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**A Comparison of Suspended Sediment and *E.coli* in Two
East Tennessee Streams**

A Thesis

Presented for the

Master of Science Degree

The University of Tennessee, Knoxville

Ann Barbour Wambersie

May 2009

Dedication

I dedicate this thesis to my parents Elizabeth and Alexander Monroe. Without their love, support, and ability to tolerate my inquisitive nature, I would not have pursued a graduate degree. I am eternally grateful for all they have given me and I would not be where I am now without their faith in my abilities. I also dedicate this to Tom Strange who has provided endless insight and support throughout this thesis process and whose continued patience throughout has helped me enormously.

Acknowledgements

I would like to thank the following persons:

Dr. Carol Harden, my major professor, for her guidance, encouragement, and tremendous amount of patience throughout the writing process. I would also like to thank her for the use of the Environmental Dynamics laboratory of the Geography Department.

Dr. Alice Layton, committee member, for all of her assistance with microbiology and her willingness to share her insight and knowledge. I would also like to thank her for conducting the *Bacteroides* analysis, allowing me to use her microbiology laboratory in the Center for Environmental Biotechnology, and providing me with supplies.

Dr. Liem Tran, committee member, for his endless help with statistics and his incredible patience with me over the past two years.

William Ryan Foster, field assistant, for all of his help, his keen ability in the field, and his overall love of nature.

I would also like to thank the EPA-funded Little River Targeted Watershed Initiative for supporting the participation of a field assistant. Funding was provided by the National Science Foundation under grant DGE-0538420.

Abstract

Two frequent water quality problems for streams on state 303(d) lists are sedimentation and bacteria. *Escherichia coli* (*E.coli*) is monitored as a widely distributed, easily cultured species that can indicate pathogenic bacterial contamination in waters designated for recreational uses. Suspended solids are also an indicator of stream health because they stress aquatic ecosystems. I investigated the relationships between concentrations and loads of suspended solids and *E. coli* over different flows in two tributary streams of the Little River (HUC 06010201) in east Tennessee. One stream, Nails Creek, flows through a rural, agricultural area, while the second, Pistol Creek, drains a dominantly urban and residential watershed. I hypothesized that relationships between *E. coli* and suspended solid concentrations would differ between the two streams.

From August 2007 through February 2008, I collected 20 water samples and suspended solids from each stream to be analyzed to determine bacterial and suspended sediment concentrations and loads, over a range of flows. I processed the samples for *E. coli*, total suspended solids (TSS), and loss-on-ignition, and used DNA analyses of *Bacteroides* to determine the percentage of bovine or human bacteria per sample. To determine relationships between the variables, I used non-parametric correlation analyses for in-stream relationships and Mann-Whitney-U tests for relationships between streams.

No relationship was found between concentrations or loads of suspended solids and *E.coli* in either Nails Creek, or Pistol Creek. However, Nails Creek had significantly higher median concentrations of *E.coli* and TSS than Pistol Creek.

Patterns of discharge, suspended solids, and *E.coli* concentrations and loads differed between the two streams, with Nails Creek samples having higher loads of TSS and *E. coli*. Pistol Creek had significantly higher TSS loads and *E.coli* loads at higher discharges than at lower discharges while there was no significant difference with discharge in Nails Creek. Nails Creek samples contained more bovine-associated fecal bacteria than human-associated fecal bacteria; Pistol Creek contained very little fecal bacteria. This study takes a step toward understanding relationships among suspended solids, bacteria, and discharge in Little River tributaries and provides a foundation for future investigations and water quality remediation efforts in the watershed.

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Chapter One

Introduction and Study Design

1.1: Introduction

Clean water is vital in everyday life. The quality of surface waters affects public health, recreational activities, and, to an extent, the quality of life.

Therefore, maintaining and improving the quality of freshwater resources is essential worldwide. The importance of clean water received public attention in the United States with the publication of Rachel Carson's Silent Spring (Carson, 1962). Environmental concerns were again raised with the burning of the Cuyahoga River in Ohio in 1969 (Cortner and Moote, 1999). These historical events helped shape the environmental movement and aided in the development of agencies dedicated to the health and safety of our nation's waters. Today, the United States recognizes the importance of protecting water resources and has, over the last four decades, adopted legislation to improve water quality (Cech, 2004).

The Clean Water Act (CWA) was developed in 1972 and finalized in 1977, following passage of amendments to the Federal Water Pollution Control Act. The Act created procedures and regulations for pollutant discharges into U.S. waters (USEPA, 2007). The CWA set limits for contaminants to maintain physical, biological, and chemical integrity of the surface waters of the U.S. To

implement the CWA, water quality standards (WQS) must be established in support of designated uses of surface waters. Once WQS are established, it is the responsibility of states to monitor rivers, lakes, bays, wetlands, estuaries, and near-shore marine waters (USEPA, 2003). If a state's waters are not meeting its WQS, the waters are considered "impaired" and placed on the state's 303(d) List, which names the impaired or "water quality limited" streams and lakes in the state (USEPA, 2003; TDEC, 2004). Once a water body is on the 303(d) List, the CWA requires that Total Maximum Daily Loads (TMDLs) be developed to restore the water to a quality appropriate for its designated use (USEPA, 2003). A TMDL is a report that quantifies a stream's or lake's pollutants, identifies the pollutants' sources, and recommends actions necessary to restore the water body to its designated use (TDEC, 2004).

Nonpoint source pollution (NPS) has been identified as one of the leading causes of pollution and water degradation throughout the country, even surpassing industrial wastewater (Gilliland and Baxter-Potter, 1987). Agricultural and urban lands are major contributors to NPS pollution and both have been linked to declines in water-quality (Larned et al., 2004). One leading NPS pollutant is an excess of sediment. The many sources of sediment in surface waters include agricultural and urban lands, land undergoing development, and streambanks (TDEC, 2006). Land-disturbing activities, such as agriculture and urban development, can have high erosion rates and add sediment to streams (Lenat and Crawford, 1994). In fact, siltation, the deposition of fine-grained sediment, is the most commonly cited water quality impairment in Tennessee, affecting over

8,000 km (5,000 mi) of streams and rivers (TDEC, 2006). Excessive siltation stresses ecosystems by altering macroinvertebrate and fish spawning habitats, and affects fish rearing and feeding behavior. Larger sediments or deeper deposits can bury invertebrates, while suspended solids affect the availability of light for photosynthesis by plants and visible light for animals (TDEC, 2006). Another common type of point and non-point source pollution is disease-causing organisms. These organisms enter waterbodies in runoff from pastures and other livestock sites, leaking septic tanks, faulty sewage lines, or sewage treatment plants. The presence of pathogenic bacteria in drinking water can lead to diseases such as gastroenteritis or giardiasis.

The watershed of the Little River, in eastern Tennessee, offers opportunities to compare the concentrations and loads of suspended solids and water-borne bacteria in tributaries draining areas with different land uses. It contains a rapidly developing urban area and a rural and agricultural landscape. Water quality in the Little River and its tributaries is threatened by suspended solids and bacteria from NPS pollution (TVA, 2003). The Little River, a tributary of the Tennessee River, was first designated as a “threatened” stream in 2000. This meant that if current conditions continued, the river was expected to become “impaired” within five years (TVA, 2003). Numerous tributaries of the Little River, including Pistol Creek, Crooked Creek, Ellejoy Creek, Short Creek, Nails Creek, Roddy Branch, Russell Branch, and the Little River embayment of Fort Loudoun Lake, were identified as having poor water quality (TVA, 2003).

The purpose of this thesis research was to identify and quantify relationships between two contaminants, pathogenic bacteria and suspended solids, in water samples taken over different flow regimes in two tributaries of the Little River. Land use in one tributary was predominantly urban, while land use in the second was predominantly rural. I monitored and analyzed relationships between the bacterium *Escherichia coli* (*E. coli*) and total suspended solids (TSS). I also sampled for *Bacterioides* to determine, through a DNA source-tracking analysis, whether cows or humans are the dominant source of bacteria in stream waters. Sampling for *Bacterioides* is supplemental to *E.coli* sampling for fecal indicators (Gentry et al., 2007). *Bacterioides* have a high degree of species specificity, which reflects differences in animal digestive systems, and, for this study, allows source identification between human and bovine digestive tracts (Layton et al., 2006a).

E.coli is an enteric bacterium that occupies the gastrointestinal tracts of all warm-blooded animals (Maier et al., 1999). This bacterium is usually classified as harmless; however, several strains are considered pathogenic. *E. coli* in surface waters indicates the potential presence of disease-causing organisms (Christensen et al., 2002). Because it is widely distributed and easily cultured, it is the primary indicator used by most agencies, such as the EPA, to determine the presence of pathogenic contamination in waters designated for recreational uses (Layton et al., 2006a). Quantitative, spatially explicit data on *E.coli* in a stream system can provide insight about possible contamination sources (USEPA, 2001).

Because *E.coli* are not free-floating in the stream, piggybacking on TSS would be a likely mode of transportation for the bacteria (Davies et al., 1995; Bai and Lung, 2005). The amount of TSS in stream waters is expected to be a function of stream discharge. Therefore, if the quantity of *E.coli* in a stream is dependent on the quantity of TSS, *E.coli* should also depend on discharge for transport capability, and the two variables, *E. coli* and TSS, should be positively correlated. Finding a positive correlation would verify this relationship for Little River tributaries and help guide land management actions for improving water quality. However, not finding a positive correlation would call attention to the need to re-evaluate the assumptions and develop a better understanding of the actual processes by which these pollutants enter and move through the streams. *E. coli* loads in Little River tributaries must be reduced to improve water quality in the River and remove its tributaries from the EPA's 303(d) List. Whether or not TSS and *E. coli* are positively correlated, this study takes an important step toward helping reduce *E. coli* loads and improve water quality in the Little River.

1.2: Study Design

In this research, I investigated the relationship between quantities of suspended solids and bacteria within two Little River tributary streams, Pistol Creek and Nails Creek, which drain watersheds containing different sets of land uses. The goal of this research was to characterize the relationships between suspended sediments and *E. coli* concentrations and loads in the two streams and provide more insight into the behavior of suspended solids and bacteria within

tributaries of the Little River watershed. The project was designed to answer the following research questions:

1. Is there a relationship between concentrations of suspended solids and of *E. coli*?
2. Do patterns of *E. coli* loads differ between two study streams?
3. Do patterns of suspended solid loads differ between two study streams?
4. Based on source-tracking analysis, does human or bovine fecal contamination have a greater presence in these two streams?

The following hypotheses are tested:

1. The first hypothesis tests for differences or correlations between the two streams and the variables *E.coli* and suspended sediment within the streams.
 - a. *E.coli* concentrations in Nails Creek differ from *E.coli* concentrations in Pistol Creek.
 - b. Suspended solid concentrations in Nails Creek differ from suspended solid concentrations in Pistol Creek.
 - c. There is a correlation between concentrations of suspended solids and *E.coli* in Nails Creek.
 - d. There is a correlation between concentrations of suspended solids and *E. coli* in Pistol Creek.
2. The second hypothesis tests whether discharge has an effect on the *E.coli*.
 - a. There is a difference in *E.coli* loads between low and high discharges in Nails Creek.

- b. There is difference in *E.coli* loads between low and high discharges in Pistol Creek.
- 3. The third hypothesis tests whether discharge has an effect on suspended sediment.
 - a. There is a difference in suspended solid loads between low and high discharges in Nails Creek.
 - b. There is a difference in suspended solid loads between low and high discharges in Pistol Creek.
- 4. Lastly, I tested whether either human-associated or bovine-associated *bacteroides* were more prevalent within each stream.
 - a. There will be a difference between the concentrations of human-associated *Bacteroides* and bovine-associated *Bacteroides* in Nails Creek.
 - b. The occurrence of Human-associated *Bacteroides* is not independent of the occurrence of bovine-associated *Bacteroides* in Pistol Creek.

The following three chapters provide detailed descriptions of the important elements in this study. Chapter two describes the physiography and the climate of the Little River watershed. Chapter Three reviews the available literature on bacteria and solids in streams and examines the roles these variables play in the environment, and Chapter Four describes in detail the methods and statistics used in this study. The final chapters are the culmination of the study: the results, discussion, conclusion, and suggestions for future research.

Chapter Two

Study Area

The Little River (HUC 06010201) is located in southeastern Tennessee, with its highest headwaters in the Great Smoky Mountains National Park (GSMNP) (Figure 1). The river drains approximately 929 km² (577 mi²) of upland elevations within parts of Knox, Blount, and Sevier Counties (TDEC, 2003). Within the Park, water quality is excellent because the area is not directly affected by the agricultural, residential, and commercial developments of the surrounding areas (Silsbee and Larson, 1982; Henry, 2005). In its upper reaches, the Little River is considered an ecoregion reference stream, a benchmark for assessing the health of other Blue Ridge Mountain watersheds, and it has been named an Outstanding National Resource (TVA, 2003).

2.1: Physiography and Geology

The Little River originates within the Blue Ridge physiographic province, Level III, ecoregion 66, crosses into the Valley and Ridge province, Level III, ecoregion 67, and flows northwestward into the Tennessee River as a perennial stream (Hart, 2006). Its watershed contains seven Level IV ecoregions: Southern Sedimentary Ridges, Limestone Valleys and Coves, Southern Metasedimentary Mountains, Southern Limestone/Dolomite Valleys and Low Rolling Hills, Southern Shale Valleys, Southern Sandstone Ridges, and Southern Dissected Ridges and Knobs (USEPA, 1987 and TDEC, 2005) (Figure 2).

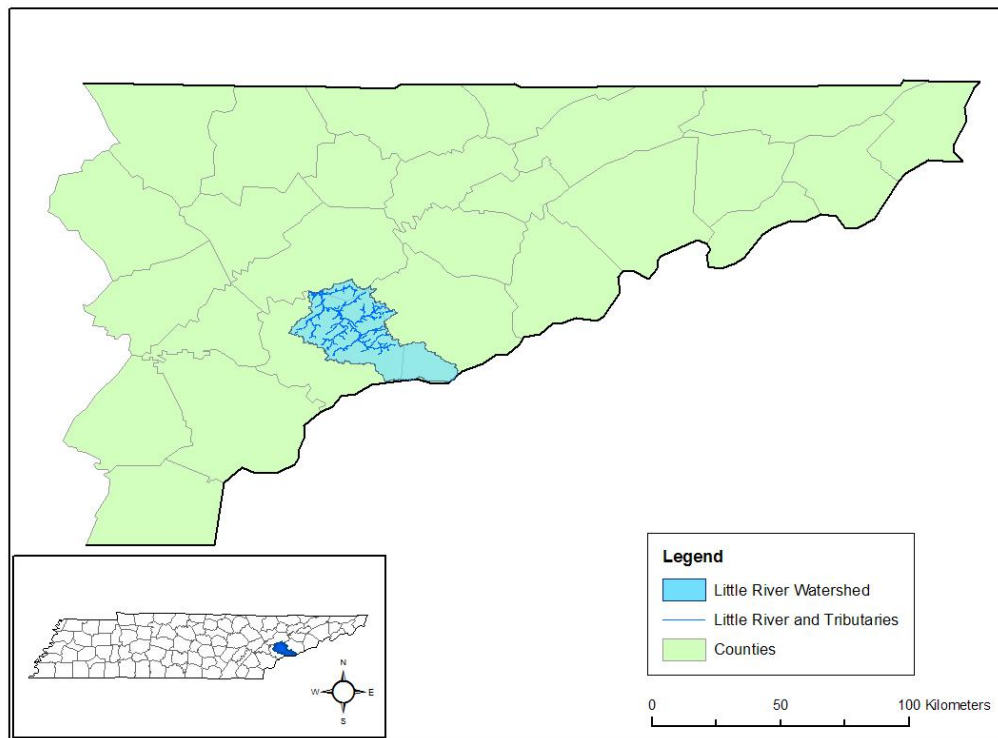


Figure 1: Location of the Little River Watershed in Eastern Tennessee.

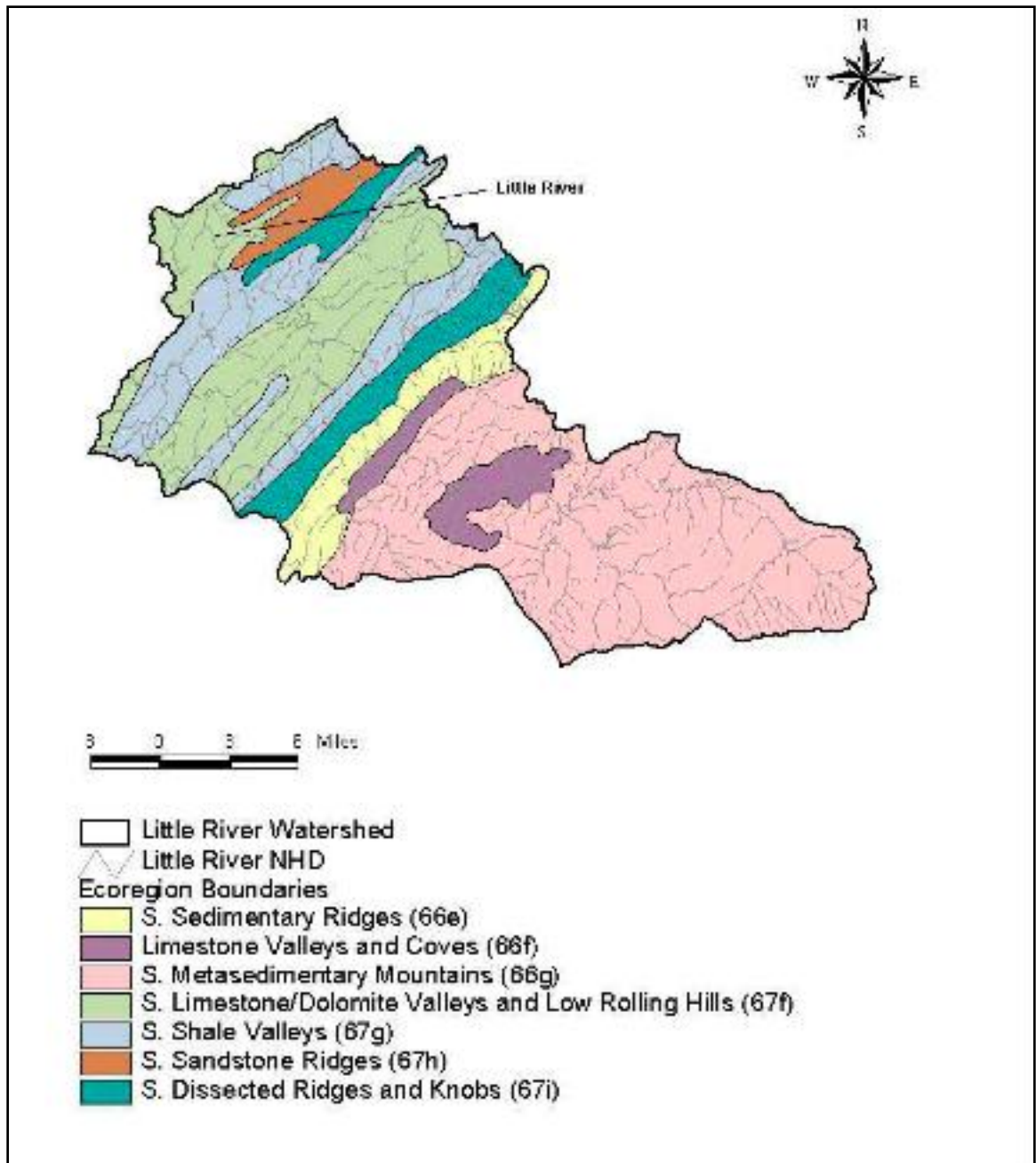


Figure 2: Level IV Ecoregions in the Little River Watershed (TDEC, 2005).

In the Southern Sedimentary Ridges, elevations range from approximately 305 to 1,372 m (1000-4500 ft) above sea level and slopes are steep. The rocks are primarily sedimentary, Cambrian-age, and are variable, with examples of shale, sandstone, conglomerate, siltstone, quartzite, and limestone (TDEC, 2005). Soils in the ecoregion tend to be friable loams and fine sandy loams containing sandstone rock fragments. The vegetation of the ecoregion generally consists of oak and oak-pine forests (TDEC, 2005). Within the Limestone Valleys and Coves ecoregion, elevations range from 457 to 762 m (1500-2500 ft) above sea level.

The Southern Metasedimentary Mountains (ecoregion 66g) are steep, and a subset of the Blue Ridge ecoregion (66). However, they are biologically different from the Blue Ridge Mountains. This ecoregion contains Clingmans Dome, the highest point in Tennessee at 2025 m or 6643 ft above sea level (National Park Service, 2008). Rocks are Precambrian in age and are predominantly metamorphic and sedimentary. The region is forested with Appalachian oak forests, northern hardwoods forests, and spruce-fir forests (TDEC, 2005). The Southern Limestone/Dolomite Valleys and Low Rolling Hills (67f) is a heterogeneous ecoregion of limestone and cherty dolomite. The topography consists of low valley and ridges, and natural vegetation is predominantly white oak forests and grassland barrens with cedar-pine glades (TDEC, 2005).

These ecoregions and their primary geologic assemblages affect certain characteristics of the Little River watershed. It is necessary to understand the geologic make-up of the watershed because the erodibility of the rocks can play a

role in the particle sizes of the suspended sediments transported within the stream (Black, 1991).

2.2: Climate and Soils

The climate in eastern Tennessee is relatively mild, with definite warm and cool seasons. Eastern Tennessee receives approximately 130 cm of rainfall per year and has, on average, 31 days above 32°C and 71 days below 0°C (National Weather Service, 2007). In Blount County, soils are predominately described as fairly deep, well-drained Inceptisols, originating from residual parent material (USDA, 1969; Hart, 2006). Soils vary throughout the Little River watershed; those that dominate the two study sites are classified in the Sequoia Litz Hamblen Series and are primarily composed of eroded silty clay and silty clay loams, shaley silty clay loams, and silt loams (USDA, 1969).

2.3: Land Use and Water Quality

Of the 1,029 km of streams (640 stream mi) within the Little River watershed, the Tennessee Department of Environment and Conservation (TDEC) has classified 354 km (220 stream mi; 34%) as impaired (USEPA 2006). The primary causes of impairment are bacteria, sediment, and habitat alteration, affecting 65%, 56%, and 34% of the 303(d)-listed streams respectively (TVA, 2008).

Designated uses of the Little River are irrigation, watering and wildlife, and recreation (TDEC, 2005). Recreational activities within the watershed, including fishing, kayaking, swimming, rafting and hiking, have grown in popularity in recent years. The Little River is not only heavily used for

recreational purposes, it is also a primary source of drinking water to local communities, and it supports many state and federally protected species (Henry, 2005). The two contaminants that are the foci of this study, bacteria and sediment, have important, negative effects on drinking water and recreation.

Currently, a number of organizations are working together to improve water quality in the Little River. The partnering organizations, headed by the Tennessee Valley Authority and the Blount County Soil Conservation District, include the U.S. Geological Survey, U.S. Fish and Wildlife Service, National Park Service, University of Tennessee, Environmental Protection Agency, and local partners such as Little River Watershed Association, Tennessee Izaak Walton League, Blount County Planning Commission the cities of Maryville and Alcoa, and citizens. Together, these groups have secured over \$3 million in grants for restoring this watershed (USEPA, 2008). Since the Little River was first identified on the 2004 303(d) list for not meeting WQS for its designated uses, numerous projects have been established to improve water quality. Results from the projects include 41 m (135 ft) of streambank restoration, and 4,726 m (15,508 ft) along streambanks of livestock-exclusion fencing created or improved (USEPA, 2008). Significant improvements have been made, but problems of suspended solids and high bacterial loads continue.

Nails Creek and Pistol Creek are two tributaries of the Little River considered high priority by the Tennessee Department of Environment and Conservation (TDEC) because of impairment by pathogens and siltation (TDEC 2005). Nails Creek has 39 km (24.5 stream mi) impaired and drains

rural/agricultural lands; Pistol Creek has 12.3 km (7.66 stream mi) impaired and drains a dominantly urban and residential watershed (TDEC, 2005). I chose to conduct this study in streams flowing through watersheds containing different land uses because numerous studies have demonstrated that surrounding landscapes and human/environment interactions affect the ecological status of streams (Ho and Tam, 1998; Young and Thackston, 1999; Shields et al., 2006). The pollutant sources in Pistol Creek, based on TDEC extrapolations from relationships determined in other stream locations, are expected to be urban runoff and/or storm sewers, while the primary source in Nails Creek is listed as pasture grazing (TDEC, 2004).

Land use varies within the watershed outside of the Great Smoky Mountains National Park. The Little River watershed contains urban and residential areas, including the towns and cities of Maryville, Alcoa, and Townsend. Much of the land within the watershed is used for agriculture or pasture. Urban and agricultural land uses are expected to negatively affect the condition of the river because of urban runoff, failing septic tanks, and increased agricultural practices (TVA, 2008). Approximately 370 km of the Little River and its tributaries are considered impaired on the EPA's 303(d) list, with bacteria and sediment being the main causes (Henry, 2005; TDEC, 2005). Nails Creek and Pistol Creek are high priority impaired streams, and the two, combined, have been found to have over 39 km impaired by *E. coli* contamination (TDEC, 2005). Understanding the relationships between *E. coli* and sediment loading within a stream should lead to better understanding of the hydrologic system and stream

morphology, and facilitate the restoration of streams in these and similar landscapes.

2.4. Site Selection

One of the important factors considered in this study is the effect of land use on the receiving streams. The two sampling sites, one on Nails Creek (NC1; Figure 3) and one on Pistol Creek (PC3; Figure 4), were chosen for this study based on a set of factors: previously determined water quality data, the presence of staff gages, and accessibility. Another factor in choosing these sites was the difference in land use surrounding the streams. PC3 is located within the greenway in Maryville and surrounded by urban or residential development. NC1, on the other hand, is predominately surrounded by agriculture, livestock or hay fields, and forested lands (Figure 5). The Nails Creek and Pistol Creek subwatersheds are similar in physiography and elevation. Nails and Pistol Creeks have similar soil assemblages, and both lie within similar ecoregions.



Figure 3: Nails Creek Sampling Site



Figure 4: Pistol Creek Sampling Site

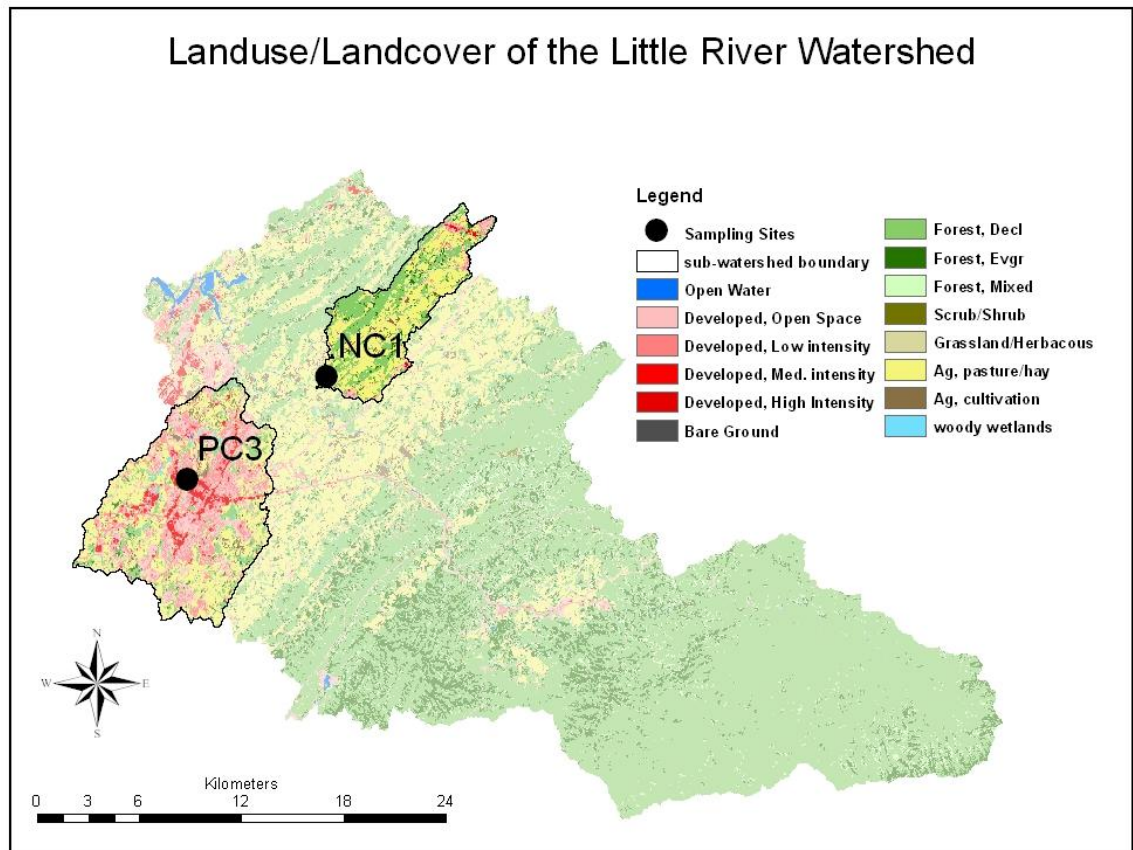


Figure 5: Land-use of Two Study Sites, NC1, near the Mouth of Nails Creek, and PC3, on Pistol Creek.

Another important factor in selecting the sites was the existence of data from prior studies. According to the TVA (2008) and Hart (2006), the four “priority” streams for assessing total suspended solids in the Little River system were Pistol Creek, Nails Creek, Ellejoy Creek, and Crooked Creek. These four streams are the leading contributors of sediment into the Little River. In previous sampling programs, from 2005 through 2007, high concentrations of total suspended solids (TSS) and of *E. coli*, surpassing Tennessee’s standards, were reported for both NC1 and PC3. Although the state of Tennessee has yet to

determine a limit for TSS, concentrations of TSS in sample means were found to be considerably higher than those at most other Little River sampling sites (TVA, 2008). Samples of 14 storm events at NC1 had extremely high concentrations of TSS, with a sample mean of approximately 5,000 mg/L and a maximum of 10,000 mg/L. Corresponding samples at PC3, had a sample mean TSS of about 400 mg/L, and a maximum of 10,000 mg/L.

The TVA collected and analyzed samples at base flow from tributaries in 2006-2007 and again found that Nails Creek (and Carr Creek) had the highest concentrations of sediments within the Little River watershed (TVA, 2008). It is important to consider the surrounding land uses when analyzing TSS concentrations. The Nails Creek watershed is predominantly rural, leading to a potentially greater susceptibility to erosion because cows in the fields trample and eat grasses that could otherwise have protected the soil, and agricultural land practices expose soil to rainfall. *E.coli* data from recent studies by the TVA have shown Nails and Pistol Creeks to have high concentrations of *E. coli* during low-flow periods. *E. coli* from samples taken at site NC1 have not only surpassed the geometric mean of 126 CFU/mg/L, but also exceeded the single grab sample standard of 941 CFU/mg/L (TVA, 2008).

The water quality of the two streams sampled in this study might be impacted by the various conditions and land-use patterns of the Little River watershed. For the purpose of this study, it is important to look at streams in different land-use regimes to understand possible differences in the in-stream relationships between *E. coli* and TSS.

Chapter Three

Review of Relevant Literature

This chapter reviews the available literature to provide background and highlight the importance of each of the variables in this study. It is essential to understand how suspended solids are transported throughout watersheds and how they degrade water quality, just as it is essential to understand where fecal bacteria come from and how they are used to indicate poor water quality. *E. coli* are not free-floating in a stream; they depend on a method of transportation to move downstream. Once fecal bacteria are carried into the stream, they may remain attached to suspended manure particles (if transported from farm land), become attached to soil particles, be trapped in soils and sediment, or be attached to subsurface solids (Pachepsky et al., 2006).

3.1: Water Quality

Section 303(d) of the Clean Water Act requires each state to list its waters for which effluent limitations are not rigorous enough to meet water quality standards, fulfill designated uses, or protect an applicable water quality standard (TDEC, 2006). The listed waters are then prioritized based upon their designated uses, and the state develops a total maximum daily load (TMDL) for each pollutant and each watershed (TDEC, 2006). TMDLs require information on the

source and the transport pathways of the pollutant (Surbeck et al., 2006). TMDLs define maximum allowable pollutant loadings of particular streams that will maintain water quality standards (WQS) and meet designated uses (USEPA, 1991). From there, the TMDL recommends regulatory actions to be taken to reduce pollution from both point and non-point sources and meet WQS (USEPA, 1991; TDEC, 2006).

3.2: Pathogens

Microorganisms are a key factor in the functionality of biotic systems, playing roles in decomposition, the nitrogen cycle, and other biogeochemical cycles (USEPA, 2001). A number of microorganisms are considered to be harmful if ingested into the human body. Microorganisms that cause sickness or even death are known as *pathogens*. Pathogens are a significant threat to those who use contaminated water sources because they are microscopic and can be transported easily in various ways, including by stormwater runoff and numerous other discharges (e.g., leaking septic tanks, agricultural runoff) (USEPA, 2001).

Once pathogens have entered a stream or other water body, they have the ability to transmit diseases to humans who have direct contact with the water, especially if that contact involves ingestion of the water itself (USEPA, 2001). In the U.S., current WQS are based on the intended use (i.e., drinking, irrigation, livestock watering, or recreational) (Jamieson et al., 2004). Many U.S. streams do not meet WQS because of pathogenic contamination, the most important criterion limiting the designated uses of recreation, public water supplies, aquifer protection, and biotic health (USEPA, 2001). Some strains of the primary

indicator microorganism used in this study, *E. coli*, can be pathogenic and, when ingested, can lead to *gastroenteritis*, causing vomiting and diarrhea (USEPA, 2001).

3.3: *Escherichia Coli*

Escherichia coli, or, *E.coli*, is a bacterium used as an indicator of fecal contamination and the potential presence of other disease-causing microorganisms in water. While it is generally non-pathogenic, it is commonly found in the feces of warm-blooded animals (USEPA, 2001). Therefore, high levels of *E. coli* in water indicate direct contact between the water and intestines, and suggest the additional presence of disease-causing bacteria, viruses, and protozoans (TVA, 2008). More than 150 pathogens, including *E. coli*, are found in manure from livestock. Common pathogens, including *E. coli*, cause a large percentage of food-borne and waterborne diseases in humans (Soupir et al., 2005).

The USEPA's *E. coli* standard for recreational freshwaters is that the geometric mean of at least five samples taken during a 30-day period must not surpass 126 *E. coli* 100 mL⁻¹, and no one sample may exceed 235 100 mL⁻¹ (Jamieson et al., 2004). However, the standard for the state of Tennessee is that *E. coli* concentrations in individual samples should not exceed 941 colony-forming units (CFU) per 100 mL⁻¹ and the geometric mean should not exceed 126 CFU/100 mL (TVA, 2008).

Details of the delivery process of *E.coli* into water sources have generated considerable research interest (National Research Council, 2001; USEPA, 2002a). In fact, an increasing amount of research is aimed at determining the influence of

watershed processes on sediment transport and fecal contamination of waterbodies (Gentry et al., 2006). This research focuses on surface water, which is more vulnerable than groundwater to fecal contamination from stormwater runoff because it lacks soil filtration (Kistemann et al., 2002). One study found that, within small streams, the amount of fecal coliform bacteria increases with suspended sediment loading (George et al., 2004). This is important because an increase in the amount of fecal coliform bacteria with an increase in suspended sediment loading would imply that both are transported by overland flow during storms and that agriculture has greater impact on fecal contamination than leaking septic systems. Or, an increase in both bacteria and suspended sediment on a low flow day might indicate that cows in the stream, stirring up sediment, are important contributors to stream water contamination. However, if bacteria were to increase without an increase of sediment, sewage or leaking septic systems would be assumed to be sources of contamination.

Understanding the spatial and temporal variability of *E.coli* in streams and relating patterns of *E.coli* concentration to sediment concentration are the main foci of this study. The amount of *E.coli* in streams has been linked to many hydrologic processes, such as stormwater runoff, seeps/springs, and turbidity (Gentry et al., 2006). Land use affects *E.coli* loading rates in streams; for example, runoff from livestock and poultry farms can be a major source of *E.coli* in streams (Soupir et al., 2005). Leaking septic tanks and sewer systems can be an important source of *E. coli* in streams in residential or urban areas (Young and

Thackston, 1999). Understanding the sources and transport mechanisms of *E. coli* is essential to the development of TMDLs.

To understand the rates and variability of *E.coli* loading in rural streams, one must first understand the many potential sources of *E. coli*. In rural areas, many different sources can contribute harmful pathogens into stream networks. Sources include agricultural runoff, cows or other animals in the streams, leaking or failed septic systems, wildlife, wastewater treatment plant discharges, and contaminated groundwater discharge, especially in limestone-rich areas. In agricultural areas, the main source of pathogenic contamination is fecal matter generated by livestock (Jamieson et al., 2004). This bacterial loading may be from point sources, such as storage facilities or feedlots, or from non-point sources, such as pastures or rangelands (Jamieson et al., 2004).

Bacterial loading should be studied at low flow, when concentrations are not diluted, and also at higher flows, when more surface runoff enters the stream. Differences in the amount and intensity of rainfall can create different situations for the transportation of *E. coli*. If rain generates overland flow, it can mobilize fine-textured soil particles and potentially high concentrations of microbial populations, which attach to the fines. Storm events that transport large amounts of sediment can mobilize and transport large quantities of pathogens (Hart, 2006). Therefore, the impacts of different flow conditions on suspended sediment and *E. coli* need to be considered in this study. The USEPA standard protocol of taking five samples in 30 days is generally implemented to avoid storms. Therefore, obtaining samples for *E. coli* in the Little River and its tributaries from a wider

range of flows would be an important contribution to knowledge of bacterial loading in streams.

3.4: Suspended Solids

Sediment, a primary component of suspended solids, is one of the most distinctive and yet complex characteristics of a river. The concentration of sediment is measured as sediment mass per unit volume of water (Kondolf and Piégay, 2003). Sedimentation is cited as a leading cause of stream impairment across the country. Sedimentation is the second largest cause of impairment in Tennessee, behind habitat alteration, with 22.5% (over 900 streams) of the rivers affected (USEPA, 2006). In-stream sediment can be measured as turbidity, suspended particles, or siltation, and is generally associated with land-disturbing activities, including development and agriculture (Bryan, 2000). Worldwide, sedimentation associated with soil erosion in the surrounding landscape has been primarily caused by deforestation and agriculture (Goudie, 2006). Most sediment is transported during storm events and carried in suspension, which is why it is important to sample in a variety of flow regimes (Lee et al., 2002).

Sediment and suspended solids are quite harmful in a stream because they damage fish and can destroy spawning habitats (Dunne and Leopold, 1978). In fact, sediment, whether in suspension in the water column or on the channel bed, can affect the behavior, health, and habitats of fish populations by damaging their gills and reducing visibility, which limits the ability to find food, and clogs spawning gravels (Ryan, 1991; Watts et al., 2003). Sediments may be contaminated with metals or chemicals and can directly affect aquatic organisms

by making sites uninhabitable for benthic organisms or by contaminating food, thereby contaminating the food chain. The latter ultimately affects human health (USEPA, 1987). Small particles, which stay suspended in streams, pose a threat to drinking water and recreational activities because they may adsorb trace metals and certain bacteria (Wilber and Hunter, 1977).

Turbidity is a measure of water clarity, classified by the ability of light to penetrate through the water (USEPA, 2002b). Higher turbidity inhibits the ability of sunlight to penetrate into the stream, and negatively affects photosynthetic processes and biodiversity in the stream. Turbidity can also affect water temperature and decomposition rates because it decreases available light (Davies-Colley et al., 1992). An increase in turbidity can also negatively affect recreational activities, such as fishing and swimming, two designated uses of the Little River Watershed (Rossi et al., 2006).

Sediment is transported in suspension or as bed load by streams and rivers. Suspended load, a main focus of this study, is carried in the flow via turbulence and generally transported for long distances before ever touching the river bed (Kondolf and Piégay, 2003). Most sediment is carried within the stream during peak or storm flows as a suspended load (Hart, 2006; Lee et al., 2002). Sediments become suspended when the vertical component of the turbulent velocity of the water is greater than the particle's settling speed (Roberts et al., 2003). When or where stream velocity decreases and the vertical component of velocity falls below the threshold for keeping particles of a given size and density in

suspension, sediments suspended within the stream settle onto the bed and greatly impact the substrate for fish, shellfish, and macroinvertebrates (Rossi et al., 2006).

Both suspended solids and *E.coli* are serious threats to water quality in the Little River. Studying water quality in two Little River tributary streams, Nails Creek and Pistol Creek, will add to current knowledge of transport mechanisms of these pollutants.

3.5: Stream Discharge

One of the most important factors influencing the mobilization and transportation of particulate and biotic water pollutants is stream discharge. An important question in this research is to understand how the two variables, TSS and *E. coli*, are affected by various flow regimes. Flow is one of the most important physical indicators of stream ecological condition (Shields et al., 2006). The five components of stream flow regimes are magnitude, frequency, duration, timing, and rate of change of hydrologic conditions (Richter et al., 1996; Poff et al., 1997; Shields et al., 2006). Storm flows increase the magnitude of runoff, which implies that storms also increase TSS and bacterial contamination in streams (Shields et al., 2006). It is therefore important to sample water at high flow.

Chapter Four

Research Methods

4.1: Field Work and Sample Collection

I took water samples for this study from August 2007 through February 2008 at two locations in Blount County, Tennessee. Nails Creek 1 (NC1), the sampling site in the more rural and agricultural of the two watersheds, is located downstream from the bridge at Andy Harris Road. Pistol Creek 3 (PC3), the site on the more urban stream, is located below the Bessemer Street Bridge. Both creeks are tributaries of the Little River and both are wadeable streams. For my field research, I visited both sites on the same day on 20 different sampling dates. I sampled for TSS using a depth-integrating sampler to gather water samples from the entire water column. For *E. coli* and *Bacteroides* I took grab samples. I used a YSI multiprobe meter to measure water quality parameters and a Marsh-McBirney flow meter to measure flow velocity.

At each visit to each site, I used a depth-integrating sampler (DH-59) to collect water in a 500-mL Nalgene bottle for analysis of total suspended solids (TSS). Depth-integrating samplers are frequently used in small, wadeable streams. The intake nozzle of the sampler is aimed into the oncoming flow. The sampler is designed so that the velocity at the nozzle intake equals the velocity of the sampled stream (Gordon et al., 2004). The sampler is slowly lowered down to the streambed and slowly brought to the surface to obtain water and suspended

sediment from the entire water column. Samples were promptly put on ice and taken back to the lab for processing. During each sampling trip I also collected one water sample at each site in a sterile, 100 mL Nalgene bottle to be tested for *E. coli* and sub-sampled for *Bacteroides*. Every fifth visit, I also obtained duplicate samples and blank samples for quality control and to ensure no outside sources of bacteria. These samples were collected by hand at the same locations. They were also promptly placed on ice and processed in the lab within six hours, as designated by the EPA (USEPA, 2000).

Once the samples were gathered, width, depth, and average velocity of each stream were measured. Sampling over a range of flow regimes during the four-month study period made it possible to relate the presence of *E. coli* and suspended sediment to different discharges.

Flow was measured using a Marsh-McBirney Model 2000 Flow Mate Current Meter. The meter is calibrated at zero velocity by being submerged in still water for approximately 20 minutes, and adjusting for zero if necessary (Marsh-McBirney, 1990). On each of the 20 days of fieldwork, the stream velocity was measured at the same cross-section of each stream.

To measure stream velocity, a tape measure was fixed across a particular cross-section on the stream. The width of the stream was then recorded, and divided into 10 increments of equal width. At each increment, three velocity readings were obtained, one every 30 seconds, with the flow meter positioned at 0.6 of the water depth. The median velocity for each increment was used in the final stream velocity calculation. The calculation is:

$$Q = \sum w_i d_i v_i \quad (\text{Equation 1})$$

Where w_i is the increment width, d_i is the depth for each increment, v_i is the median value of the velocity readings for that increment, and Q is the total discharge in cubic feet per second (cfs).

I used a YSI 556 MPS multi-probe meter to measure four water quality parameters—temperature, dissolved oxygen (DO), pH, and specific conductance—during every sample collection. Before leaving for the field each day, I calibrated the YSI meter. In the field, I placed the YSI probe in a flowing section of the stream upstream from the site of the velocity measurement. Water quality readings were recorded for each sampling date at each stream after at least 20 minutes. Finally, stream stage was also measured at a constant location at each site at a TVA-installed staff gage. This helped identify low, average, or high flow.

4.2: Lab Methods and Analysis

Two different laboratories were used in the processing of these samples. I processed *E. coli* in the University of Tennessee's Center for Environmental Biotechnology in the Science and Engineering Research Facility. In this lab, I analyzed *E. coli* samples according to the HACH Analytical Procedures for total coliforms and *E. coli*. For recreational use waters, the required sample size is 10-mL (HACH, 1999). I created triplicate 10 mL filtered samples for each field sample obtained. I processed the samples using the membrane filtration (simultaneous detection) method, which uses ColiBlue24 for the detection of at least 95% of all *E.coli* in the stream. The samples were filtered and added to the ColiBlue24 Broth using a sterilized Field Vacuum Support, syringe, sterile

forceps, membrane filter, funnels, and a Petri dish for each sample. The samples were then placed in an incubator set at 35°C for 24 hours. The resultant number of *E. coli* colonies on the filters was counted, and the sample placed back in the incubator for another 48 hours and recounted. I counted both red- and blue-stained colonies in each filtered sample. *E.coli* colonies appeared as blue spots, and total coliform was recorded as the total of red and blue colonies. Once I had counted the samples twice, I wrapped them, labeled them, and placed them in a walk-in-freezer. Later, I calculated Coliform concentration as density per membrane, using the equation:

$$\text{Coliform colonies per 100 mL} = (\text{coliform colonies counted/ mL of original sample filtered}) \times 100 \quad (\text{Equation 2})$$

To calculate *E. coli* loads, I used the following equation:

$$E.coli \text{ loads} = (\text{Coliform colonies per 100 mL} * 10) * Q \quad (\text{Equation 3})$$

in which multiplying the *E. coli* count by 10 converts the data from per 100 mL to per L; and in which Q is the discharge in liters per second.

In the Environmental Dynamics Lab in Burchfiel Geography Building, I analyzed samples for total suspended solids (TSS) according to standard methods (American Public Health Association, 1999). I used the following materials: 47 µm glass-fiber Millipore filters, aluminum weigh boats, glassware (1000 mL suction flask), Millipore vacuum filtration apparatus, tweezers, drying oven at 103-105°C, desiccators, and analytical balance.

For the TSS laboratory procedure, I first rinsed the filters with de-ionized water and suctioned the water from the filters. I dried the filters in a 103-105°C

oven until a constant weight was obtained, then I weighed them on the analytical balance. I placed one filter per sample on the vacuum apparatus. I hand-shook the samples to ensure thorough mixing, then measured a 50 mL sample from each of the 500 mL bottles, poured the sample on the filter, and applied suction. Each used filter was returned to the oven at 103-105°C for one hour to remove all moisture. I re-weighed the samples on the analytical balance. Finally, TSS, in g L^{-1} , was calculated by the following equation:

$$\text{TSS} = ([A-B] * 1000)/C \quad (\text{Equation 4})$$

where A is the oven-dried weight in g of the sediment-carrying filter, B is the dry weight (g) of the rinsed filter, and C is the volume of the filtered water sample (50 mL). Once the TSS analyses were completed, the bottles were returned to the refrigerator overnight so that particles would settle to the bottom. The next morning, clear water was decanted and discarded, and the remainder of each sample analyzed for loss-on-ignition (LOI).

LOI is a widely used method for estimating the organic content of sediments (Heiri et al., 2001). Organic material in a sample begins to burn off at 200°C, and is then completely ignited by approximately 550°C (Dean, 1974). The difference between the dry weight of the sample and the final, post-ignition weight of the sample represents the amount of organic matter in the sample. Therefore, the dry mass after ignition represents the inorganic suspended sediment content (Dean, 1974).

The first step in conducting LOI is to weigh crucibles on an analytical balance to determine their clean weight. I then poured the remaining sediment

from the sample bottles into individual crucibles, placed the crucibles into an oven pre-set at 105°C, removed them after one hour of oven-drying, and placed them in a desiccator to cool to room temperature. The dry samples were weighed on the analytical balance; their oven-dry weight is used as the basis for all LOI calculations (Dean, 1974). Next, the dried samples were placed in a muffle furnace at 550°C for one hour, cooled, and re-weighed. The following equation was used to calculate LOI:

$$LOI_{550} = ((DW_{105} - DW_{550}) / DW_{105}) * 100 \quad (\text{Equation 5})$$

where LOI_{550} represents LOI at 550°C. DW_{105} is the dry weight of the sample before combustion and DW_{550} is the dry weight of the sample after heating to 550°C (both in g; Heiri et al., 2001).

Another important analytical step was to organize the discharge data between high and low flows. I decided to categorize discharges into five different categories, based on a report from America's Clean Water Foundation (Cleland, 2003). Because Nails and Pistol Creeks are not regularly gaged, I created flow percentiles based on ten years of daily stream flow samples from a nearby USGS gaging station on the Little River in Maryville (03498500). I assumed that the discharge percentile for Pistol and Nails Creek would be the same as that in the Little River on the same date. Following Cleland (2003), I defined high flows as those exceeded 0-10% of the time, moist conditions as those exceeded 11-40% of the time, mid-range flows as those exceeded 41-60% of the time, dry conditions as those exceeded 61-90% of the time, and low flows as those exceeded 91-100%

of the time. Conducting this step allowed me to compare TSS and *E.coli* in the two streams at different flows.

4.3: Statistics

Data were entered into Microsoft Excel and then exported to the statistical analysis program SPSS. SPSS, the Statistical Package for the Social Sciences, provides a multifaceted means of working with numerical datasets (Clifford and Valentine, 2003). My first step was to create histograms of the data to illustrate their distributions. The histograms showed outliers in two variables, mean *E. coli* and TSS. I ran a one-sample Kolmogorov-Smirnov goodness-of-fit test to see if the data were normal. A goodness-of-fit test compares an observed frequency distribution with an expected frequency distribution to check the validity of the assumption of normality (McGrew and Monroe, 2000). I log-transformed data that did not meet the test for normality. Using log-normalized data, I ran a Pearson's correlation analysis to determine the presence and strength of relationships between different variables. For comparison, I ran a Kendall's tau-b correlation non-parametric tests on the original data. A correlation analysis measures the association between pairs of variables by statistically determining the strength and the directions between the variables (McGrew and Monroe, 2000). The pairs of data that I analyzed for correlation were (per site):

- *E.coli* concentrations and discharge
- *E.coli* load and discharge
- *E.coli* concentration and suspended sediment concentration
- Suspended sediment concentrations and discharge
- Suspended sediment loading and discharge

The results of these correlations are presented in the appendices.

To compare the same variable between the two streams (e.g., *E.coli* concentrations between Nails and Pistol Creek) I ran non-parametric tests to determine whether the samples came from the same distribution. I ran the Mann-Whitney-U test as well as the two-sample Kolmogorov-Smirnov test. Scatter diagrams of TSS and *E.coli* relationships within the two study streams are in the appendix.

4.4: *Bacteroides*

The analysis of *Bacteroides* is an important step in this project. Bacteria belonging to the genus *Bacteroides* are used as an alternative fecal indicator to *E.coli* and can be used to for water quality when determining health risks. *Bacteroides* can be used because they have a high degree of species specificity, make up a large portion of fecal bacteria, and have little potential for growth in the environment (Gentry et al., 2007). *Bacteroides* were analyzed using AllBac (for total fecal mass) and BoBac (for bovine-associated fecal mass) real-time polymerase chain reaction (PCR) assays developed by Layton et al. (2006a). The bacteria detection limit for the PCR assays is 1.0 mg/L. Any data falling below the detection limit are considered invalid.

To determine whether there was a significant difference between bovine-associated *Bacteroides* and human-associated *Bacteroides* in each stream, I froze two 1 mL capsules of each sample from all sampling dates. Later, Dr. Alice Layton performed source-tracking DNA analysis on *Bacteroides* in each sample. Once the data were obtained, I ran a Mann-Whitney-U test for Nails Creek and

planned to run a Chi-square test for Pistol Creek to determine whether a significant difference was present within each stream.

Chapter Five

Results

5.1: Goal and Hypotheses

The goal of this research was to determine what relationships existed among the variables *E.coli*, total suspended solids (TSS), and stream discharge in two low-order tributaries of the Little River in east Tennessee. The research tested the following hypotheses:

1. The first hypothesis is broken into four parts. These four parts test for the existence of differences or correlations between the two streams based on suspended solids and *E.coli*, and between suspended solids and *E.coli* within each stream.

a. H_0 : *E.coli* concentrations in Nails Creek do not differ from *E.coli* concentrations in Pistol Creek.

H_a : *E.coli* concentrations in Nails Creek differ from *E.coli* concentrations in Pistol Creek.

b. H_0 : Suspended solid concentrations in Nails Creek do not differ from suspended solid concentrations in Pistol Creek.

H_a : Suspended solid concentrations in Nails Creek differ from suspended solid concentrations in Pistol Creek.

c. H_0 : There is no correlation between concentrations of suspended solids and *E.coli* in Nails Creek.

H_a : There is a correlation between concentrations of suspended solids and *E.coli* in Nails Creek.

d. H_0 : There is no correlation between concentrations of suspended solids and *E. coli* in Pistol Creek.

H_a : There is a correlation between concentrations of suspended solids and *E. coli* in Pistol Creek.

2. The second hypothesis seeks to determine whether discharges, low or high, have an effect on *E.coli* within each stream.

a. H_0 : There is no difference in *E.coli* loads between low and high discharges in Nails Creek.

H_a : There is a difference in *E.coli* loads between low and high discharges in Nails Creek.

b. H_0 : There is no difference in *E.coli* loads between low and high discharges in Pistol Creek.

H_a : There is difference in *E.coli* loads between low and high discharges in Pistol Creek.

3. I also wanted to see if suspended solids were affected by discharge (low or high).

a. H_0 : There is no difference in suspended solid loads between low and high discharges in Nails Creek.

H_a : There is a difference in suspended solid loads between low and high discharges in Nails Creek.

b. H_0 : There is no difference in suspended solid loads between low and high discharges in Pistol Creek.

H_a : There is a difference in suspended solid loads between low and high discharges in Pistol Creek.

4. Finally, I wanted to determine which fecal bacteria (human-associated *Bacteroides* or bovine-associated *Bacteroides*) were more prevalent within each stream.

a. H_0 : There will be no difference between the concentrations of human-associated *Bacteroides* and bovine-associated *Bacteroides* in Nails Creek.

H_s : There will be a difference between the concentrations of human-associated *Bacteroides* and bovine-associated *Bacteroides* in Nails Creek.

b. H_0 : The occurrence of Human-associated *Bacteroides* is independent of the occurrence of bovine-associated *Bacteroides* in Pistol Creek.

H_a : The occurrence of Human-associated *Bacteroides* is not independent of the occurrence of bovine-associated *Bacteroides* in Pistol Creek

5.2: Data Analysis

5.2.1: Data Preparation

I collected data from August 2007 through February 2008. The first steps in data analysis were to calculate descriptive statistics of the variables, check to see that the data met expectations, and test for normality. Appendices to this thesis contain the raw data (Appendices I and II), descriptive statistics

(Appendices V and VI), and tests for normality of the distributions of variables in each stream (Appendix VIII).

An initial examination of the data showed two extremely high values that I considered suspicious. The first of these was a large TSS value in Nails Creek. This value did not correspond to a high TSS value in Pistol Creek on the same date, nor was it obtained at a time of high discharge or high turbidity in Nails Creek. The high value (3.0 mg/L, or 160.0 mg/s) is three times greater than the next highest TSS value in Nails Creek (1.0 mg/L, or 61.0 mg/s). This value may be an error, but, because of the varying nature of sediment in streams, as well as outside factors such as cow, dogs or other creatures (all witnessed in stream) disrupting the bed sediment, I am considering the point valid. The second unusually high value, which is very noticeable in Figure 7, is an extremely high discharge value for Pistol Creek during a very dry time of year, when the corresponding discharge of Nails Creek was very low. This datum may have been caused by regulation of the dam upstream of the site or perhaps by a local rainstorm. I have also considered this a valid point.

Quality control procedures for *E.coli* data required that I check the similarity of sample values with duplicate values and document that blank samples were free of *E.coli*. A table showing mean *E.coli* concentrations for all samples and duplicates as well as the blank counts is located in Appendix VII. All of the blank samples had zero *E. coli*, verifying that no outside bacteria had entered the samples.

Once I had collected and analyzed the raw data, I conducted correlation analyses to test for relationships among the parameters and Mann-Whitney U tests to determine whether the samples came from the same or different distributions of data. The raw data for loads and concentrations of TSS, *E.coli*, and discharge were not normally distributed. I ran parametric correlations on log-normalized data and non-parametric correlations on the raw data, and compared the results.

5.2.2: Discharge Data

One of the primary purposes of this study was to understand how different pollutants, specifically suspended solids and fecal bacteria, respond to various flow regimes. Nails and Pistol Creeks are not gaged, so I used information from the nearby USGS gage on the Little River at Maryville to view the broader patterns of discharge during the time of this study. Figure 6 illustrates the range of discharges in the Little River during the months August, 2007 through February, 2008, when the samples for this thesis were obtained in Nails and Pistol Creeks. Figure 7 shows discharge by flow-duration percentile for tributary sites NC1 and PC3 over the months in which I took water samples. Figure 7 shows that most of my samples were taken within the low flow conditions. Table 1 shows descriptive statistics for the low and high discharge groups for each stream.

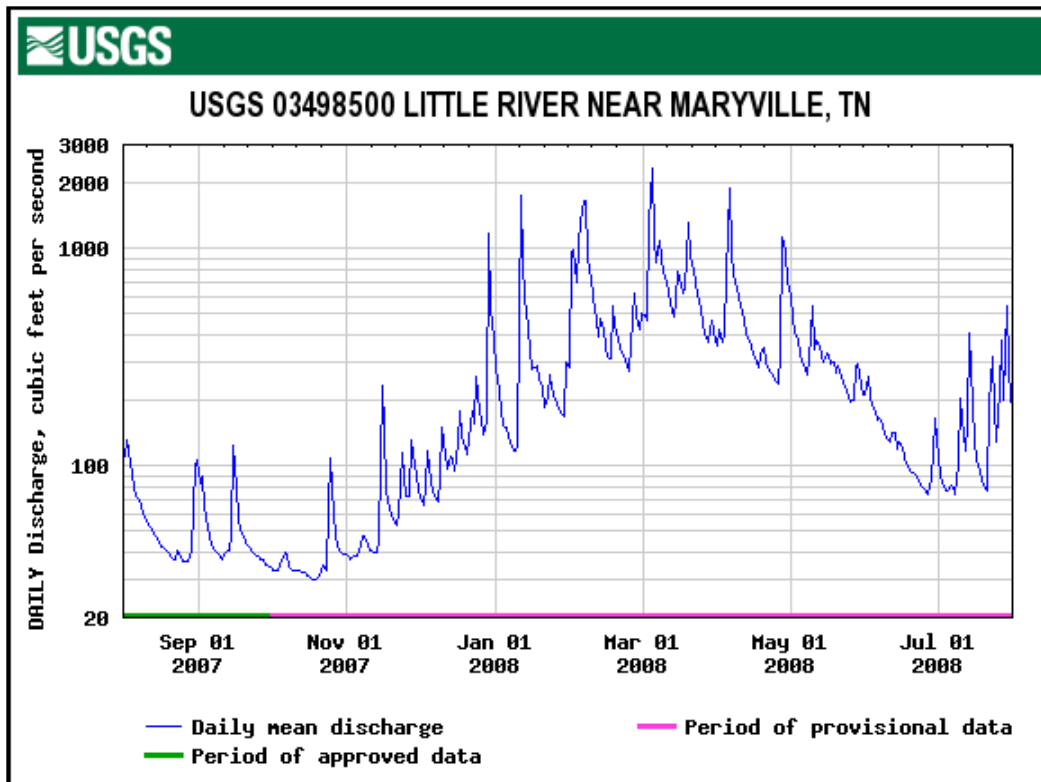


Figure 6: USGS Daily Discharge of the Little River at Maryville, TN.

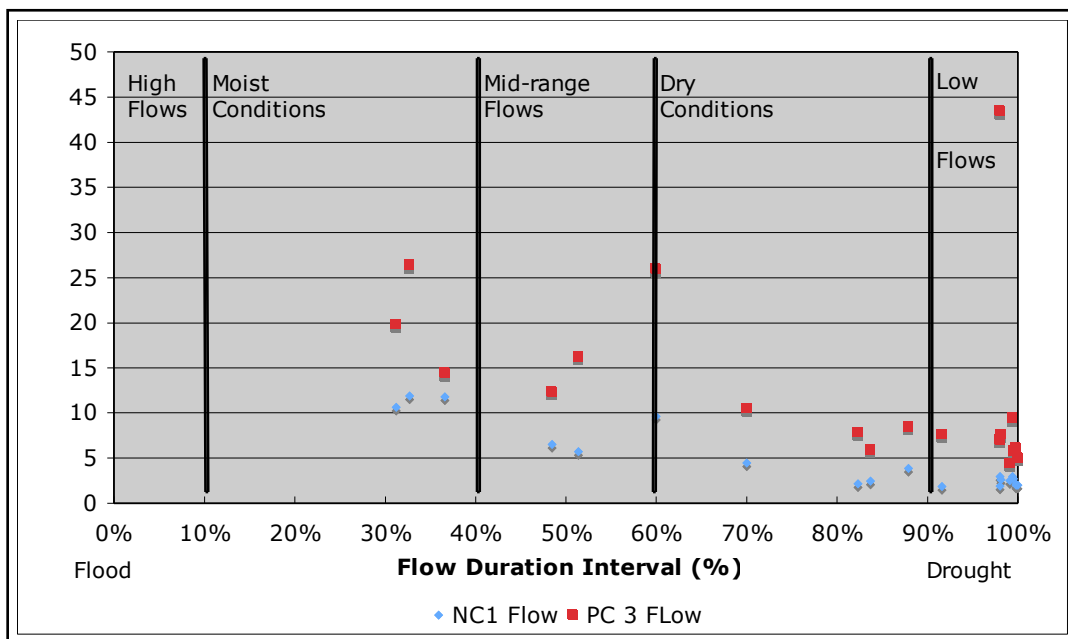


Figure 7: Discharges of Nails and Pistol Creeks Shown by Flow Conditions in the Little River at Maryville on the Same Dates (following method of Cleland, 2003).

Table 1: Descriptive Statistics for Low and High Discharge Groups in Nails Creek (NC) and Pistol Creek (PC)

Discharge (cfs)	Descriptive Statistics				
	N	Minimum	Maximum	Mean	Std. Deviation
NC Low Discharge	14	1.8	4.4	2.6	0.8
NC High Discharge	6	5.7	11.9	9.4	2.7
PC Low Discharge	14	4.4	43.5	9.6	9.9
PC High Discharge	6	12.3	26.4	19.2	6.0

One thing that immediately stands out in Figure 7 is one extremely high discharge in Pistol Creek, noted earlier as a suspicious data point. The extremely high Pistol Creek measurement was taken on September 9th, 2007, in a severe drought. Management of the dam upstream of the Pistol Creek site could have caused this high discharge in Pistol Creek when discharge in the Little River was low.

None of the discharges measured falls into the “high flows” (exceeded only 0-10% of the time) category, but about one-quarter of them had a flow duration interval less than 60%, meaning 60% or fewer of the daily discharges for the past 10 years surpassed these measurements. To characterize all flows as either low or high, I followed the example of Cleland (2003) but I split the data into two groups. I categorized discharges surpassed at least 60% of the time within the last 10 years as “low flow,” and less frequent discharges as “high.” In other words, I grouped the Cleland categories labeled “high flows,” “moist conditions,” and “mid-range flows” together as “high flow” conditions, and lumped “dry conditions” and “low flows” together as “low flow” conditions.

5.3: Hypothesis 1: Relationships between TSS and *E.coli* in and Between Streams

Alternative hypothesis 1a was that *E.coli* concentrations in Nails Creek differ from *E.coli* concentrations in Pistol Creek. Alternative hypothesis 1b was that suspended solid concentrations in Nails Creek differ from suspended solid concentrations in Pistol Creek. Medians of the two variables for each stream are shown in Table 2 and the data are graphed in Figures 8 and 9. Nails Creek had greater median concentrations of both *E. coli* and TSS and a much greater range of values. Furthermore, Nails Creek had a higher concentration of *E. coli* and of TSS in 19 of the 20 samples, or 95% of the time. Results of the Mann-Whitney U test, shown in Table 3, indicate that the null hypotheses for both 1a and 1b can be rejected.

Table 2: Medians of *E.coli* and TSS Concentrations in Both Sample Streams

	Nails Creek	Pistol Creek
Median <i>E.coli</i> (cfu/100mL)	715.8	251.7
Median TSS (mg/L)	90.0	20.0

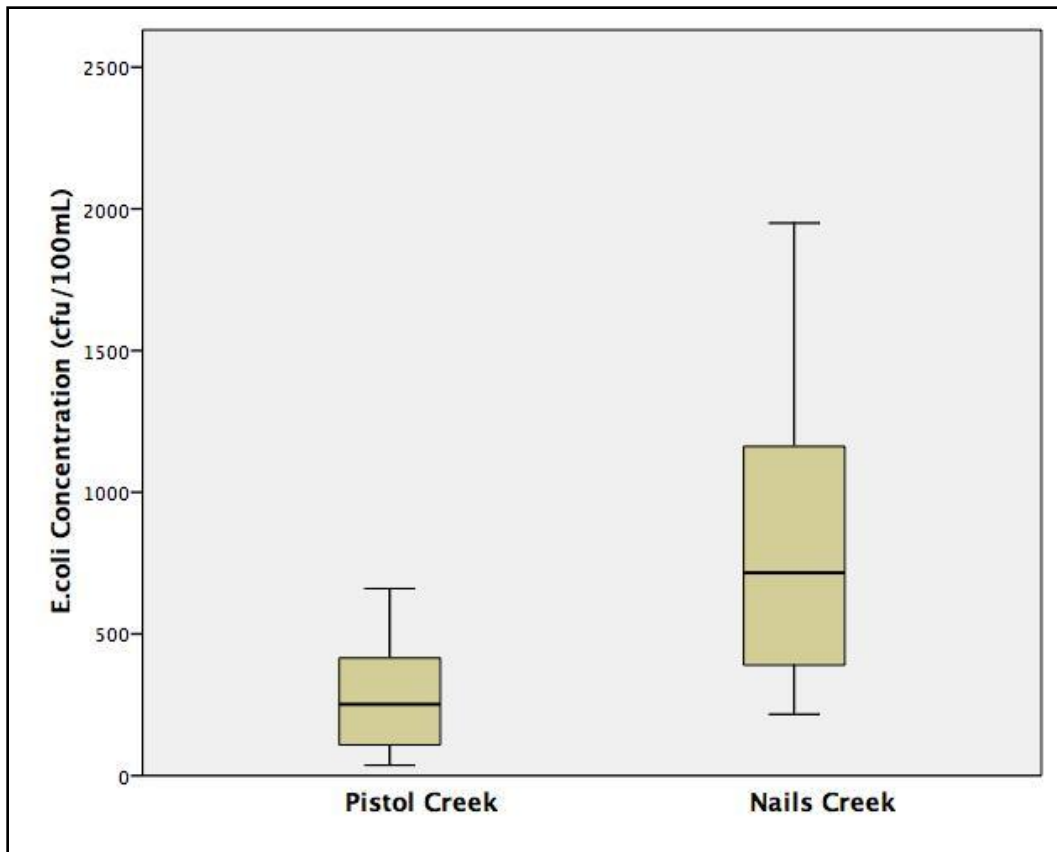


Figure 8: Box Plot of *E. coli* Concentrations in the Two Sample Streams.

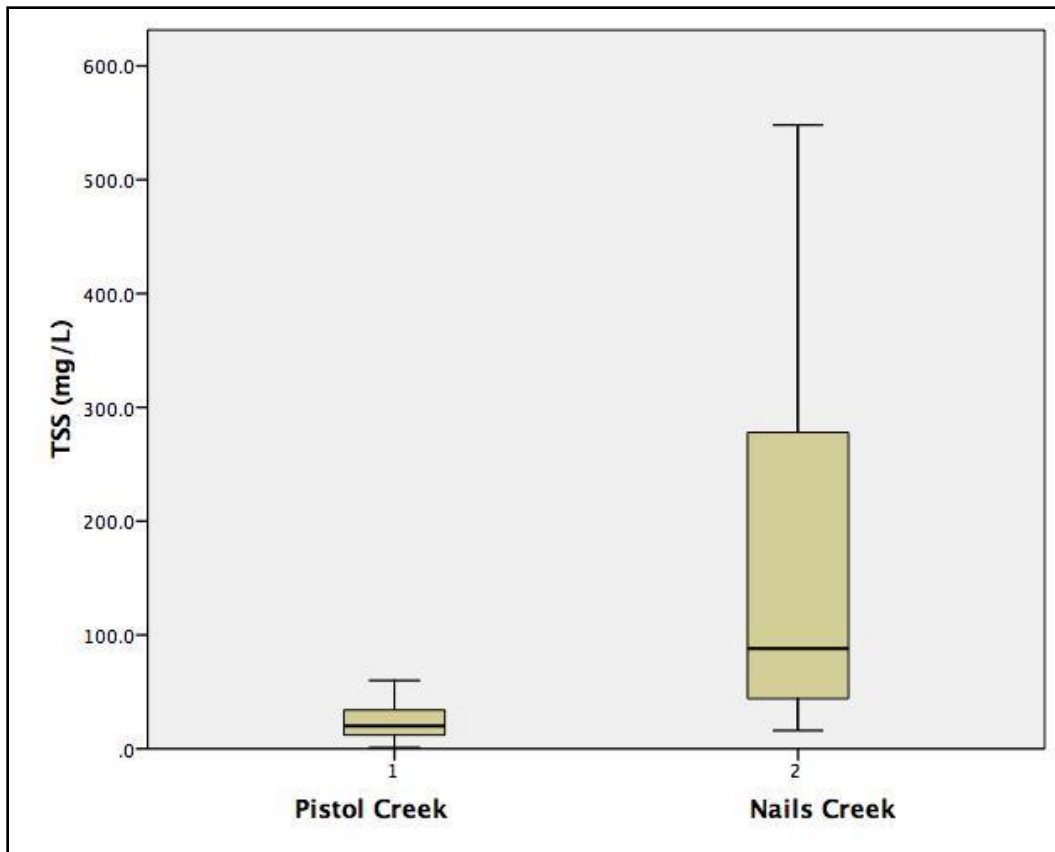


Figure 9: Box Plot of TSS Concentrations in the Two Sample Streams

Table 3: Mann-Whitney Tests of *E.coli* and TSS between Nails Creek and Pistol Creek.

Mann-Whitney Test		
	<i>E.coli</i> Concentration	TSS Concentration
Mann-Whitney U	73.5	45.0
Wilcoxon W	283.5	255.0
Z	-3.4	-4.2
Asymp. Sig. (2-tailed)	.001	.000

Alternative hypothesis 1c stated that concentrations of TSS and *E.coli* would be correlated in Nails Creek. Alternative hypothesis 1d made the same statement for Pistol Creek. Because the data were not normally distributed, I did this analysis in two ways. Tables 4 and 5 show Pearson correlation matrices of log-normalized data, and Tables 6 and 7 show the results of non-parametric (Kendall's tau) correlations of the original data, for comparison.

Correlation matrices for the parametric and non-parametric correlations show no significant correlations between concentrations of TSS or *E.coli* in Nails Creek. Therefore, the null hypothesis for 1b, that there are no correlations between *E.coli* and TSS in Nails Creek, cannot be rejected.

For Pistol Creek, neither the non-parametric correlation nor the log-transformed correlation shows a significant relationship between concentrations of TSS and of *E.coli*. Thus, for Pistol Creek, the null hypothesis cannot be rejected.

Table 4. Correlation Matrix for Nails Creek¹

Nails Creek Correlations:	logTSS mg/L	log<i>e.coli</i> cfu/100mL
logTSS(mg/L): Pearson Correlation Sig. (2-tailed) N	1.000 20	
Log<i>E. Coli</i> (cfu/100mL): Pearson Correlation Sig. (2-tailed) N	0.012 0.961 20	1.000 20

¹ Relationships of interest are shown in the non-shaded cells.

Table 5: Correlation Matrix for Pistol Creek²

Pistol Creek Correlations:	logTSS mg/L	loge.coli cfu/100mL
TSS Log: Pearson Correlation Sig. (2-tailed) N	1.000 20	
LogE. Coli Concentration Sig. (2-tailed) N	0.301 0.198 20	1.000 20

Table 6. Non-Parametric Correlation Matrix for Nails Creek²

Kendalls Tau_b	TSS (mg/L)	E.coli (cfu/100mL)
TSS(mg/L): Correlation Coefficient Sig. (2-tailed) N	1.000 20	
E. coli(cfu/100mL): Correlation Coefficient Sig. (2-tailed) N	0.095 0.559 20	1.000 20

Table 7. Non-Parametric Correlation Matrix for Pistol Creek²

Kendalls Tau_b	TSS (mg/L)	E.coli (cfu/100mL)
TSS(mg/L): Correlation Coefficient Sig. (2-tailed) N	1.000 20	
E. coli(cfu/100mL): Correlation Coefficient Sig. (2-tailed) N	0.200 0.227 20	1.000 20

² Relationships of interest are shown in the non-shaded cells.

5.4: Hypothesis 2: *E. coli* Loads at Different Flows for Each Stream

The second set of alternative hypotheses for this study was that there is a difference between *E. coli* loads at low discharges and *E. coli* loads at high discharges in Nails Creek and in Pistol Creek. Nails Creek has the highest maximum loads, but median *E. coli* loads are similar in both creeks. To test these hypotheses, I separated the data into two groups for each stream: *E. coli* loads at high flows and low flows. Figures 10 and 11 show box plots of *E. coli* loads in high and low discharges in each stream and Table 8 shows the descriptive statistics. I ran a Mann-Whitney U test for each stream to determine whether there was a difference in *E. coli* loads between the two discharge groups. Table 9 shows the results of the Mann-Whitney tests.

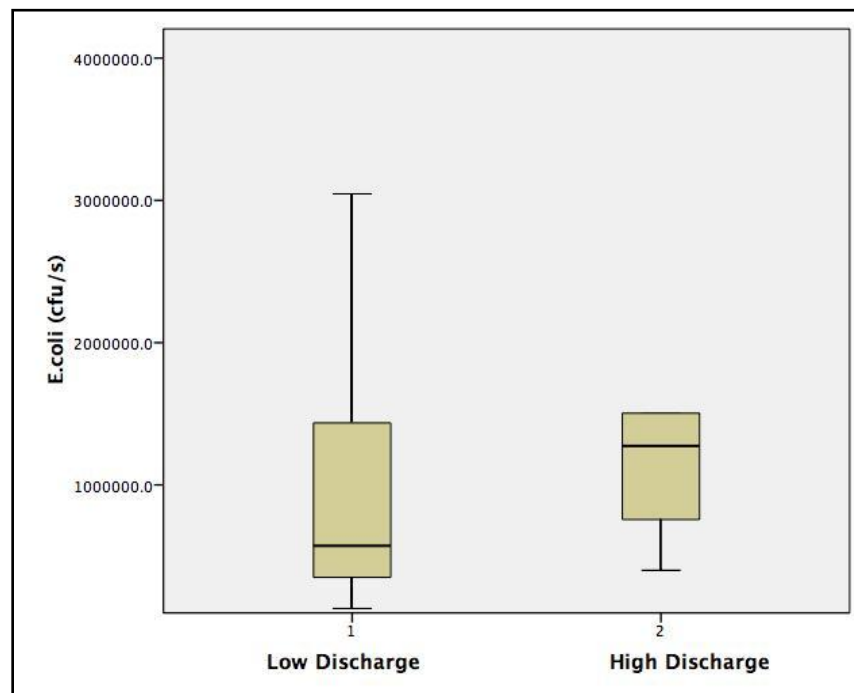


Figure 10: Box Plot of *E. coli* Loads in Nails Creek

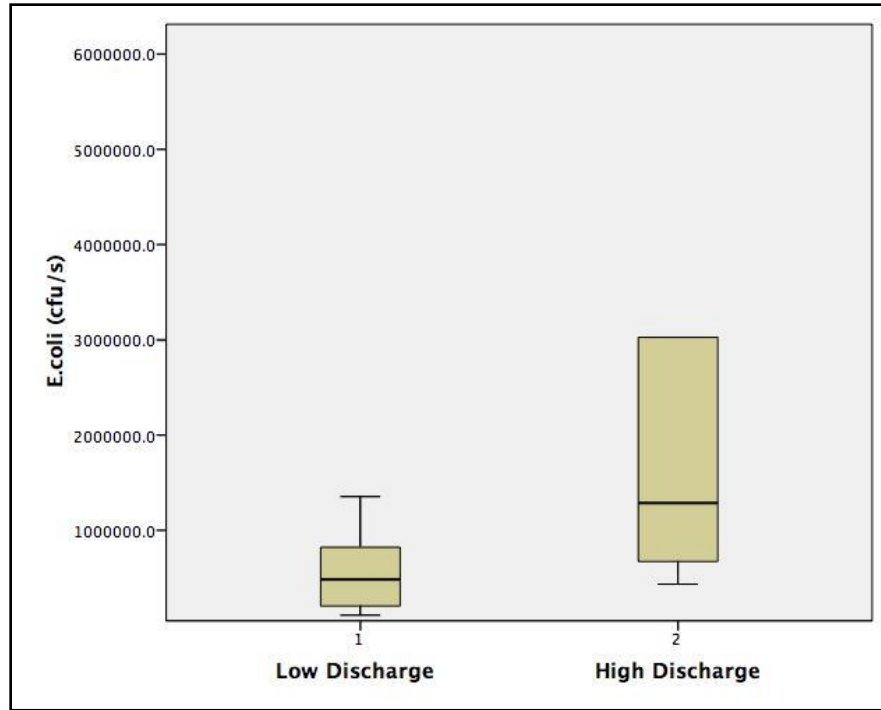


Figure 11: Box Plot of *E.coli* Loads in Pistol Creek.

Table 8: Descriptive Statistics for *E.coli* Loads (cfu/s) at High and Low Flows in Each Stream.

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
NC <i>E.coli</i> (Low Discharge)	14	1.31×10^5	3.04×10^6	8.74×10^5	8.20×10^5
NC <i>E.coli</i> (High Discharge)	6	4.00×10^5	3.28×10^7	6.34×10^6	1.29×10^7
PC <i>E.coli</i> (Low Discharge)	14	1.08×10^5	1.35×10^6	5.52×10^5	3.64×10^5
PC <i>E.coli</i> (High Discharge)	6	4.34×10^5	6.25×10^7	1.15×10^7	2.49×10^7

Table 9: Mann-Whitney Test for *E.coli* at High and Low Flows in Both Streams

	NC <i>E. coli</i> loads	PC <i>E. coli</i> loads
Mann-Whitney U	24	15
Wilcoxon W	129	120
Z	-1.485	-2.227
Asymp. Sig (2-tailed)	0.138	0.026

The Mann-Whitney test results indicate no significant difference in *E.coli* loads in Nails Creek between high or low discharges. Therefore, the null hypothesis is not rejected for Nails Creek. In Pistol Creek, *E.coli* loads at low flows differ significantly from *E.coli* loads at high flows. Thus, the null hypothesis is rejected for Pistol Creek.

5.5: Hypothesis 3: TSS Loads at Different Discharges

The third set of alternative hypotheses was that TSS loads at low discharges would differ from TSS loads at high discharges in each stream. To determine whether TSS loads in each stream differed between high and low discharges, I divided the samples based on the dates corresponding to the lower and higher discharges of the Little River at Maryville. Descriptive statistics for TSS at high and low flows for each stream are shown in Table 10. I created box plots for TSS at low and high discharges for each stream (Figures 12 and 13) and ran a Mann-Whitney-U test to compare the medians and distributions of the two sample groups to determine whether they differed. Results of the Mann-Whitney-U test (Table 11) indicate that, for Nails Creek, TSS loading at low flow did not differ significantly from TSS loading at high flow; the null hypothesis cannot be rejected. For Pistol Creek, the Mann-Whitney U test showed a significant difference between TSS loads at higher flows and lower flows; therefore, the null hypothesis for Pistol Creek can be rejected.

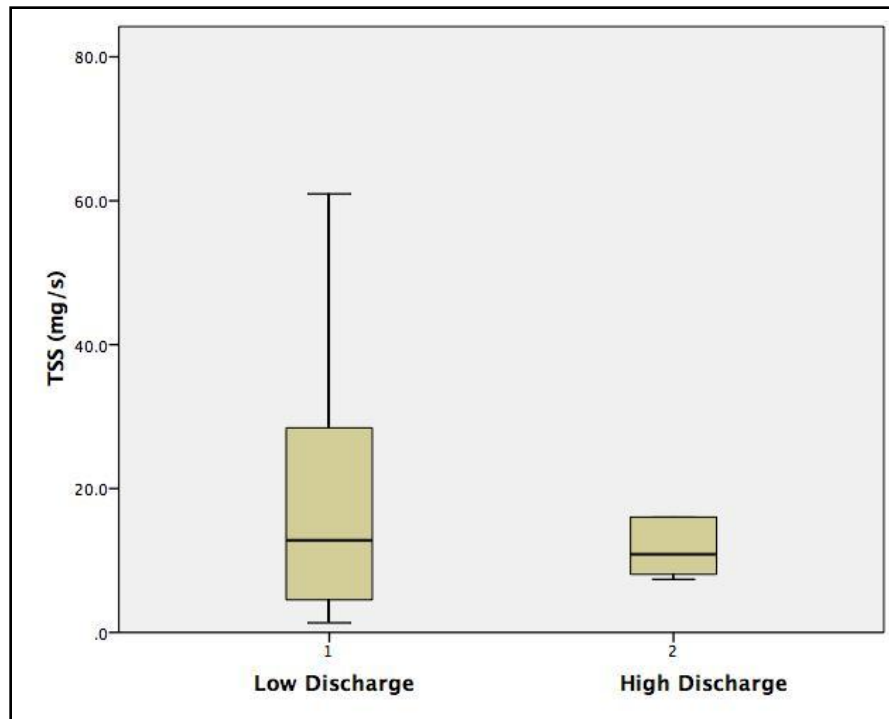


Figure 12: Box Plot of TSS Loads in Nails Creek at Different Discharges

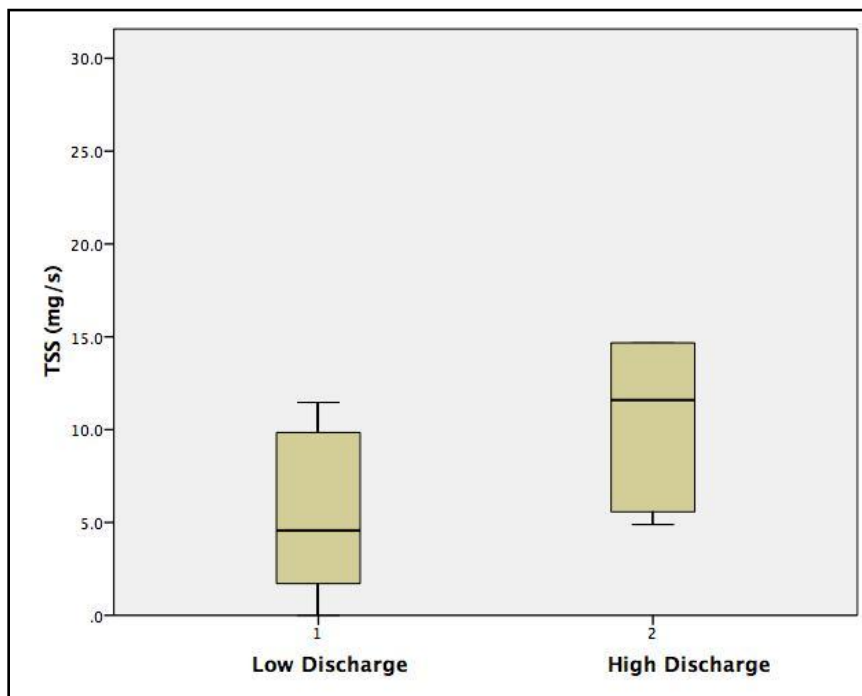


Figure 13: Box Plot of TSS Loads at High and Low Discharges in Pistol Creek.

Table 10: Descriptive Statistics for TSS Loads (mg/s) in Both Streams at High and Low Discharges

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
NC TSS Loads (Low Discharge)	14	1.3	160.0	27.5	41.9
NC TSS Loads (High Discharge)	6	7.4	37.1	15.1	11.2
PC TSS Loads (Low Discharge)	14	0	11.5	5.5	3.9
PC TSS Loads (High Discharge)	6	4.9	61.7	18.3	21.6

Table 11: Mann-Whitney Test for TSS at Different Discharges

	NC TSS Loads	PC TSS Loads
Mann-Whitney U	40	13
Wilcoxon W	145	118
Z	-0.165	-2.392
Asymp. Sig (2-tailed)	0.869	0.017

5.6: Loss on Ignition

One other factor I addressed in this study was to determine what percentage of the TSS was organic. Understanding this can help determine the source for the sediment. I used loss-on-ignition, as described in Chapter 4, to estimate the percentage (by mass) of organic matter in the suspended sediment samples. The percentage of organic material in Nails Creek is generally higher than the percentage in Pistol Creek. In Nails Creek, the percentage ranged from 0.08% to 4.5%. The percentage of organic material in Pistol Creek ranged from 0.09% to 0.6%. These low percentages indicate that most of the suspended material in both streams is inorganic. Three samples from Nails Creek had more

than 1% organic material, while the organic content of suspended sediment in Pistol Creek was always less than 1%. Each of the three times the organic content of suspended sediment in Nails Creek was greater than 1% was during low flow. The loss-on-ignition data are located in Appendices III and IV.

5.7: Hypothesis 4: Human and Bovine Sources of *Bacteroides*

The final research question sought to determine whether human- or bovine-associated fecal matter was more prevalent in each of the study streams. To answer that question, I used data provided by Dr. Layton, University of Tennessee, Center for Environmental Biotechnology, after she measured the concentrations of *Bacteroides* DNA from the water samples. I graphed human- and bovine-associated fecal bacteria against discharge for both study sites to visualize which source, human- or bovine-associated *Bacteroides*, appeared to dominate and whether the occurrence of *Bacteroides* was affected by discharge. I ran a Mann-Whitney-U test to see if concentrations of human- and bovine-associated *Bacteroides* differed significantly in Nails Creek. An alternate hypothesis was that there would be a significant difference between bovine- and human-associated *Bacteroides* in Nails Creek. For Pistol Creek, most of the *Bacteroides* in the samples were below the detection limit (below 1.0 mg/L). Because of this, I created a 2x2 table for comparison to show the occurrence (low or high) of human-associated *Bacteroides* versus the occurrence (low or high) of bovine-associated *Bacteroides*. I graphed human- and bovine-associated bacteria within the streams to illustrate their relationship with discharge in Figures 14 and 15. They show that Pistol Creek had much less bacteria than Nails Creek. Table

12 shows the results of the Mann-Whitney-U test (Nails Creek) and Table 13 shows comparison tables for Pistol Creek.

The Mann-Whitney-U test showed a significant difference between human- and bovine-associated *Bacteroides* in Nails Creek; therefore, I rejected the null hypothesis that there is no difference. In Pistol Creek, because I could not conduct a statistical analysis, no statement can be made regarding the null hypothesis, which addresses whether or not human-associated and bovine-associated *Bacteroides* occur independently.

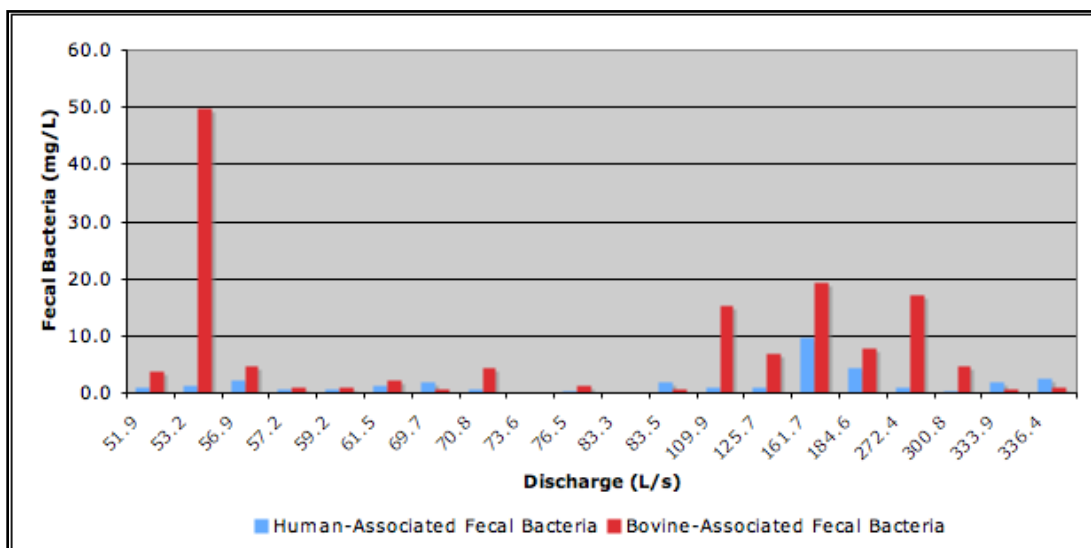


Figure 14: Human- and Bovine-Associated Bacteria in Nails Creek Compared to Discharge at Time of Sampling.

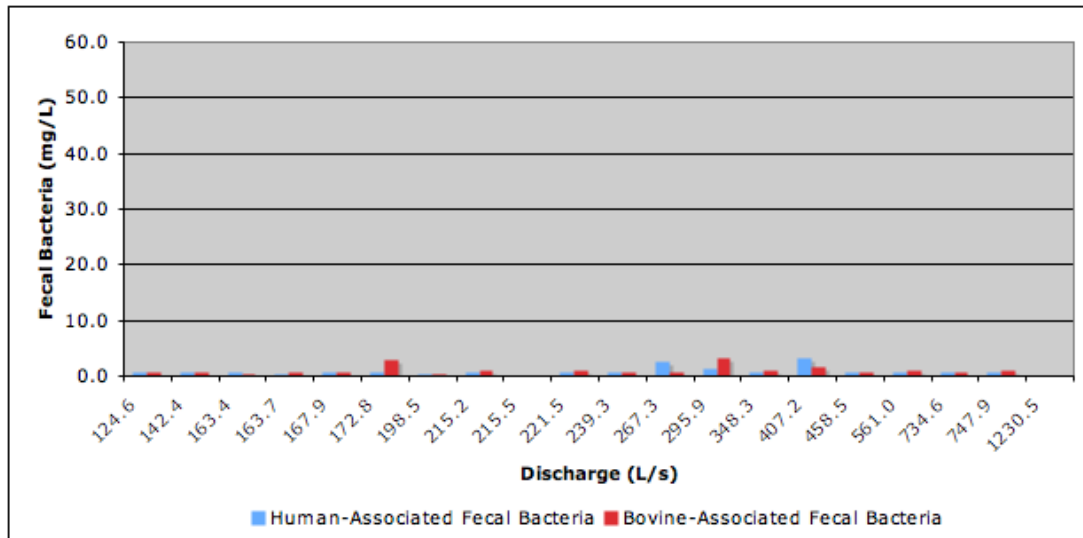


Figure 15: Human- and Bovine-Associated Bacteria in Pistol Creek Compared to Discharge at Time of Sampling.

Table12: Mann-Whitney-U Test of *Bacteroides* in Nails Creek

Test Statistics	
	NC <i>Bacteroides</i>
Mann-Whitney U	44
Wilcoxon W	135
Z	-2.29
Asymp. Sig. (2-tailed)	.022

Table 13: Comparison of Pistol Creek *Bacteroides*

Human and Bovine-Associated <i>Bacteroides</i>				
		Human-Associated	Bovine-Associated	Total
Low Occurrence	Count	1	2	3
High Occurrence	Count	2	2	4
Total	Count	3	4	7

5.8: Other Results

Although it was not one of the original hypotheses, I wanted to learn whether there was a difference between human-associated and bovine-associated *Bacteroides* at different flows. I divided the human-associated bacteria data between low and high discharge groups and the bovine-associated bacteria between low and high discharge groups. I ran a Mann-Whitney-U test for the human-associated *Bacteroides* data separated into low and high discharges as well as the bovine-associated *Bacteroides* data separated into low and high discharges in Nails Creek. Table 14 shows the results of the test after each variable was separated into the low and high discharge groups. In Pistol Creek, again because most of the data were below the detection limit, I created another 2x2 table to show the distribution of the *Bacteriodes* data over the two flows, as shown in Tables 15 and 16. For Nails Creek, the results indicate there are no significant differences in the occurrence of human- and bovine-associated *Bacteroides* at different discharges.

Table 14: Mann-Whitney-U Test for *Bacteroides* at different flows in Nails Creek.

Test Statistics		
	Human	Bovine
Mann-Whitney U	9.0	16.5
Wilcoxon W	45.0	61.5
Z	-1.621	-.801
Asymp. Sig. (2-tailed)	.105	.423

Table 15: Matrix of Human-associated *Bacteroides* and Discharge in Pistol Creek

Human –Associated Bacteroides				
		Low Discharge	High Discharge	Total
Low Occurrence	Count	1	0	1
High Occurrence	Count	1	1	2
Total	Count	2	1	3

Table 16: Matrix of Bovine-associated *Bacteroides* and Discharge in Pistol Creek

Bovine –Associated Bacteroides				
		Low Discharge	High Discharge	Total
Low Occurrence	Count	1	1	2
High Occurrence	Count	2	0	2
Total	Count	3	1	4

5.9: Summary

In summary, there was sufficient evidence to support the alternative hypotheses (1a, 1b) that *E.coli* and TSS concentrations were significantly different between Nails and Pistol Creek. Nails Creek water samples contained more *E.coli* and TSS than Pistol Creek. There was no correlation between *E.coli* and TSS concentrations or between *E.coli* and TSS loads in Nails Creek. There was not sufficient evidence to reject the null hypothesis that there was no correlation between *E.coli* and TSS in Pistol Creek. In Nails Creek, there was no significant difference in *E.coli* or TSS loads between low and high discharges.

There were significant differences in *E.coli* loads and TSS loads between low and high discharges in Pistol Creek. Finally, bovine-associated *Bacteroides* were significantly more prevalent than human-associated *Bacteriodes* in Nails Creek. No statistical tests were conducted between human-associated and bovine-associated *Bacteriodes* in Pistol Creek, where the occurrence of fecal bacteria was very low in all samples.

Chapter Six

Discussion and Conclusion

6.1: Discussion

My goal for this research was to determine whether relationships were present and, if so, the strengths of the relationships between the variables *E.coli* and suspended sediment in two tributary streams of the Little River, in east Tennessee. Hypotheses tested in this study were developed from expectations based on previous studies. I expected Nails Creek to have far more suspended solids and *E.coli* than Pistol Creek, based on a study conducted by the TVA that measured suspended sediment in rising state samplers in the Little River Watershed (TVA, 2008). The TVA concluded that Nails Creek had the highest mean of suspended solids of the tributaries in the Little River watershed; it also determined that Nails Creek had more *E.coli* than Pistol Creek. Previous studies have also suggested a strong relationship between TSS and *E.coli*. Based on the presumed need of *E.coli* to attach to particles for transportation, I expected to find a strong relationship between suspended solids and *E.coli*. My assumptions for *Bacteroides* data were based on land-use conditions. Because the Nails Creek site is directly downstream from a cattle farm, I expected bovine-associated fecal bacteria to be present much more often and in much greater quantities than human-associated fecal bacteria in Nails Creek. On the other hand, because Pistol Creek is located in an urban area, I expected human-associated fecal bacteria in Pistol Creek to be more prevalent than bovine-associated fecal bacteria. In this

chapter, I discuss the research questions I posed for this project and compare the results to those from previous studies.

6.1.1: Is There a Relationship between Concentrations of Suspended Solids and *E. coli*?

To determine whether a relationship existed between *E.coli* and suspended sediment concentrations, I posed a three-part hypothesis, testing first to determine whether concentrations and loads of suspended solids and *E.coli* differed between the two streams. The null hypothesis was rejected; there is significant evidence that the concentrations and loads of *E.coli* and TSS differed between the two streams. Nails Creek had far higher concentrations of *E.coli* and TSS than Pistol Creek and much lower flows than Pistol Creek. As the more rural of the two watersheds, the Nails Creek watershed contains more locations, particularly farms with cattle, which could contribute sediment and bacteria. One potential explanation for lower TSS and *E. coli* loads in Pistol Creek is that the Pistol Creek site is located downstream of a dam, which would be expected to control discharge and cause sediments and bacteria to settle out. This result is consistent with the outcome of the TVA (2008) study.

Secondly, I tested whether a correlation was present between suspended solids and *E.coli* within each stream. I found no significant relationship between *E.coli* and suspended sediment concentrations in Nails Creek. The results for Pistol Creek were inconclusive: one test detected a relationship while the other did not.

The lack of correlation between *E.coli* and TSS might be attributed to methods of transport in the stream other than piggybacking on suspended sediment. Another explanation may be that the amount of sediment required by *E.coli* must be a very small fraction of that measured as TSS. In Pistol Creek, which also showed no significant relationships between TSS and *E. coli*, the upstream dam may affect the relationship between TSS and *E. coli*. Obtaining more samples would help verify this finding and identify patterns of the two variables in relation to a wider spectrum of discharge regimes.

A strong relationship between suspended solids and *E.coli* had been assumed to exist at the start of this research because *E. coli* are understood to not be free-floating in streams. Bacteria are often associated with fine, cohesive sediment, such as silt and clay sized particles (Gannon et al., 1983; Auer and Niehaus, 1993; Jamison et al., 2005). Other studies have analyzed relationships between suspended solids and *E.coli* and have contributed different insights. For example, Fries et al. (2006) discovered that bacteria settle quickly to the bottom and are more likely attached to sediment after a heavy rainstorm. The study by Fries et al. (2006) demonstrated the importance of sampling during or soon after a storm event to measure the influx of bacteria from the rain event before the *E.coli* settle out. Similarly, Schillinger and Gammon (1985) discovered that bacteria are more likely to be attached to sediment in stormwater than at base flow. Mahler et al. (2000) discovered that when bacteria were present, attachment rates of bacteria to sediment vary from 5%–100%, or from almost none to all, in karst environments. The research sites on Nails and Pistol Creek are located within a

limestone-dominated landscape, so perhaps karst has an effect on bacterium-to-sediment attachment in the study area. One interesting result, obtained by Jamieson et al. (2005), was that the transport properties of the suspended solids would, for the most part, control the movement of bacteria attached to suspended solids. Therefore, a significant relationship between *E.coli* and suspended solids would depend on the mobility of the suspended solids within the stream. The absence of the expected relationship between suspended solids and *E.coli* in this thesis research supports recent findings by other researchers regarding the complexity of the *E.coli* and sediment relationship in stream water.

I was able to obtain one set of samples during a storm. The storm event clearly stands out in the discharge, TSS, and *E.coli* data. This is an important sample point because storm event data are scarce. The storm event samples have much higher concentrations of *E.coli* and TSS than samples collected at base flows. Even though it is much more challenging to gather storm data, these data indicate that concentrations of suspended solids and fecal bacteria transported during storm events are orders of magnitude higher than those at base flow. Correlation analyses run with and without data from the storm event, however, produced the same outcomes of no significant relationships between TSS and *E.coli*.

Two other data points were considered suspicious, and correlation analyses were run with and without these points. One was a very high discharge value in Pistol Creek during a very dry time of year. The other was a very high TSS value in Nails Creek that did not correspond to high turbidity or a high TSS

value in Pistol Creek. The high discharge could have been caused by management of the upstream dam, or possibly by a local shower. The very high TSS value could have been caused by a number of different sources disrupting the sediment, such as cows or dogs in the stream. Outcomes of correlation analyses were not sensitive to these suspicious points.

6.1.2 Do Patterns of Discharge Affect *E. coli* Loads within the Two Study Streams?

The second research question addressed whether loads of *E.coli* differed between high and low discharges. In Nails Creek, there was no significant difference in *E.coli* loads between low or high discharges, suggesting that, in Nails Creek, *E.coli* are flow-independent. In Pistol Creek, there was a significant difference between loads of *E.coli* at low and high discharges. Higher TSS values corresponded with higher discharge values, suggesting that *E.coli* may be flow dependent in Pistol Creek.

In 2000, the Little River was designated a “threatened stream,” and by 2004 the EPA had placed segments of the river on its 303(d) list for not meeting Water Quality Standards. Since then, many initiatives have sprung up to monitor and restore the river to a healthy state. The data from this research help show the conditions during the sample year, and provide additional data for evaluating the effects of efforts to improve water quality. In late summer, 2006, Dr. Alice Layton sampled site PC3 five times and calculated the geometric mean of the *E.coli* concentrations (Layton, 2006). Dr Layton also sampled NC1 for *E.coli* in

1998, 2003, and 2006. Comparing these results to my data (Tables 20 and 21), *E.coli* loads appear to have increased in Pistol Creek since 2006.

For Nails Creek, the 1998 and 2003 sample means were calculated from only three samples. The concentrations of *E.coli* in Nails Creek decreased between 2006 and 2007/2008. This may be attributed to the drought in the summer of 2007, to restoration initiatives in the Little River Watershed, or to the season in which the samples were taken. Higher concentrations of *E.coli* are more commonly found in the summer. During the fall and winter months, more precipitation falls, possibly creating a dilution effect, and cattle in areas surrounding the stream are less active or less prevalent. The mean of the 2007/2008 data includes *E.coli* measured in the winter, which could decrease the geometric mean. Although these small datasets are too small to provide concrete evidence that the presence of *E.coli* in the stream has changed over the various testing years, this thesis research provides new data documenting *E.coli* patterns over a span of months. This study of *E.coli* and TSS over a period of several months can serve as a foundation for future studies that continue to determine the impact of seasonality on the two variables and their varying relationships with each other.

Table 17: Comparison of Mean *E.coli* Concentrations in Pistol Creek, 2006-2008

Pistol Creek	Summer 2006 (n=5)	Summer 2007-Winter 2008 (n=20)
<i>E.coli</i> Geometric Mean (Cfu/100mL)	504	246.2

Data sources: Summer 2006: Layton, 2006b; 2007-2008: this thesis research

Table 18: Comparison of Mean *E.coli* Concentrations in Nails Creek, 1998, 2003, 2006-2008.

Nails Creek	1998 (n=3)	2003 (n=3)	Summer 2006 (n=5)	Summer 2007– Winter 2008 (n=20)
<i>E.coli</i> Geometric Mean (Cfu/100mL)	731	884	1937	796.8

Data sources: 1998, 2003, and 2006: Layton, 2006b; 2007-2008: this thesis research

6.1.3: Do Sediment Loads in These Streams Change with Different Patterns of Discharge?

My third question asked whether TSS loads differed significantly between low and high discharges. There was no significant difference between TSS loads at different discharges in Nails Creek, but there was a significant difference between TSS loads at different discharges in Pistol Creek.

Flow independence of TSS and *E.coli* in Nails Creek indicates that both bacteria and suspended solids may have alternative ways to enter the stream rather than in overland flow from storm events. A similar study, conducted in Pennsylvania found no significant difference between TSS and discharge (Carline and Walsh, 2007). Their study, like this thesis research, was also conducted during a drought year, which may have had an influence on the transport of suspended solids. Cows in the streams and alongside the stream banks might contribute an equal amount or even more solids and fecal bacteria into the stream than runoff from rainstorms. In Pistol Creek, both TSS and *E.coli* loads were significantly different at different discharges, indicating that both contaminants may be flow dependent. Sampling in this research was conducted during a drought year, with only one storm sample, but six of the 20 samples did fall into the “high flow” category. Ellison and Brett (2006), in a study conducted in

Washington, found that flow state (high or low flow) had the greatest impact on suspended solids and that base or low discharges contained the highest concentrations of TSS. These results differ from the results I obtained in Nails Creek, where flow appeared to have no effect on TSS. Ellison and Brett's study found dilution to be a controlling factor for TSS concentrations, but my data appear to suggest a more complicated set of factors.

Because suspended solids have been cited as one of the leading causes of pollution in the Little River Watershed, it is therefore necessary to compare the results obtained in this thesis research with those of previous studies. It is important to note that samples from earlier studies were obtained during storm flows. Data from 2003-2004 (Hart, 2006) and from 2006 (TVA, 2008) are from samples obtained during rising stages of flow. Tables 22 and 23 show the comparison.

Table 19: Comparison of Mean TSS Concentrations in Pistol Creek, 2006-2008

Pistol Creek	2003-2004 (n=28)	2006 (n=14)	Summer 2007-Winter 2008 (n=20)
TSS Geometric Mean (mg/L)	136	400	17.1

Data sources: 2003-2004: Hart, 2006; 2006: TVA, 2008; 2007-2008: this thesis research

Table 20: Comparison of Mean TSS Concentrations in Nails Creek, 2006-2008

Nails Creek	2003- 2004 (n=28)	2006 (n=14)	Summer 2007- Winter 2008 (n=20)	Storm Sample Fall 2007 (n=1)
TSS Geometric Mean (mg/L)	1696	5000	100.6	2296

Data sources: 2003-2004: Hart, 2006; 2006: TVA, 2008; 2007-2008: this thesis research

6.1.4: Based on Source-Tracking Analysis, Do Human or Bovine Bacteria Have a Greater Impact on These Two Streams?

My final research question addressed which source of fecal bacteria (human or bovine) had a greater presence in the two streams. The *Bacteroides* analysis proved interesting and useful. In Pistol Creek, very low amounts of human and bovine-associated fecal bacteria were present. In the few samples with detectable Bacteriodes, neither contributing factor (human-associated nor bovine-associated) appeared to dominate. For Pistol Creek to have similar amounts of both human and bovine bacteria, one would assume that bovine-associated *E.coli* must have the capacity to travel to the PC3 site, which is downstream from the city of Maryville. The closest farm with cattle is approximately 5 km upstream from the PC3 site. The presence of bovine-associated Bacteriodes at PC3 indicates that more sampling should be conducted upstream from the city to better assess the contribution of bovine-associated bacteria in Pistol Creek. One study using *Bacteroides* analysis (Layton et al., 2006) determined that relationships between *Bacteroides* and factors such as turbidity, *E.coli*, and flow are spatially dependent within a stream. Therefore, more measurements throughout both Nails and Pistol Creeks are necessary to validate the findings of this thesis.

The presence of human-associated fecal bacteria differed significantly from the presence of bovine-associated fecal bacteria in Nails Creek. The total concentration attributable to human feces was 33.1 mg/L, while the total concentration of bovine feces was 141.4 mg/L. The much larger presence of bovine-associated fecal bacteria was expected because the research site on Nails

Creek is located directly downstream from a farm, and Nails Creek and its tributaries flow through other pastures containing livestock, too. Cows in the stream may contribute large quantities of bovine bacteria. I often witnessed cows in the stream near or in the Nails Creek site during or right before sampling.

In Pistol Creek, I had wanted to test whether there was a significant difference between the amount of human-associated and bovine-associated fecal bacteria; however, because most of the results were below the detection limit, I could make no definitive statements. More data from more samples will be required to allow researchers to determine whether the occurrence of human- and bovine-associated fecal bacteria differs significantly and whether low or high flow has a significant effect on *Bacteroides* in Pistol Creek.

6.2: Further Discussion

The two sampling sites in this study were chosen for the differences in the land-use characteristics of their contributing areas. I wondered whether either predominant land use, agriculture or urban, would affect suspended sediment and *E. coli* concentrations. I found that these streams contained varying amounts of TSS and *E.coli* and differed from one another. Discharge at the Nails Creek site was consistently less than the discharge at the Pistol Creek site, yet samples from Nails Creek had far higher concentrations and greater loads of suspended sediments and *E.coli*. Differences in concentrations of TSS and *E.coli* may reflect differences in land use, but differences in the surrounding land uses of the two streams may be just one of many possible factors explaining why these two streams are so different.

Because this research produced a small database (two sampling sites with 20 samples each), I can make only preliminary inferences regarding how the variables, bacteria and suspended solids, are affected by discharge or by each other. With more samples over a longer period of time, significant correlations might emerge. This study provides a small window into how suspended solids and *E. coli* interact within the Little River watershed. More samples should be collected over multiple years and through a spectrum of discharges to better understand the relationship between bacteria and sediment in a stream. One study concluded that the presence of *E.coli* within a stream at base flow is influenced by the stream's capability to flush water through after a storm (higher *E.coli* presence in faster-draining sub-basins) (Gentry et al., 2006). Therefore, for future studies, it will be extremely important to gather data before, during, and after storm events to understand how bacteria and suspended solids react to various stages in water level and discharge.

6.3: Conclusions

I undertook this research to investigate whether a relationship was present between *E. coli* and total suspended solid concentrations and loads in two tributaries of the Little River. I also wanted to determine how the loads and concentrations of the two variables related to or were affected by stream discharge. Would they increase as discharge increased or would they be diluted with the influx of more water? I chose to study suspended solids and bacteria because they are two of the primary causes for listing the Little River as a threatened stream on the EPA 303(d) list (TDEC, 2008). I hypothesized that

relationships between *E. coli* and suspended solid concentrations would differ between the two streams. I suspected this because of the differences in land use within the watersheds. I chose to study relationships between TSS and *E.coli* to test the assumption that, because bacteria must attach to something, concentrations and loads of bacteria would be related to those of TSS.

After collecting 20 samples from each site, conducting the necessary lab work, and analyzing the data, I found no significant correlation between concentrations and loads of *E.coli* and TSS in either stream. Nails Creek has more *E.coli*, more TSS, and a lower discharge than Pistol Creek. Discharge has more influence on TSS and *E.coli* in Pistol Creek than in Nails Creek. In Pistol Creek, on average, higher amounts of TSS and of *E.coli* were measured during higher flows, which not only indicates a need for sediment control practices in developing as well in agricultural areas, but also for inspections of sewer systems that may be backing up or leaking during high flow conditions.

The results of this thesis contribute new data to the small but growing body of information available on the water quality of tributaries of the Little River and add to existing knowledge of *E.coli* and TSS in Little River tributary streams, especially in winter months. Results obtained in this study do not support all of the hypotheses, which were based on information from previous studies. Given the small dataset and the fact that the research was conducted in an unusually dry year, it remains possible that studies conducted over a longer period of time might find a direct relationship between suspended sediment and *E.coli* in the streams. Future research should include analysis of more peak flow samples and further

examination of spatial and temporal differences between *E.coli* and TSS concentrations.

The results of this study have important implications for individuals and agencies in positions to influence land management and water quality improvement in these watersheds. One of the primary results of this study is that *E.coli* and bovine-associated *Bacteroides* are prevalent at both low and high flows in Nails Creek, which implies that storm flows are not the primary source for bacteria entering the stream; farmers in the Nails Creek watershed should limit or restrict cows from accessing the stream. In Pistol Creek, high or storm flows carried high levels of TSS and *E.coli*, and both bovine-associated and human-associated fecal bacteria were found in the stream. These results suggest a need for more attention to potential sources of bacteria and sediment, as well as strategic sampling above and below the potential sources in storm conditions to identify contributing locations.

Within both Pistol and Nails Creek, high concentrations of TSS were found in both low and high flows and in no particular season. More attention should also be given to residential development in the areas surrounding all streams within the Little River Watershed to ensure that land management practices are put into place. Finally, high levels of *E.coli* were found in both streams, and both contained human-associated bacteria and bovine-associated bacteria, which means sewer and septic systems need to be monitored and checked for leaks and more sampling needs to be conducted throughout both streams to identify source locations.

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Appendices

Appendix I. Raw Data: Pistol Creek

Site_ID	Date	Gage Height (ft)	Discharge (L/s)	Discharge (cfs)	Temperature (°C)	Specific Conductance (mS/cm)	pH	Dissolved Oxygen (%)	Dissolved Oxygen (mg/L)	Turbidity (NTU)
PC3	8/27/07	0.54	267	9.4	22.95	0.467	7.93	104	8.9	3.55
PC3	9/14/07	1.3	1231	43.5	22.77	0.441	6.98	81.1	7.0	5.27
PC3	9/20/07	0.5	216	7.6	20.83	0.414	7.68	93.1	8.3	2.55
PC3	10/3/07	0.48	173	6.1	19.53	0.418	7.71	97.6	9.0	3.75
PC3	10/4/07	0.48	163	5.8	20.72	0.41	7.72	97	8.7	3.32
PC3	10/9/07	0.5	164	5.8	20.96	0.413	7.67	96.5	8.6	6.64
PC3	10/18/07	0.48	142	5.0	19.79	0.419	7.73	93.3	8.5	2.79
PC3	10/25/07	0.49	168	5.9	16.87	0.365	7.46	87.9	8.5	1.92
PC3	11/1/07	0.5	125	4.4	14.69	0.424	7.72	99.6	10.1	2.49
PC3	11/8/07	0.5	199	7.0	11.46	0.401	7.55	105.5	11.5	2.8
PC3	11/15/07	0.83	735	25.9	13.56	0.239	7.21	86.6	9.0	
PC3	11/29/07	0.55	239	8.5	10.97	0.431	7.53	103.3	11.4	3.88
PC3	12/4/07	0.54	221	7.8	10.34	0.406	7.47	104.1	11.6	3.53
PC3	12/6/07	0.53	215	7.6	9.21	0.458	7.51	113.2	13	2.94
PC3	1/17/08	0.73	459	16.2	8.13	0.367	7.35	111.9	13.2	24.5
PC3	1/28/08	0.6	296	10.5	9.19	0.445	7.64	123.8	14.2	4.34
PC3	1/30/08	0.65	348	12.3	10.18	0.369	7.5	123.9	13.9	7.5
PC3	2/14/08	0.88	748	26.4	8.73	0.421	7.24	110.2	12.8	8.79
PC3	2/19/08	0.79	561	19.8	9.51	0.426	7.32	112.7	12.9	9.21
PC3	2/21/08	0.73	407	14.4	9.76	0.439	7.39	110.8	12.6	7.48

Appendix I. Raw Data Cont. Pistol Creek *E.coli* counts (cfu/100mL)

Site_ID	Date	Count 1	Count 2	Count 3
PC3	8/27/07			
PC3	9/14/07	11	11	11
PC3	9/20/07	0	14	9
PC3	10/3/07	39	36	71
PC3	10/4/07	53	68	73
PC3	10/9/07	44	27	35
PC3	10/18/07	54	41	44
PC3	10/25/07	28	32	23
PC3	11/1/07	32	31	24
PC3	11/8/07	24	19	23
PC3	11/15/07	924	702	927
PC3	11/29/07	40	34	29
PC3	12/4/07	6	28	34
PC3	12/6/07	9	10	7
PC3	1/17/08	89	47	62
PC3	1/28/08	1	0	10
PC3	1/30/08	42	34	34
PC3	2/14/08	23	9	20
PC3	2/19/08	13	9	14
PC3	2/21/08	8	12	12

Appendix II. Raw Data: Nails Creek

Site ID	Date	Gage Height (ft)	Discharge (L/s)	Discharge (cfs)	Temperature (°C)	Specific Conductance (mS/cm)	pH	Dissolved Oxygen (%)	Dissolved Oxygen (mg/L)	Turbidity (NTU)
NC1	8/27/07	0.85	84	3.0	23.2	0.391	7.41	139.0	11.9	9.68
NC1	9/14/07	0.96	83	2.9	20.69	0.321	7.59	85.5	7.7	
NC1	9/20/07	0.91	74	2.6	19.27	0.352	7.84	102.3	9.4	12.1
NC1	10/3/07	0.88	57	2.0	17.63	0.345	7.73	102.9	9.8	33.7
NC1	10/4/07	0.89	76	2.7	19.72	0.343	7.68	98.5	9.0	8
NC1	10/9/07	0.89	59	2.1	20.72	0.35	7.71	100.2	9.0	22.9
NC1	10/18/07	0.98	57	2.0	19.23	0.352	7.65	97.2	9.0	29.4
NC1	10/25/07	0.92	70	2.5	16.26	0.349	7.42	88.1	8.6	12.2
NC1	11/1/07	0.89	70.8	2.5	12	0.351	7.56	99.8	10.7	8.96
NC1	11/8/07	0.9	53	1.9	9.6	0.274	7.61	116.4	13.3	
NC1	11/15/07	1.22	272	9.6	12.86	0.337	7.34	93.8	9.9	8.58
NC1	11/29/07	0.93	110	3.9	7.91	0.334	7.52	118.9	14.1	59.4
NC1	12/4/07	0.92	61	2.2	7.32	0.347	7.6	127.5	15.3	16.1
NC1	12/6/07	0.91	52	1.8	6.61	0.348	7.7	133.6	16.3	17.2
NC1	1/17/08	1.03	162	5.7	6.28	0.351	7.38	117.0	14.5	34
NC1	1/28/08	0.98	126	4.4	5.45	0.354	7.29	122.0	15.4	8.36
NC1	1/30/08	1.04	185	6.5	7.53	0.357	7.52	121.9	14.6	6.78
NC1	2/14/08	1.26	336	11.9	8.29	0.339	7.51	120.1	14.1	9.08
NC1	2/19/08	1.22	301	10.6	8.26	0.339	7.59	123.3	14.5	9.58
NC1	2/21/08	1.17	334	11.8	7.7	0.354	7.4	122.5	14.6	8.13

Appendix II. Raw Data Cont. Nails Creek *E.coli* counts (cfu/100mL)

Site ID	Date	Count 1	Count 2	Count 3
NC1	8/27/07			
NC1	9/14/07	26	26	26
NC1	9/20/07	347	34	204
NC1	10/3/07	60	56	54
NC1	10/4/07	430	390	375
NC1	10/9/07	42	95	78
NC1	10/18/07	114	79	76
NC1	10/25/07	75	81	84
NC1	11/1/07	97	90	76
NC1	11/8/07	371	341	364
NC1	11/15/07	1356	1308	954
NC1	11/29/07	141	132	145
NC1	12/4/07	52	47	72
NC1	12/6/07	10	32	34
NC1	1/17/08	94	87	98
NC1	1/28/08	68	37	35
NC1	1/30/08	25	14	26
NC1	2/14/08	30	41	23
NC1	2/19/08	55	69	25
NC1	2/21/08	24	22	22

Appendix III. Processed Data: Pistol Creek

Date	<i>E.coli</i> (cfu/100mL)	Mean	Standard Deviation	<i>E.coli</i> (cfu/s)	TSS (mg/L)	TSS (mg/s)	Human Bacteria (mg/L)	Bovine Bacteria (mg/L)	Human Bacteria (mg/s)	Bovine Bacteria (mg/s)	Percent Organic (LOI)
8/27/07	77.00	36.80	2.01	205852.42	1.0	267.3	2.6	0.5	699.5	133.7	0.07
9/14/07	110.00	11.00	0.00	1353554.40	8.0	9844.0			0.0	0.0	0.14
9/20/07	76.67	7.67	7.09	165228.32	16.0	3448.2			0.0	0.0	0.01
10/3/07	486.67	48.67	19.40	840726.40	28.0	4837.1	0.5	2.8	86.4	485.5	0.01
10/4/07	646.67	64.67	10.41	1056694.72	1.0	163.4	0.5	0.4	81.7	68.6	0.60
10/9/07	353.33	35.33	8.50	578369.92	20.0	3273.8	0.3	0.5	45.8	81.8	0.64
10/18/07	463.33	46.33	6.81	660016.48	12.0	1709.4	0.7	0.5	94.5	71.2	0.01
10/25/07	276.67	27.67	4.51	464627.36	60.0	10076.3	0.5	0.5	84.0	84.0	0.25
11/1/07	290.00	29.00	4.36	361363.20	92.0	11463.9	0.5	0.8	62.3	97.1	0.63
11/8/07	220.00	22.00	2.65	436751.04	8.0	1588.2	0.2	0.2	36.5	33.5	0.03
11/15/07	8510.00	851.00	129.05	62516230.08	84.0	61708.1	0.5	0.5	367.3	367.3	0.35
11/29/07	343.33	34.33	5.51	821610.40	44.0	10529.4	0.5	0.5	119.7	119.7	0.06
12/4/07	226.67	22.67	14.74	501981.44	36.0	7972.6	0.5	1.0	110.7	229.2	0.05
12/6/07	86.67	8.67	1.53	186534.40	20.0	4304.6	0.6	0.8	125.2	175.8	0.12
1/17/08	660.00	66.00	21.28	3026105.28	32.0	14672.0	0.7	0.5	333.5	229.3	0.18
1/28/08	36.67	3.67	5.51	108512.80	24.0	7102.7	1.3	3.3	395.1	972.4	0.45
1/30/08	366.67	36.67	4.62	1277232.00	16.0	5573.4	0.5	0.9	174.2	319.9	0.04
2/14/08	173.33	17.33	7.37	1296414.08	16.0	11966.9	0.5	0.9	374.0	668.1	0.02
2/19/08	120.00	12.00	2.65	673223.04	20.0	11220.4	0.5	0.9	280.5	487.0	0.42
2/21/08	106.67	10.67	2.31	434391.04	12.0	4886.9	3.1	1.5	1256.8	627.4	0.30

Appendix IV. Processed Data: Nails Creek

Date	<i>E.coli</i> (cfu/100mL)	Mean	Standard Deviation	<i>E.coli</i> (cfu/s)	TSS (mg/L)	TSS (mg/s)	Human Bacteria (mg/L)	Bovine Bacteria (mg/L)	Human Bacteria (mg/s)	Bovine Bacteria (mg/s)	Percent Organic (LOI)
8/27/07	715.00	26.52	2.64	597339.60	16.0	1336.7	2.0	0.6	164.7	49.2	0.47
9/14/07	260.00	26.00	0.00	216478.08	220.0	18317.4					0.50
9/20/07	1950.00	195.00	156.69	1435824.00	36.0	2650.8					0.26
10/3/07	566.67	56.67	3.06	324169.60	440.0	25170.8	0.5	0.8	28.6	45.3	0.61
10/4/07	3983.33	398.33	28.43	3045816.00	220.0	16822.1	0.4	1.1	31.1	87.2	0.80
10/9/07	716.67	71.67	27.06	424186.40	56.0	3314.6	0.5	1.0	29.6	60.5	0.21
10/18/07	896.67	89.67	21.13	510411.36	120.0	6830.8	2.3	4.8	128.8	271.3	0.22
10/25/07	800.00	80.00	4.58	557337.60	2296.0	159955.9	1.7	0.5	119.9	34.8	2.54
11/1/07	876.67	87.67	10.69	620680.00	64.0	4531.2	0.6	4.4	43.1	312.9	0.01
11/8/07	3586.67	358.67	15.70	1909598.72	96.0	5111.2	1.2	49.9	61.5	2655.9	0.11
11/15/07	12060.00	1206.00	219.55	32856071.04	136.0	37051.6	1.0	17.2	277.8	4690.4	0.16
11/29/07	1393.33	139.33	6.66	1531016.96	80.0	8790.5	1.0	15.1	105.2	1659.9	4.49
12/4/07	570.00	57.00	13.23	350290.08	992.0	60962.8	1.2	2.1	73.0	126.4	1.18
12/6/07	253.33	25.33	13.32	131435.01	548.0	28431.5	1.1	3.9	54.9	199.9	0.51
1/17/08	930.00	93.00	5.57	1503876.96	60.0	9702.4	9.6	19.3	1549.2	3124.4	0.22
1/28/08	466.67	46.67	18.50	586790.40	336.0	42248.9	1.0	6.8	122.3	861.1	0.26
1/30/08	216.67	21.67	6.66	400067.20	40.0	7385.9	4.4	7.6	813.7	1408.2	0.18
2/14/08	313.33	31.33	9.07	1054183.68	24.0	8074.6	2.5	1.0	826.2	338.8	0.31
2/19/08	496.67	49.67	22.48	1493766.72	40.0	12030.3	0.4	4.7	130.0	1407.0	0.09
2/21/08	226.67	22.67	1.15	756823.68	48.0	16026.9	1.9	0.5	626.5	177.8	0.15

Appendix V. Pistol Creek Descriptive Statistics:

	N	Minimum	Maximum	Mean	Std. Deviation
Discharge (L/s)	20	0.125	1.231	.35	.28
<i>E. coli</i> (cfu/100mL)	20	37	8510	681.52	1851.92
<i>E. coli</i> (cfu/s)	20	108512.80	62516230.08	3848270.92	13824487.51
TSS (mg/L)	20	1.0	92.0	27.5	25.13
TSS (mg/s)	20	163.4	61708.1	9330.43	13040.18

Appendix VI. Nails Creek Descriptive Statistics:

	N	Minimum	Maximum	Mean	Std. Deviation
Discharge (L/s)	20	0.052	0.336	0.131	0.099
<i>E. coli</i> (cfu/100mL)	20	217	12060	1563.92	2680.759
<i>E. coli</i> (cfu/s)	20	131435.01	32856071.04	2515308.143	7177382.531
TSS (mg/L)	20	16	2296.0	293.4	528.38
TSS (mg/s)	20	1336.7	159955.9	23737.35	35583.27

Appendix VII. Blanks and Duplicate Data:

Date	NC1 <i>E.coli</i> Count Mean (cfu/100mL)	NC1 Duplicate Mean (cfu/100mL)	PC3 <i>E.coli</i> Count Mean (cfu/100mL)	PC3 Duplicate Mean (cfu/100mL)	Blank Count (cfu/100mL)
10/09/07	71.7	105.7	35.3	35.7	0
11/15/07	1206	926	851	681	0
1/28/08	46.7	74	3.7	7	0
2/21/08	22.7	19.7	10.7	16.7	0

Appendix VIII: One-Sample K-S Test

One-Sample Kolmogorov-Smirnov Test

		PC Discharge (L/s)	PC <i>E.coli</i> (cfu/100mL)	PC <i>E.coli</i> (cfu/s)	PC TSS (mg/L)	PC TSS(mg/s)	NC Discharge (L/s)	NC <i>E.coli</i> (cfu/100mL)	NC <i>E.coli</i> (cfu/s)	NC TSS (mg/L)	NC TSS (mg/s)
N		20	20	20	20	20	20	20	20	20	20
Normal Parameters ^a	Mean	.35385	681.52	3.8483E6	27.5	9330.43	.13115	1563.92	2.5153E6	293.4	23737.35
	Std. Deviation	.277944	1851.916	1.38245E7	25.13	13040.18	.099426	2680.759	7.17738E6	528.38	35583.27
Most Extreme Differences	Absolute	.232	.455	.474	.217	.320	.282	.343	.434	.305	.265
	Positive	.232	.455	.474	.217	.320	.282	.343	.434	.305	.261
	Negative	-.205	-.364	-.393	-.146	-.241	-.213	-.308	-.370	-.300	-.265
Kolmogorov-Smirnov Z		1.039	2.033	2.119	.972	1.431	1.263	1.536	1.939	1.365	1.183
Asymp. Sig. (2-tailed)		.230	.001	.000	.301	.033	.082	.018	.001	.048	.122
a. Test distribution is Normal.											

Appendix IX: Correlation Analysis: Pistol Creek.

Appendix IX.1: Logged Data Correlations

		LogPC <i>E.coli</i> (cfu/100mL)	LogPC TSS(mg/L)	LogPC TSS(mg/s)	LogPC <i>E.coli</i> (cfu/s)
LogPC <i>E.coli</i> (cfu/100mL)	Pearson Correlation	1	.301	.070	.882**
	Sig. (2-tailed)		.198	.770	.000
	N	20	20	20	20
LogPC TSS(g/L)	Pearson Correlation	.301	1	.825**	.264
	Sig. (2-tailed)	.198		.000	.261
	N	20	20	20	20
LogPC TSS(g/s)	Pearson Correlation	.070	.825**	1	.247
	Sig. (2-tailed)	.770	.000		.294
	N	20	20	20	20
LogPC <i>E.coli</i> (cfu/s)	Pearson Correlation	.882**	.264	.247	
	Sig. (2-tailed)	.000	.261	.294	
	N	20	20	20	20

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

			PC Discharge (L/s)	PC <i>E.coli</i> (cfu/100mL)	PC <i>E.coli</i> (cfu/s)	PC TSS (mg/L)	PC TSS (mg/L)
Kendall's tau b	PC Discharge (L/s)	Correlation Coefficient	1.000	-.158	.253	-.059	.389*
		Sig. (2-tailed)	.	.330	.119	.720	.016
		N	20	20	20	20	20
	PC <i>E.coli</i> (cfu/100mL)	Correlation Coefficient	-.158	1.000	.589**	.198	.158
		Sig. (2-tailed)	.330	.	.000	.228	.330
		N	20	20	20	20	20
	PC <i>E.coli</i> (cfu/s)	Correlation Coefficient	.253	.589**	1.000	.016	.316
		Sig. (2-tailed)	.119	.000	.	.922	.052
		N	20	20	20	20	20
	PC TSS (g/L)	Correlation Coefficient	-.059	.198	.016	1.000	.563**
		Sig. (2-tailed)	.720	.228	.922	.	.001
		N	20	20	20	20	20
	PC TSS (g/s)	Correlation Coefficient	.389*	.158	.316	.563**	1.000
		Sig. (2-tailed)	.016	.330	.052	.001	.
		N	20	20	20	20	20
*, Correlation is significant at the 0.05 level (2-tailed).							
**, Correlation is significant at the 0.01 level (2-tailed).							

Appendix IX.2: Correlations without outliers

			Discharge (L/s)	<i>E.coli</i> Concentration	<i>E.coli</i> Load	TSS Concentration	TSS Load
Kendall's tau_b	Discharge (L/s)	Correlation Coefficient	1.000	-.263	.193	-.143	.345*
		Sig. (2-tailed)	.	.115	.248	.399	.039
		N	19	19	19	19	19
	<i>E.coli</i> Concentration	Correlation Coefficient	-.263	1.000	.544**	.119	.064
		Sig. (2-tailed)	.115	.	.001	.482	.700
		N	19	19	19	19	19
	<i>E.coli</i> Load	Correlation Coefficient	.193	.544**	1.000	-.084	.240
		Sig. (2-tailed)	.248	.001	.	.622	.151
		N	19	19	19	19	19
	TSS Concentration	Correlation Coefficient	-.143	.119	-.084	1.000	.525**
		Sig. (2-tailed)	.399	.482	.622	.	.002
		N	19	19	19	19	19
	TSS Load	Correlation Coefficient	.345*	.064	.240	.525**	1.000
		Sig. (2-tailed)	.039	.700	.151	.002	.
		N	19	19	19	19	19
*. Correlation is significant at the 0.05 level (2-tailed).							
**. Correlation is significant at the 0.01 level (2-tailed).							

Appendix X. Correlation Analysis: Nails Creek
Appendix X.1. Logged Correlation

		LogNC <i>E.coli</i> (cfu/100mL)	LogNC TSS (mg/s)	LogNC TSS(mg/L)	LogNC <i>E.coli</i> (cfu/s)
LogNC <i>E.coli</i> (cfu/100mL)	Pearson Correlation	1	-.057	.012	.829**
	Sig. (2-tailed)		.811	.961	.000
	N	20	20	20	20
LogNC TSS(g/s)	Pearson Correlation	-.057	1	.862**	-.024
	Sig. (2-tailed)	.811		.000	.919
	N	20	20	20	20
LogNC TSS(g/L)	Pearson Correlation	.012	.862**	1	-.251
	Sig. (2-tailed)	.961	.000		.285
	N	20	20	20	20
	Sig. (2-tailed)	.325	.352	.905	.922
	N	20	20	20	20
LogNC <i>E.coli</i> (cfu/s)	Pearson Correlation	.829**	-.024	-.251	
	Sig. (2-tailed)	.000	.919	.285	
	N	20	20	20	20

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix X.2. Non-Parametric Correlation

			NC Discharge (L/s)	NC <i>E.coli</i> (cfu/100mL)	NC <i>E.coli</i> (cfu/s)	NC TSS(mg/L)	NC TSS(mg/s)
Kendall's tau b	NC Discharge (L/s)	Correlation Coefficient	1.000	-.121	.322*	-.377*	-.005
		Sig. (2-tailed)	.	.455	.048	.021	.974
		N	20	20	20	20	20
	NC <i>E.coli</i> (cfu/100mL)	Correlation Coefficient	-.121	1.000	.558**	.095	-.074
		Sig. (2-tailed)	.455	.	.001	.559	.650
		N	20	20	20	20	20
	NC <i>E.coli</i> (cfu/s)	Correlation Coefficient	.322*	.558**	1.000	-.159	-.116
		Sig. (2-tailed)	.048	.001	.	.330	.475
		N	20	20	20	20	20
	NC TSS (g/L)	Correlation Coefficient	-.377*	.095	-.159	1.000	.635**
		Sig. (2-tailed)	.021	.559	.330	.	.000
		N	20	20	20	20	20
	NC TSS (g/s)	Correlation Coefficient	-.005	-.074	-.116	.635**	1.000
		Sig. (2-tailed)	.974	.650	.475	.000	.
		N	20	20	20	20	20
*. Correlation is significant at the 0.05 level (2-tailed).							
**. Correlation is significant at the 0.01 level (2-tailed).							

Appendix X.3. No Outlier Correlation

			Discharge (L/s)	<i>E.coli</i> Concentration	<i>E.coli</i> Load	TSS Concentration	TSS Load
Kendall's tau_b	Discharge (L/s)	Correlation Coefficient	1.000	-.211	.282	-.422*	-.013
		Sig. (2-tailed)	.	.208	.093	.015	.940
		N	19	19	19	18	18
	<i>E.coli</i> Concentration	Correlation Coefficient	-.211	1.000	.509**	.059	-.203
		Sig. (2-tailed)	.208	.	.002	.733	.240
		N	19	19	19	18	18
	<i>E.coli</i> Load	Correlation Coefficient	.282	.509**	1.000	-.204	-.203
		Sig. (2-tailed)	.093	.002	.	.240	.240
		N	19	19	19	18	18
	TSS Concentration	Correlation Coefficient	-.422*	.059	-.204	1.000	.594**
		Sig. (2-tailed)	.015	.733	.240	.	.000
		N	18	18	18	19	19
	TSS Load	Correlation Coefficient	-.013	-.203	-.203	.594**	1.000
		Sig. (2-tailed)	.940	.240	.240	.000	.
		N	18	18	18	19	19

Appendix XI: Mann-Whitney Tests:

Pistol Creek Ranks

	Percentile	N	Mean Rank	Sum of Ranks
Discharge	1	14	7.9	111
	2	6	16.5	99
	Total	20		
<i>E.coli</i> Load	1	14	8.6	120
	2	6	15	90
	Total	20		
TSS Load	1	14	8.4	118
	2	6	15.3	92
	Total	20		

Pistol Creek Test Statistics^b			
	Discharge	E.coli	TSS
Mann-Whitney U	6.0	15.0	13.0
Wilcoxon W	111.0	120.0	118.0
Z	-2.969	-2.227	-2.392
Asymp. Sig. (2-tailed)	.003	.026	.017
Exact Sig. [2*(1-tailed Sig.)]	.002 ^a	.026 ^a	.015 ^a
a. Not corrected for ties.			
b. Grouping Variable: percentile			

Nails Creek Ranks				
	Percentile	N	Mean Rank	Sum of Ranks
<i>E.coli</i> Load	1	14	9.2	129
	2	6	13.5	81
	Total	20		
TSS Load	1	14	10.4	145
	2	6	10.8	65
	Total	20		
Discharge	1	14	7.5	105
	2	6	17.5	105
	Total	20		

Nails Creek Test Statistics ^b			
	E.coli Load	TSS Load	Discharge
Mann-Whitney U	24.0	40.0	.0
Wilcoxon W	129.0	145.0	105.0
Z	-1.485	-.165	-3.465
Asymp. Sig. (2-tailed)	.138	.869	.001
Exact Sig. [2*(1-tailed Sig.)]	.153 ^a	.904 ^a	.000 ^a
a. Not corrected for ties.			
b. Grouping Variable: percentile			

Appendix XII: Both Streams Mann-Whitney U Test

Ranks				
	Site	N	Mean Rank	Sum of Ranks
Discharge	PC	20	27.50	550.00
	NC	20	13.50	270.00
	Total	40		
<i>E.coli</i> Concentration	PC	20	14.18	283.50
	NC	20	26.82	536.50
	Total	40		
<i>E.coli</i> Load	PC	20	19.25	385.00
	NC	20	21.75	435.00
	Total	40		
TSS Concentration	PC	20	12.75	255.00
	NC	20	28.25	565.00
	Total	40		
TSS Load	PC	20	16.80	336.00
	NC	20	24.20	484.00
	Total	40		

Test Statistics ^b					
	Discharge	E.coli Concentration	E.coli Load	TSS Concentration	TSS Load
Mann-Whitney U	60.000	73.500	175.000	45.000	126.000
Wilcoxon W	270.000	283.500	385.000	255.000	336.000
Z	-3.787	-3.422	-.676	-4.197	-2.002
Asymp. Sig. (2-tailed)	.000	.001	.499	.000	.045
Exact Sig. [2*(1-tailed Sig.)]	.000 ^a	.000 ^a	.512 ^a	.000 ^a	.046 ^a
a. Not corrected for ties.					
b. Grouping Variable: Site_ID					

Appendix XIII: *Bacteroides*

NC <i>Bacteroides</i> Correlations					
			TSS Concentrations	Human Bacteria	Bovine Bacteria
Kendall's tau b	TSS Concentrations	Correlation Coefficient	1.000	-.120	-.013
		Sig. (2-tailed)	.	.493	.939
		N	20	18	18
	Human Bacteria	Correlation Coefficient	-.120	1.000	.074
		Sig. (2-tailed)	.493	.	.675
		N	18	18	18
	Bovine Bacteria	Correlation Coefficient	-.013	.074	1.000
		Sig. (2-tailed)	.939	.675	.
		N	18	18	18

Nails Creek Log Correlations

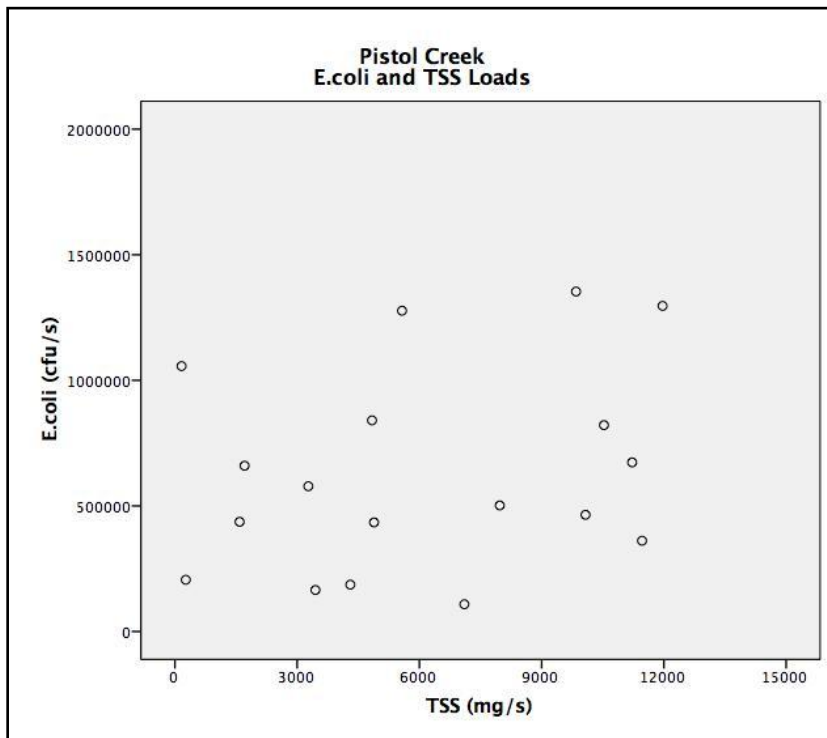
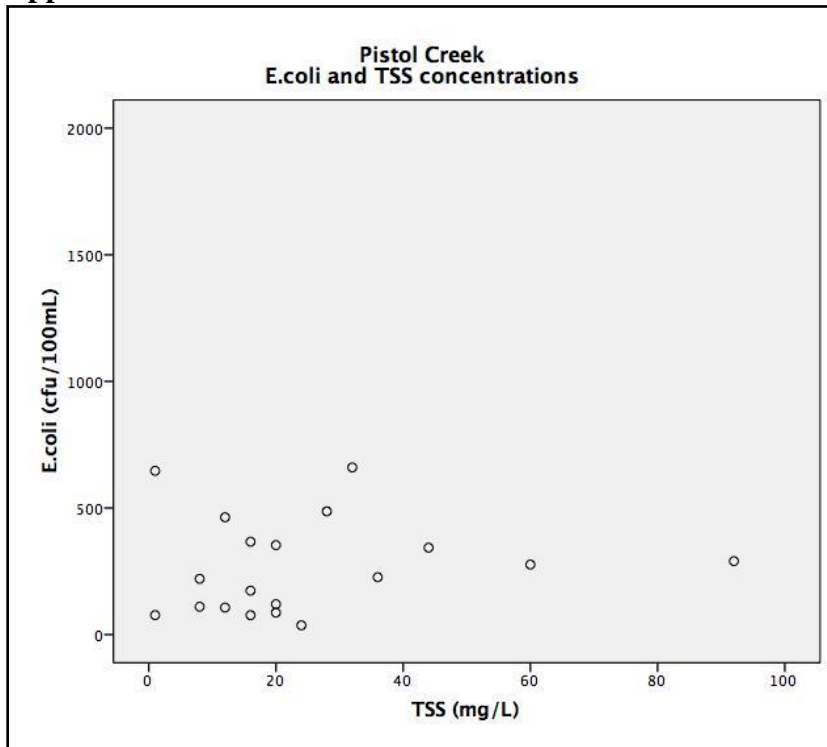
		LogTSS Concentration	LogHuman Bacteria	LogBovine Bacteria
LogTSS Concentration	Pearson Correlation	1.000	-.222	-.118
	Sig. (2-tailed)		.375	.640
	N	20.000	18	18
LogHuman Bacteria	Pearson Correlation	-.222	1.000	.196
	Sig. (2-tailed)	.375		.437
	N	18	18.000	18
LogBovine Bacteria	Pearson Correlation	-.118	.196	1.000
	Sig. (2-tailed)	.640	.437	
	N	18	18	18.000

Nails Creek *Bacteroides* Load Correlations

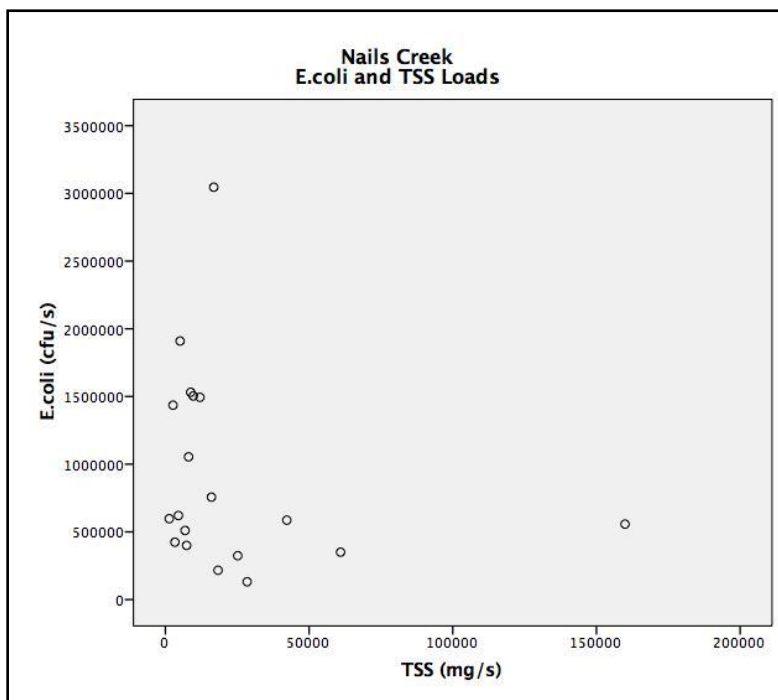
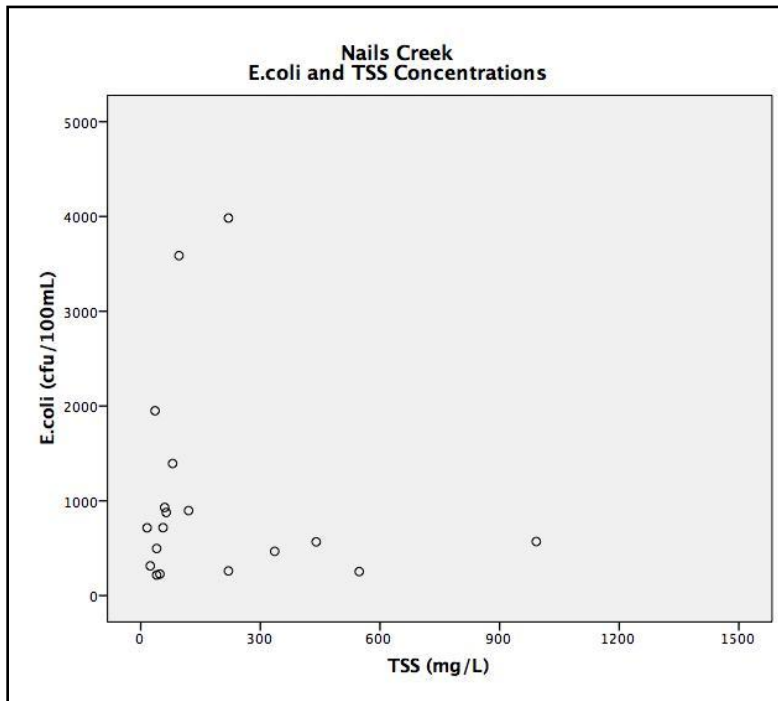
		TSS Load	Bovine Bacteria	Human Bacteria
Kendall's tau b TSS Load	Correlation Coefficient	1.000	-.072	-.007
	Sig. (2-tailed)	.	.677	.970
	N	20	18	18
Bovine Bacteria	Correlation Coefficient	-.072	1.000	.359*
	Sig. (2-tailed)	.677	.	.037
	N	18	18	18
Human Bacteria	Correlation Coefficient	-.007	.359*	1.000
	Sig. (2-tailed)	.970	.037	.
	N	18	18	18

*. Correlation is significant at the 0.05 level (2-tailed).

Appendix XIV: Pistol Creek Scatter Plots



Appendix XV: Nails Creek Scatter Plots



Vita

Ann Barbour Wambersie was born and raised in Richmond, Virginia in 1983. Following in her mother's footsteps, she attended the University of Mary Washington, in Fredericksburg, Virginia where she graduated with a Bachelor of Arts degree in Geography in 2006. She attended the University of Tennessee in Knoxville, Tennessee and graduated in May of 2009 with a Master of Science degree in Geography. She hopes to continue studying water quality and environmental degradation and to pursue a career in water resources.