Erlenmeyer flasks were inoculated with 500 mL of water collected from the Mill Creek tributary at Hardester Hollow (water was collected after a storm event to ensure a high initial concentration of *E. coli*). Each experiment was started the day following the collection of the contaminated water (used for inoculation) from Hardester Hollow (the water was stored in the fridge overnight to prevent an initial die-off). At the start of each experiment, the membrane filtration method was used to determine the initial *E. coli* concentration of the inoculum water.

The nine flasks were fixed with a foam stopper and aerated to prevent excessive evaporation and die-off due to anoxic conditions in the flask (which are not present in the natural stream system). Each flask was also wrapped in aluminum foil to control for and prevent UV light exposure, which could damage or kill the bacterial cells. After preparing the flasks, each was placed under one of three temperature treatments, cool temperature (in fridge, about 8°C), room temperature (on lab counter, about 24°C), or body temperature (in incubator set to 37°C). Three flasks were placed under each treatment condition, which allowed for multiple samples to be taken and analyzed to ensure accuracy in the sampling procedure. During each sampling interval, 10 mL samples were taken from each flask and processed using the membrane filtration method to determine *E. coli* concentration. Over a three-month period, numerous sampling intervals were completed (one sample each of the first five days and then one sample per week for the remainder of the experiment), with data being recorded for each flask. The *E. coli* concentrations of the triplicate samples from each temperature treatment were averaged and this average was used to calculate the decay rate of *E. coli* concentration at each temperature (decay rates are calculated as $\log_{10}$(cfu/100 mL) per day).

3.4.3. Sedimentation and Adsorption. It may be unclear at first how the processes of sedimentation and adsorption are able to affect the survival of *E. coli* in aquatic systems. These processes are primarily associated with the transport (i.e., dynamics) and ultimate storage of *E. coli* in the bottom sediment of a stream or pond. However, the attachment to solid surfaces (e.g., stream sediments) and storage in sediment reservoirs could provide beneficial living conditions, such as protection from sunlight and predators and additional food (i.e., organic matter) and nutrients, to *E. coli* and other fecal pathogens, which could in turn lead to increased survival times in aquatic systems.

To test the effect of sedimentation and adsorption on the storage and consequential survival of *E. coli* in streams, a mesocosm experiment was designed using four one-meter plastic gutters (Figure 3.3). The main goal of the experiment was to determine if a major trend existed between the removal of *E. coli* from the water column, due to sedimentation and adsorption, and the sediment size of the stream bottom. Three mesocosms were used as experimental groups, each had a different size sediment for a stream bottom, and the fourth mesocosm served as a control, which did not have any stream substrates (i.e., was left empty). The first experimental stream bottom consisted of sand (considered fine sediments), the second consisted of aquarium gravel (considered coarser pebbles), and the last consisted of stream substrate from Mill Creek, which included a mixture of large cobbles, coarse rocks and pebbles, and fine sands and silt. *E. coli* was expected to preferentially bind to smaller particles (i.e., fine sediments). Thus, the mesocosm with the sandy stream bottom was expected to remove the most *E. coli*, followed by the Mill Creek substrate (i.e., sand and gravel), and the aquarium gravel would remove the least.

Figure 3.3. Sedimentation and adsorption experimental setup. Four gutter mesocosms with different sediment bottoms were used as treatments in this experiment. From top to bottom: (1) Mill Creek stream substrate, (2) aquarium gravel, (3) sand, and (4) control (no sediment).

Survival can also be examined by disturbing the sediment bottoms of the mesocosms after conducting the experiment. It is expected that *E. coli* will adhere to the particles in the mesocosms and become stored in the bottom (where it will have improved survival conditions). In theory this would mean that the resuspension of the sediment from a mesocosm (taken at the end of the experiment) would contain an *E. coli* concentration that is close to the initial sample taken from the water column of the mesocosm at the start of the experiment (the resuspension will be slightly less than the initial sample due to some expected die-off of *E. coli* in the mesocosms). Also, it is expected that the resuspension would have an *E. coli* concentration that is greater than or equal to the final concentration in the control mesocosm, which had no sediment to improve survival. These two results would display that the processes of adsorption and sedimentation play some protective role, which leads to improved survival of *E. coli* and other fecal pathogens in aquatic systems.

A series of problems occurred during this experiment, which resulted in major revisions to the experimental design. Initially, contaminated water, used for inoculation of the mesocosms, was collected from the field after storms, like the sunlight and temperature experiments. However, after the water was circulated through the aquarium pumps for eight to twelve hours, the research team noticed a surprising trend, in which *E. coli* concentrations were increasing instead of decreasing in the mesocosms. Two possible reasons for this increase in concentration were proposed: (1) *E. coli* was growing in the mesocosms or (2) circulation in the mesocosms may be freeing attached *E. coli* from fine sediment and fecal particles that were present in the water collected from the field. The research team considered growth to be the less likely explanation because the gutter mesocosms did not provide optimal growing conditions for *E. coli*. Thus, the research team decided to investigate if using unattached or free-floating *E. coli* to inoculate the water would stop this trend. To do this, *E. coli* from Hardester Hollow was sampled and plated on modified mTEC agar. A single colony was collected from the plate and grown in Tryptic soy broth for 24 hours at 37°C in a shaking incubator. A dilution of this *E. coli* media was then made and used to inoculate twelve liters of autoclaved stream water, which was distributed into the mesocosms. Inoculating the mesocosms with autoclaved stream water that was contaminated with grown free-floating *E. coli* did not result in an increase in *E. coli* concentration at eight to twelve hours into the experiment. An added benefit to using the grown *E. coli* method was it allowed the research team to autoclave the water used for the inoculum.

In addition to discovering that free-floating *E. coli* needed to be used in gutter mesocosm experiments that were carried out for longer than six hours, the research team noticed that the heat from the running aquarium pumps could cause the water in the mesocosm to become very warm after six to twelve hours. To solve this issue, the experiments were conducted in a temperature controlled room, which was set to either 8°C or 15°C depending on the temperature of the stream that was being tested. The research team also decided to conduct the sedimentation and adsorption gutter mesocosm experiments for 48 hours instead of the original 24 hours because results were inconclusive after only 24 hours of incubation in the mesocosms. Contamination from external sources also presented a problem in these gutter mesocosm experiments. To prevent contamination the following protocol was taken before and during experiments to ensure that only the *E. coli* added to the inoculum was present: mesocosms (i.e., gutters) were sterilized with ethanol, all sediments were autoclaved, the stream water used for inoculum was autoclaved, sterile conical tubes were used to collect samples, and the mesocosms were covered with aluminum foil at all times except when sampling was being performed.

The best results were collected using the following experimental design, which was corrected for the problems explained above. To start each experiment, the gutter mesocosms were prepared by sterilizing the gutters, setting up the aquarium pumps and hoses, and washing, autoclaving, and adding the treatment sediments. A filter screen was placed under the aquarium pumps to prevent the sediment in the mesocosms from clogging and stopping the pumps during the experiment. An inoculum (12 liters total) of autoclaved stream water and *E. coli*, grown overnight in Tryptic soy broth, was then made (an initial *E. coli* concentration of 500 to 1000 cfu/100 mL was preferred), thoroughly mixed in a carboy, and three liters were distributed to each mesocosm. The experiments were conducted for 48 hours in a temperature controlled room (set at either 8°C or 15°C). Duplicate 10 mL samples were collected in sterile conical tubes from the water column, near the middle of the mesocosm, at the following times: 0 (initial sample), 1, 2, 4, 8, 12, 24, 36, and 48 hours from the start of the experiment. The samples were immediately processed for turbidity and *E. coli* concentration, using a Hach meter and the membrane filtration method, respectively. The *E. coli* concentrations of the duplicate samples were averaged and used to calculate the percent of *E. coli* removed from each mesocosm and the decay rates during the 48-hour experiment.

At the end of the experiment, the sediment bottom of each mesocosm was emptied into a five-gallon bucket and mixed using a cordless power drill with a spiral paint mixer, like the sediment disturbance tests performed at Mill Creek. A 10 mL sample was taken from each resuspension and processed for turbidity and *E. coli* concentration. The effect on survival was analyzed by comparing the

E. coli concentration of each sediment resuspension sample to the initial and final *E. coli* concentrations of its corresponding experimental mesocosm and the control mesocosm.

4. RESULTS

4.1. MILL CREEK WATERSHED HYDROLOGICAL SURVEY

The main goals of the Mill Creek watershed hydrological survey were to determine the extent of *E. coli* contamination in Mill Creek, explore the possible sources and dynamics of *E. coli*, and to assess if a threat to human health exists. Two sites in Mill Creek, Hardester Hollow and the picnic area, were the primary sites for accomplishing these goals. The site at Hardester Hollow was by far the most impacted of all the sites sampled in the Mill Creek watershed. Hardester Hollow was near a cow pasture and thus was known to be contaminated by *E. coli* from nonpoint sources. This allowed the research team to study the degree of contamination and dynamics of *E. coli* in streams, which can be directly related to nonpoint sources (no point sources of fecal pollution were identified in the Mill Creek watershed). The picnic area site has the most recreation in the stream in the watershed, given fishermen and swimmers may be in danger of encountering fecal pathogens. Thus, the picnic area was studied to assess if a threat to human health existed in Mill Creek.

4.1.1. Water Quality. Simple linear regression models (and global F-tests) were used to relate *E. coli* concentration to other water quality indicators (e.g., water temperature, dissolved oxygen, specific conductivity, and turbidity), which were measured at sampling sites in the Mill Creek watershed. Water temperature and dissolved oxygen had no significant correlation to *E. coli* concentration at Hardester Hollow (p-value = 0.481 and 0.172, respectively) or the Mill Creek picnic area (p-value = 0.758 and 0.414, respectively). However, a significant negative correlation was observed between specific conductivity and *E. coli* concentration at Hardester Hollow and the Mill Creek picnic area (p-value = 0.002 and 0.001, respectively) (Figures 4.1 and 4.2). Turbidity was also found to be significantly related to *E. coli* concentration at both sampling sites (p-values < 0.001). Increasing turbidity levels in the stream were strongly correlated with increasing *E. coli* concentration (Figures 4.3 and 4.4).

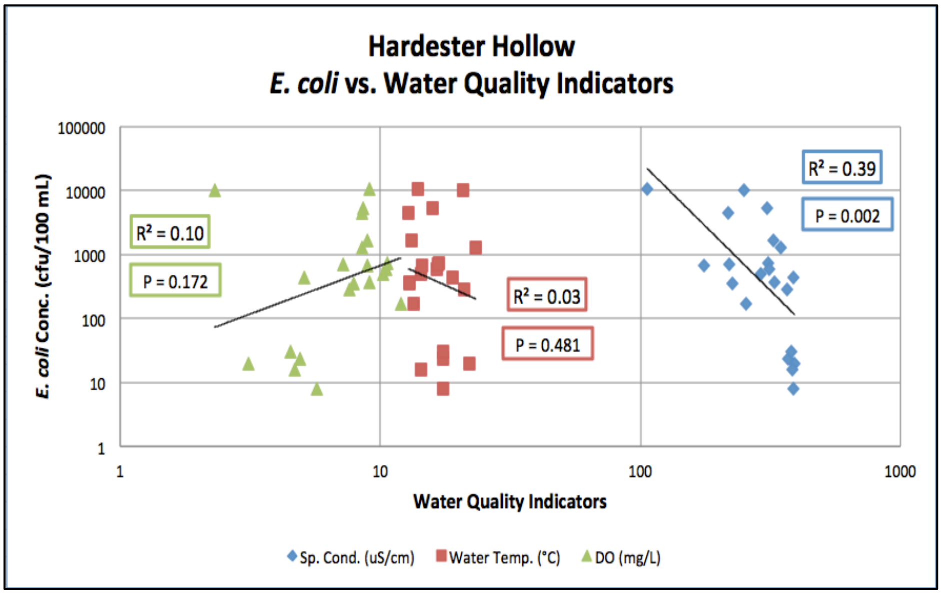


Figure 4.1. E. coli vs. water quality indicators at Hardester Hollow. Scatterplot showing regression of E. coli concentration (cfu/100 mL) with specific conductivity (μS/cm), water temperature (°C), and dissolved oxygen (DO) (mg/L). Both axes are on log scale. Blue: “Sp. Cond.” R-sq. = 0.391. Red: “Water Temp.” R-sq. = 0.026. Green: “DO” R-sq. = 0.096. A significant inverse relationship was observed between E. coli concentration and specific conductivity (P = 0.002). No significant relationship existed with water temperature or DO (P = 0.481 and P = 0.172, respectively).

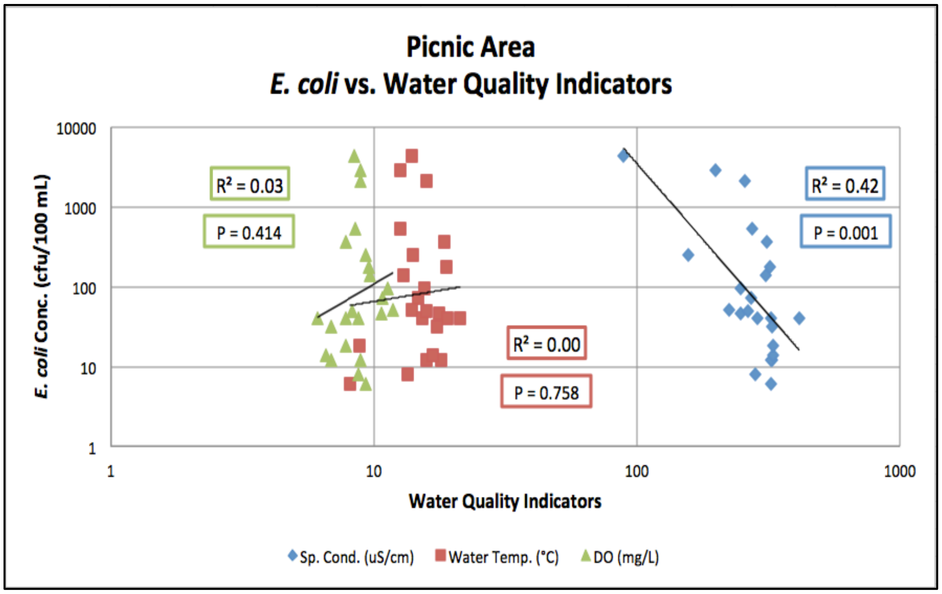


Figure 4.2. E. coli vs. water quality indicators at Picnic Area. Scatterplot showing regression of E. coli concentration (cfu/100 mL) with specific conductivity (µS/cm), water temperature (°C), and dissolved oxygen (DO) (mg/L). Both axes are on log scale. Blue: “Sp. Cond.” R-sq. = 0.417. Red: “Water Temp.” R-sq. = 0.005. Green: “DO” R-sq. = 0.032. A significant inverse relationship was observed between E. coli concentration and specific conductivity (P = 0.001). No significant relationship existed with water temperature or DO (P = 0.758 and P = 0.414, respectively).

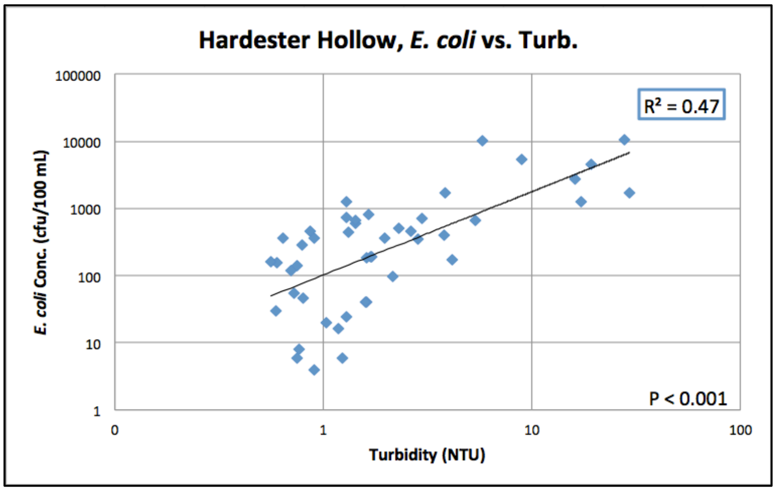


Figure 4.3. E. coli vs. turbidity at Hardester Hollow. Scatterplot showing regression of E. coli concentration (cfu/100 mL) with turbidity (NTU). Both axes are on log scale. A significant direct relationship was observed between E. coli concentration and turbidity (p-value < 0.001). R-sq. = 0.468.

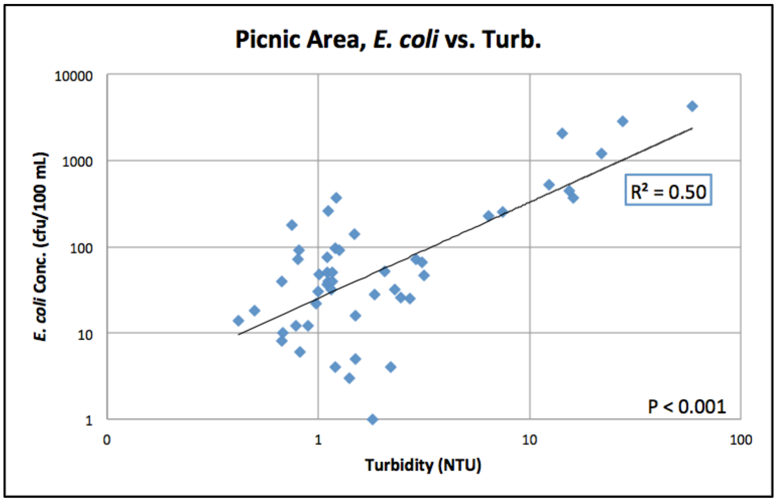


Figure 4.4. E. coli vs. turbidity at Picnic Area. Scatterplot showing regression of E. coli concentration (cfu/100 mL) with turbidity (NTU). Both axes are on log scale. A significant direct relationship was observed between E. coli concentration and turbidity (p-value < 0.001). R-sq. = 0.503.

4.1.2. Stream Flow. Large amounts of rainfall during storm events can result in surface runoff, which is the main source of nonpoint source pollution in aquatic systems. Thus, surface runoff is related to stormflow conditions and can lead to increased levels for water quality indicators such as discharge, turbidity, and *E. coli* concentration. Water samples collected within 24 hours of the Mill Creek watershed receiving at least a half-inch (or 12.5 mm) of rain were considered stormflow samples. All other water samples were recorded as measures of water quality during baseflow.

All sampling sites in Mill Creek had higher observed *E. coli* concentrations during stormflow conditions than during baseflow (Figure 4.5). Comparing baseflow and stormflow samples collected at the Mill Creek picnic area showed that the average *E. coli* concentration was greater after storm events than at baseflow (one-sided, two-sample independent t-test with unequal variance, p-value < 0.001). In fact, about a one log difference in median *E. coli* concentration during sampled stormflow and baseflow conditions was detected at the picnic area (Figure 4.6). The relationship between turbidity and *E. coli* concentration was further investigated during baseflow and stormflow conditions to determine the effect of stream flow on other water quality indicators.

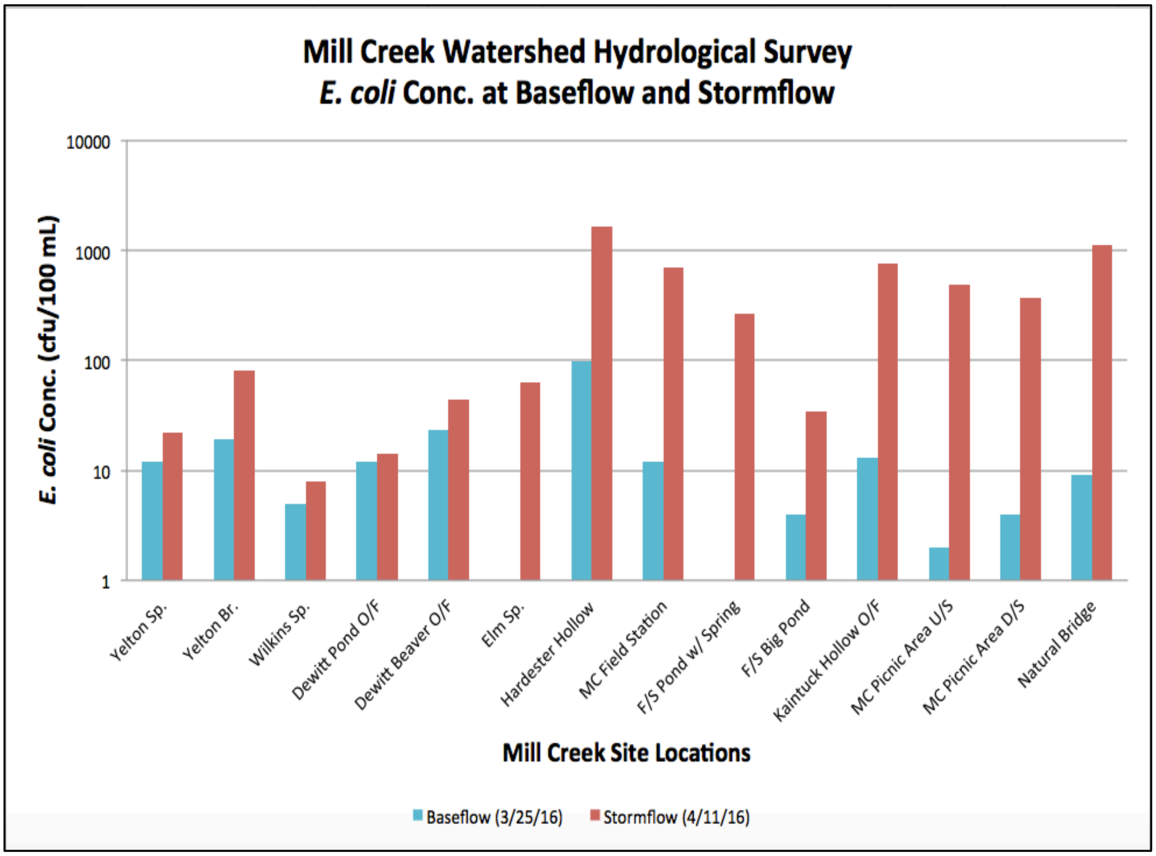


Figure 4.5. Bar chart of E. coli concentrations at Mill Creek sites during baseflow and stormflow. Blue = baseflow sampling on 3/25/2016. Red = stormflow sampling on 4/11/2016. The y-axis is on log scale. E. coli concentrations at all sampled sites in Mill Creek are higher after storms than during baseflow.

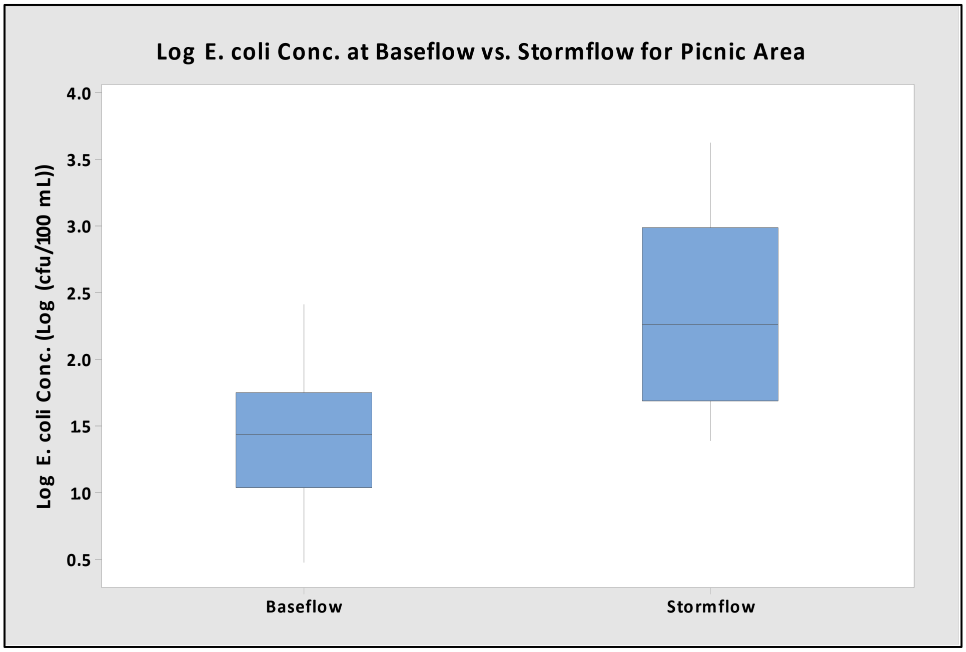
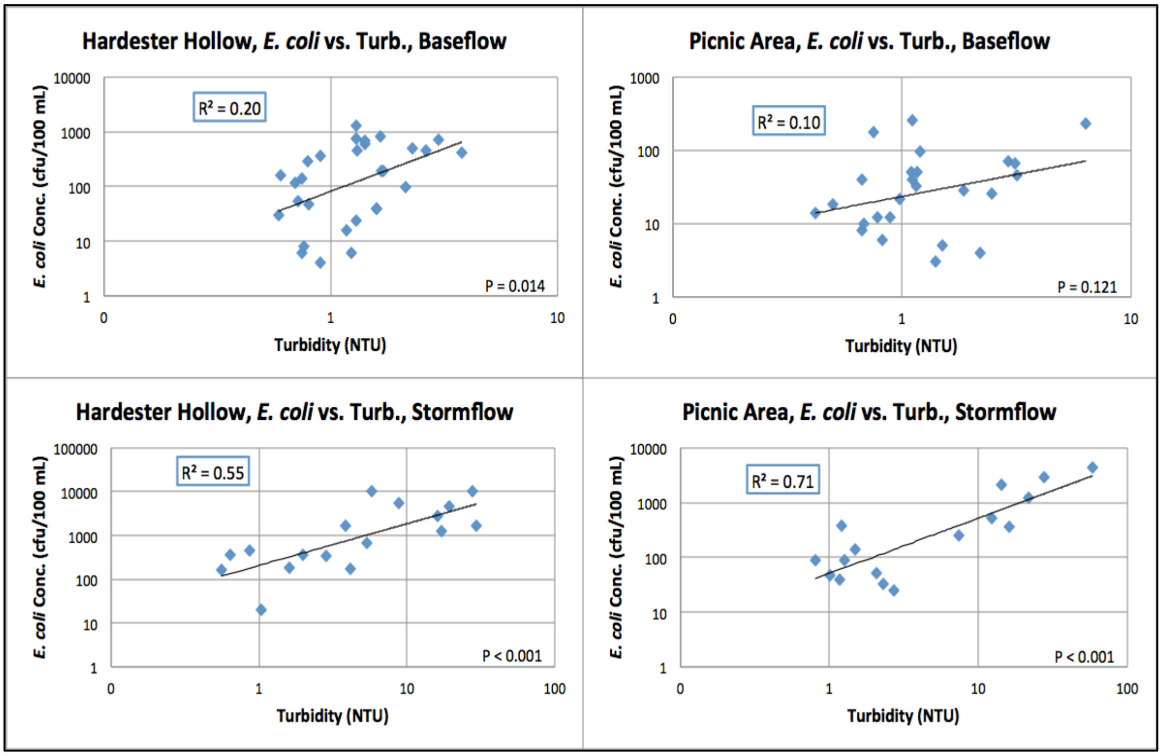


Figure 4.6. E. coli concentration during baseflow and stormflow at Picnic Area. Box plot comparing log E. coli concentration ($\log_{10}$(cfu/100 mL)) of baseflow and stormflow samples collected at the Mill Creek Picnic Area site.

Figure 4.7 displays four linear regressions between *E. coli* concentration and turbidity for Hardester Hollow and the picnic area. Simple linear regressions indicated that significant relationships exist between *E. coli* concentration and turbidity at both sites during stormflow conditions (p-values < 0.001). This relationship between *E. coli* concentration and turbidity was also significant for baseflow samples at Hardester Hollow (p-value = 0.014), but not at the picnic area (p-value = 0.121). Greater variation in the relationship between *E. coli* concentration and turbidity was observed for baseflow data, suggesting that factors other than turbidity, surface runoff, or rainfall (i.e., indicators of nonpoint source pollution) may have more influence on the presence of *E. coli* at a site during baseflow. Such factors may include: location near known sources of *E.*

coli (e.g., Hardester Hollow), direct inputs of fecal matter from animals, or recreational sediment disturbances. Nevertheless, a strong linear relationship between *E. coli* concentration and turbidity was clearly seen during stormflow conditions, indicating that major inputs of *E. coli* detected at sites in Mill Creek are in fact related to stormwater runoff and nonpoint sources of fecal pollution.


Hardester Hollow, *E. coli* vs. Turb., Baseflow

Picnic Area, *E. coli* vs. Turb., Baseflow

Hardester Hollow, *E. coli* vs. Turb., Stormflow

Picnic Area, *E. coli* vs. Turb., Stormflow

Figure 4.7. E. coli vs. turbidity during baseflow and stormflow at Hardester Hollow and Picnic Area. Scatterplots showing regression of E. coli concentration (cfu/100 mL) with turbidity (NTU). Both axes are on log scale. Top-Left: during baseflow at Hardester Hollow; R-sq. = 0.205. Bottom-Left: during stormflow at Hardester Hollow; R-sq. = 0.554. Top-Right: during baseflow at Picnic Area; R-sq. = 0.101. Bottom-Right: during stormflow at Picnic Area; R-sq. = 0.712.

Stream discharge increases with stormwater runoff and could have been used to differentiate stormflow and baseflow conditions instead of rainfall (24-hour rainfall totals of at least 0.5” or 12.5 mm, prior to a sample, were considered stormflow conditions). However, like other small order streams, the stream discharge at Mill Creek is generally low and can rise rapidly with rain, but will also quickly recede and return to low flow once it stops raining. Consequently, if the discharge was not measured during or immediately after a storm event, then it was possible to see no change, which makes using stream discharge problematic for determining if a sample was taken during stormflow or baseflow conditions. The change in stream discharge was mostly dependent on how long a storm event lasted and the total amount of rainfall it delivered. Thus, total amount of rainfall was used to distinguish stormflow and baseflow samples.

Stream discharge was measured at the Mill Creek picnic area during each sampling trip and compared to turbidity and *E. coli*. Stream discharge had a significant positive correlation to turbidity and *E. coli* concentration at the Mill Creek picnic area (simple linear regression, global F-tests, p-values < 0.001) (Figures 4.8 and 4.9). The relationship between turbidity and discharge was very strong (R-sq. = 0.806), which implied that a primary source of turbidity in the stream was related to surface runoff. *E. coli* concentration and discharge were not as strongly related (R-sq. = 0.468), suggesting factors other than stream flow are involved, but stormwater runoff and turbidity remain the strongest predictors of *E. coli* concentration.

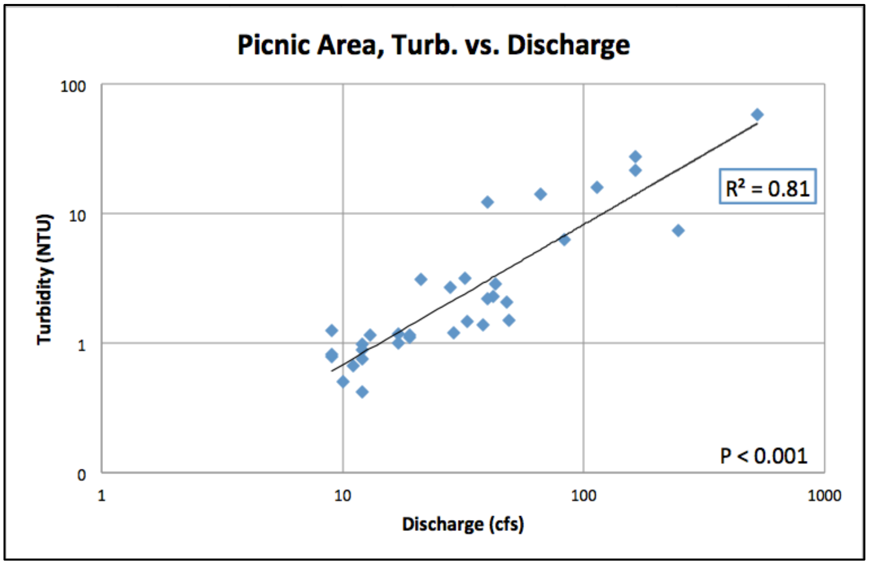


Figure 4.8. Turbidity vs. discharge at Picnic Area. Scatterplot showing regression of turbidity (NTU) with discharge (cfs). Both axes are on log scale. A significant direct relationship was observed between turbidity and discharge (p-value < 0.001). R-sq. = 0.806.

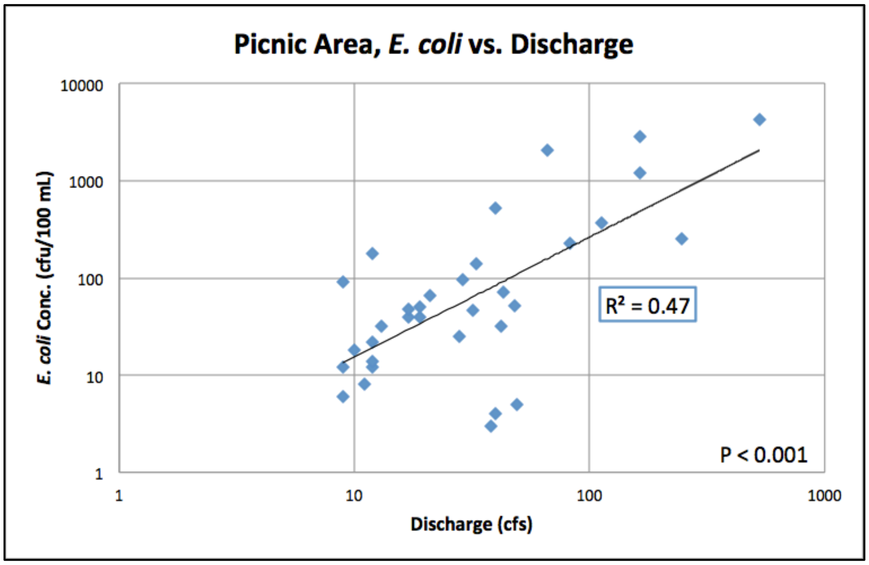


Figure 4.9. E. coli vs. discharge at Picnic Area. Scatterplot showing regression of E. coli concentration (cfu/100 mL) with discharge (cfs). Both axes are on log scale. A significant direct relationship was observed between E. coli concentration and discharge (p-value < 0.001). R-sq. = 0.468.

A storm hydrograph, which includes trends for *E. coli* concentration and turbidity, was constructed from data collected at the Mill Creek picnic area before, during, and after a storm event on April 26th and 27th, 2017 (Figure 4.10). During the rising limb of the storm hydrograph, the first flush (i.e., initial amount of surface runoff) resulted in a significant rise in *E. coli* concentration and turbidity. Both of which peaked around the same time as discharge. However, while discharge slowly declined during the falling limb of the storm hydrograph, *E. coli* concentration and turbidity rapidly returned to pre-storm levels. The storm hydrograph further illustrates the strong relationships observed between stream discharge (i.e., stormwater runoff), turbidity, and *E. coli* concentration.

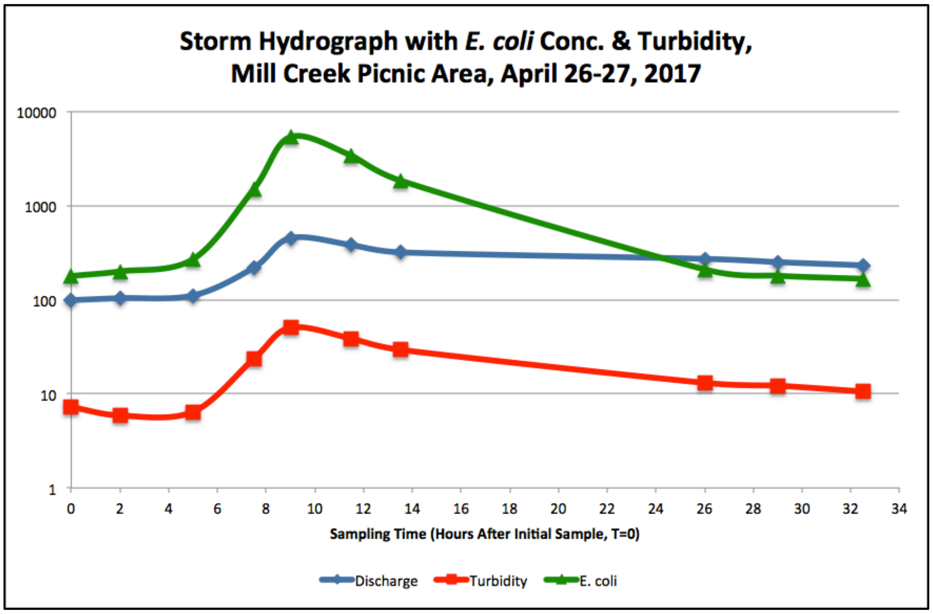


Figure 4.10. Storm hydrograph at Picnic Area. Line graph displaying E. coli concentration in green (cfu/100 mL), turbidity in red (NTU), and discharge in blue (cfs), which were recorded over time (33 hours total) during a storm event (from 4/26/2017 at 6 am (T = 0) to 4/27/2017 at 2:30 pm (T = 32.5)). Y-axis is on log scale.

4.2. SEDIMENT RESERVOIRS

Sediment disturbance samples, collected at the Mill Creek picnic area, revealed that a higher concentration of *E. coli* was stored in the sediment than was originally sampled from the water column. The results were statistically significant (at $\alpha = 0.05$) to conclude that disturbing the sediment leads to a greater average log *E. coli* concentration than samples collected from the water column (one-sided, paired t-test, p-value < 0.001). Approximately a one log difference in median *E. coli* concentration was observed between samples collected from the water column before and after disturbing the sediment (Figure 4.11). Results remained consistent and significant (p-value < 0.001) for samples that were only collected and analyzed after storm events (Figure 4.12).

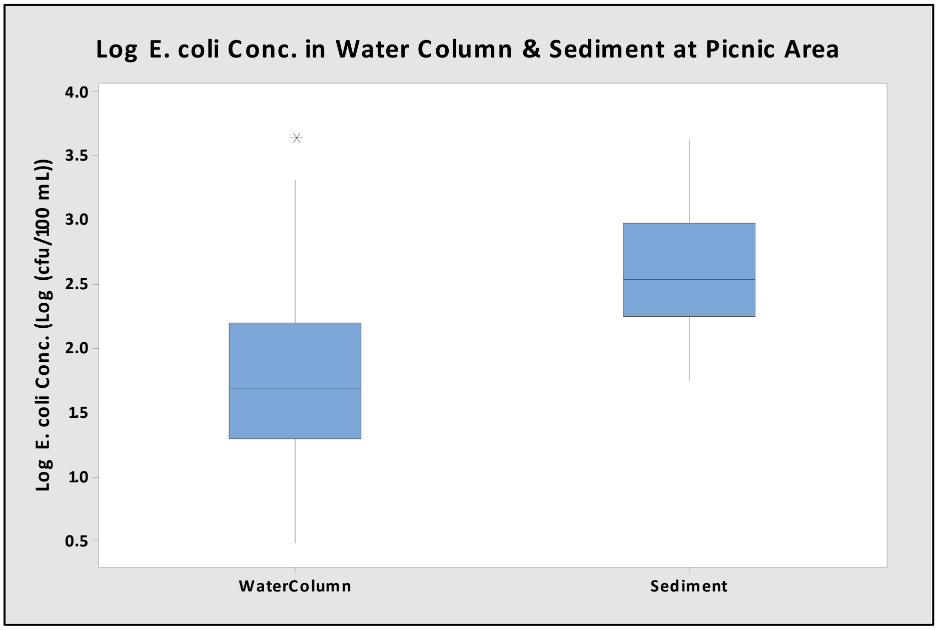


Figure 4.11. E. coli concentration in water column and sediment at Picnic Area. Box plot comparing log E. coli concentration ($\log_{10}$(cfu/100 mL)) of water and sediment samples collected at the Mill Creek Picnic Area site.

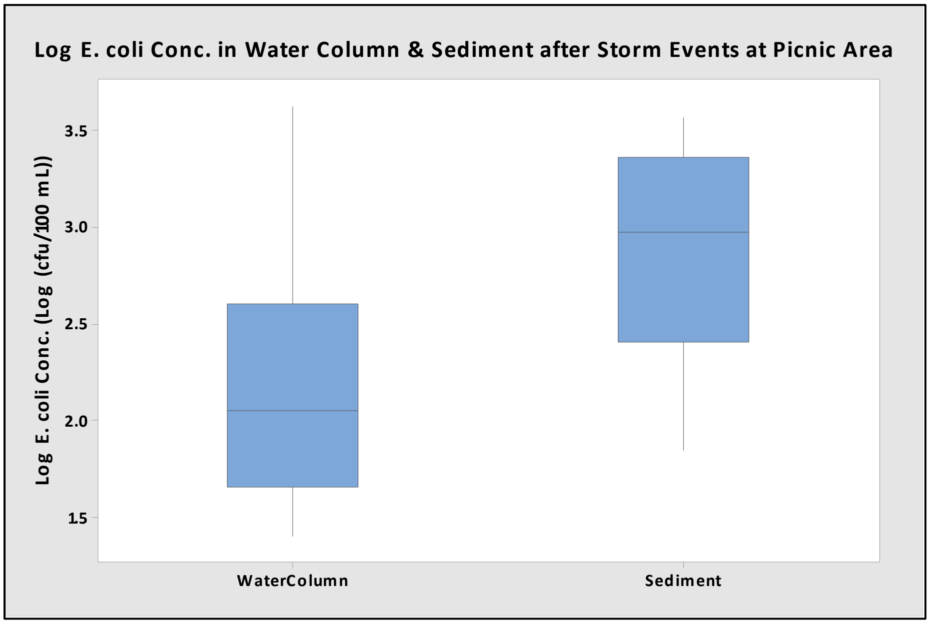


Figure 4.12. E. coli concentration in water column and sediment after storm events at Picnic Area. Box plot comparing log E. coli concentration ($\log_{10}$(cfu/100 mL)) of water and sediment samples collected after storm events at the Mill Creek Picnic Area site.

4.3. ENVIRONMENTAL FACTORS AFFECTING E. COLI SURVIVAL

A variety of environmental factors (e.g., physical, chemical, and biological) impact the survival of *E. coli* and other pathogenic microbes in streams and sediments. The main in-stream factors include: solar radiation, water temperature, sedimentation, adsorption, predation, stream vegetation, and nutrient availability. Also, other factors can affect *E. coli* and microbe survival outside of streams, such as the local hydrology, geology, soil characteristics, and the presence (or lack) of riparian vegetation.

4.3.1. Solar Radiation. Solar radiation is known to have a strong inactivation effect on bacteria, including *E. coli*. To test this effect, a two-hour sunlight inactivation experiment in two gutter mesocosms, shade (control) and sun (experiment), was conducted. Within two hours of being exposed to sunlight, only 3.2% of the initial population of *E. coli* in the mesocosm had survived (initial concentration was 500 cfu/100 mL; final concentration was 16 cfu/100 mL). In the control mesocosm, which was kept in the shade, 95% of the initial population of *E. coli* had survived (initial concentration was 484 cfu/100 mL; final concentration was 460 cfu/100 mL) (Figures 4.13 and 4.14). A one-sided, two-sample independent t-test with equal variance was used to determine that there was statistically significant evidence to conclude (at $\alpha = 0.05$) that the average percent survival of *E. coli* concentration in the mesocosm kept in the shade was greater than the mesocosm exposed to the sun after the two-hour sunlight inactivation experiment ($p\text{-value} < 0.001$). Thus, sunlight did in fact have a strong inactivation effect on *E. coli*. To further explain the effect of sunlight on *E. coli* survival, the decay rate of the *E. coli* concentration in the two mesocosms used during the two-hour sunlight inactivation experiment was calculated. The control (shade) mesocosm had a decay rate of $-0.011 \log_{10}(\text{cfu}/100\text{ mL})$ per hour, which was much less than the decay rate of $-0.735 \log_{10}(\text{cfu}/100\text{ mL})$ per hour for the mesocosm kept in the sun.

4.3.2. Water Temperature. The survival and persistence of *E. coli* and other fecal coliform bacteria is believed to be higher at lower water temperatures. To further investigate this idea, flasks of *E. coli* contaminated water were stored at three different temperatures (8°C, 24°C, and 37°C) and the *E. coli* concentrations were monitored over a three-month period. The overall survival time (in days) and decay rate of the *E. coli* bacteria were determined. At 37°C, *E. coli* persisted for only eight to ten days and had the greatest average decay rate, which was –0.291 $\log_{10}$(cfu/100 mL) per day. At 24°C, the survival time of *E. coli* improved to between 35 and 41 days, which decreased the average decay rate to –0.071 $\log_{10}$(cfu/100 mL) per day. *E. coli* persisted the longest, over 86 days, and had the lowest average decay rate, –0.020 $\log_{10}$(cfu/100 mL) per day, at 8°C.

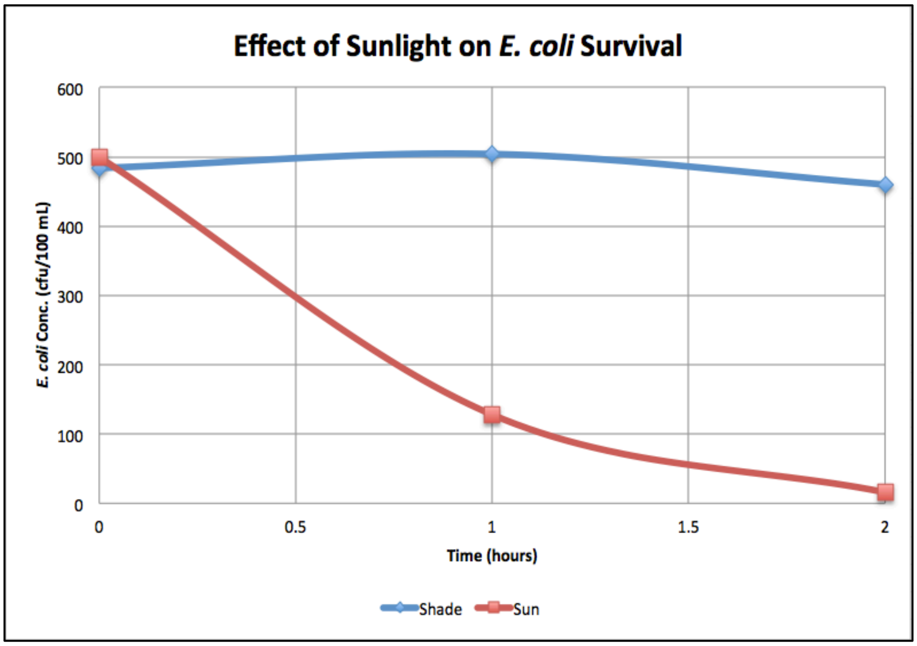


Figure 4.13. Effect of sunlight on E. coli survival. Line graph displaying the change in E. coli concentration over two hours in sun (red) and shade (blue) gutter mesocosms.

Figure 4.15 displays the change in *E. coli* concentration at each temperature during the three-month experiment. The *E. coli* concentration drastically declined during the first ten days of the experiment in all three temperature conditions. Specifically, on day ten, only about 1% of the initial concentration of *E. coli* remained at 37°C, about 10% remained at 24°C, and about 30% remained at 8°C. After the initial ten days of the experiment, the decay rate appears to be reduced at 24°C and 8°C, which results in much longer survival times for *E. coli* kept at these temperatures than at 37°C. Colder water temperatures appear to improve the survival of *E. coli*.

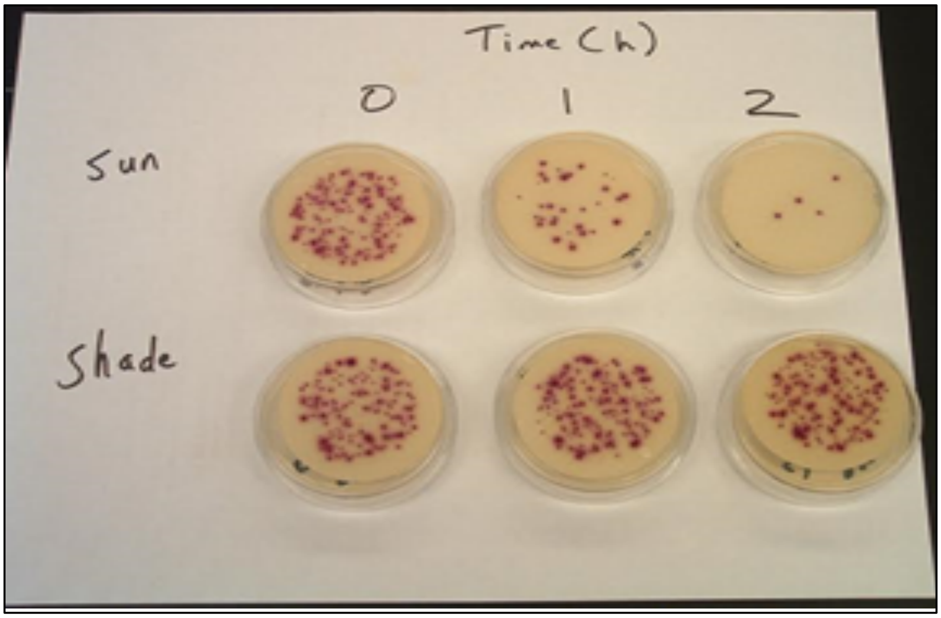


Figure 4.14. Sunlight inactivation of E. coli. Image of E. coli, grown on modified mTEC agar plates, which were collected from gutter mesocosms kept in the sun or shade (control) during a two-hour incubation period.

4.3.3. Sedimentation and Adsorption. Due to the higher concentrations of *E. coli* observed in the stream sediment at Mill Creek, the research team designed a mesocosm experiment to test the processes believed to be responsible for these sediment stores. *E. coli* is known to adhere to solid particles in streams (a process called adsorption), which is expected to lead to the removal of *E. coli* from the water column via sedimentation. *E. coli* survives longer in the stream bottom than in the water column due to the extra protection and possible food (i.e., organic matter) and nutrients provided by the sediment. Thus, the processes of sedimentation and adsorption could be linked to *E. coli* survival and persistence in the stream and sediment at Mill Creek.

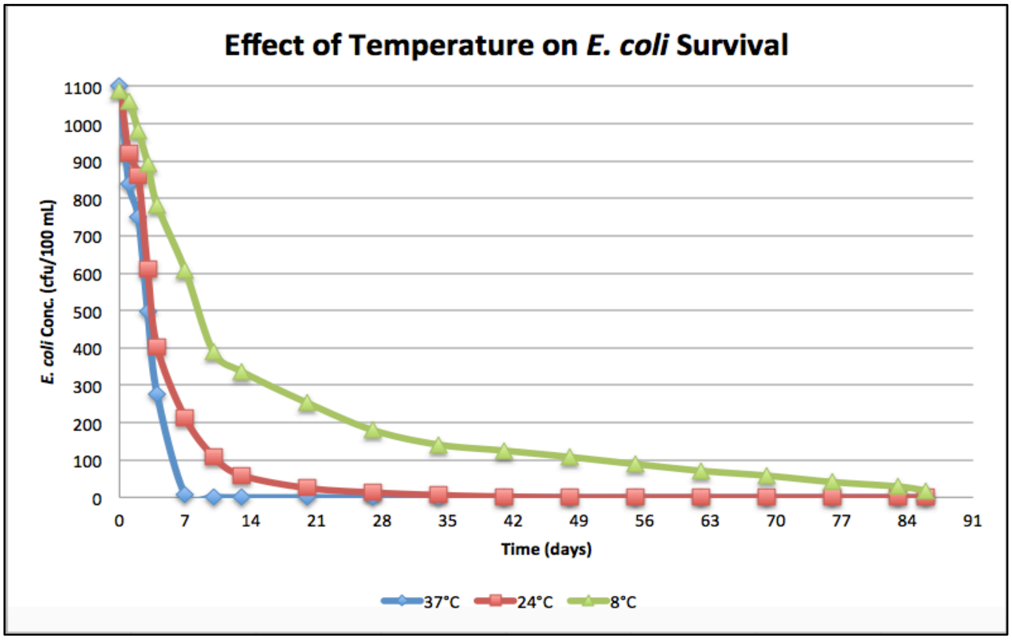


Figure 4.15. Effect of temperature on E. coli survival. Line graph displaying the change in E. coli concentration over time (in days) in flasks kept at 8°C (green), 24°C (red), and 37°C (blue). E. coli survived the longest (86 days) at 8°C.

The main goal of the experiment was to determine if the particle size of the stream bottom influenced the removal rate of *E. coli* via sedimentation and adsorption. Temperature was also a factor in this experiment and trials were performed at 15°C and 8°C (which represented seasonal stream temperatures at Mill Creek). For experiments conducted at 15°C, the average percent of *E. coli* lost in each mesocosm was: 36% (–0.004) for the control, 84.4% (–0.012) for sand, 90.5% (–0.020) for aquarium gravel, and 83.8% (–0.013) for Mill Creek substrate (the average decay rate (i.e., removal rate) of *E. coli* concentration in $\log_{10}$(cfu/100 mL) per hour is provided in parentheses). Results were similar at 8°C, in which the average percent of *E. coli* removed in each mesocosm was: 21.7% (–0.002) for control, 96.7% (–0.033) for sand, 77.2% (–0.015) for aquarium gravel, and 82.3% (–0.016) for Mill Creek substrate.

A two-way ANOVA test and Tukey pairwise comparisons were used to analyze the effect of particle size and temperature on the percent of *E. coli* removed from each mesocosm. The global F-test determined that there was no significant interaction between particle size and temperature on the average percent of *E. coli* removed from each mesocosm (p-value = 0.406). Thus, the global F-test was then used to test the main effects. The effect of temperature was not statistically significant (p-value = 0.732); however, there was statistically significant evidence to conclude (at $\alpha = 0.05$) that particle size influenced the average percent of *E. coli* removed from each mesocosm (p-value = 0.002). Tukey pairwise comparisons were conducted on particle size and concluded that sand (P = 0.002), aquarium gravel (P = 0.006), and Mill Creek substrate (P =

0.008) all differed from the control mesocosm in the percent of *E. coli* removed, but were not significantly different from one another (P = 0.835 for aquarium gravel and sand; P = 0.739 for Mill Creek substrate and sand; and P = 0.997 for Mill Creek substrate and aquarium gravel).

Figure 4.16 displays the results from an experiment conducted on July 18th and 19th, 2017 at 8°C, which seems to suggest that during the 48-hour experiment a difference in *E. coli* removal between particle sizes can be observed and may possibly exist. However, the high variation between results from all the experiments performed led to the conclusion, by the two-way ANOVA test, that the size of the particles did not lead to statistically significant differences in *E. coli* removal. From the results the research team concluded that temperature had no observed effect on *E. coli* removal in the gutter mesocosms, but the presence of stream sediment did. Thus, adsorption and sedimentation are important in removal of *E. coli* from the water column and in the formation of sediment reservoirs.

The results of the sediment disturbances, which were performed in each experimental mesocosm after completing the 48-hour experiment, were inconclusive. The hope was that by disturbing and sampling the resuspension from each mesocosm, the research team could have provided further evidence of the sediment’s ability to protect *E. coli* and improve survival. The following inductive arguments were used to conclude that the presence of sediment in the experimental mesocosms had improved the survival of *E. coli* during the 48-hour experiment: (1) the sediment will store and protect most *E. coli* in the mesocosm,

(2) the protected *E. coli* can be resuspended from the sediment after 48 hours, and (3) more *E. coli* will die-off when sediment is not present (i.e., in the control).

The *E. coli* concentration sampled from a mesocosm's sediment resuspension was never close to the initial concentration in that mesocosm and was never greater than or equal to the final concentration in the control mesocosm for that experiment. The best results showed that the research team was only able to resuspend 40 percent of the initial *E. coli* concentration from the sediment. Thus, the inductive arguments listed above were not supported by the results and can be considered weak arguments.

One supportive trend was consistently observed in every experiment conducted. The *E. coli* concentration of the sediment resuspension was always greater than the final concentration sampled from the water in each mesocosm (this result applied to all three sediment bottom treatments). This trend allowed the research team to infer that more *E. coli* was present in the sediment bottom of each mesocosm than the water column at the end of the 48-hour experiment. However, this result alone does not definitively confirm or deny that the sediment is protecting *E. coli* and improving its survival in the mesocosms. Therefore, the research team concluded that the effect of sedimentation and adsorption on *E. coli* survival was uncertain based on these results and will require further research in the future.

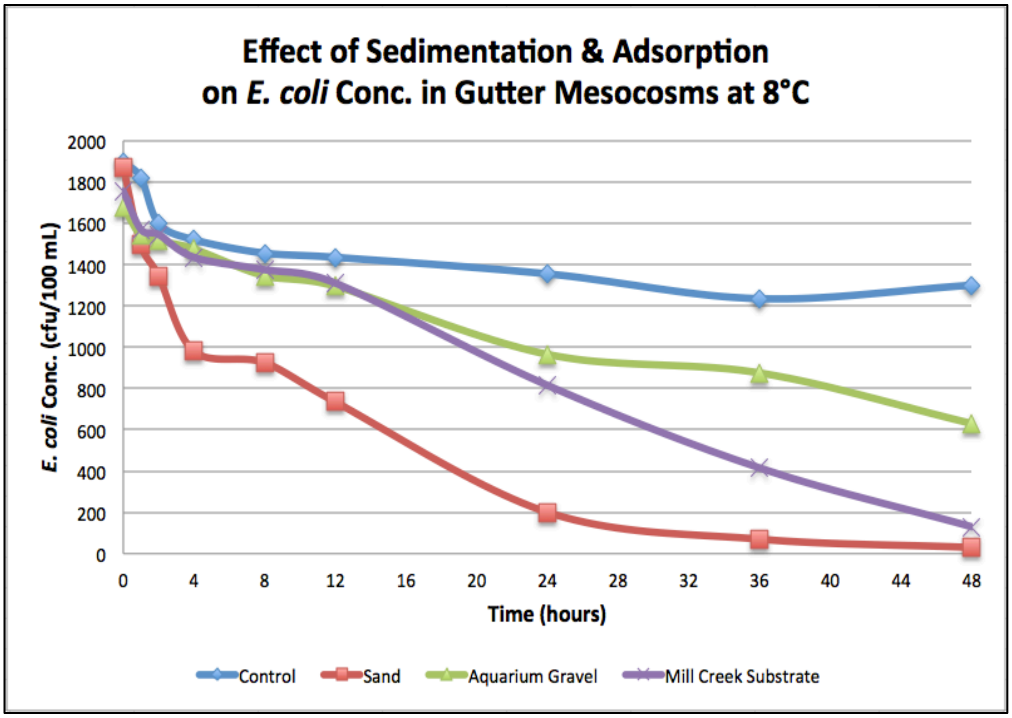


Figure 4.16. Effect of sedimentation and adsorption on E. coli concentration in gutter mesocosms at 8°C. Line graph displaying the change in E. coli concentration over 48 hours in gutter mesocosms with varying sediment bottoms: control/no sediment (blue), sand (red), aquarium gravel (green), and substrate from Mill Creek (purple). This experiment was conducted in a temperature controlled room that was set at 8°C.

4.4. LEVELS OF FECAL POLLUTION IN SURFACE WATERS

A one-way ANOVA test was used to analyze the difference in *E. coli* concentration between water samples collected from springs, ponds, and streams. There was statistically significant evidence to conclude (at $\alpha = 0.05$) that the average log *E. coli* concentration differed between springs, ponds, and streams in the sampled population (one-way ANOVA, Tukey pairwise comparisons, adjusted p-values were: $P = 0.004$ for ponds and springs; $P < 0.001$ for streams and springs; and $P < 0.001$ for streams and ponds). Streams had the highest average log *E. coli* concentration and springs had the lowest (Figure 4.17). The *E. coli* concentration of sampled streams and ponds was highly varied, including multiple upper-end outliers in ponds, which displayed the strong association between *E. coli* concentration and nonpoint source pollution in these surface water sources. *E. coli* concentrations were generally lower when sampled during baseflow conditions (i.e., no rainfall) while higher concentrations were recorded in samples taken during and after storm events.

Figure 4.18 is a box plot displaying the difference in log *E. coli* concentration of water samples collected from the rural watershed of Mill Creek and the urban watershed of Deible Branch in Rolla, Missouri. A greater than one log difference in median *E. coli* concentration was observed between the two sites. The average log *E. coli* concentration of the urban stream site was greater than the rural stream site (one-sided, two-sample independent t-test with unequal variance, p-value < 0.001). Urban watersheds are expected to possess additional nonpoint sources of fecal pollution and more direct pathways for stormwater runoff (e.g., storm drains and roads), which would explain the greater *E. coli* concentrations seen in the results.

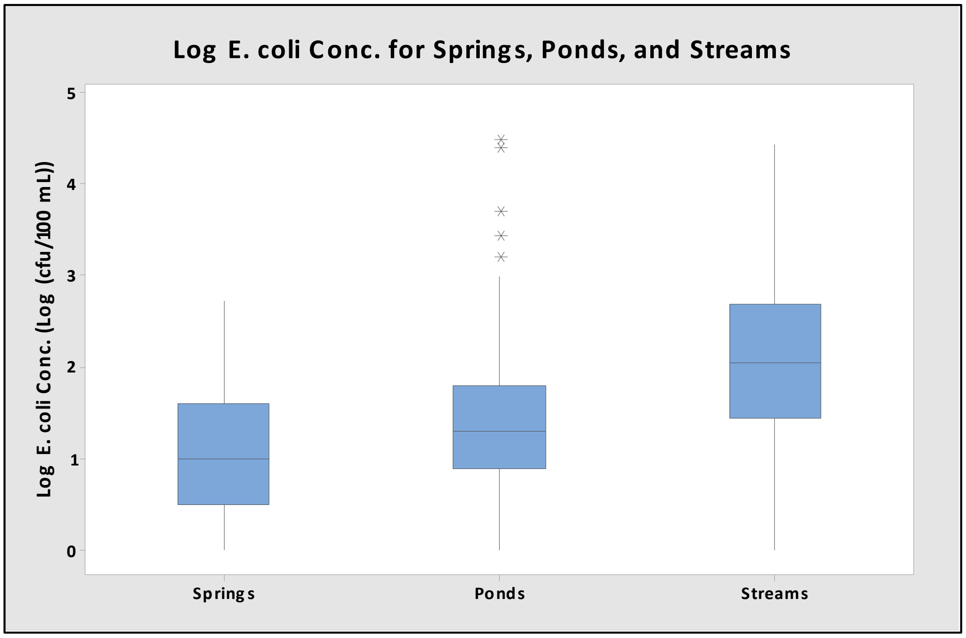


Figure 4.17. E. coli concentration of sampled springs, ponds, and streams. Box plot comparing log E. coli concentration ($\log_{10}$(cfu/100 mL)) of water samples collected from springs, ponds, and streams at Mill Creek and near the Missouri S&T campus in Rolla, Missouri.

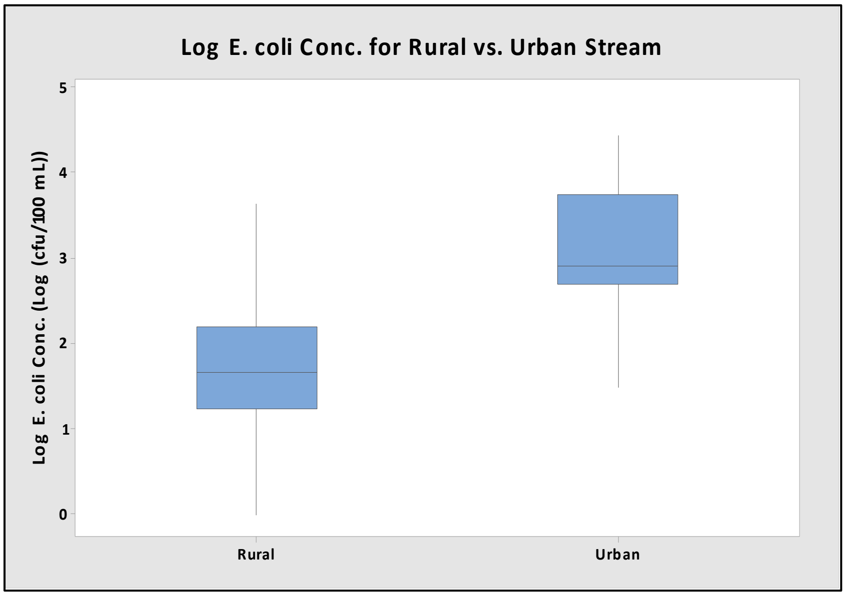


Figure 4.18. E. coli concentration of a rural and urban stream. Box plot comparing log E. coli concentration ($\log_{10}$(cfu/100 mL)) of water samples collected from the rural watershed of Mill Creek and the urban watershed of Deible Branch in Rolla, Missouri.

5. DISCUSSION

5.1. MILL CREEK WATERSHED HYDROLOGICAL SURVEY

In 2012, the EPA updated the recreational water quality criteria recommendations, which were designed to protect human health in waters designated for primary contact recreational use (e.g., swimming, bathing, surfing, water skiing, tubing, water play by children, and similar water contact activities where immersion and ingestion are likely). For culturable *E. coli*, the EPA recommended that a geometric mean of 126 cfu/100 mL and a statistical threshold value (this represents a value that should not be exceeded by more than 10 percent of samples taken) of 410 cfu/100 mL should not be surpassed during any 30-day interval (U.S. EPA, Recreational Water Quality Criteria: 2012 Report, 2012). Conforming to this recommendation would ensure that the public is protected from exposure to harmful levels of fecal pathogens and would maintain an estimated illness rate, due to gastrointestinal diseases, of less than 36 per 1,000.

During the Mill Creek watershed hydrological survey, the *E. coli* concentration, recorded at multiple sites, often surpassed the EPA's recreational water quality criteria recommendation. The site at Hardester Hollow was especially impacted by fecal contamination, most likely due to the close proximity to a cattle pasture. Hardester Hollow and other sites exceeded the EPA's recommendation for recreational use most often after strong storm events. These results indicated that areas of the Mill Creek watershed were most likely threatened by fecal pollution from nonpoint sources. Thus, a concern to public health may exist, specifically for the recreational use of the watershed following storm events.

Current methods for evaluating the threat of fecal pollution in water sources involves the growth and enumeration of fecal indicator bacteria. Culture-based methods are time-consuming, requiring at least 24 hours to perform the bacterial analysis in a laboratory. This 24-hour delay presents a problem for agencies that are responsible for protecting human health and safety at recreational water areas (i.e., beaches and national/state parks). Thus, a faster method is needed to determine if a water source is polluted by fecal bacteria and should be closed to the public. New techniques and methods are being developed to rapidly detect, identify, and quantify waterborne pathogens in water sources. These rapid methods include nucleic acid-based, immunology-based, and biosensor-based detection methods, which provide more accurate, sensitive, specific, and time-effective results than culture-based methods (Deshmukh et al. 2016).

Water quality sampling at sites in the Mill Creek watershed revealed the possibility of using other water quality characteristics to predict the concentration of *E. coli*. Lower specific conductivity and higher turbidity were determined to be significantly associated with higher concentrations of *E. coli* at sampled sites. Specific conductivity and turbidity levels are also known to be related to rainfall and storm runoff in streams, which supported the idea that nonpoint sources of fecal pollution were primarily impacting the sites at Mill Creek. Thus, this prediction may not apply to water sources with known point sources of fecal pollution. In addition, sites with consistently low turbidity and *E. coli* concentrations, such as springs, did not show a strong relationship, but these sites are of less concern due to the low values of *E. coli*. Specific conductivity and turbidity can be directly measured from the water source and a prediction of *E. coli* levels can be immediately made. This prediction will be less accurate than DNA methods (i.e., qPCR), but detecting fecal pathogen DNA requires more expensive equipment.

A significant relationship between *E. coli* concentration and stream discharge (or stream flow) was observed during the hydrological survey of Mill Creek. Stream discharge is known to increase because of surface runoff during storm events. Thus, two important inferences can be made: (1) *E. coli* enters streams with surface runoff (i.e., nonpoint sources of fecal pollution are present) and (2) *E. coli* concentrations, and the threat of fecal pollution, are highest in streams after storm events. A study conducted by Knierim et al. (2015) monitored the water quality of a spring and stream in northwestern Arkansas and observed a significant relationship between stream discharge and *E. coli* concentration. The results from the research on Mill Creek are similar to the results of Knierim et al. (2015) and validate that fecal pathogens from nonpoint sources are more abundant after storm events than during baseflow.

Davis et al. (2005) proposed that *E. coli* concentrations in springs increase rapidly, peak with the peak of the storm pulse, and decline rapidly. A modified storm hydrograph was constructed to relate stream flow to other water quality indicators measured at Mill Creek, specifically turbidity and *E. coli* concentration, and the results confirmed this proposal by Davis et al. (2005). The rise in *E. coli* concentration and turbidity corresponded with the first flush of surface runoff (i.e., during the rising limb of the storm hydrograph) at Mill Creek. All three indicators (stream discharge, turbidity, and *E. coli* concentration) peaked at the same time during the storm and strong positive correlations between the indicators were observed. *E. coli* concentration and turbidity levels also decreased before the stream discharge of Mill Creek did. Due to the strong relationships observed between stream discharge, turbidity, and *E. coli* concentration, the use of stream discharge and turbidity as predictors of *E. coli* concentration are possible.

The sediment disturbance samples collected at Mill Creek supported the findings of Stephenson and Rychert (1982). They suggested that stream sediments could improve the survival of *E. coli*, which would lead to the formation of sediment reservoirs of *E. coli* in the stream. The *E. coli* concentration of the sediment at Mill Creek was consistently greater than the water column (about a one log difference in median *E. coli* concentration was observed at the picnic area). Thus, it could be inferred that the *E. coli* concentrations of the sediment, determined from the sediment disturbance samples, were greater because of the existence of these sediment reservoirs of *E. coli* in Mill Creek.

The disturbance of sediment reservoirs, by storms or human recreational activity, is potentially an additional major source of *E. coli* and fecal pathogens in surface water sources. However, the amount of sediment disturbance required to release a dangerous level of *E. coli* (indicating a dangerous level of fecal pathogens) from this reservoir is still relatively unknown. In this study, a power drill and mixer were used to achieve repeatable experimental results. The *E. coli* concentrations of the sediment disturbance samples often surpassed the EPA's recreational water quality criteria recommendation for statistical threshold value (i.e., 410 cfu/100 mL). The power drill and mixer may disturb the sediment more than storm flows or human recreational activity and thus lead to overestimations of *E. coli* released from the sediment by these more natural causes.

Three main conclusions were established from the water quality sampling and fieldwork conducted during the hydrological survey of the Mill Creek watershed. First, *E. coli* concentrations, collected at sampling sites in the Mill Creek watershed, were always higher following storm events. In fact, samples collected after storm events revealed that the *E. coli* concentration can surpass the EPA's recommendation for safe use of recreational water sources (i.e., a potential threat to human health from fecal pollution can exist). Second, strong direct relationships were observed between stream discharge, turbidity, and *E. coli* concentration, which suggested that fecal pathogens enter the Mill Creek watershed with surface runoff after storm events. In other words, nonpoint sources of fecal pollution were determined to be primarily impacting the water quality at sampling sites in the Mill Creek watershed (no point sources of fecal pollution were identified). Due to these strong direct relationships, stream discharge and turbidity were also determined to be predictors of *E. coli* concentration (and thus could be used to assess the risk of contracting a waterborne disease) in water sources known to be only contaminated by nonpoint sources of fecal pollution, such as Mill Creek. Third, *E. coli* concentrations in the sediment were determined to be greater than in the overlying water, which indicated the presence of sediment reservoirs of *E. coli* in Mill Creek. The disturbance of these reservoirs, from recreation or subsequent storms, could elevate the levels of *E. coli* in streams and cause a health risk.

5.2. ENVIRONMENTAL FACTORS AFFECTING E. COLI SURVIVAL

The survival experiments, performed in gutter and flask mesocosms, have reinforced previous research results and led to the development of new ideas about the persistence of *E. coli* in streams, such as Mill Creek. The results of the sunlight mesocosm experiments were convincing, clearly verifying that sunlight does effectively lower the survival of *E. coli* in water. The temperature experiments were also successful, confirming that colder water temperatures improved *E. coli* survival. *E. coli* could survive for at least three months in water kept at 8°C. However, the results of the sediment mesocosm experiments were less conclusive and led to more questions than answers. Several potential problems, both known and unknown, existed in the experimental design, which brought into question the experiment’s ability to generate accurate and reproducible results. Regardless of the issues encountered, the sediment experiments still enabled the research team to uncover some clues about the dynamics of fecal pollution.

5.2.1. Solar Radiation. Gutierrez-Cacciabue et al. (2016) used microcosm bags, made from cellulose dialysis tubing, to determine the inactivation rate (or decay rate) of *E. coli* in light and dark conditions. In the study multiple microcosm bags were inoculated with *E. coli* and placed in two glass containers filled with river water to simulate a natural stream environment. One glass container was exposed to sunlight, but the other was covered by a black bag to avoid sunlight exposure. Gutierrez-Cacciabue et al. (2016) found that the *E. coli* exposed to sunlight suffered an immediate inactivation (i.e., 99.9% of *E. coli* was inactivated within 4 hours). The decay rate of the *E. coli* kept in the dark was –0.021 $\log_{10}$(cfu/100 mL) per hour. *E. coli* kept in the dark had a much slower rate of decay than *E. coli* exposed to the sun.

The results of the sunlight mesocosm experiments agreed with the results from the study performed by Gutierrez-Cacciabue et al. (2016). In the mesocosm exposed to direct sunlight, it only took 2 hours for 99.9% of *E. coli* to be inactivated, which resulted in a decay rate of –0.735 $\log_{10}$(cfu/100 mL) per hour. The *E. coli* in the mesocosm that was kept in the shade had a decay rate of –0.011 $\log_{10}$(cfu/100 mL) per hour, which was much slower than the decay rate of *E. coli* that was exposed to the sun. Both studies confirmed that sunlight has a strong negative effect on the survival of *E. coli*, displaying that a 3-log reduction in *E. coli* concentration can be achieved in less than six hours of direct sunlight exposure in mesocosm gutters or microcosm bags. This indicates that other factors must protect *E. coli* and fecal pathogens in the natural environment from immediate inactivation by solar radiation.

5.2.2. Water Temperature. Numerous studies, including lab and field experiments, have been performed on the effect of water temperature on *E. coli* survival. Decay rates for *E. coli* and other fecal coliforms were reported to range from –0.026 to –0.72 $\log_{10}$(cfu/100 mL) per day in rivers with annual average temperatures ranging from 8°C to 20°C (Sherer et al. 1992, Howell et al. 1996, Easton et al. 2005, Servais et al. 2007). The range in decay rates observed in the field is very large, most likely due to the difficulty associated with isolating a single variable in field studies (i.e., factors other than water temperature are likely involved). Nevertheless, the same conclusion can be drawn from such lab and field data. Colder water temperatures are related to slower decay rates and longer survival times for *E. coli*.

In the temperature experiments conducted in flask mesocosms, higher incubation temperatures (37°C) experienced largely decreased bacterial survival (average decay rate was –0.291 $\log_{10}$(cfu/100 mL) per day). On the opposite end of the spectrum, flasks treated under cold conditions (8°C) displayed prolonged survival (average decay rate was –0.020 $\log_{10}$(cfu/100 mL) per day) as *E. coli* could be detected for over 86 days. The 24°C treatment, as expected, fell in the middle of the other treatments (average decay rate was –0.071 $\log_{10}$(cfu/100 mL) per day). The average decay rates obtained from the temperature experiments in flask mesocosms were comparable to the decay rates found by researchers performing similar lab experiments and supported the same conclusions.

Garzio-Hadzick et al. (2010) designed an experiment utilizing flow-through chambers (used to simulate stream conditions) to examine the survival of *E. coli* at different stream temperatures. The following decay rates were presented in the results: at 4°C the decay rates ranged from –0.0169 to –0.0233 $\log_{10}$(cfu/100 mL) per day; at 14°C the decay rates ranged from –0.0754 to –0.138 $\log_{10}$(cfu/100 mL) per day; at 24°C the decay rates ranged from –0.110 to –0.346 $\log_{10}$(cfu/100 mL) per day. The average decay rates determined by the flask mesocosm experiments fit within the ranges of the decay rates for the corresponding temperature conditions (i.e., cold: 4-8°C; warm: 14-24°C, and hot: 24-37°C) used by Garzio-Hadzick et al. (2010), but roughly a ten-degree difference in temperature existed. Thus, Garzio-Hadzick et al. (2010) found slightly higher decay rates at cooler temperatures than were observed in the flask mesocosms. These slight differences in observed decay rates could be due to possible differences in experimental design. For example, the flow-through chambers had a constant circulation of water that could have influenced the survival of *E. coli* differently from the stagnant conditions maintained in the flask mesocosms. Regardless of these slight variations, both experiments showed that the coldest temperature condition had the smallest decay rate and the warmest temperature had the greatest decay rate.

An earlier study by Jameson et al. (2002) proposed that the die-off (i.e., decay rate) of *E. coli* and other fecal coliforms approximately doubles with every 10°C increase in water temperature. The average decay rates determined from the flask mesocosms at 8°C and 24°C seem to support this proposed pattern. The average decay rate at 8°C was –0.020 $\log_{10}$(cfu/100 mL) per day, which if the proposed pattern by Jameison et al. (2002) were true would predict that the decay rate at 28°C should be approximately –0.080 $\log_{10}$(cfu/100 mL) per day. At 24°C the average decay rate was determined to be –0.071 $\log_{10}$(cfu/100 mL) per day, which seems reasonable because this value is slightly less than the predicted decay rate at 28°C. However, the proposed pattern was not supported at 37°C, which had an average decay rate of –0.291 $\log_{10}$(cfu/100 mL) per day. Jameison et al. (2002) would have predicted a decay rate of about –0.160 $\log_{10}$(cfu/100 mL) per day at 38°C based on their proposed pattern, which is much less than the actual average decay rate measured at 37°C. This difference suggests that at warmer temperatures other factors, such as oxygen concentration and bacterial metabolism, may result in greater decay rates of *E. coli*.

An interesting trend in decay rate was observed in the flask mesocosms over the experimental period. At the beginning of the experiment, the initial rate of bacterial decay was high. During the first five days of the trial the *E. coli* concentration of all three temperature treatments decreased dramatically. The research team believes that this initial die-off represents the death phase of bacterial growth, in which death is occurring from the buildup of metabolic waste or lack of available nutrients (i.e., starvation). This period was followed by a gradual leveling out in decay rates throughout the remainder of the experiment.

A study by Flint (1987) suggests that bacteria, including *E. coli*, lower their metabolic activity and enter an inactive state at colder temperatures. At 37°C, *E. coli* never enters this inactive state, but instead keeps metabolizing and accumulates metabolic wastes. This results in faster cell death at this temperature and the observation of a high decay rate throughout the remainder of the experimental period. On the other hand, inactive *E. coli* cells do not metabolize and are able to persist for a longer time in the flasks. Thus, the observation of decay rates leveling off and becoming lower after the initial die-off is seen at lower temperatures. The persistence of *E. coli* kept in flasks at 8°C and 24°C, was assumed to be linked to the amount of inactive *E. coli* in the flask. Thus, some *E. coli* is assumed to be inactive at 24°C (i.e., persistence was longer than 37°C, but not as long as 8°C), and the most *E. coli* was assumed to become dormant and inactive at 8°C (i.e., longest observed persistence).

According to Jameison et al. (2002), *E. coli* could survive for over 100 days in a water-soil mixture kept at 10°C. The combination of colder stream temperatures and additional protection, from solar radiation and predation, provided by the soil could result in this long persistence time for *E. coli*. The research team observed a similar result, in which *E. coli* in a flask mesocosm kept in the fridge at 8°C was able to survive for 86 days. Thus, depending on the stream temperature, it may be possible for viable *E. coli* to survive in sediment reservoirs in streams for between two to three months (60-90 days). This is concerning because these sediment reservoirs of *E. coli* pose additional risks to the public.

5.2.3. Sedimentation and Adsorption. The research team designed a mesocosm experiment to study the processes of sedimentation and adsorption. Adsorption attaches *E. coli* to solid particles in streams, which leads to the removal of *E. coli* from the water column via sedimentation. The processes of sedimentation and adsorption are believed to be responsible for the formation of sediment stores at Mill Creek. Other studies have found that the surfaces of stream sediments, soil particles, and algae provide higher concentrations of organic matter and nutrients as well as additional protection from sunlight and predators, which can prolong the survival of *E. coli* in aquatic environments (Sherer et al. 1992, Byappanahalli et al. 2003). Thus, *E. coli* persistence in the stream and sediment at Mill Creek could also be linked to the processes of sedimentation and adsorption.

The presence of a sediment bottom in the experimental mesocosms did lead to a statistically significant increase in *E. coli* removal when compared to the control mesocosm, which suggests that the processes of sedimentation and adsorption do in fact play a major role in the formation of sediment reservoirs. Unfortunately, a statistically significant difference in removal rate was not observed based on particle size. It was also surprising that temperature had no meaningful effect on *E. coli* removal in the gutter mesocosms, which suggests that perhaps the gutter mesocosm experiment was not conducted for a long enough period or the enumeration method was not sensitive enough to detect differences (or trends) in *E. coli* in the water column of each mesocosm. Also, there were potentially too many sources of unknown error in the mesocosms to provide meaningful results on *E. coli* adsorption to different size particles. A revision to the gutter mesocosm experiment is required, or a totally different experimental design is needed, to further test the role of particle size on the removal rate of *E. coli* in streams.

From all mesocosm experiments performed on sedimentation and adsorption, the average final concentration only accounted for 72% of the initial concentration in the control mesocosms. Thus, 28% of *E. coli* concentration in the control mesocosm (and thus all mesocosms) was unaccounted for and indicates that factors other than the experimental variable (i.e., type of sediment bottom) may have influenced results. In addition, sediment disturbance samples, performed at the end of the 48-hour experiment, did not recover the initial *E. coli* concentration in the mesocosm. Thus, the answer to this important question is needed to better understand the results of these experiments: where did the *E. coli* go in the mesocosms? A few possibilities include: (1) the *E. coli* died, (2) the *E. coli* was filtered out by the sediment or aquarium pump, (3) the *E. coli* was stored in the sediment and remained tightly attached to particles even after disturbing the sediment, or (4) a combination of these possibilities. Determining the final fate of the *E. coli* would reveal if any factors other than the various sediments are influencing the removal of *E. coli* in the mesocosms, which would assist in accurately interpreting the results of these experiments.

The sediment mesocosm experiments were unable to support the idea that the attachment to sediments improves the survival and persistence of *E. coli* in streams. However, other studies, such as Gutierrez-Maccabee et al. (2016), have confirmed that the sediment is indeed protecting *E. coli* and improving its survival. Gutierrez-Cacciabue et al. (2016) utilized microcosm bags to investigate the rate of sunlight inactivation of culturable *E. coli*. In the study, microcosm bags were not only placed in light and dark treatment conditions, but some were filled with solid particles and others were not, so the effect of solid particles on sunlight inactivation could be evaluated. Microcosm bags filled with solid particles had lower inactivation rates, in both sunlight and dark conditions. This indicated that the sediment was not only protecting *E. coli* from harmful solar radiation, but also influences other factors (e.g., predation or nutrient availability) to improve *E. coli* survival in the dark.

Similar results were observed in a study by Anderson et al. (2005). They examined fecal coliforms from different sources (dog feces, untreated wastewater, and sediment from a chronically contaminated stream bank) to determine if various strains (or phylotypes) of fecal coliforms exhibit greater persistence than others in aquatic environments. The experiment was conducted in outdoor mesocosms, which were constructed to simulate the natural environment of a stream. Samples were taken from the water column (i.e. grab samples) and sediment (i.e., core samples and sonication). A membrane filtration method was then used to determine the fecal coliform concentration in the water column and sediment, which was tracked over a one-month period and the change over time was used to calculate a decay rate. The overall decay rate for fecal coliforms in the sediment (–0.02 $\log_{10}$(cfu/100 mL) per day) was much lower than the overall decay rate for fecal coliforms in the water column (–0.24 $\log_{10}$(cfu/100 mL) per day). Once again, this result indicated that the sediment is indeed protecting *E. coli* and improving its survival in the experiment, a result that is expected to also exist in streams and other aquatic systems.

The first experimental design for the sediment mesocosm experiments was like the sunlight mesocosm experiments. The sunlight mesocosm experiments were working well and obtaining expected results; therefore, a similar setup was considered a good starting point for the sediment mesocosm experiments. The first few experiments in the sediment mesocosms only led to unexpected and surprising results. Specifically, *E. coli* concentrations in the mesocosms were increasing during the 48-hour experimental period instead of declining. The research team was expecting to see a decline in *E. coli* over time due to the physical removal processes of sedimentation and adsorption.

These unexpected results were not considered a new discovery, but instead brought into question the experimental design of the sediment mesocosm experiments. The research team discovered (through experimentation) that using *E. coli* grown in Tryptic soy broth, to inoculate the water, stopped the observed increase in *E. coli* concentration during the experiment. Contaminated water from the field was collected and used as an inoculum in the sunlight mesocosm experiment; consequently, contaminated water from the field was also used in the first sediment mesocosm experiments. Grown *E. coli* was considered to be free-floating or unattached, but the *E. coli* in the water collected from the field was likely to be attached to solid particles. Thus, the research team believes that the unexpected increase in *E. coli* concentration was due to attached *E. coli* becoming unattached due to circulation by the aquarium pump in the gutter mesocosms (this trend was not observed in the control mesocosm of the sunlight mesocosm experiments because they were only conducted for two hours).

The growth of *E. coli* in the mesocosms was also considered and could not be disproven. However, fecal indicator bacteria are not expected to grow in aquatic environments because the growing conditions are inadequate. Some researchers in the field have disagreed with this expectation for *E. coli* and instead have argued that *E. coli* can grow in aquatic environments under certain conditions (Sherer et al. 1992, Moreira et al. 2012). This has brought into question the feasibility of *E. coli* as a fecal indicator bacteria. Nevertheless, there is also support in the literature for the research team's belief that *E. coli* was becoming unattached from particles during the 48-hour experiment and leading to the increased concentrations of *E. coli* detected. Some researchers have argued that current enumeration methods may underestimate the amount of fecal pollution in streams (Ervin et al. 2013). This is because these methods are unable to distinguish between bacteria that are attached and unattached to solid particles. In other words, what appears to be one *E. coli* colony on a modified m-TEC plate may be 10 colonies attached to a single solid particle.

5.3. IMPLICATIONS AND FUTURE RESEARCH

From this study, a better understanding of the sources, dynamics, and persistence of *E. coli* in Mill Creek was achieved. The research team believed that *E. coli* was primarily entering the stream, some attached to solid particles, via surface runoff during storm events (i.e., originated from nonpoint sources of fecal pollution). This resulted in the observation of strong relationships between stream discharge, turbidity, and *E. coli* concentration. After entering the stream, it was possible for *E. coli* to become stored in the sediment bottom. This storage resulted in the formation of sediment reservoirs of *E. coli*, which were observed by the research team in Mill Creek. *E. coli* can persist in the sediment reservoirs of streams for 60 to 90 days, depending on the stream's temperature (colder temperatures have been linked to longer survival times). These sediment reservoirs also improve the survival of *E. coli* in streams by protecting them from predators and harmful UV radiation. The existence of sediment reservoirs in aquatic environments is a concern because elevated levels of *E. coli* in the water column have been shown to result from disturbing these sediment reservoirs. Thus, sediment reservoirs can prolong the potential for waterborne disease outbreak.

It was determined in this study that Mill Creek is often contaminated by fecal pollution from nonpoint sources and would be considered impaired by EPA standards (i.e., not safe for its designated uses). In fact, the *E. coli* concentration of water samples collected from sites in Mill Creek, especially after storm events, often surpassed the EPA's recreational water quality criteria recommendation. Results of this study also suggested that fecal pollution can impact rural watersheds to a similar degree as urban watersheds. Thus, more routine monitoring of water quality is needed in rural watersheds, such as Mill Creek. Also, more public awareness for the possible risks associated with fecal contamination of water resources is needed worldwide to reduce the number of deaths per year due to waterborne diseases. The results of this study may help to establish better guidelines for safe use of water resources in rural areas, which are known to be impaired by nonpoint sources of fecal pollution.

Future research should continue to build upon the foundation of information gathered by this study on the sources, dynamics, and survival of *E.*

coli in Mill Creek. The major sources of fecal pollution in Mill Creek were only assumed based on weekly *E. coli* concentrations at various site locations and physical observations of the watershed. Microbial source tracking needs to be performed to check these assumptions and precisely determine the sources of fecal pollution in Mill Creek. It is possible that some point sources of fecal pollution from leaking septic tanks are present in Mill Creek, but were never detected by the research team during this study.

A faster and easier method of assessing the risk of fecal pollution in streams would be detecting indicators of *E. coli*. Turbidity is one such indicator that is known to be related to *E. coli* concentration and can be quickly measured in the field at the stream site. The ability of specific conductivity to serve as another potential indicator of *E. coli* concentration was analyzed in this study. The measure of specific conductivity in a stream indicates the amount of stormflow, which can be used to infer the *E. coli* concentration and thus the risk of fecal pollution. For example, a lower specific conductivity reading indicates more rain and storm runoff, which results in more *E. coli* (stormflows are known to be directly related to higher *E. coli* concentrations). The sample size of data used in the analysis was small, which could have influenced the results. Additional data needs to be collected on specific conductivity and its relationship to *E. coli* concentration to further assess the use of specific conductivity as an accurate indicator of fecal pollution risk in streams.

Understanding the movement and storage (i.e., dynamics) of *E. coli* in water resources is key to establishing guidelines for minimizing human contact with fecal pathogens and thus lowering the threat of waterborne disease. Little is known about how far downstream, from the site of origin, *E. coli* can travel in the water column before becoming stored in the sediment. An experiment designed to track *E. coli* as it travels downstream from the cow pasture near Hardester Hollow could provide an answer to this important question with implications for protecting downstream areas from upstream sources of fecal pollution. It would also be beneficial to know, what percentage of *E. coli* that enters a stream can become stored in the sediment and possibly be resuspended later? Further research on the amount of force required to resuspend a dangerously high level of *E. coli* from the sediment would assist in assessing the risk of these potentially dangerous sediment disturbances being caused by human activity at recreational areas (i.e., beaches).

The effect of other environmental factors, including predation, vegetation, and nutrient availability, on *E. coli* survival still need to be investigated. Also, the effect of sunlight and water temperature on *E. coli* survival could be tested in the field to confirm that the results from the mesocosms support the findings in natural systems.

Water is an essential natural resource for sustaining life. Thus, providing the growing human population with an adequate supply of water while preserving high water quality is a major goal for maintaining a sustainable future. Water resources in the U.S., and presumably the world, are primarily impacted by fecal pollutants. One way to reduce this impact is to study the sources, dynamics, and persistence of *E. coli* (a fecal indicator organism) in aquatic environments.

Understanding the major pathway that fecal contaminants use to enter water sources will allow for the development of better remediation options, such as trapping sediments before they can reach streams or lakes. Also, finding and establishing faster indicators of fecal pollution risk and becoming more aware of the environmental factors involved in prolonging the persistence of fecal pathogens in water sources will assist in planning for and preventing future outbreaks of waterborne diseases. The goal of providing the world with clean water can be achieved by placing more emphasis on the identification and mitigation of water pollutants from both point and nonpoint sources.

APPENDIX A.

SAMPLING SITES AND FIELD DATA

Site 1: Yelton Spring (Mill Creek Watershed)

Date	Storm Event (> 0.5" of rain)	Water T. (°C)	DO (mg/L)	Sp. Cond. (µS/cm at 25°C)	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
9/3/15		-	-	-	1.8	20
9/29/15		-	-	-	1.7	2
10/24/15		-	-	-	0.8	96
11/6/15		-	-	-	0.7	50
11/18/15	✔	-	-	-	15.9	300
1/29/16		-	-	-	2.6	12
2/3/16		-	-	-	2.3	2
2/10/16		-	-	-	1.9	5
2/18/16		-	-	-	1.7	5
3/2/16		-	-	-	1.6	3
3/10/16	✔	-	-	-	1.3	4
3/14/16		-	-	-	2.23	63
3/25/16		-	-	-	2.02	12
4/6/16		-	-	-	2.63	42
4/11/16	✔	-	-	-	2.54	22
4/20/16		13.6	7.4	265	2.88	18
4/27/16		13.8	8.3	275	1.44	0
5/2/16	✔	13.4	8.9	283	1.44	4
5/10/16		13.9	8.5	289	1.83	4
5/17/16	✔	13.5	7.5	295	1.12	36
5/24/16	✔	13.7	7.7	262	3.98	78
6/2/16		-	-	-	8.22	135
6/22/16		-	-	-	2.36	18
6/28/16		14.9	6.2	-	2.26	8
7/4/16	✔	-	-	-	2.37	6
7/18/16		-	-	-	1.6	11
7/25/16		-	-	-	1.95	296
8/1/16	✔	-	-	-	1.54	164
8/16/16		-	-	-	1.29	441
8/23/16		18.4	7	378	1.2	160
9/9/16	✔	16.4	7.7	433	1.65	540
9/16/16	✔	14.2	7.4	298	2.99	140
9/22/16		-	-	-	-	55
9/29/16		13.8	7.2	302	1.42	100
10/3/16		13.9	7	312	2.11	48
10/14/16		13.7	5.8	296	1.72	16
10/21/16	✔	13.7	5.9	293	1.93	48
10/27/16		13.9	6.5	295	0.98	20
11/4/16		13.9	6.7	321	1.5	54
11/15/16		13.6	6.6	308	0.94	18
11/29/16		-	-	-	1.06	18
12/6/16		13.5	5.1	332	0.78	0
12/12/16		13.4	4.6	343	1.22	0
1/17/17	✔	-	-	-	-	-

Site 2: Wilkins Spring (Mill Creek Watershed)

Date	Storm Event (> 0.5" of rain)	Water T. (°C)	DO (mg/L)	Sp. Cond. (µS/cm at 25°C)	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
9/3/15		-	-	-	1.4	5
9/29/15		-	-	-	1	4
10/24/15		-	-	-	0.7	0
11/6/15		-	-	-	0.9	42
11/18/15	✔	-	-	-	-	-
1/29/16		-	-	-	2.8	0
2/3/16		-	-	-	2.3	4
2/10/16		-	-	-	1.9	4
2/18/16		-	-	-	2	0
3/2/16		-	-	-	1.5	1
3/10/16	✔	-	-	-	1.5	5
3/14/16		-	-	-	1.7	3
3/25/16		-	-	-	2.3	5
4/6/16		-	-	-	2.43	43
4/11/16	✔	-	-	-	2.76	8
4/20/16		13.1	7.6	276	2.8	10
4/27/16		13.6	7.75	284	2.09	4
5/2/16	✔	13	8.5	285	2.61	12
5/10/16		13.7	8.6	295	1.64	4
5/17/16	✔	13.1	8.5	311	1.29	2
5/24/16	✔	13.6	8.5	299	2.45	4
6/2/16		-	-	-	5.16	80
6/22/16		-	-	-	3.51	29
6/28/16		14.4	5.75	-	2.32	13
7/4/16	✔	-	-	-	-	-
7/18/16		-	-	-	1.94	4
7/25/16		-	-	-	-	-
8/1/16	✔	-	-	-	-	-
8/16/16		-	-	-	1.77	6
8/23/16		-	-	-	-	-
9/9/16	✔	-	-	-	1.81	0
9/16/16	✔	13.8	7.1	314	1.26	80
9/22/16		-	-	-	-	10
9/29/16		14	7.8	310	1.65	56
10/3/16		14.6	7.2	297	0.75	8
10/14/16		13.8	5.63	309	1.2	10
10/21/16	✔	13.8	5.1	309	1.18	0
10/27/16		14.1	6.9	311	1.03	16
11/4/16		13.8	6.4	310	1.5	38
11/15/16		13.8	6.7	310	1.4	12
11/29/16		-	-	-	0.85	2
12/6/16		-	-	-	-	-
12/12/16		13.7	6.1	314	0.76	4
1/17/17	✔	-	-	-	-	-

Site 3: Dewitt Pond Outflow (Mill Creek Watershed)

Date	Storm Event (> 0.5" of rain)	Water T. (°C)	DO (mg/L)	Sp. Cond. (µS/cm at 25°C)	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
9/3/15		-	-	-	1.6	7
9/29/15		-	-	-	2.6	9
10/24/15		-	-	-	2.9	10
11/6/15		-	-	-	1.2	104
11/18/15	✔	-	-	-	-	-
1/29/16		-	-	-	3.8	0
2/3/16		-	-	-	2.4	8
2/10/16		-	-	-	1.8	4
2/18/16		-	-	-	2.1	2
3/2/16		-	-	-	2	3
3/10/16	✔	-	-	-	1.7	29
3/14/16		-	-	-	1.61	85
3/25/16		-	-	-	2.41	12
4/6/16		-	-	-	7.31	27
4/11/16	✔	-	-	-	3.28	14
4/20/16		15.2	2.8	283	3.8	0
4/27/16		20.6	6	297	5.43	0
5/2/16	✔	14.5	3.1	292	2.41	16
5/10/16		18	3.4	305	5.45	8
5/17/16	✔	13	3.7	284	1.49	5
5/24/16	✔	17.6	6.1	262	7.2	20
6/2/16		-	-	-	4.91	40
6/22/16		-	-	-	3.4	16
6/28/16		15.8	8.7	-	1.73	12
7/4/16	✔	-	-	-	-	-
7/18/16		-	-	-	2.45	10
7/25/16		-	-	-	-	-
8/1/16	✔	-	-	-	-	-
8/16/16		-	-	-	1.34	0
8/23/16		14.7	8.7	312	1.18	4
9/9/16	✔	-	-	-	1.03	10
9/16/16	✔	15	8.8	315	1.13	10
9/22/16		-	-	-	-	10
9/29/16		14.2	9.1	311	1.69	32
10/3/16		15.1	9.1	305	1.78	24
10/14/16		14.3	6.75	306	1.24	14
10/21/16	✔	14	6.5	308	1.37	20
10/27/16		15	6.3	311	1.56	52
11/4/16		14.9	6.7	316	1.6	36
11/15/16		13.8	7.5	312	1.77	10
11/29/16		-	-	-	1.44	2
12/6/16		-	-	-	-	-
12/12/16		11.4	6.2	318	0.98	0
1/17/17	✔	-	-	-	-	-

Site 4: Yelton Branch (Mill Creek Watershed)

Date	Storm Event (> 0.5" of rain)	Water T. (°C)	DO (mg/L)	Sp. Cond. (µS/cm at 25°C)	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
9/3/15		-	-	-	0.9	75
9/29/15		-	-	-	1.7	428
10/24/15		-	-	-	1.1	256
11/6/15		-	-	-	1.7	450
11/18/15	✔	-	-	-	14.9	290
1/29/16		-	-	-	3.2	16
2/3/16		-	-	-	1.9	6
2/10/16		-	-	-	1.6	8
2/18/16		-	-	-	1.7	16
3/2/16		-	-	-	1.2	3
3/10/16	✔	-	-	-	2.3	19
3/14/16		-	-	-	2.04	76
3/25/16		-	-	-	1.86	19
4/6/16		-	-	-	3.08	51
4/11/16	✔	-	-	-	5.83	80
4/20/16		14.2	9.2	266	2.7	20
4/27/16		16	14.5	273	1.06	10
5/2/16	✔	13.6	11.2	281	1.07	32
5/10/16		16.5	12.5	284	0.93	64
5/17/16	✔	12.9	9.5	292	1.21	90
5/24/16	✔	14.3	9.8	268	3.48	100
6/2/16		-	-	-	8.1	100
6/22/16		-	-	-	2.28	58
6/28/16		19.1	10.8	255	1.77	122
7/4/16	✔	-	-	-	2.07	50
7/18/16		-	-	-	1.26	36
7/25/16		-	-	-	2.07	796
8/1/16	✔	-	-	-	1.02	230
8/16/16		-	-	-	2.29	60
8/23/16		20.5	8.2	306	2.54	132
9/9/16	✔	-	-	-	2.16	140
9/16/16	✔	21.3	6.6	291	2.39	1,270
9/22/16		-	-	-	-	155
9/29/16		18.5	8.4	305	0.84	40
10/3/16		18.1	8.2	317	2.36	60
10/14/16		16.5	5.8	327	1.24	32
10/21/16	✔	15.3	6.1	294	1.83	292
10/27/16		18.2	6.2	314	0.94	82
11/4/16		17.7	5.4	324	1.29	6
11/15/16		13.5	7.4	331	1.32	4
11/29/16		-	-	-	1.23	18
12/6/16		6.6	7.4	325	0.92	50
12/12/16		4.9	11.4	333	0.59	6
1/17/17	✔	-	-	-	0.76	4

Site 5: Elm Spring (Mill Creek Watershed)

Date	Storm Event (> 0.5" of rain)	Water T. (°C)	DO (mg/L)	Sp. Cond. (µS/cm at 25°C)	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
9/3/15		-	-	-	0.5	12
9/29/15		-	-	-	1.2	8
10/24/15		-	-	-	0.4	0
11/6/15		-	-	-	0.8	6
11/18/15	✔	-	-	-	19	250
1/29/16		-	-	-	1.8	0
2/3/16		-	-	-	1	1
2/10/16		-	-	-	0.9	0
2/18/16		-	-	-	2.1	1
3/2/16		-	-	-	1.2	2
3/10/16	✔	-	-	-	1.5	0
3/14/16		-	-	-	2.52	7
3/25/16		-	-	-	1.47	0
4/6/16		-	-	-	2.1	3
4/11/16	✔	-	-	-	12.9	63
4/20/16		11.3	7.4	320	2.65	6
4/27/16		11.9	7.5	371	0.91	4
5/2/16	✔	11.6	8.1	298	5.87	32
5/10/16		12.6	6.9	369	1.11	4
5/17/16	✔	12.2	7.3	406	0.92	2
5/24/16	✔	12.4	7	392	1.35	30
6/2/16		-	-	-	2.91	20
6/22/16		-	-	-	1.05	2
6/28/16		14.2	4.9	419	0.83	0
7/4/16	✔	-	-	-	2.68	70
7/18/16		-	-	-	1.46	31
7/25/16		-	-	-	0.9	14
8/1/16	✔	-	-	-	0.63	8
8/16/16		-	-	-	0.96	25
8/23/16		21.5	8.8	345	2.64	12
9/9/16	✔	13.1	5.8	266	0.51	10
9/16/16	✔	13.1	6.4	344	1.19	70
9/22/16		-	-	-	-	45
9/29/16		13.1	5.5	420	0.75	12
10/3/16		14.2	4.7	418	0.71	12
10/14/16		13.3	5.6	288	0.6	2
10/21/16	✔	13.1	6.2	378	1.94	44
10/27/16		13.4	6.2	408	1.44	96
11/4/16		13.2	6.7	426	2.9	112
11/15/16		13.1	6.7	436	3.23	6
11/29/16		-	-	-	0.65	0
12/6/16		12.8	4	431	0.53	2
12/12/16		12.9	5.5	436	0.71	2
1/17/17	✔	-	-	-	4.89	8

Site 6: Hardester Hollow at Cow Pasture (Mill Creek Watershed)

Date	Storm Event (> 0.5" of rain)	Water T. (°C)	DO (mg/L)	Sp. Cond. (µS/cm at 25°C)	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
9/3/15		-	-	-	0.6	157
9/29/15		-	-	-	0.9	4
10/24/15		-	-	-	0.75	6
11/6/15		-	-	-	0.9	358
11/18/15	✔	-	-	-	17.2	1,270
1/29/16		-	-	-	1.6	40
2/3/16		-	-	-	0.7	118
2/10/16		-	-	-	0.75	141
2/18/16		-	-	-	0.8	46
3/2/16		-	-	-	0.72	54
3/10/16	✔	-	-	-	1.6	186
3/14/16		-	-	-	1.59	40
3/25/16		-	-	-	2.14	98
4/6/16		-	-	-	1.68	191
4/11/16	✔	-	-	-	29.4	1,704
4/20/16		14.3	10.3	291	2.3	502
4/27/16		16.8	10.6	310	1.29	742
5/2/16	✔	13.4	12	256	4.15	170
5/10/16		16.5	10.5	312	1.42	591
5/17/16	✔	12.9	9.1	328	1.97	365
5/24/16	✔	15.9	8.6	307	8.87	5,360
6/2/16		-	-	-	3.77	405
6/22/16		-	-	-	1.7	190
6/28/16		21.1	7.6	367	0.79	282
7/4/16	✔	-	-	-	2.83	345
7/18/16		-	-	-	1.42	668
7/25/16		-	-	-	1.65	808
8/1/16	✔	-	-	-	0.86	460
8/16/16		-	-	-	2.63	455
8/23/16		23.3	8.5	347	1.29	1,264
9/9/16	✔	22	3.1	393	1.03	20
9/16/16	✔	20.9	2.3	250	5.75	10,160
9/22/16		-	-	-	-	120
9/29/16		18.9	5.1	389	1.31	448
10/3/16		17.4	4.9	370	1.29	24
10/14/16		-	-	-	-	-
10/21/16	✔	12.9	7.9	225	0.64	360
10/27/16		17.4	4.5	382	0.59	30
11/4/16		17.5	5.7	389	0.76	8
11/15/16		14.4	4.7	386	1.18	16
11/29/16		-	-	-	1.23	6
12/6/16		-	-	-	-	-
12/12/16		-	-	-	-	-
1/17/17	✔	-	-	-	0.56	160
3/25/17	✔	13.2	8.9	324	3.85	1,695
4/5/17	✔	12.8	8.6	218	19.2	4,540
4/22/17	✔	-	-	-	16	2,780
4/29/17	✔	14	9.1	106	27.6	10,500

Site 7: Pond with Spring at Field Station (Mill Creek Watershed)

Date	Storm Event (> 0.5" of rain)	Water T. (°C)	DO (mg/L)	Sp. Cond. (µS/cm at 25°C)	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
9/3/15		-	-	-	2.4	53
9/29/15		-	-	-	4.1	120
10/24/15		-	-	-	1.4	0
11/6/15		-	-	-	1.3	24
11/18/15	✓	-	-	-	21	170
1/29/16		-	-	-	2	12
2/3/16		-	-	-	1.1	4
2/10/16		-	-	-	1.4	1
2/18/16		-	-	-	0.8	0
3/2/16		-	-	-	1.5	0
3/10/16	✓	-	-	-	1.7	25
3/14/16		-	-	-	2.72	7
3/25/16		-	-	-	1.59	1
4/6/16		-	-	-	2.45	8
4/11/16	✓	-	-	-	19.5	264
4/20/16		13.8	9.8	300	3.2	976
4/27/16		16.7	9.7	361	7.66	408
5/2/16	✓	12.8	10	272	5.46	70
5/10/16		14.4	9.6	357	2.55	354
5/17/16	✓	12.9	9.5	392	2.09	360
5/24/16	✓	14.6	10	385	6.03	30,000
6/2/16		-	-	-	3.37	80
6/22/16		-	-	-	4.62	64
6/28/16		18.1	6.8	444	4.55	44
7/4/16	✓	-	-	-	4.81	590
7/18/16		-	-	-	3.31	98
7/25/16		-	-	-	2.93	600
8/1/16	✓	-	-	-	2.47	2,710
8/16/16		-	-	-	2.44	50
8/23/16		19	10.4	408	1.52	20
9/9/16	✓	-	-	-	2.22	360
9/16/16	✓	17.3	8.8	447	3.44	50
9/22/16		-	-	-	-	20
9/29/16		-	-	-	-	-
10/3/16		18.5	6.9	402	1.57	14
10/14/16		15.2	6.8	438	2.1	14
10/21/16	✓	14.9	6.8	435	1.65	12
10/27/16		15.4	7.7	422	2.25	20
11/4/16		15.2	7.2	416	1.76	14
11/15/16		12.2	8.1	429	1.34	8
11/29/16		-	-	-	1.31	2
12/6/16		8.7	7	435	2.1	4
12/12/16		7.5	10.5	439	0.76	2
1/17/17	✓	-	-	-	0.62	4

Site 8: Big Pond at Field Station (Mill Creek Watershed)

Date	Storm Event (> 0.5" of rain)	Water T. (°C)	DO (mg/L)	Sp. Cond. (µS/cm at 25°C)	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
9/3/15		-	-	-	1.2	123
9/29/15		-	-	-	1.6	44
10/24/15		-	-	-	3.2	8
11/6/15		-	-	-	1.2	204
11/18/15	✔	-	-	-	8.7	340
1/29/16		-	-	-	3.6	0
2/3/16		-	-	-	2	2
2/10/16		-	-	-	2.6	0
2/18/16		-	-	-	1.7	0
3/2/16		-	-	-	1.4	0
3/10/16	✔	-	-	-	3.8	33
3/14/16		-	-	-	2.9	18
3/25/16		-	-	-	1.57	4
4/6/16		-	-	-	2.17	4
4/11/16	✔	-	-	-	6.42	34
4/20/16		18.4	6.1	277	2.4	10
4/27/16		22.1	3.75	305	4.91	30
5/2/16	✔	18.3	4.5	313	14.4	64
5/10/16		22.3	5.2	330	3.01	54
5/17/16	✔	16.9	5.8	330	3.15	1,620
5/24/16	✔	22	5.8	333	3.55	5,000
6/2/16		-	-	-	3.41	290
6/22/16		-	-	-	5.91	42
6/28/16		32	5.9	357	9.1	142
7/4/16	✔	-	-	-	4.57	110
7/18/16		-	-	-	10.7	6
7/25/16		-	-	-	-	-
8/1/16	✔	-	-	-	-	-
8/16/16		-	-	-	2.56	40
8/23/16		26.5	8.7	262	3.02	16
9/9/16	✔	-	-	-	3.43	180
9/16/16	✔	25.9	8.4	241	1.64	180
9/22/16		-	-	-	-	0
9/29/16		-	-	-	-	-
10/3/16		20.1	5.9	270	1.81	14
10/14/16		18.5	6.5	263	1.96	30
10/21/16	✔	18.8	5.7	269	2.42	16
10/27/16		20.4	6.4	279	2.28	118
11/4/16		19.9	5.7	289	1.94	26
11/15/16		14	7.9	304	0.77	20
11/29/16		-	-	-	1.42	10
12/6/16		5.8	8.7	320	1.16	2
12/12/16		4.3	11	331	1.03	18
1/17/17	✔	-	-	-	-	-

Site 9: Stream at Field Station (Mill Creek Watershed)

Date	Storm Event (> 0.5" of rain)	Water T. (°C)	DO (mg/L)	Sp. Cond. (µS/cm at 25°C)	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
9/3/15		-	-	-	0.7	48
9/29/15		-	-	-	1.9	88
10/24/15		-	-	-	0.9	64
11/6/15		-	-	-	0.7	64
11/18/15	✔	-	-	-	16.25	620
1/29/16		-	-	-	2.3	12
2/3/16		-	-	-	1.55	10
2/10/16		-	-	-	1.3	10
2/18/16		-	-	-	1.45	15
3/2/16		-	-	-	2.1	11
3/10/16	✔	-	-	-	1.45	50
3/14/16		-	-	-	1.97	32
3/25/16		-	-	-	1.62	6
4/6/16		-	-	-	1.75	35
4/11/16	✔	-	-	-	14	712
4/20/16		14.5	10	268	4.11	71
4/27/16		16.8	11.5	281	1.65	42
5/2/16	✔	13.7	11.3	281	2.41	58
5/10/16		17.5	10.6	290	1.79	45
5/17/16	✔	13	9.6	300	1.6	163
5/24/16	✔	15.5	9.2	274	5.49	670
6/2/16		-	-	-	6.32	105
6/22/16		-	-	-	1.6	65
6/28/16		20.4	7.8	282	3.52	188
7/4/16	✔	-	-	-	2.74	115
7/18/16		-	-	-	1.21	44
7/25/16		-	-	-	1.56	488
8/1/16	✔	-	-	-	1.24	110
8/16/16		-	-	-	2.67	70
8/23/16		17.4	9.4	317	1.09	160
9/9/16	✔	-	-	-	0.64	60
9/16/16	✔	17.3	8.2	312	1.23	990
9/22/16		-	-	-	-	70
9/29/16		-	-	-	-	-
10/3/16		17.4	8.2	340	1.92	18
10/14/16		15.4	6.7	321	1.14	130
10/21/16	✔	15.2	6.7	321	0.83	48
10/27/16		16.7	5.8	324	0.79	28
11/4/16		16.3	6.2	324	1.17	32
11/15/16		14.1	7.5	323	0.66	16
11/29/16		-	-	-	1.05	4
12/6/16		9.6	7.3	320	0.55	20
12/12/16		9.3	9.2	326	1.02	24
1/17/17	✔	-	-	-	0.9	36

Site 10: Kaintuck Hollow's Natural Bridge (Mill Creek Watershed)

Date	Storm Event (> 0.5" of rain)	Water T. (°C)	DO (mg/L)	Sp. Cond. (µS/cm at 25°C)	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
9/3/15		-	-	-	-	-
9/29/15		-	-	-	0.7	55
10/24/15		-	-	-	2	28
11/6/15		-	-	-	0.6	68
11/18/15	✔	-	-	-	11.1	185
1/29/16		-	-	-	0.75	2
2/3/16		-	-	-	0.7	1
2/10/16		-	-	-	0.8	9
2/18/16		-	-	-	1.8	5
3/2/16		-	-	-	0.65	0
3/10/16	✔	-	-	-	1.65	60
3/14/16		-	-	-	1.19	9
3/25/16		-	-	-	0.94	7
4/6/16		-	-	-	1.33	17
4/11/16	✔	-	-	-	18.7	1,120
4/20/16		13.5	8.1	262	1.3	33
4/27/16		17.4	8.5	283	1	25
5/2/16	✔	13.2	10.6	236	1.18	40
5/10/16		14.8	10.4	289	0.48	138
5/17/16	✔	12.3	9.4	306	1.63	212
5/24/16	✔	15	8.9	277	4.3	860
6/2/16		-	-	-	0.9	70
6/22/16		-	-	-	0.49	66
6/28/16		22.3	7.1	337	0.5	91
7/4/16	✔	-	-	-	7.01	205
7/18/16		-	-	-	0.55	70
7/25/16		-	-	-	0.94	560
8/1/16	✔	-	-	-	0.74	170
8/16/16		-	-	-	0.99	410
8/23/16		18.6	9.3	319	0.73	776
9/9/16	✔	-	-	-	0.89	110
9/16/16	✔	19.6	6.8	288	3.08	3,380
9/22/16		-	-	-	-	55
9/29/16		17.7	8.1	323	0.68	200
10/3/16		18.2	7.2	330	0.79	32
10/14/16		16.1	6.9	333	0.79	28
10/21/16	✔	15.5	7.4	288	2.43	168
10/27/16		17.7	5.7	328	0.5	6
11/4/16		16.2	5.6	329	0.47	36
11/15/16		13.2	7.6	331	0.53	16
11/29/16		-	-	-	0.55	16
12/6/16		7.5	7.3	331	0.6	6
12/12/16		7.4	9.5	330	0.64	8
1/17/17	✔	-	-	-	3.55	56
4/5/17	✔	12.1	9.2	120	20.4	660
4/22/17	✔	-	-	-	16.4	850
4/29/17	✔	13.9	9.0	81	26.2	3,030

Site 11: Kaintuck Hollow Outflow (Mill Creek Watershed)

Date	Storm Event (> 0.5" of rain)	Water T. (°C)	DO (mg/L)	Sp. Cond. (µS/cm at 25°C)	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
9/3/15		-	-	-	0.4	43
9/29/15		-	-	-	1	4
10/24/15		-	-	-	0.4	6
11/6/15		-	-	-	0.5	36
11/18/15	✔	-	-	-	12.8	290
1/29/16		-	-	-	0.7	4
2/3/16		-	-	-	1.2	16
2/10/16		-	-	-	0.9	2
2/18/16		-	-	-	0.9	12
3/2/16		-	-	-	0.4	0
3/10/16	✔	-	-	-	1.2	57
3/14/16		-	-	-	1.02	31
3/25/16		-	-	-	1.04	13
4/6/16		-	-	-	1.13	33
4/11/16	✔	-	-	-	18.9	752
4/20/16		14	9.2	292	0.92	100
4/27/16		16.7	10	321	0.52	12
5/2/16	✔	13.5	10.3	286	0.83	28
5/10/16		15.5	8.2	329	0.58	26
5/17/16	✔	12.9	9.6	334	0.83	170
5/24/16	✔	15.9	8.6	289	5.74	2,040
6/2/16		-	-	-	0.92	70
6/22/16		-	-	-	0.49	70
6/28/16		19.9	7.2	390	0.9	52
7/4/16	✔	-	-	-	2.48	145
7/18/16		-	-	-	0.82	0
7/25/16		-	-	-	0.67	94
8/1/16	✔	-	-	-	0.83	60
8/16/16		-	-	-	0.68	70
8/23/16		19.2	7.8	378	1.12	164
9/9/16	✔	-	-	-	0.7	30
9/16/16	✔	19.1	7	372	0.95	570
9/22/16		-	-	-	-	5
9/29/16		18.2	7.2	373	1.16	20
10/3/16		18.6	7.5	313	0.8	16
10/14/16		16.6	6.2	387	0.69	38
10/21/16	✔	16	6.4	335	1.43	40
10/27/16		17.2	5.2	366	0.77	10
11/4/16		16.8	5.7	376	0.85	12
11/15/16		13.5	7.2	380	0.45	2
11/29/16		-	-	-	0.47	10
12/6/16		10	7	380	0.57	2
12/12/16		9.3	9.7	382	0.41	4
1/17/17	✔	-	-	-	-	-

Site 12: Upstream Picnic Area (Mill Creek Watershed)

Date	Storm Event (> 0.5" of rain)	Water T. (°C)	DO (mg/L)	Sp. Cond. (µS/cm at 25°C)	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
9/3/15		-	-	-	0.8	72
9/29/15		-	-	-	1.1	76
10/24/15		-	-	-	1.1	36
11/6/15		-	-	-	1	30
11/18/15	✔	-	-	-	15.4	440
1/29/16		-	-	-	1.8	0
2/3/16		-	-	-	1.5	16
2/10/16		-	-	-	1.2	4
2/18/16		-	-	-	1.3	12
3/2/16		-	-	-	1.2	2
3/10/16	✔	-	-	-	1.8	44
3/14/16		-	-	-	1.94	41
3/25/16		-	-	-	1.83	2
4/6/16		-	-	-	1.71	56
4/11/16	✔	-	-	-	14.2	488
4/20/16		14.7	11.5	271	1.96	56
4/27/16		17.9	10.9	283	2.09	92
5/2/16	✔	13.9	11.4	223	2.12	40
5/10/16		15.5	11.4	294	1.36	76
5/17/16	✔	13.1	10.5	303	1.45	175
5/24/16	✔	15.9	10	253	14.1	2,810
6/2/16		-	-	-	5.7	80
6/22/16		-	-	-	1.19	58
6/28/16		21.1	8.7	288	0.98	54
7/4/16	✔	-	-	-	2.69	125
7/18/16		-	-	-	0.95	22
7/25/16		-	-	-	0.95	380
8/1/16	✔	-	-	-	0.98	90
8/16/16		-	-	-	1.19	20
8/23/16		18.8	9.3	317	0.9	184
9/9/16	✔	-	-	-	0.57	130
9/16/16	✔	18.4	8	310	1.15	410
9/22/16		-	-	-	-	55
9/29/16		18	9.1	324	0.51	12
10/3/16		18.3	8.9	411	0.56	26
10/14/16		15.8	6.8	324	0.66	44
10/21/16	✔	15.3	6.1	298	0.79	56
10/27/16		17.4	6.9	327	0.52	30
11/4/16		16.7	6.1	327	0.8	20
11/15/16		13.5	8.6	325	0.53	20
11/29/16		-	-	-	0.49	20
12/6/16		8.8	8.8	285	0.86	22
12/12/16		8.1	9.2	327	0.39	8
1/17/17	✔	-	-	-	0.97	32

Site 13: Downstream Picnic Area (Mill Creek Watershed)

Date	Storm Event (> 0.5" of rain)	Water T. (°C)	DO (mg/L)	Sp. Cond. (µS/cm at 25°C)	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
9/3/15		-	-	-	-	-
9/29/15		-	-	-	-	-
10/24/15		-	-	-	-	-
11/6/15		-	-	-	-	-
11/18/15	✓	-	-	-	-	-
1/29/16		-	-	-	-	-
2/3/16		-	-	-	-	-
2/10/16		-	-	-	-	-
2/18/16		-	-	-	1.5	5
3/2/16		-	-	-	1.4	3
3/10/16	✓	-	-	-	2.3	32
3/14/16		-	-	-	2.46	26
3/25/16		-	-	-	2.19	4
4/6/16		-	-	-	1.85	28
4/11/16	✓	-	-	-	16	368
4/20/16		14.7	10.8	272	2.9	72
4/27/16		17.7	10.7	248	3.18	46
5/2/16	✓	13.9	11.8	224	2.07	52
5/10/16		15.5	11.3	247	1.2	96
5/17/16	✓	13	9.7	310	1.48	140
5/24/16	✓	15.8	8.9	258	14.2	2,080
6/2/16		-	-	-	6.37	230
6/22/16		-	-	-	3.11	66
6/28/16		21.3	7.8	288	1.11	40
7/4/16	✓	-	-	-	2.71	25
7/18/16		-	-	-	0.98	22
7/25/16		-	-	-	1.11	260
8/1/16	✓	-	-	-	0.81	90
8/16/16		-	-	-	1.1	50
8/23/16		18.8	9.6	321	0.75	176
9/9/16	✓	-	-	-	1.26	90
9/16/16	✓	18.5	7.8	312	1.21	370
9/22/16		-	-	-	-	25
9/29/16		18	8.9	325	0.89	12
10/3/16		19	8.7	413	0.67	40
10/14/16		15.8	6.9	324	0.78	12
10/21/16	✓	15.3	6.1	324	1.17	40
10/27/16		17.3	6.9	327	1.15	32
11/4/16		16.8	6.6	328	0.42	14
11/15/16		13.5	8.7	283	0.67	8
11/29/16		-	-	-	0.68	10
12/6/16		8.8	7.8	328	0.5	18
12/12/16		8.1	9.3	324	0.82	6
1/17/17	✓	-	-	-	1.01	48
3/25/17	✓	12.6	8.5	274	12.3	528
4/5/17	✓	12.6	8.9	199	27.5	2,850
4/22/17	✓	-	-	-	21.8	1,220
4/29/17	✓	13.9	8.4	89	58.7	4,300

Stream Discharge Readings at Mill Creek Picnic Area

Date	Storm Event (> 0.5" of rain)	Discharge (cfs)	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
2/18/16		49	1.5	5
3/2/16		38	1.4	3
3/10/16	✔	42	2.3	32
3/25/16		40	2.19	4
4/11/16	✔	113	16	368
4/20/16		43	2.9	72
4/27/16		32	3.18	46
5/2/16	✔	48	2.07	52
5/10/16		29	1.2	96
5/17/16	✔	33	1.48	140
5/24/16	✔	66	14.2	2,080
6/2/16		83	6.37	230
6/22/16		21	3.11	66
6/28/16		19	1.11	40
7/4/16	✔	28	2.71	25
7/18/16		12	0.98	22
8/23/16		12	0.75	176
9/9/16	✔	9	1.26	90
9/29/16		12	0.89	12
10/14/16		9	0.78	12
10/21/16	✔	17	1.17	40
10/27/16		13	1.15	32
11/4/16		12	0.42	14
11/15/16		11	0.67	8
12/6/16		10	0.5	18
12/12/16		9	0.82	6
1/17/17	✔	17	1.01	48
3/25/17	✔	40	12.3	528
4/5/17	✔	164	27.5	2,850
4/22/17	✔	164	21.8	1,220
4/29/17	✔	526	58.7	4,300

Sediment Disturbance Sampling at Mill Creek Picnic Area

Date	Storm Event (> 0.5" of rain)	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
2/18/16		720	100
3/2/16		408	56
3/10/16	✔	607	150
3/14/16		814	150
3/25/16		614	75
4/6/16		278	180
4/11/16	✔	492	1,180
4/20/16		789	200
4/27/16		846	200
5/2/16	✔	967	250
5/10/16		816	170
5/17/16	✔	1,080	260
5/24/16	✔	1,480	3,440
6/2/16		771	350
6/22/16		1,380	370
6/28/16		1,440	350
7/4/16	✔	585	700
7/18/16		853	550
7/25/16		536	1,010
8/1/16	✔	521	470
8/16/16		100	650
8/23/16		169	180
9/9/16	✔	491	2,630
9/16/16	✔	790	2,230
9/29/16		1,200	500
10/14/16		627	250
10/21/16	✔	1,270	1,160
10/27/16		1,230	4,240
11/4/16		1,020	780
11/15/16		1,290	240
12/6/16		1,420	220
12/12/16		1,320	90
1/17/17	✔	759	70
3/25/17	✔	961	950
4/29/17	✔	898	3,720

Site 14: Wagner Hollow (Mill Creek Watershed)

Date	Storm Event (> 0.5" of rain)	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
5/10/16		1.17	532
5/17/16	✔	2.63	425
5/24/16	✔	33.5	14,880
6/2/16		3.08	210
6/22/16		1.53	204
6/28/16		0.86	276
7/4/16	✔	4.43	390
7/18/16		0.85	205
7/25/16		1.55	728
8/1/16	✔	1.40	280
8/16/16		0.84	60
8/23/16		0.99	312
9/9/16	✔	0.68	170
9/16/16	✔	24.00	9,360
9/29/16		0.92	8
10/3/16		0.78	70
10/14/16		0.50	12
10/21/16	✔	0.54	52
10/27/16		0.44	28
11/4/16		1.14	44
11/15/16		0.44	12
11/29/16		0.48	8
12/6/16		0.29	2
12/12/16		0.46	2
1/17/17	✔	0.92	16

Site 15: Deible Branch (City of Rolla, MO, near ACORN Trail)

Date	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
5/17/16	12.3	7,900
5/24/16	259.0	28,000
6/22/16	3.22	512
10/25/16	1.9	700
10/27/16	1.8	270
11/1/16	1.58	150
11/3/16	8.46	10,000
11/8/16	3.48	2,920
11/10/16	1.14	500
11/15/16	2.74	30
11/17/16	0.99	820
1/17/17	13.8	496
4/5/17	47.7	6,350
4/22/17	25.2	4,740
4/29/17	47.8	4,040

Site 16: West Inflow to Frisco Pond in Schuman Park (City of Rolla, MO)

Date	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
5/17/16	25.2	4,100
5/24/16	33.9	18,000
10/25/16	7.55	120
10/27/16	3.04	150
11/1/16	6.88	820
11/3/16	10.6	5,400
11/8/16	22.4	7,600
11/10/16	8.79	320
11/15/16	2.7	80
11/17/16	2.39	20
1/17/17	27.5	480

Site 17: North Inflow to Frisco Pond in Schuman Park (City of Rolla, MO)

Date	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
10/25/16	6.58	490
10/27/16	5.5	300
11/1/16	5.37	16,000
11/3/16	3.33	3,750
11/8/16	53.8	10,480
11/10/16	5.92	1,100
11/15/16	1.61	430
11/17/16	3.09	590
1/17/17	2.87	2,624

Site 18: Frisco Pond in Schuman Park (City of Rolla, MO)

Date	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
5/24/16	9.54	24,800
6/22/16	9.69	236
10/25/16	3.05	30
10/27/16	4.96	110
11/1/16	4.4	60
11/3/16	1.62	90
11/8/16	3.31	100
11/10/16	1.48	30
11/15/16	1.53	10
11/17/16	1.43	30
1/17/17	6.24	64

Site 19: Lion’s Club Park Pond (City of Rolla, MO)

Date	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
10/25/16	3.26	20
10/27/16	2.43	40
11/1/16	4.65	10
11/3/16	2.13	280
11/8/16	1.62	0
11/10/16	3.26	20
11/15/16	1.53	10
11/17/16	1.26	10

Site 20: Rolla Lodge Pond in Ber Juan Park (City of Rolla, MO)

Date	Turbidity (NTU)	*E. coli* Conc. (cfu/100 mL)
10/25/16	1.51	40
10/27/16	2.21	70
11/1/16	2.35	40
11/3/16	1.44	20
11/8/16	9.93	10
11/10/16	2.41	70
11/15/16	1.3	10
11/17/16	1.61	0

APPENDIX B.

SAMPLING TRIP WEATHER AND STREAM CONDITIONS

Procedure:

For each sampling trip (listed below), weather conditions and rainfall totals for the Phelps County area (includes the city of Rolla, MO, and Mill Creek watershed) were retrieved from the weather station at the Rolla National Airport in Vichy, MO (http://w1.weather.gov/data/obhistory/KVIH.html). Stream discharge for the Little Piney Creek at Newburg, MO, was also recorded for each sampling trip, retrieved from USGS National Water Information System (https://waterdata.usgs.gov/nwis/uv?06932000).

9/3/15 Trip

Weather- Clear Skies, High- 93°F, Low- 67°F, Avg. Humidity- 67

Rainfall in Last 48 Hours- 0.00 in.

Rainfall in Last 24 Hours- 0.00 in.

Stream Discharge at Time of Sampling- 77 cubic ft. / second (cfs)

Peak Discharge from Rainfall- N/A

Gage Height at Time of Sampling- 1.99 ft.

Peak Gage Height from Rainfall- N/A

9/29/15 Trip

Weather- Mostly Cloudy, High- 83°F, Low-61°F, Avg. Humidity- 88

Rainfall in Last 48 Hours- 0.00 in.

Rainfall in Last 24 Hours- 0.00 in.

Stream Discharge at Time of Sampling- 64 cubic ft. / second

Peak Discharge from Rainfall- N/A

Gage Height at Time of Sampling- 1.86 ft.

Peak Gage Height from Rainfall- N/A

10/24/15 Trip

Weather- Overcast, High- 66°F, Low- 45°F, Avg. Humidity- 80

Rainfall in Last 48 Hours- 0.15 in.

Rainfall in Last 24 Hours- 0.00 in.

Stream Discharge at Time of Sampling- 63 cubic ft. / second

Peak Discharge from Rainfall- 64 cubic ft. /second

Gage Height at Time of Sampling- 1.85 ft.

Peak Gage Height from Rainfall- 1.86 ft.

11/6/15 Trip

Weather- Clear, High- 64°F, Low- 42°F, Avg. Humidity- 66

Rainfall in Last 48 Hours- 0.00 in.

Rainfall in Last 24 Hours- 0.00 in.

Stream Discharge at Time of Sampling- 82 cubic ft. / second

Peak Discharge from Rainfall- N/A

Gage Height at Time of Sampling- 2.03 ft.

Peak Gage Height from Rainfall- N/A

11/18/15 Trip

Weather- Mostly Cloudy, High- 60°F, Low- 43°F, Avg. Humidity- 73

Rainfall in Last 48 Hours- 6.25 in.

Rainfall in Last 24 Hours- 5.12 in.

Stream Discharge at Time of Sampling- 1750 cubic ft. / second

Peak Discharge from Rainfall- 12,900 cubic ft. /second

Gage Height at Time of Sampling- 6.47 ft.

Peak Gage Height from Rainfall- 13.06 ft.

1/29/16 Trip

Weather- Clear, High- 58°F, Low- 23°F, Avg. Humidity- 66

Rainfall in Last 48 Hours- 0.00 in.

Rainfall in Last 24 Hours- 0.00 in.

Stream Discharge at Time of Sampling- 134 cubic ft. / second

Peak Discharge from Rainfall- N/A

Gage Height at Time of Sampling- 2.52 ft.

Peak Gage Height from Rainfall- N/A

2/3/16 Trip **Time: 11am – 2pm**

Weather- Overcast; Temp: High- 39°F, Low- 25°F; Avg. Humidity- 71

Rainfall in Last 48 Hours- 0.21 in.

Rainfall in Last 24 Hours- 0.00 in.

Stream Discharge at Time of Sampling- 123 cubic ft. / second

Peak Discharge from Rainfall- 134 cubic ft. / second

Gage Height at Time of Sampling- 2.44 ft.

Peak Gage Height from Rainfall- 2.51 ft.

2/10/16 Trip Time: 11am – 2pm

Weather- Overcast/Snow; Temp: High- 28°F, Low- 12°F; Avg. Humidity- 73

Rainfall in Last 48 Hours- 0.00 in.

Rainfall in Last 24 Hours- 0.00 in.

Stream Discharge at Time of Sampling- 106 cubic ft. / second

Peak Discharge from Rainfall- N/A

Gage Height at Time of Sampling- 2.31 ft.

Peak Gage Height from Rainfall- N/A

Note- Roughly an inch of snowfall.

2/18/16 Trip Time: 8am – 11am

Weather- Fair & Breezy; Temp: High- 52°F, Low- 39°F; Avg. Humidity- 64

Rainfall in Last 48 Hours- 0.45 in.

Rainfall in Last 24 Hours- 0.00 in.

Stream Discharge at Time of Sampling- 144 cubic ft. / second

Peak Discharge from Rainfall- 158 cubic ft. / second

Gage Height at Time of Sampling- 2.57 ft.

Peak Gage Height from Rainfall- 2.65 ft.

3/2/2016 Trip

Weather- Scattered Clouds, High- 48°F, Low- 31°F, Avg. Humidity- 63

Rainfall in Last 48 Hours- 0.08 in.

Rainfall in Last 24 Hours- 0.08 in.

Stream Discharge at Time of Sampling- 120 cubic ft. / second

Peak Discharge from Rainfall- N/A

Gage Height at Time of Sampling- 2.43 ft.

Peak Gage Height from Rainfall- N/A

3/10/16 Trip

Weather- Overcast, High- 55°F, Low- 40°F, Avg. Humidity- 87

Rainfall in Last 48 Hours- 1.06 in.

Rainfall in Last 24 Hours- 0.64 in.

Stream Discharge at Time of Sampling- 150 cubic ft. / second

Peak Discharge from Rainfall- 150 cubic ft. / second

Gage Height at Time of Sampling- 2.62 ft.

Peak Gage Height from Rainfall- 2.62 ft.

3/14/16 Trip

Weather- Mostly Cloudy & Fog, High- 64°F, Low- 50°F, Avg. Humidity- 95

Rainfall in Last 48 Hours- 0.09 in.

Rainfall in Last 24 Hours- 0.05 in.

Stream Discharge at Time of Sampling- 333 cubic ft. / second

Peak Discharge from Rainfall- 344 cubic ft. / second

Gage Height at Time of Sampling- 3.48 ft.

Peak Gage Height from Rainfall- 3.52 ft.

3/25/16 Trip Time: 9am-11:30am

Weather- Overcast, High- 43°F, Low- 34°F, Avg. Humidity- 77

Rainfall in Last 48 Hours- 0.27 in.

Rainfall in Last 24 Hours- 0.00 in.

Stream Discharge at Time of Sampling- 128 cubic ft. / second

Peak Discharge from Rainfall- 143 cubic ft. / second

Gage Height at Time of Sampling- 2.48 ft.

Peak Gage Height from Rainfall- 2.58 ft.

4/6/16 Trip Time: 9am-11:30am

Weather- Cloudy & Rain, High- 60°F, Low- 48°F, Avg. Humidity- 71

Rainfall in Last 48 Hours- 0.00 in.

Rainfall in Last 24 Hours- 0.00 in.

Rainfall at Time of Sampling- 0.23 in.

Stream Discharge at Time of Sampling- 122 cubic ft. / second

Peak Discharge from Rainfall- 131 cubic ft. / second

Gage Height at Time of Sampling- 2.44 ft.

Peak Gage Height from Rainfall- 2.51 ft.

**4/11/16 Trip		Time: 7am-12:30pm**

Weather- Cloudy & Rain, High- 61°F, Low- 53°F, Avg. Humidity- 88

Rainfall in Last 48 Hours- 1.28 in.

Rainfall in Last 24 Hours- 1.28 in.

Rainfall at Time of Sampling- 0.37 in.

Stream Discharge at Time of Sampling- 761 cubic ft. / second

Peak Discharge from Rainfall- 788 cubic ft. / second

Gage Height at Time of Sampling- 4.72 ft.

Peak Gage Height from Rainfall- 4.78 ft.

**4/20/16 Trip		Time: 11am-2:00pm**

Weather- Mostly Cloudy, High- 64°F, Low- 55°F, Avg. Humidity- 86

Rainfall in Last 48 Hours- 0.04 in.

Rainfall in Last 24 Hours- 0.04 in.

Stream Discharge at Time of Sampling- 131 cubic ft. / second

Peak Discharge from Rainfall- 133 cubic ft. / second

Gage Height at Time of Sampling- 2.51 ft.

Peak Gage Height from Rainfall- 2.52 ft.

**4/27/16 Trip		Time: 11am-2:00pm**

Weather- Sunny, High- 73°F, Low- 57°F, Avg. Humidity- 85

Rainfall in Last 48 Hours- 0.45 in.

Rainfall in Last 24 Hours- 0.45 in.

Stream Discharge at Time of Sampling- 116 cubic ft. / second

Peak Discharge from Rainfall- 123 cubic ft. / second

Gage Height at Time of Sampling- 2.40 ft.

Peak Gage Height from Rainfall- 2.45 ft.

5/2/16 Trip **Time: 11am-2:00pm**

Weather- Cloudy, High- 54°F, Low- 46°F, Avg. Humidity- 82

Rainfall in Last 48 Hours- 1.53 in.

Rainfall in Last 24 Hours- 0.76 in.

Stream Discharge at Time of Sampling- 125 cubic ft. / second

Peak Discharge from Rainfall- 205 cubic ft. / second

Gage Height at Time of Sampling- 2.46 ft.

Peak Gage Height from Rainfall- 2.92 ft.

5/9/16 Trip **Time: 9am-3:00pm**

Weather- Few Showers, High- 66°F, Low- 62°F, Avg. Humidity- 83

Rainfall in Last 48 Hours- 0.00 in.

Rainfall in Last 24 Hours- 0.00 in.

Rainfall at Time of Sampling- 0.09 in.

Stream Discharge at Time of Sampling- 96 cubic ft. / second

Peak Discharge from Rainfall- N/A

Gage Height at Time of Sampling- 2.25 ft.

Peak Gage Height from Rainfall- N/A

5/10/16 Trip Time: 9am-3:00pm

Weather- Sunny, High- 75°F, Low- 55°F, Avg. Humidity- 88

Rainfall in Last 48 Hours- 0.31 in.

Rainfall in Last 24 Hours- 0.31 in.

Stream Discharge at Time of Sampling- 108 cubic ft. / second

Peak Discharge from Rainfall- 110 cubic ft. / second

Gage Height at Time of Sampling- 2.35 ft.

Peak Gage Height from Rainfall- 2.36 ft.

5/16/16 Trip Time: 9am-12:00pm

Weather- Few Showers, High- 55°F, Low- 48°F, Avg. Humidity- 84

Rainfall in Last 48 Hours- 0.32 in.

Rainfall in Last 24 Hours- 0.32 in.

Rainfall at Time of Sampling- 0.11 in.

Stream Discharge at Time of Sampling- 88 cubic ft. / second

Peak Discharge from Rainfall- 89 cubic ft. / second

Gage Height at Time of Sampling- 2.19 ft.

Peak Gage Height from Rainfall- 2.20 ft.

5/17/16 Trip Time: 11am-3:00pm

Weather- Cloudy, Light Rain, High- 52°F, Low- 46°F, Avg. Humidity- 94

Rainfall in Last 48 Hours- 1.52 in.

Rainfall in Last 24 Hours- 1.52 in.

Rainfall at Time of Sampling- 0.32 in.

Stream Discharge at Time of Sampling- 130 cubic ft. / second

Peak Discharge from Rainfall- 136 cubic ft. / second

Gage Height at Time of Sampling- 2.49 ft.

Peak Gage Height from Rainfall- 2.54 ft.

5/24/16 Trip Time: 4:30pm-7:30pm

Weather- Cloudy, Light Rain, High- 72°F, Low- 59°F, Avg. Humidity- 88

Rainfall in Last 48 Hours- 0.67 in.

Rainfall in Last 24 Hours- 0.67 in. (Note- storms from 10am-4pm on 5/24/16)

Stream Discharge at Time of Sampling- 138 cubic ft. / second

Peak Discharge from Rainfall- 153 cubic ft. / second

Gage Height at Time of Sampling- 2.55 ft.

Peak Gage Height from Rainfall- 2.64 ft.

6/2/16 Trip Time: 4:30pm-6:30pm

Weather- Clear, High- 80°F, Low- 63°F, Avg. Humidity- 76

Rainfall in Last 48 Hours- 0.03 in.

Rainfall in Last 24 Hours- 0.01 in. (Note- heavy rain (> 1”) on May 28-29, 2016)

Stream Discharge at Time of Sampling- 201 cubic ft. / second

Peak Discharge from Rainfall- 1,230 cubic ft. / second

Gage Height at Time of Sampling- 2.90 ft.

Peak Gage Height from Rainfall- 5.66 ft.

6/22/16 Trip Time: 5:30pm-7:30pm

Weather- Sunny, High- 96°F, Low- 70°F, Avg. Humidity- 67

Rainfall in Last 48 Hours- 0.02 in.

Rainfall in Last 24 Hours- 0.02 in.

Stream Discharge at Time of Sampling- 95 cubic ft. / second

Peak Discharge from Rainfall- 97 cubic ft. / second

Gage Height at Time of Sampling- 2.14 ft.

Peak Gage Height from Rainfall- 2.16 ft.

6/28/16 Trip Time: 1:30pm-3:30pm

Weather- Sunny, High- 86°F, Low- 66°F, Avg. Humidity- 67

Rainfall in Last 48 Hours- 0.47 in.

Rainfall in Last 24 Hours- 0.01 in.

Stream Discharge at Time of Sampling- 92 cubic ft. / second

Peak Discharge from Rainfall- 117 cubic ft. / second (6/27/16 1:00am CDT)

Gage Height at Time of Sampling- 2.12 ft.

Peak Gage Height from Rainfall- 2.30 ft.

7/4/16 Trip Time: 3:30pm-5:30pm

Weather- Cloudy, Light Rain, High- 79°F, Low- 68°F, Avg. Humidity- 92

Rainfall in Last 48 Hours- 2.86 in.

Rainfall in Last 24 Hours- 1.36 in.

Stream Discharge at Time of Sampling- 197 cubic ft. / second

Peak Discharge from Rainfall- 266 cubic ft. / second (7/4/16 10:00am CDT)

Gage Height at Time of Sampling- 2.77 ft.

Peak Gage Height from Rainfall- 3.10 ft.

7/18/16 Trip Time: 12:00pm-2:00pm

Weather- Sunny, High- 89°F, Low- 73°F, Avg. Humidity- 78

Rainfall in Last 48 Hours- 0.00 in.

Rainfall in Last 24 Hours- 0.00 in.

Stream Discharge at Time of Sampling- 103 cubic ft. / second

Peak Discharge from Rainfall- N/A

Gage Height at Time of Sampling- 2.20 ft.

Peak Gage Height from Rainfall- N/A

7/25/16 Trip Time: 9:00am-11:00am

Weather- Cloudy, Light Rain, High- 81°F, Low- 71°F, Avg. Humidity- 88

Rainfall in Last 48 Hours- 0.11 in.

Rainfall in Last 24 Hours- 0.11 in.

Stream Discharge at Time of Sampling- 96 cubic ft. / second

Peak Discharge from Rainfall- 110 cubic ft. / second

Gage Height at Time of Sampling- 2.15 ft.

Peak Gage Height from Rainfall- 2.25 ft.

8/1/16 Trip **Time: 3:00pm-5:00pm**

Weather- Thunderstorms, High- 73°F, Low- 68°F, Avg. Humidity- 95

Rainfall in Last 48 Hours- 1.36 in.

Rainfall in Last 24 Hours- 1.14 in.

Stream Discharge at Time of Sampling- 111 cubic ft. / second

Peak Discharge from Rainfall- 113 cubic ft. / second

Gage Height at Time of Sampling- 2.26 ft.

Peak Gage Height from Rainfall- 2.27 ft.

8/16/16 Trip **Time: 10:00am-1:00pm**

Weather- Partly Cloudy, High- 78°F, Low- 66°F, Avg. Humidity- 92

Rainfall in Last 48 Hours- 0.40 in.

Rainfall in Last 24 Hours- 0.17 in. (Note- over 2" of rainfall over the last 5 days)

Stream Discharge at Time of Sampling- 92 cubic ft. / second

Peak Discharge from Rainfall- 283 cubic ft. / second (8/12/16 7:45pm CDT)

Gage Height at Time of Sampling- 2.20 ft.

Peak Gage Height from Rainfall- 3.26 ft.

8/23/16 Trip **Time: 12:00pm-3:00pm**

Weather- Cloudy, Light Rain, High- 75°F, Low- 62°F, Avg. Humidity- 88

Rainfall in Last 48 Hours- 0.06 in.

Rainfall in Last 24 Hours- 0.06 in.

Stream Discharge at Time of Sampling- 83 cubic ft. / second

Peak Discharge from Rainfall- 86 cubic ft. / second (8/23/16 5:00pm CDT)

Gage Height at Time of Sampling- 2.13 ft.

Peak Gage Height from Rainfall- 2.15 ft.

9/9/16 Trip Time: 3:00pm-6:00pm

Weather- Partly Sunny, High- 86°F, Low- 71°F, Avg. Humidity- 89

Rainfall in Last 48 Hours- 0.76 in.

Rainfall in Last 24 Hours- 0.58 in.

Stream Discharge at Time of Sampling- 81 cubic ft. / second

Peak Discharge from Rainfall- 93 cubic ft. / second

Gage Height at Time of Sampling- 2.11 ft.

Peak Gage Height from Rainfall- 2.21 ft.

9/16/16 Trip Time: 3:00pm-6:00pm

Weather- Rain, High- 76°F, Low- 66°F, Avg. Humidity- 94

Rainfall in Last 48 Hours- 1.61 in.

Rainfall in Last 24 Hours- 1.58 in.

Stream Discharge at Time of Sampling- 113 cubic ft. / second

Peak Discharge from Rainfall- 220 cubic ft. / second (9/16/16 9:00pm CDT)

Gage Height at Time of Sampling- 2.36 ft.

Peak Gage Height from Rainfall- 2.98 ft.

9/22/16 Trip **Time: 10:00am-1:00pm**

Weather- Sunny, High- 66°F, Low- 62°F, Avg. Humidity- 71

Rainfall in Last 48 Hours- 0.00 in.

Rainfall in Last 24 Hours- 0.00 in.

Stream Discharge at Time of Sampling- 91 cubic ft. / second

Peak Discharge from Rainfall- N/A

Gage Height at Time of Sampling- 2.19 ft.

Peak Gage Height from Rainfall- N/A

9/29/16 Trip **Time: 2:00pm-5:00pm**

Weather- Sunny, Clear, High- 73°F, Low- 48°F, Avg. Humidity- 74

Rainfall in Last 48 Hours- 0.00 in.

Rainfall in Last 24 Hours- 0.00 in.

Stream Discharge at Time of Sampling- 77 cubic ft. / second

Peak Discharge from Rainfall- N/A

Gage Height at Time of Sampling- 2.08 ft.

Peak Gage Height from Rainfall- N/A

10/3/16 Trip **Time: 11:00am-2:00pm**

Weather- Overcast, High- 73°F, Low- 53°F, Avg. Humidity- 86

Rainfall in Last 48 Hours- 0.00 in.

Rainfall in Last 24 Hours- 0.00 in.

Stream Discharge at Time of Sampling- 67 cubic ft. / second

Peak Discharge from Rainfall- N/A

Gage Height at Time of Sampling- 1.99 ft.

Peak Gage Height from Rainfall- N/A

10/14/16 Trip Time: 3:00pm-6:00pm

Weather- Mostly Cloudy, High- 69°F, Low- 48°F, Avg. Humidity- 83

Rainfall in Last 48 Hours- 0.02 in.

Rainfall in Last 24 Hours- 0.00 in.

Stream Discharge at Time of Sampling- 74 cubic ft. / second

Peak Discharge from Rainfall- N/A

Gage Height at Time of Sampling- 1.96 ft.

Peak Gage Height from Rainfall- N/A

10/21/16 Trip Time: 4:00pm-7:00pm

Weather- Sunny, Clear, High- 58°F, Low- 37°F, Avg. Humidity- 79

Rainfall in Last 48 Hours- 2.43 in.

Rainfall in Last 24 Hours- 1.22 in.

Stream Discharge at Time of Sampling- 107 cubic ft. / second

Peak Discharge from Rainfall- 231 cubic ft. / second (10/20/16 3:00am CDT)

Gage Height at Time of Sampling- 2.24 ft.

Peak Gage Height from Rainfall- 2.97 ft.

10/27/16 Trip Time: 2:00pm-5:00pm

Weather- Sunny, Clear, High- 69°F, Low- 54°F, Avg. Humidity- 83

Rainfall in Last 48 Hours- 0.03 in.

Rainfall in Last 24 Hours- 0.16 in.

Stream Discharge at Time of Sampling- 86 cubic ft. / second

Peak Discharge from Rainfall- 86 cubic ft. / second (No increase in discharge)

Gage Height at Time of Sampling- 2.06 ft.

Peak Gage Height from Rainfall- 2.07 ft.

11/4/16 Trip Time: 4:00pm-7:00pm

Weather- Sunny, Clear, High- 68°F, Low- 42°F, Avg. Humidity- 84

Rainfall in Last 48 Hours- 0.14 in.

Rainfall in Last 24 Hours- 0.00 in.

Stream Discharge at Time of Sampling- 78 cubic ft. / second

Peak Discharge from Rainfall- 86 cubic ft. / second (No increase in discharge)

Gage Height at Time of Sampling- 2.00 ft.

Peak Gage Height from Rainfall- 2.06 ft.

11/15/16 Trip Time: 2:00pm-5:00pm

Weather- Sunny, Clear, High- 68°F, Low- 36°F, Avg. Humidity- 82

Rainfall in Last 48 Hours- 0.00 in.

Rainfall in Last 24 Hours- 0.00 in.

Stream Discharge at Time of Sampling- 75 cubic ft. / second

Peak Discharge from Rainfall- N/A

Gage Height at Time of Sampling- 1.97 ft.

Peak Gage Height from Rainfall- N/A

11/29/16 Trip Time: 2:00pm-5:00pm

Weather- Sunny, Clear, High- 57°F, Low- 39°F, Avg. Humidity- 73

Rainfall in Last 48 Hours- 0.17 in.

Rainfall in Last 24 Hours- 0.14 in.

Stream Discharge at Time of Sampling- 74 cubic ft. / second

Peak Discharge from Rainfall- 76 cubic ft. / second (No increase in discharge)

Gage Height at Time of Sampling- 1.96 ft.

Peak Gage Height from Rainfall- 1.98 ft.

12/6/16 Trip Time: 11:00am-2:00pm

Weather- Cloudy, Light Rain, High- 39°F, Low- 32°F, Avg. Humidity- 96

Rainfall in Last 48 Hours- 0.18 in.

Rainfall in Last 24 Hours- 0.00 in.

Stream Discharge at Time of Sampling- 71 cubic ft. / second

Peak Discharge from Rainfall- 75 cubic ft. / second (No increase in discharge)

Gage Height at Time of Sampling- 1.94 ft.

Peak Gage Height from Rainfall- 1.97 ft.

12/12/16 Trip Time: 10:00am-1:00pm

Weather- Sunny, Clear, High- 35°F, Low- 26°F, Avg. Humidity- 81

Rainfall in Last 48 Hours- 0.03 in.

Rainfall in Last 24 Hours- 0.03 in.

Stream Discharge at Time of Sampling- 64 cubic ft. / second

Peak Discharge from Rainfall- N/A

Gage Height at Time of Sampling- 1.92 ft.

Peak Gage Height from Rainfall- N/A

1/17/17 Trip Time: 8:00am-11:00am

Weather- Overcast, High- 42°F, Low- 39°F, Avg. Humidity- 93

Rainfall in Last 48 Hours- 0.84 in.

Rainfall in Last 24 Hours- 0.53 in.

Stream Discharge at Time of Sampling- 95 cubic ft. / second

Peak Discharge from Rainfall- 99 cubic ft. / second (1/16/17 7:00pm CDT)

Gage Height at Time of Sampling- 2.19 ft.

Peak Gage Height from Rainfall- 2.22 ft.

3/25/17 Trip Time: 9:00am-12:00pm

Weather- Thunderstorm, High- 62°F, Low- 48°F, Avg. Humidity- 95

Rainfall in Last 48 Hours- N/A

Rainfall in Last 24 Hours- 1.12 in.

Stream Discharge at Time of Sampling- 147 cubic ft. / second

Peak Discharge from Rainfall- 277 cubic ft. / second (3/25/17 7:30pm CDT)

Gage Height at Time of Sampling- 2.55 ft.

Peak Gage Height from Rainfall- 3.20 ft.

4/5/17 Trip Time: 12:00pm-3:00pm

Weather- Rain, High- 61°F, Low- 40°F, Avg. Humidity- 84

Rainfall in Last 48 Hours- N/A

Rainfall in Last 24 Hours- 0.98 in.

Stream Discharge at Time of Sampling- 971 cubic ft. / second

Peak Discharge from Rainfall- 1,670 cubic ft. / second (4/5/17 3:30am CDT)

Gage Height at Time of Sampling- 5.12 ft.

Peak Gage Height from Rainfall- 6.35 ft.

4/22/17 Trip Time: 9:00am-12:00pm

Weather- Rain, High- 55°F, Low- 41°F, Avg. Humidity- 78

Rainfall in Last 48 Hours- N/A

Rainfall in Last 24 Hours- 0.97 in.

Stream Discharge at Time of Sampling- 544 cubic ft. / second

Peak Discharge from Rainfall- 776 cubic ft. / second (4/22/17 2:00pm CDT)

Gage Height at Time of Sampling- 4.04 ft.

Peak Gage Height from Rainfall- 4.67 ft.

4/29/17 Trip Time: 9:00am-12:00pm

Weather- Thunderstorm, High- 67°F, Low- 53°F, Avg. Humidity- 100

Rainfall in Last 48 Hours- N/A

Rainfall in Last 24 Hours- 1.05 in.

Stream Discharge at Time of Sampling- 2,660 cubic ft. / second

Peak Discharge from Rainfall- 13,900 cubic ft. / second (4/29/17 11:00pm CDT)

Gage Height at Time of Sampling- 7.57 ft.

Peak Gage Height from Rainfall- 13.40 ft.

APPENDIX C.

DATA FROM MESOCOSM EXPERIMENTS

Solar Radiation – Experiment #1

	E. coli Conc. cfu/100 mL		Log *E. coli* Conc. $\log_{10}$(cfu/100 mL)		K-values Based On Slopes $\log_{10}$(cfu/100 mL) per hour	
Hours	Shade	Sun	Shade	Sun	Shade	Sun
0	484	500	2.7	2.7	–0.011	–0.735
1	504	128	2.7	2.1	R-sq. Values	
2	460	16	2.7	1.2	Shade	Sun
					0.3086	0.9871
% Survival	95.0%	3.2%				

Solar Radiation – Experiment #2

	E. coli Conc. cfu/100 mL		Log *E. coli* Conc. $\log_{10}$(cfu/100 mL)		K-values Based On Slopes $\log_{10}$(cfu/100 mL) per hour	
Hours	Shade	Sun	Shade	Sun	Shade	Sun
0	1530	1430	3.2	3.2	–0.008	–0.790
1	1580	400	3.2	2.6	R-sq. Values	
2	1520	10	3.2	1.0	Shade	Sun
3	1460	10	3.2	1.0	0.5085	0.8848
% Survival	95.4%	0.70%				

Water Temperature – Experiment #1

Days	0	1	5	8	12	16
	10/26/16	10/27/16	10/31/16	11/3/16	11/7/16	11/11/16
37.1	1500	700	10	10	0	0
37.2	1500	740	50	20	0	0
37.3	1500	890	30	0	0	0
Avg.	**1500**	**776.7**	**30**	**10**	**0**	**0**
24.1	1500	940	190	180	30	28
24.2	1500	840	300	150	80	32
24.3	1500	960	240	70	80	32
Avg.	**1500**	**913.3**	**243.3**	**133.3**	**63.3**	**30.7**
8.1	1500	1400	770	440	390	230
8.2	1500	1340	710	560	410	270
8.3	1500	1310	560	540	410	360
Avg.	**1500**	**1350**	**680**	**513.3**	**403.3**	**286.7**

Days	21	35	41	47	89
	11/16/16	11/30/16	12/6/16	12/12/16	1/23/17
37.1	0	0	0	0	0
37.2	0	0	0	0	0
37.3	0	0	0	0	0
Avg.	**0**	**0**	**0**	**0**	**0**
24.1	8	2	0	0	0
24.2	10	0	0	0	0
24.3	12	2	0	0	0
Avg.	**10**	**1.3**	**0**	**0**	**0**
8.1	200	104	68	80	15
8.2	240	52	104	68	7
8.3	190	80	104	100	26
Avg.	**210**	**78.7**	**92**	**82.7**	**16**

	E. coli Conc. cfu/100 mL			Log *E. coli* Conc. $\log_{10}$(cfu/100 mL)		
Days	37°C	24°C	8°C	37°C	24°C	8°C
0	1500	1500	1500	3.2	3.2	3.2
1	777	913	1350	2.9	3.0	3.1
5	30	243	680	1.5	2.4	2.8
8	10	133	513	1.0	2.1	2.7
12	0	63	403	0.0	1.8	2.6
16	0	31	287	0.0	1.5	2.5
21	0	10	210	0.0	1.0	2.3
35	0	1	79	0.0	0.4	1.9
41	0	0	92	0.0	0.0	2.0
47	0	0	83	0.0	0.0	1.9
89	0	0	16	0.0	0.0	1.2
% Survival at Day 8	0.67%	8.89%	34.22%			
% Survival at Day 12	0.00%	4.22%	26.89%			

K-values Based On Slopes $\log_{10}$(cfu/100 mL) per day		
37°C	24°C	8°C
–0.279	–0.079	–0.021
R-sq. Values		
37°C	24°C	8°C
0.9744	0.9585	0.91876

Water Temperature – Experiment #2

Days	0	1	2	3	4	7	10	13
	6/16/17	6/17/17	6/18/17	6/19/17	6/20/17	6/23/17	6/26/17	6/29/17
37.1	1100	950	680	590	320	0	0	0
37.2	1180	800	750	380	160	0	0	0
37.3	1020	770	820	520	350	20	4	0
Avg.	**1100**	**840**	**750**	**497**	**277**	**7**	**1**	**0**
24.1	1060	980	860	600	400	220	100	72
24.2	1150	930	920	550	370	180	80	40
24.3	1120	850	800	680	440	240	150	64
Avg.	**1110**	**920**	**860**	**610**	**403**	**213**	**110**	**59**
8.1	1080	980	950	800	670	500	300	270
8.2	1050	1040	890	820	800	570	380	320
8.3	1140	1160	1100	1060	880	750	490	420
Avg.	**1090**	**1060**	**980**	**893**	**783**	**607**	**390**	**337**

Days	20	27	34	41	48	55	62	69
	7/6/17	7/13/17	7/20/17	7/27/17	8/3/17	8/10/17	8/17/17	8/24/17
37.1	0	0	0	0	0	0	0	0
37.2	0	0	0	0	0	0	0	0
37.3	0	0	0	0	0	0	0	0
Avg.	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
24.1	28	12	6	2	0	0	0	0
24.2	14	8	2	0	0	0	0	0
24.3	32	18	10	4	1	0	0	0
Avg.	**25**	**13**	**6**	**2**	**0**	**0**	**0**	**0**
8.1	180	130	106	98	82	68	54	42
8.2	220	170	132	112	118	88	68	58
8.3	360	240	184	166	124	110	90	74
Avg.	**253**	**180**	**141**	**125**	**108**	**89**	**71**	**58**

Days	76	83	86
	8/31/17	9/7/17	9/10/17
37.1	0	0	0
37.2	0	0	0
37.3	0	0	0
Avg.	**0**	**0**	**0**
24.1	0	0	0
24.2	0	0	0
24.3	0	0	0
Avg.	**0**	**0**	**0**
8.1	29	20	11
8.2	42	27	17
8.3	53	39	25
Avg.	**41**	**29**	**18**

	E. coli Conc. cfu/100 mL			Log *E. coli* Conc. $\log_{10}$(cfu/100 mL)		
Days	37°C	24°C	8°C	37°C	24°C	8°C
0	1100	1110	1090	3.0	3.0	3.0
1	840	920	1060	2.9	3.0	3.0
2	750	860	980	2.9	2.9	3.0
3	497	610	893	2.7	2.8	3.0
4	277	403	783	2.4	2.6	2.9
7	7	213	607	0.9	2.3	2.8
10	1	110	390	0.3	2.0	2.6
13	0	59	337	0.0	1.8	2.5
20	0	25	253	0.0	1.4	2.4
27	0	13	180	0.0	1.1	2.3
34	0	6	141	0.0	0.8	2.2
41	0	2	125	0.0	0.5	2.1
48	0	0	108	0.0	0.0	2.0
55	0	0	89	0.0	0.0	2.0
62	0	0	71	0.0	0.0	1.9
69	0	0	58	0.0	0.0	1.8
76	0	0	41	0.0	0.0	1.6
83	0	0	29	0.0	0.0	1.5
86	0	0	18	0.0	0.0	1.3
% Survival at Day 7	0.64%	19.19%	55.69%			
% Survival at Day 10	0.09%	9.91%	35.78%			
% Survival at Day 13	0.00%	5.32%	30.92%			

K-values Based On Slopes $\log_{10}$(cfu/100 mL) per day		
37°C	24°C	8°C
–0.303	–0.063	–0.018
R-sq. Values		
37°C	24°C	8°C
0.9461	0.9644	0.96277

Sedimentation and Adsorption – Experiment #1

Date: 6/13/17

Tested in a Climate Controlled Room, Set at 15°C

Turbidity (NTU)

	Time (hours)						
	0	1	2	4	8	12	24
Control	5.74	7.65	6.51	6.38	6.89	4.84	4.00
Sand	21.3	13.1	12.4	13.8	6.48	5.24	7.82
Gravel	6.36	4.82	3.38	5.74	6.15	4.17	5.01
Mill Creek	7.24	5.05	4.36	5.98	4.54	4.53	5.88

E. coli Concentration (cfu per 100 mL)

	Time (hours)							
	0	1	2	4	8	12	24	Disturbed
Control-1	390	450	450	390	500	430	390	N/A
Control-2	420	560	450	470	400	480	390	
Avg.	**405**	**505**	**450**	**430**	**450**	**455**	**390**	
Sand-1	410	330	410	400	320	290	230	420
Sand-2	430	240	350	450	590	350	390	
Avg.	**420**	**285**	**380**	**425**	**455**	**320**	**310**	
Gravel-1	300	440	430	440	320	190	270	180
Gravel-2	510	380	400	400	360	430	190	
Avg.	**405**	**410**	**415**	**420**	**340**	**310**	**230**	
Mill Creek-1	510	550	370	530	350	210	80	140
Mill Creek-2	290	360	360	470	350	200	150	
Avg.	**400**	**455**	**365**	**500**	**350**	**205**	**115**	

	E. coli Conc. cfu/100 mL				Log *E. coli* Conc. $\log_{10}$(cfu/100 mL)			
Hours	Control	Sand	Gravel	Mill Creek	Control	Sand	Gravel	Mill Creek
0	405	420	405	400	2.6	2.6	2.6	2.6
1	505	285	410	455	2.7	2.5	2.6	2.7
2	450	380	415	365	2.7	2.6	2.6	2.6
4	430	425	420	500	2.6	2.6	2.6	2.7
8	450	455	340	350	2.7	2.7	2.5	2.5
12	455	320	310	205	2.7	2.5	2.5	2.3
24	390	310	230	115	2.6	2.5	2.4	2.1
% Removed	3.7%	26.2%	43.2%	71.3%				

K-values Based On Slopes $\log_{10}$(cfu/100 mL) per hour			
Control	Sand	Gravel	Mill Creek
–0.002	–0.003	–0.011	–0.025
R-sq. Values			
Control	Sand	Gravel	Mill Creek
0.2471	0.1324	0.9611	0.8908

Sedimentation and Adsorption – Experiment #2

Date: 6/20/17 | Tested in a Climate Controlled Room, Set at 15°C

Turbidity (NTU)

	Time (hours)								
	0	1	2	4	8	12	24	48	Disturbed
Control	7.76	6.61	7.19	6.12	6.04	6.6	5.56	5.64	N/A
Sand	16.7	15.4	11.4	8.0	6.13	3.8	2.38	2.02	107
Gravel	8.06	5.7	5.85	5.54	5.28	3.73	3.93	2.28	70.4
Mill Creek	9.73	7.62	6.3	6.6	5.5	4.65	4.41	1.82	181

E. coli Concentration (cfu per 100 mL)

	Time (hours)								
	0	1	2	4	8	12	24	48	Disturbed
Control-1	270	200	250	210	400	190	350	240	N/A
Control-2	290	230	250	240	450	160	290	250	
Avg.	**280**	**215**	**250**	**225**	**425**	**175**	**320**	**245**	
Sand-1	310	170	80	70	170	260	160	30	40
Sand-2	300	190	130	100	80	190	140	40	
Avg.	**305**	**180**	**105**	**85**	**125**	**225**	**150**	**35**	
Gravel-1	290	140	180	180	150	150	120	10	0
Gravel-2	360	260	170	210	160	260	90	20	
Avg.	**325**	**200**	**175**	**195**	**155**	**205**	**105**	**15**	
Mill Creek-1	370	270	320	290	520	700	670	40	200
Mill Creek-2	230	300	200	260	550	560	700	70	
Avg.	**300**	**285**	**260**	**275**	**535**	**630**	**685**	**55**	

	E. coli Conc. cfu/100 mL				Log *E. coli* Conc. $\log_{10}$(cfu/100 mL)			
Hours	Control	Sand	Gravel	Mill Creek	Control	Sand	Gravel	Mill Creek
0	280	305	325	300	2.4	2.5	2.5	2.5
1	215	180	200	285	2.3	2.3	2.3	2.5
2	250	105	175	260	2.4	2.0	2.2	2.4
4	225	85	195	275	2.4	1.9	2.3	2.4
8	425	125	155	535	2.6	2.1	2.2	2.7
12	175	225	205	630	2.2	2.4	2.3	2.8
24	320	150	105	685	2.5	2.2	2.0	2.8
48	245	35	15	55	2.4	1.6	1.2	1.7
% Removed	12.5%	88.5%	95.4%	81.7%				

K-values Based On Slopes $\log_{10}$(cfu/100 mL) per hour			
Control	Sand	Gravel	Mill Creek
0.000	–0.012	–0.023	–0.012
R-sq. Values			
Control	Sand	Gravel	Mill Creek
0.0014	0.4890	0.9081	0.3049

Sedimentation and Adsorption – Experiment #3

Date: 7/11/17

Tested in a Climate Controlled Room, Set at 15°C

Turbidity (NTU)

	Time (hours)									
	0	1	2	4	8	12	24	36	48	Disturbed
Control	9.21	6.52	5.22	4.07	4.14	6.57	2.22	3.19	2.14	N/A
Sand	28.4	19.7	20.1	18.9	22.8	19.4	14.8	16	2.74	116
Gravel	12.3	6.58	4.42	6.67	8.62	7.54	7.67	3.34	4.54	118
Mill Creek	10.5	7.09	5.4	6.17	6.69	5.74	4.79	3.7	2.46	151

E. coli Concentration (cfu per 100 mL)

	Time (hours)									
	0	1	2	4	8	12	24	36	48	Disturbed
Control-1	690	400	540	470	570	300	370	260	210	N/A
Control-2	690	500	470	540	460	330	570	220	350	
Avg.	**690**	**450**	**505**	**505**	**515**	**315**	**470**	**240**	**280**	
Sand-1	630	510	370	400	270	290	220	180	120	130
Sand-2	530	430	380	340	340	240	360	230	110	
Avg.	**580**	**470**	**375**	**370**	**305**	**265**	**290**	**205**	**115**	
Gravel-1	580	470	430	560	350	170	180	90	110	150
Gravel-2	660	370	490	390	260	250	180	110	70	
Avg.	**620**	**420**	**460**	**475**	**305**	**210**	**180**	**100**	**90**	
Mill Creek-1	640	580	510	550	520	420	340	310	110	180
Mill Creek-2	780	550	520	490	360	420	380	270	90	
Avg.	**710**	**565**	**515**	**520**	**440**	**420**	**360**	**290**	**100**	

	E. coli Conc. cfu/100 mL				Log *E. coli* Conc. $\log_{10}$(cfu/100 mL)			
Hours	Control	Sand	Gravel	Mill Creek	Control	Sand	Gravel	Mill Creek
0	690	580	620	710	2.8	2.8	2.8	2.9
1	450	470	420	565	2.7	2.7	2.6	2.8
2	505	375	460	515	2.7	2.6	2.7	2.7
4	505	370	475	520	2.7	2.6	2.7	2.7
8	515	305	305	440	2.7	2.5	2.5	2.6
12	315	265	210	420	2.5	2.4	2.3	2.6
24	470	290	180	360	2.7	2.5	2.3	2.6
36	240	205	100	290	2.4	2.3	2.0	2.5
48	280	115	90	100	2.4	2.1	2.0	2.0
% Removed	59.4%	80.2%	85.5%	85.9%				

K-values Based On Slopes $\log_{10}$(cfu/100 mL) per hour			
Control	Sand	Gravel	Mill Creek
–0.007	–0.011	–0.017	–0.013
R-sq. Values			
Control	Sand	Gravel	Mill Creek
0.6334	0.8652	0.9202	0.8748

Sedimentation and Adsorption – Experiment #4

Date:	7/18/17	Tested in a Climate Controlled Room, Set at 8°C

Turbidity (NTU)

	Time (hours)								
	0	1	2	4	8	12	24	36	48	Disturbed
Control	5.15	4.82	4.42	3.51	3.41	2.19	2.88	1.28	1.51	N/A
Sand	17.1	16.7	11.6	7.01	9.13	8.65	3.9	2.35	2.6	310
Gravel	4.61	3.58	4.18	2.36	2.93	1.97	2.04	1.67	1.39	145
Mill Creek	5.65	4.56	4.38	4.52	3.7	4.09	2.54	2.04	1.64	135

E. coli Concentration (cfu per 100 mL)

	Time (hours)									
	0	1	2	4	8	12	24	36	48	Disturbed
Control-1	1870	1890	1510	1580	1380	1490	1440	1210	1370	N/A
Control-2	1920	1750	1690	1460	1530	1380	1270	1260	1230	
Avg.	**1895**	**1820**	**1600**	**1520**	**1455**	**1435**	**1355**	**1235**	**1300**	
Sand-1	1840	1580	1100	1020	910	770	200	80	30	30
Sand-2	1900	1410	1590	940	940	700	200	60	30	
Avg.	**1870**	**1495**	**1345**	**980**	**925**	**735**	**200**	**70**	**30**	
Gravel-1	1670	1580	1550	1430	1310	1340	900	770	610	690
Gravel-2	1690	1500	1480	1520	1380	1250	1030	980	650	
Avg.	**1680**	**1540**	**1515**	**1475**	**1345**	**1295**	**965**	**875**	**630**	
Mill Creek-1	1880	1470	1560	1370	1360	1300	740	500	140	150
Mill Creek-2	1630	1670	1530	1500	1390	1320	890	330	120	
Avg.	**1755**	**1570**	**1545**	**1435**	**1375**	**1310**	**815**	**415**	**130**	

	E. coli Conc. cfu/100 mL				Log *E. coli* Conc. log$_{10}$(cfu/100 mL)			
Hours	Control	Sand	Gravel	Mill Creek	Control	Sand	Gravel	Mill Creek
0	1895	1870	1680	1755	3.3	3.3	3.2	3.2
1	1820	1495	1540	1570	3.3	3.2	3.2	3.2
2	1600	1345	1515	1545	3.2	3.1	3.2	3.2
4	1520	980	1475	1435	3.2	3.0	3.2	3.2
8	1455	925	1345	1375	3.2	3.0	3.1	3.1
12	1435	735	1295	1310	3.2	2.9	3.1	3.1
24	1355	200	965	815	3.1	2.3	3.0	2.9
36	1235	70	875	415	3.1	1.9	2.9	2.6
48	1300	30	630	130	3.1	1.5	2.8	2.1
% Removed	31.4%	98.4%	62.5%	92.6%				

K-values Based On Slopes log$_{10}$(cfu/100 mL) per hour			
Control	Sand	Gravel	Mill Creek
–0.003	–0.037	–0.008	–0.021
R-sq. Values			
Control	Sand	Gravel	Mill Creek
0.6874	0.9928	0.9848	0.9378

Sedimentation and Adsorption – Experiment #5

Date: 8/1/17

Tested in a Climate Controlled Room, Set at 8°C

Turbidity (NTU)

	Time (hours)									
	0	1	2	4	8	12	24	36	48	Disturbed
Control	2.8	2.94	3.16	3.29	1.65	1.59	1.71	1.56	1.49	N/A
Sand	12.2	9.87	7.45	5.21	4.79	4.36	4.34	3.94	3.59	712
Gravel	4.38	5.37	2.84	2.76	1.97	1.54	1.78	2.04	1.7	461
Mill Creek	5.68	3.78	3.15	3.02	2.72	2.3	2.22	2.06	1.83	541

E. coli Concentration (cfu per 100 mL)

	Time (hours)									
	0	1	2	4	8	12	24	36	48	Disturbed
Control-1	250	280	170	290	360	190	190	260	140	N/A
Control-2	250	220	260	330	230	240	320	250	300	
Avg.	**250**	**250**	**215**	**310**	**295**	**215**	**255**	**255**	**220**	
Sand-1	420	390	390	390	230	210	90	10	10	170
Sand-2	370	300	200	350	190	140	120	40	30	
Avg.	**395**	**345**	**295**	**370**	**210**	**175**	**105**	**25**	**20**	
Gravel-1	390	360	290	350	270	370	110	110	10	140
Gravel-2	340	360	410	470	440	380	170	60	50	
Avg.	**365**	**360**	**350**	**410**	**355**	**375**	**140**	**85**	**30**	
Mill Creek-1	260	290	270	200	280	270	200	160	90	280
Mill Creek-2	310	190	230	270	250	380	210	120	70	
Avg.	**285**	**240**	**250**	**235**	**265**	**325**	**205**	**140**	**80**	

	E. coli Conc. cfu/100 mL				Log *E. coli* Conc. $\log_{10}$(cfu/100 mL)			
Hours	Control	Sand	Gravel	Mill Creek	Control	Sand	Gravel	Mill Creek
0	250	395	365	285	2.4	2.6	2.6	2.5
1	250	345	360	240	2.4	2.5	2.6	2.4
2	215	295	350	250	2.3	2.5	2.5	2.4
4	310	370	410	235	2.5	2.6	2.6	2.4
8	295	210	355	265	2.5	2.3	2.6	2.4
12	215	175	375	325	2.3	2.2	2.6	2.5
24	255	105	140	205	2.4	2.0	2.1	2.3
36	255	25	85	140	2.4	1.4	1.9	2.1
48	220	20	30	80	2.3	1.3	1.5	1.9
% Removed	12.0%	94.9%	91.8%	71.9%				

K-values Based On Slopes $\log_{10}$(cfu/100 mL) per hour			
Control	Sand	Gravel	Mill Creek
–0.001	–0.028	–0.022	–0.010
R-sq. Values			
Control	Sand	Gravel	Mill Creek
0.0692	0.9687	0.9380	0.8034

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VITA

Kaleb Colt Bassett was born in Rolla, Missouri. He graduated from Waynesville High School in 2010 and began studying biology at Missouri State University in Springfield, Missouri. After attending MSU for three semesters, Kaleb returned home to Waynesville where he spent eight months working for his family's construction company. In August 2012 he started college again at the Missouri University of Science and Technology in Rolla, Missouri. Here, he continued with his degree in biology, but changed his emphasis area to secondary education. During his undergraduate training at Missouri S&T, Kaleb worked with Professor Terry Wilson as an undergraduate teaching assistant in general biology and biodiversity lab courses. In 2013, while working as a teaching assistant, Kaleb met and worked with Travis Thompson, a former Missouri S&T graduate student. Travis introduced him to Dr. Dev Niyogi and he started to assist in freshwater ecology research in Dr. Niyogi's lab at Missouri S&T. During his senior year, Kaleb collaborated with the U.S. Forest Service in Rolla, Missouri, and helped set up a long-term temperature monitoring study in several streams in the Ozarks. After receiving his B.A. in Biological Sciences in 2015, Kaleb continued his graduate studies at the Missouri University of Science and Technology under the advising of Dr. Dev Niyogi. He received his M.S. degree in Applied and Environmental Biology from Missouri S&T in December 2017. Kaleb moved back to Springfield, Missouri, in January 2018 and began his career as a teacher with Springfield Public Schools.