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Storm Sampling to Assess Inclement Weather Impacts on Water Quality in a Karst Watershed: Sinking Creek, Watauga Watershed, East Tennessee

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Storm Sampling to Assess Inclement Weather Impacts on Water Quality in a Karst Watershed:

Sinking Creek, Watauga Watershed, East Tennessee

A thesis

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the faculty of the Department of Geosciences

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Master of Science in Geosciences, Geospatial Analysis

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by

Porcha McCurdy

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Keywords: *E. coli,* inclement weather, turbidity, electrical conductivity, karst

ABSTRACT

Storm Sampling to Assess Inclement Weather Impacts on Water Quality in a Karst Watershed: Sinking Creek, Watauga Watershed, East Tennessee

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Escherichia coli changes in Sinking Creek, an impaired water body in the Watauga watershed of northeast Tennessee, were assessed during storm events using water samples collected with ISCO automated samplers during eight storms at two locations. Turbidity and electrical conductivity (EC) data loggers were deployed in the creek, and dissolved oxygen (DO) was measured in situ to test the stream's water quality and reaction to inclement weather. Cotton fabric was deployed at both locations and sent to an external lab to test for the presence of Optical Brighteners (OB), which are indicators of residential wastewater. *E. coli* and turbidity at the creek generally increased within 2.5 hours of a rain event, remaining above the single sample standard for several hours during the storm. At the spring, *E. coli* became elevated within 30 minutes of precipitation onset, but generally decreased below the standard during the event.

DEDICATION

This thesis is dedicated to my parents Jackie and Harold, and to my partner Ryan. I am grateful for their support and continued encouragement.

ACKNOWLEDGEMENTS

I want to thank my thesis advisor, and committee chair Dr. Ingrid Luffman for her guidance through this process; her time, commitment and encouragement have aided me in completing this process. I would like to thank my committee members Dr. Andrew Joyner and Dr. Kurt Maier for providing me with help and feedback. I am thankful to for assistance that I received from several students in the ETSU Department of Geosciences. I want express gratitude to my good friends Monica Ayala and Montana Kruske who assisted me in the field. This research would have been very difficult to achieve with funding assistance from Dr. Bob Lauf and the Mayo Educational Foundation, and I am very grateful for their contributions.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. INTRODUCTION

Water Quality

Water quality in the United States has been impacted by channelization, oxygen depletion, and agricultural runoff, resulting in the presence of fecal coliforms in various rivers and loss of habitat for aquatic life (USEPA 2016a). When assessing water quality of streams and rivers, agriculture and industry are regarded as the foremost contributors of pollution in the U.S resulting in sediment, pathogens, and nutrients in water bodies (USEPA 2017). Some common contaminants that can be present in water bodies occur in nature such as uranium and radon, but others such as pesticides, fertilizers, heavy metals, and pathogens have anthropogenic origins (Centers for Diseases Control and Prevention 2014; USEPA 2018a).

The two common types of impairment in Tennessee are bacterial contamination and sedimentation (TDEC 2014). The Watauga watershed (HUC06010103) in east Tennessee is impaired due to *Escherichia coli* (*E. coli*) (141 miles impaired) and excess siltation (159 miles impaired) (TDEC 2016). The 305(b) and 303(d) reports are required by the Clean Water Act to ensure all water bodies of each state are evaluated; the assessment is usually conducted every two years (USEPA 2018b). The 305(b) report is an assessment of water bodies within each state; the U.S. water bodies that do not meet water quality standards for their intended use are added to the 303(d) list. A Total Maximum Daily Load (TMDL) report details how much of a pollutant is permissible in a water body and is prepared by the state for each type of impairment in a watershed (USEPA 2018c). Sinking Creek was added to the 303(d) list as impaired for *E. coli* in 1998 (Craig et al. 2004) and has continued to be listed to date (TDEC 2018).

Sinking Creek Introduction

The TMDL report for *E. coli* in the Watauga watershed indicates that 17 water bodies are listed as impaired due to *E. coli.* The Watauga watershed TMDL for sedimentation indicates that 15 water bodies are impaired due to excess siltation. Sinking Creek is a small tributary of the Watauga River located in northeast Tennessee (Washington and Carter County) and is one of the streams impaired for *E. coli* (TDEC 2018). Geologically, Sinking Creek flows through karst topography consisting of limestone and dolomite. The dissolution of limestone in the area has resulted in the presence of multiple springs (Moore 2006). Karst systems occur when water from the surface seeps into subsurface carbonate rocks such as limestone and dolostone (Boyer and Pasquarell 1999). When precipitation occurs, rainfall infiltrates the soil and interacts with organic systems. As the water percolates into the carbonate rocks, it flows through fractures in the rock and the limestone is dissolved. It eventually intersects the water table or contacts an aquitard and flows horizontally through the overlying rocks and emerges as a spring (USGS 2020a). Springs travel through the subsurface and may be a reflection of groundwater quality. Both groundwater and surface water are related in karst regions as contaminated surface water may enter groundwater systems through sinkholes and openings in carbonate rocks (USGS 2016).

E. coli

E. coli is a fecal coliform, rod-shaped bacterium that lives within the digestive tract of all mammals (Oliver 2016), and some strains may cause illnesses such as urinary tract infections, respiratory issues, and foodborne illnesses (Gould 2010). *E. coli* can be contracted through direct contact, exposure to impaired water or by consumption of contaminated foods. Most research regarding *E. coli* focuses on the exposure that may be caused by swimming and other recreational activities in impaired water bodies (CDC 2014), and most strains of *E. coli* bacteria are harmless and necessary for digestion. The Shiga-toxin *E. coli* (STEC) strain O157: H7, however, has been linked to illness and fatality due to kidney failure (CDC 2014). Approximately 265,000 cases of STEC occur each year in the U.S. and about 36% of these can be attributed to *E. coli* O157 (CDC 2014).

Siltation

Siltation is the suspension of silt-sized particles in water and can result in loss of biological integrity (TDEC 2016). Excess siltation can lead to the death of aquatic organisms by interfering with filter-feeding processes, while also inhibiting aquatic vegetation from receiving adequate sunlight for photosynthesis (Edwards 1968). Siltation can be caused by soil erosion due to agricultural practices such as overgrazing (Fleischner 1994). Periods of heavy rain and snowmelt aid in sediment pollution via runoff in river systems (Gardner 1950; Gillespie1981; Owens et al. 1982) and, in combination with overgrazing, can lead to high turbidity concentrations in streams (Agouridis et al. 2005).

When analyzing water quality, testing for total suspended solids (TSS) is integral in assessing water quality (Wu et al. 2014). Although an efficient practice, measuring TSS can sometimes prove difficult, as it must be measured in a lab by filtering water samples and drying and weighing the constituents (Oram 2020). Turbidity, however, can easily be measured through the use of data loggers that operate by measuring scattered light from particles (LaMotte 2020). Turbidity and TSS are related in that they both access solids that may be in a waterbody. They have been shown to have a positive correlation (Wu et al. 2014), therefore, measuring turbidity is a suitable surrogate for measuring suspended particles in water (Göransson et al. 2012, USGS 2020b). Under normal river flow conditions, turbidity concentrations are usually no more than 10

Nephelometric Turbidity Units (NTU, a standard of measuring turbidity equal to Formazin Turbidity Units (FTU), which are used in this study). During precipitation events, turbidity may increase to 1,000 NTU due to the high influx of surface water runoff and the transport of sediment from the stream channel (USGS 2020b; USEPA 2012).

Runoff Effects

During inclement weather, pollutants such as sediment and pathogens are more prone to being washed into surface water such as rivers and streams via runoff. Runoff may result from excess precipitation, melting ice/snow from mountains, or residual water from irrigation that flows into bodies of water and drainage systems (USGS 2020c). A factor influenced by runoff is the 'first flush' which is when the highest amount of a constituent, such as nitrates, *E. coli,* or turbidity, is expected to be present in a waterbody during an inclement weather event (Lee et al. 2002). In addition to runoff, the first flush is dependent upon rainfall amount, duration of the storm event, temperature, antecedent precipitation, land use, and topographic slope (Lee et al. 2002; USGS 2020c). Precipitation and antecedent precipitation influence runoff differently, antecedent conditions control how saturated or unsaturated the soil is which influences the soil infiltration capacity (Chen and Chang 2014). Alterations in land use such as deforestation, increasing agricultural areas, and urbanization influence runoff (Sajikumar and Remya 2015). The relationship between slope and runoff depends on the slope incline and soil permeability; therefore, if there is low infiltration due to an impermeable soil, higher runoff can be expected (Mu et al. 2015).

Water quality concerns arise when contaminants such as fertilizers, excess sediment, and pathogens from the surrounding areas are incorporated into the runoff and enter the surface and groundwater. A common misconception is that once water passes through the ground and emerges at springs, it has been filtered and “purified” by the minerals in the rock, making it safe. Filtration through the rock may remove some particles from the water, but chemicals from anthropogenic origins such as fertilizers and bacteria can still be present (Waller 1982).

E. coli may be transported to streams from impaired surface runoff during inclement weather events. Nonpoint sources of *E. coli* include agricultural and urban runoff (Teague et al. 2009). Urban runoff consists of stormwater from residential areas or developed locations that enter streams through storm sewer outfalls or direct surface runoff; fecal coliform bacteria from humans, pets, or wild animals are usually components (Benham et al. 2006). Agricultural land use in Northeast Tennessee mainly consists of pastures for cows; during precipitation events runoff from pastures may flow into streams, contributing fecal bacterial (Boyer and Pasquarell 1999).

Surface runoff may also contain high concentrations of nitrates in streams that affect aquatic plants and algae by increasing growth rates and altering dissolved oxygen (DO) concentrations. Excessive concentrations of algae limit the amount of sunlight that can penetrate water, which in turn can result in plant decay (Senn et al. 2017). Bacteria present in the runoff may also feed on plant decay and multiply, while also consuming the available DO. Negative impacts of lower DO concentrations are that aquatic species experience stress, and their ability to reproduce is limited (USEPA 2016b).

Storm Sampling

Storm sampling refers to a process when a designated flow or precipitation threshold is used to initiate the collection of water samples over certain time intervals (Harmel et al. 2003). Two methods of storm sampling are manual and automated. First, manual sampling involves collecting grab or integrated samples. Grab samples are usually taken in triplicate and consist of sample collection in a chosen location of the water body. Grab sampling provides more flexibility with regard to sample collection intervals (Harmel et al. 2010) because it is dependent on when the person decides to collect samples, and it also allows one to observe temporal changes in the water quality. Unless grab samples are collected in multiple locations across the stream, limitations in spatial variability can occur (Harmel et al. 2010). Grab sampling can also pose safety risks as personnel may be subjected to dangerous weather conditions and high stream flows while sampling (Harmel et al. 2006).

The other method of manual sampling is integrated sampling, which involves collecting samples across the river channel; it offers a way to capture cross-sectional variations in water quality. This method, however, is very time-consuming and may be more difficult because of the locations of the collection points; this can be mitigated by utilizing specifically designated samplers such as the DH- 81(USGS Instrument) (Harmel et al. 2010), which operates by taking constant water samples while the instrument is navigated through the cross-section. Cross-sectional variability from integrated sampling is useful for larger rivers due to the greater influx of constituents (Harmel et al. 2010) and is the best technique to provide cross-sectional stream variability when sampling sediment concentrations (Harmel et al. 2006). Difficulties associated with integrated sampling are that it usually requires at least eight cross-sectional samples to be collected to obtain the most accurate results (Harmel et al. 2010), and during storm events, this could be challenging due to weather conditions.

The second method, automated sampling, is the most predominant storm sampling technique because it doesn’t require high personnel involvement (Harmel et al. 2010). Automated sampling works by deploying the sampler at a fixed location and by programming it to begin collection at a certain time or volumetric flow. With this method, temporal variations in samples are easily identified as each sample comes from the same location, however, spatial variations across and along a stream reach cannot be captured due to the samplers fixed location unless multiple samplers are used (Harmel et al. 2010). A suggestion for mitigating spatial variation losses are to utilize a vertical intake and place it in a location with representative flow; the problem with this is that vertical intakes are not very accessible (Harmel et al. 2010). Automated sampling is a more convenient method than manual sampling because the samplers can be set for specific start times and sampling intervals, and it is appropriate for small streams that have fluctuations in flow (Robertson and Roerish 1999). Automated sampling has been used to sample for dissolved material (Ging 1999), urban stormwater runoff (Leecaster et al. 2002), and inorganic nitrogen content in wetlands during inclement weather conditions (Kearney et al. 2013).

To ensure proper functionality when deploying the automated sampler for collection, it should be positioned in a location to collect well-mixed flow in the stream, possibly at the midpoint of the stream cross-section (Harmel et al. 2006) or at the thalweg. ISCO automated samplers are very flexible and allow many different sampling strategies, therefore, a method of sampling must be selected. Sampling options may include selecting a minimum flow threshold to collect flow interval samples or choosing a time interval approach (Harmel et al. 2003). A minimum flow threshold is developed to trigger the ISCO to collect samples depending on a pre-determined minimum flow value. Problems related to minimum flow involve possible difficulties with keeping the intake submerged below water level during minimum flow conditions. Time and flow interval methods involve programming the sampler to collect at timed intervals or by utilizing the minimum flow threshold (Harmel et al. 2003). The benefit of using the flow interval approach is that more samples will be taken during storm events as water levels increase. The time interval approach has an easier setup and doesn't require hydrologic inputs; however, downfalls of this method are that depending on the sample collection frequency, the number of samples collected can greatly vary (Harmel et al. 2003).

Once a time or flow interval has been chosen, a method of discrete or composite sampling must be selected. Discrete sampling involves collecting only one water sample in each bottle and follows either the time-interval or volumetric flow approach (King and Harmel 2003). The benefit of discrete sampling is that the samples are not combined, and each sample is collected in a single ISCO bottle; the drawback, however, is that for a given sampling interval, the maximum sampling duration will be reduced as each bottle is only used once (King and Harmel 2003). Composite sampling means that multiple samples are included in each bottle which permits an extended sampling duration; it can be either time-based or flow-based (King and Harmel 2003). A downfall of this method, however, is that it is more difficult to associate the concentration of constituents in the water sample to specific time periods of the events because samples are combined (King and Harmel 2003). Storm sampling intervals for automated samplers are typically 15 minutes (Leecaster et al. 2002; King and Harmel 2003), or 30 minutes (Harmel et al. 2006; Harmel 2010).

Automated storm sampling for *E. coli* is complex because of the potential for cross-contamination. When water samples are collected with automated samplers, each sample travels through the ISCO tubing and is dispensed into one of the sample bottles. During sampling events, the same equipment tubing is utilized and could result in residual *E. coli* concentrations left behind (Hathaway et al. 2014). In an effort to assess the potential for and degree of cross-contamination of samples through the sampler tubing and intake, Line et al. (2008) collected samples of distilled water with the automated sampler after a fecal coliform collection event.

Results indicated 12 cfu/100 mL of fecal coliforms were present in the distilled water samples (EPA standard for a single sample is 941cfu/100 mL). Positioning the ISCO sampler so that the tubing is sloped toward the intake during sample collection aids in tube drainage and limits contamination of tubing with fecal bacteria (Hathaway et al. 2014). Before and after each sample collection, the ISCO automated sampler purges the tubing line (Harmel et al. 2003) and this is useful in decreasing bacterial cross-contamination (Hathaway et al. 2014).

Identifying Sources of E. coli

Determining the source of anthropogenic pollutants like *E. coli* can be difficult due to various origins of the bacteria. Optical brighteners (OB) are synthetic chemicals and are good indicators used to identify anthropogenic sources of *E. coli*. They are included in detergents to enhance colors and are also utilized in textiles and paper manufacturing (Poiger et al. 1998; Floreguerra 2003). OB from detergents are discharged in effluent and can be released into surface and groundwater from septic tank leaks (Hartel et al. 2007a, b) or direct discharge. Runoff originating from residential areas may contain OBs from laundry wastewater, while surface runoff from agricultural areas generally does not contain OBs. Optical brighteners can, therefore, serve as an indicator of an anthropogenic source of impairment. Tavares et al. (2008) conducted a study to measure tidal creeks (North Carolina) in an area undergoing urbanization and found a significant correlation ($p = 0.0248$) between the fecal coliforms and OBs. When observed under a black light, OBs fluoresce. Complications identifying the presence of OBs exist because other material, such as residuals from paper production and organic material, may also fluoresce (Gregor et al. 2002). Because OB fluoresce at a wavelength of 415 – 445 nm, a spectrofluorophotometer may be used to distinguish between the presence of OB and other fluorescent materials (Tavares et al. 2008), and an even narrower range of 415 – 422 nm has been used by Ozark Underground Laboratories, Inc., a Missouri-based company that specializes in groundwater dye tracing.

Other methods to identify origins of *E. coli* include DNA ribo-typing and assessment of antibiotic resistance. DNA ribo-typing involves characterizing *E. coli* strains based on their serotypes, which are distinguishing variations among a species (Martin et al. 1996). *E. coli* strains are divided into O (~183 antigens), K (~80 antigens), and H (~53 antigens) (Delannoy et al. 2017); and differentiation of the serotypes within these groups can help determine the source of *E. coli* contamination in water bodies. After strains are determined, they can be combined in an *E. coli* library. An *E. coli* library is a database of wildlife within the watershed, and although it can be a very intensive method, it may help determine origins of the *E. coli* (Lu et al. 2005; Wilkison and Davis 2010). Antibiotic resistance patterns have also been used to distinguish between human and non-human sources; limitations with this method are that it is difficult to account for all species in a location, and therefore some strains identified in water samples are left unaccounted for (Hagedorn et al. 1999).

E. coli and Precipitation

Increased runoff during periods of heavy precipitation has been linked to decreased water quality. Subsequently, after inclement weather events more waterborne outbreaks have been documented globally (Cann et al. 2012; Tornevi et al. 2014). This is a problem in all countries regardless of developmental status (Cann et al. 2012, Gleason and Fagliano 2017). During intense inclement weather events water treatment facilities can be inundated and lead to contamination of drinking water supplies. Locations that utilize non-disinfected groundwater as a source of drinking water have also been affected by elevated gastrointestinal (GI) outbreaks after a rainfall event, further proof that the rocks are not sufficient in filtering out the bacteria (Gleason and Fagliano 2017).

In particular, *E. coli* concentrations in surface water have been correlated to 7-day antecedent precipitation (Chen and Chang 2014), and wet conditions (Wittman et al. 2013). A peak in *E. coli* was observed two days after an inclement weather event began (Tornevi et al. 2014). Land use is also a determining factor in the relationship between precipitation and *E. coli*. In forested and residential areas, *E. coli* is more highly correlated to precipitation during the dry season, while in urban, forested, and agricultural lands the highest correlation between *E. coli* and precipitation occurs during the wet season (Chen and Chang 2014). *E. coli* concentrations are also influenced by temperature; during the summer when temperatures are higher, lower concentrations have been recorded; however, low rainfall amounts during the summer in some locations have also resulted in higher concentrations of *E. coli* (Chen and Chang 2014). Even during normal precipitation and runoff conditions, *E. coli* concentrations may be high enough to have adverse effects on the population (Wittman et al. 2013).

Precipitation and Turbidity

Turbidity in a small watershed in Tennessee, USA, peaked approximately 3 to 4 hours after a precipitation event had begun (Luffman 2016), and there was a positive correlation between turbidity and precipitation (Hamilton and Luffman 2009). In forested areas, the turbidity was affected by infiltration, therefore decreasing the sediment load in surface water (Chen and Chang 2014). Higher turbidity has been associated with a region’s wet season (Maillard and Santos 2008). Turbidity concentration during inclement weather events is influenced by discharge as well, with more intensive storm events causing increased turbidity concentrations (Göransson et al. 2013).

Study Area

The Watauga watershed is located in northeastern Tennessee and northwestern North Carolina; it extends throughout parts of Washington, Unicoi, Sullivan, Johnson, and Carter counties (TDEC 2015). Sinking Creek (TN06010103046) flows through both Washington and Carter counties, and in both counties, 10 river miles are impaired due to *E. coli* from municipal areas and grazing in riparian zones (TDEC 2018) (Figure 1). The location has a Cfa, or humid subtropical, climate type (Kottek et al. 2006), receiving an average of 104 cm (41 in.) of rainfall annually (National Weather Service 2020). Approximately 3.3 miles from the East Tennessee State University (ETSU) campus, Sinking Creek flows through Jacob's Nature Park (36.32°N, 82.32°W), a Johnson City park. In 2014 the Boone Watershed Partnership (local nonprofit) was awarded an EPA 319 grant that would be used to tackle sources of impairment at Sinking Creek (TDEC 2015). The project was divided into two phases, with the first focusing on connecting residents who were on separate sewer systems to the cities' sewer system; it also involved repairs made to existing septic systems and one cattle exclusion project (TDEC 2015). The second phase of the project involved transforming the wetland area of Sinking Creek into a natural arboretum that could be used for recreation as well as scientific learning, which is now known as Jacob's Nature Park at Sinking Creek. Within the park, a spring has been located and will be analyzed along with the creek during inclement weather events to better understand the water quality and its variability under these conditions.

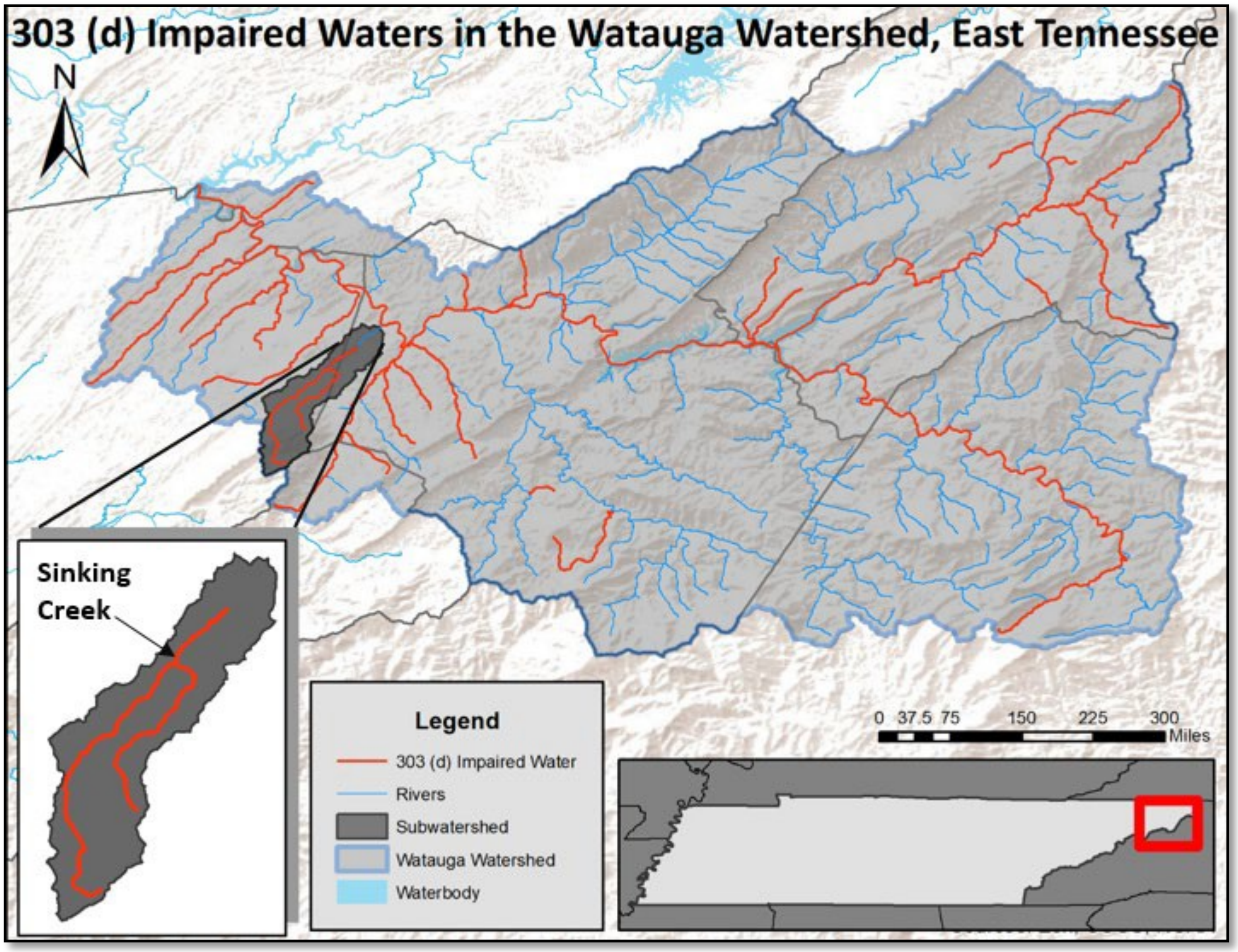

Figure 1. Study site and impaired water bodies within the Watauga watershed (HUC 06010103)

Sinking Creek Studies

This study area was selected, in part, because of a wealth of prior research on Sinking Creek. Dulaney (2003) identified locations in Sinking Creek with high concentrations of fecal coliform bacteria. The highest concentrations of fecal coliform in water were measured at sites with the largest amount of agricultural land cover (Dulaney 2003). Mitigation strategies such as creating vegetation buffer zones to limit the transport of fecal coliforms to water bodies and streams were suggested (Dulaney 2003). Floresguerra (2003) identified parameters influencing water quality in Sinking Creek. These parameters included *E. coli*, nitrates, and OB with the objective of locating non-point sources of pollution. Floresguerra (2003) also analyzed water samples for OB; results indicated possible OB presence in highly forested areas, with higher concentrations during the fall months. The variables were selected for their utility in distinguishing point and nonpoint sources of *E. coli*. The OB results were not conclusive as the concentrations measured could have been be attributed to the breakdown of organic material such as chlorophyll and fulvic acids (Alhjjar et al. 1990).

Luffman (2016) completed a pilot study in Sinking Creek to analyze the relationship between precipitation and turbidity. Data were collected over three inclement weather events. Turbidity peaked three to four hours after onset of precipitation. Due to the short sampling period, a longer sampling duration was suggested to adequately analyze the relationship between the variables (Luffman 2016).

Models predicting the presence of fecal indicators based on ecological drivers in Sinking Creek were developed using MaxEnt (maximum entropy modelling) to understand if chemical and microbial variables could predetermine if an area may be impaired by *E. coli* (Gilfillan et al. 2018a, b). Research by Hall et al. (2014) aimed to determine if the presence of *E. coli* O157:H7 could be confirmed at Sinking Creek. She was, however, unable to confirm the presence of the pathogen, and explained that it was likely due to the interference of the soil water chemistry of the stream with her testing methods.

Previous research at Sinking Creek from Gilfillan (2018a, b) and Hall et al. (2011) has indicated that *E. coli* concentrations in Sinking Creek were impacted by seasonal variations as well as surface run off from pastoral areas. Gilfillan (2018b) concluded that summer months, specifically August, resulted in the highest concentration of fecal coliforms, but stressed that bacterial counts during other months should not be neglected because concentrations may still be high. Gilfillan (2018b) indicated that seasonality and agriculture in the watershed were the main contributors of *E. coli* despite the fact that developed land made up a larger portion of the watershed. *E. coli* concentrations were higher in downstream regions, consistent with the results from Floresguerra (2003) research indicated higher *E. coli* concentrations in downstream regions, where were likely influenced by organic pollution. Similarly, to Gilfillan (2018a, b), her research concluded that *E. coli* concentrations were higher in the summer months, and that they were likely due to agricultural activity and seasonally lower rainfall.

Research Questions

Previous research at Sinking Creek has provided relevant information about the water quality such as when turbidity can be expected to increase, possible locations of where OB are present, as well as locations along the creek that have the highest runoff input from agricultural areas. There are, however, still unanswered questions about the stream. Although the previous methods focus on water quality indicators, there is not a substantial amount of information regarding how *E. coli,* EC, turbidity, and DO vary during inclement weather events. There is also uncertainty as to whether OB are present in the stream. Therefore, the research questions to be evaluated in this study are:

1. What is the relationship between **inclement weather** events and water quality [*E. coli*, turbidity, DO, EC] in surface and groundwater at Sinking Creek?
2. What is the relationship between **water quality parameters** [*E. coli*, turbidity, and EC]?
3. What is the timing of the **first flush** for *E. coli*, turbidity, and EC?
4. Can **anthropogenic sources** of *E. coli* (spring and creek) be confirmed through the presence of Optical Brighteners?

CHAPTER 2. METHODOLOGY

Overview

Storm sampling involves the collection of water samples during inclement weather events to understand how water quality parameters respond to precipitation. Inclement weather events with an expected precipitation duration of four to five hours were chosen. Storm sampling was conducted at two locations in Jacob's Nature Park in Johnson City TN, a spring and Sinking Creek which flows through the park. The two locations were chosen because storm runoff into the creek would be indicative of surface water quality while the spring would provide insight on the water quality of groundwater. Sampling locations were based on finding sites where automated samplers could be safely deployed, with preference for locations that concealed the samplers from most foot traffic at the park to limit the potential for tampering.

At the creek where water samples were collected to analyze for *E. coli,* turbidity, and EC, data loggers were deployed and DO measurements were taken *in situ*. In the spring, water samples were collected to test for *E. coli* and DO measurements were taken *in situ*. The *E. coli* samples were compared to 941 colony-forming units/100 mL (cfu/100 mL), which is the maximum water quality standard for *E. coli* in surface water. OB were examined at both sites to determine if effluent from anthropogenic sources are the origin of *E. coli* at the creek and spring. In addition to OB analysis, land cover assessment was conducted for the Sinking Creek watershed upstream of the study sites. The objective was to quantify agricultural, residential (developed), and forested land use, to help determine which areas may be contributing to *E. coli* concentrations at the study sites.

Storm Selection

Eight storm events were sampled, and the selection of storm events was dependent on the expected duration of precipitation. At least four to five hours of continuous precipitation was desired to trigger storm sampling based on Luffman (2016), which indicated that turbidity in Sinking Creek peaked between three and four hours after precipitation began. The ETSU Geosciences weather station (https://www.wunderground.com/weather/us/tn/johnson-city/KTNJOHNS46), located ~3.3 miles away, was utilized for meteorological data such as forecast, radar, and precipitation rate and precipitation accumulations.

Data Loggers

An Aquatec AQUAlogger 210 turbidity data logger and a HOBO ONSET Fresh Water Conductivity Data Logger (U24-001) were deployed in the creek bed and were securely tied to a tree along the bank of the creek. The turbidity data logger was stabilized with rocks to keep it in an upright position so that the sensor would be near the top of the stream; therefore, reducing deposition of sediment on the optical window which would skew results. The conductivity data logger was placed in PVC housing for protection. Both data loggers were programmed to collect measurements at 15-minute intervals. Dissolved oxygen was measured *in situ* at both study sites for storms 1 – 6 with an Oakton DO 6+ Dissolved Oxygen Meter with NIST – Traceable Calibrations. After experiencing calibration issues following replacement of the electrode solution in the meter's probe, a HACH Dissolved Oxygen test kit (0-15 mg/L Model AV) was used for storms 7 and 8.

Water samples were collected using ISCO 6712 automated samplers at the two sites. At the creek site, an ISCO sampler with a 25 ft. hose was placed on a ledge adjacent to a bridge that spans the creek (Figure 2 A). This enabled the sampler to be elevated above the flood zone. At the creek location, the ISCO's polypropylene strainer was placed in the middle of the creek. It was elevated from the channel bed with rocks to limit sediment intake from the creek bed and weighted down with rocks to keep it stable during high velocity flow events. At the spring site, an ISCO sampler with a 6 ft. hose was placed immediately adjacent to the spring and was concealed by vegetation (Figure 2 B). Due to a much lower water discharge at the spring than at the creek, water samples drawn from the spring were noticeably more turbid. A small trench was dug to provide sufficient water depth to allow placement of rocks to elevate the strainer and limit sediment intake, while keeping the intake below water. Rocks were also placed on top of the strainer to restrict mobility during storm events.

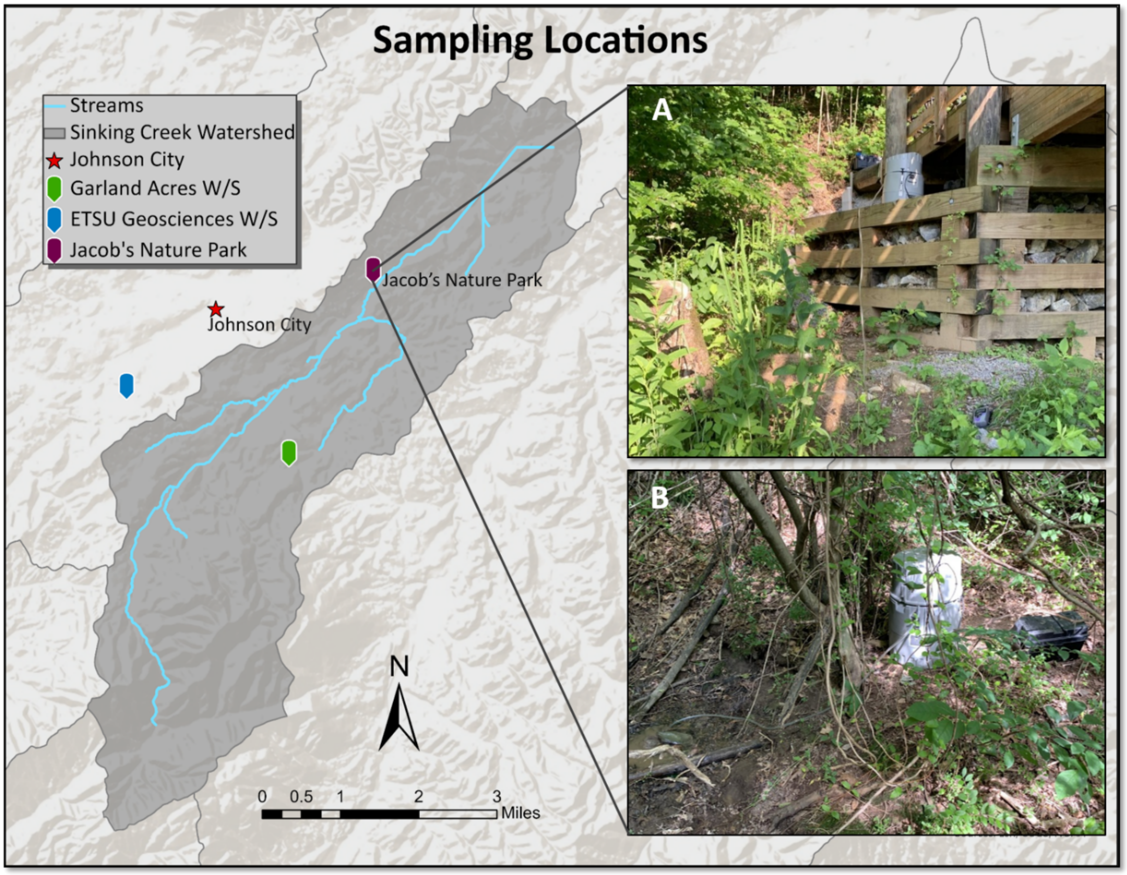

Figure 2. Sinking Creek ISCO sampler location. A) ISCO location at creek. B) ISCO location at spring.

For most storms, the ISCOs were transported to Jacob's Nature Park a day prior to the inclement weather event. If the forecast changed, only slight adjustments in timing would be needed since the equipment was already on site. Two car batteries were charged before each sampling event and were used as the power source for the ISCOs. Shortly before the start of sampling, ice was added to each ISCO tub to ensure the water samples remained cold and to preserve the samples by limiting the growth of *E. coli* bacteria (Anderson and Rounds 2010). Following the methods of Harmel and King (2003), samplers were programmed to start at a specific time and collect discrete, 1000 mL samples every 30 minutes sequentially. The full volumetric capacity of the sample bottles (1000 mL), was chosen to acquire a representative sample and of the 1000 mL collected. Sampling events ranged from 5 hours to 16 hours depending on storm duration. For longer storms, at the 5-hour mark of sample collection, samples were retrieved from both locations and transported to the East Tennessee State University Department of Geosciences Hydrology Laboratory so that they could be processed within 6 hours. The samplers were then reset and restocked with clean sterile bottles and ice. For each round of sampling, the water samples for the creek and spring were transported to the lab in separate ice-filled coolers to prevent cross-contamination (Tavares et al. 2008).

Laboratory Analysis

Water samples were processed for *E. coli* using the Colilert Quanti-Tray method. The Colilert method uses a reagent that contains two enzymes that can metabolize carbon sources in both coliforms and more specifically *E. coli.* The metabolization from the reagent causes the water samples to turn yellow if coliforms are present and a fluorescent cell indicates the presence of *E. coli* (IDEXX 2020). For each sample, one packet of reagent was added to 100 mL of water in a glass Erlenmeyer flask that was triple washed in a 10% bleach solution, triple rinsed in tap water, and triple rinsed with Deionized water. Each sample was swirled to dissolve the reagent before pouring it into the foil Quanti-Trays. Each tray was labeled with the date, time processed, site location, and storm event, and sealed in a Quanti-Tray sealer. Trays were incubated in a Fisher Scientific Isotemp oven at 35°C for 24 hours. At the end of the incubation period, trays were retrieved from the oven and an ultra-violet light was used to count the number of large and small cells that fluoresced, indicating the presence of *E. coli*. A Colilert Most Probable Number (MPN) table was consulted to obtain the number of *E. coli* colony forming units present per 100 mL.

OA/QC

Quality Assurance and Quality Control (QA/QC) methods involved filling two ISCO bottles with approximately 500 mL of deionized (DI) water to serve as lab and trip blanks. An approximate volume of 500 mL was chosen because this was the amount of water to be transported to the lab during storm sampling events. The lab blank remained in the hydrology lab refrigerator, while the trip blank was placed in a cooler along with ice and transported to Jacob's Park during equipment deployment and sample collection events. Both the lab and trip blank were tested for fecal coliforms and *E. coli* using the same methods as the samples collected with the ISCOs.

The QA/QC procedure for the 48 1-liter polyethylene ISCO bottles involved triple washing each bottle with a 10% bleach and tap water solution, triple rinsing with tap water, and lastly triple rinsing with DI water. This was done between each sampling event as the bottles were reused. The lower tub of the automatic samplers was sanitized with tap water and a 10% bleach solution and after cleansing, each ISCO tub was filled with 24 clean bottles, for water sample collection. Other equipment essentials included gloves that were used to decrease cross-contamination between water sample retrieval at the spring and creek.

Optical Brighteners

Optical brightener samples were collected for two separate storm events on November 18, 2019 and December 15, 2019, using eight squares of cotton test fabric provided by Ozark

Underground Laboratory, Inc. During each of the two storms, four pieces of the fabric were placed at Sinking Creek, two were anchored together at the creek, and two at the spring by tightening cable ties around the fabric. The fabric was placed in locations that would receive representative stream flow for both the creek and spring. Following instructions provided by Ozark Underground Laboratory, the fabric squares were deployed at the sites between four to seven days. During retrieval, the fabric squares were rinsed in creek or spring water according to the location of deployment. Both fabrics from the creek were placed in a single whirl-pak bag, this was also done for the spring. Pertinent information such as site name, date, and time of deployment and collection were recorded on the bag.

The cotton test fabrics were frozen and mailed to Ozark Underground Laboratory for processing and analysis. Duplicate cotton test fabrics were deployed at each site so that the fabric piece with the best fluorescence intensity within the appropriate wavelength for OBs was analyzed. The lab utilized a black light to determine areas on the cotton test fabric with high OB intensity. A solid sample holder in a Shimadzu RF 5301 spectrofluorophotometer was used to analyze the samples behind quartz glass using the following settings: a 17 nm bandwidth separation with synchronous scan, excitation slits at 5 nm, and emission slits at 2 nm.

Statistical Methods

EC and turbidity data were collected at intervals of 15 minutes, therefore, prior to analysis the data were aggregated to 30 minutes in Microsoft Excel to match the water samples collected for *E. coli* analysis. Similarly, to EC and turbidity data, precipitation data downloaded from the ETSU weather station were aggregated from the collection interval of 5 minutes to 30 minutes. After the data were aggregated, up to six hours of antecedent precipitation prior to storm sampling was used to create at table that would allow cross-correlation of the water quality parameters with antecedent precipitation. The relationships between *E. coli,* turbidity, and EC were analyzed using the Spearman correlation coefficient in IBM SPSS Statistics 25 (IBM 2017). The Spearman correlation coefficient was selected because the data were non-parametric. The Spearman test was run for the parameters during each individual storm as well as the combined storms and they were tested for two-tailed significance. To compare variability in *E. coli* and DO at both the creek and spring, Mann Whitney U tests were conducted to compare means and determine statistical significance.

CHAPTER 3. RESULTS

Description of Storms

During storm 1 on June 18, 2019 precipitation began at 2:45 pm (Figure 3), and storm sampling began at 1:00 pm. Turbidity initially was very low, <10 FTU, one hour after the storm began it increased and peaked at 125 FTU. EC had a baseline of ~270 µS/cm prior to precipitation and decreased to 150 µS/cm 1.5 hours after the storm began. *E. coli* at the creek was initially between 200 to 400 cfu/100 mL, concentrations dramatically increased approximately one hour after the start of precipitation and reached the upper limit of 1011 cfu/100 mL for the Colilert Quanti-tray method. *E. coli* at the creek remained elevated for the duration of storm sampling, a total of approximately four hours. *E. coli* at the spring began at a baseline of 40 cfu/100 mL and began increasing 30 minutes after precipitation began. *E. coli* at the spring peaked at 960 cfu/ 100 mL, one hour after the storm began and after peaking, the concentrations steadily declined for the remainder of the storm. Both *E. coli* at the creek and spring increased beyond 941 cfu/100 mL, which is the single sample standard for a non-recreational body of water.

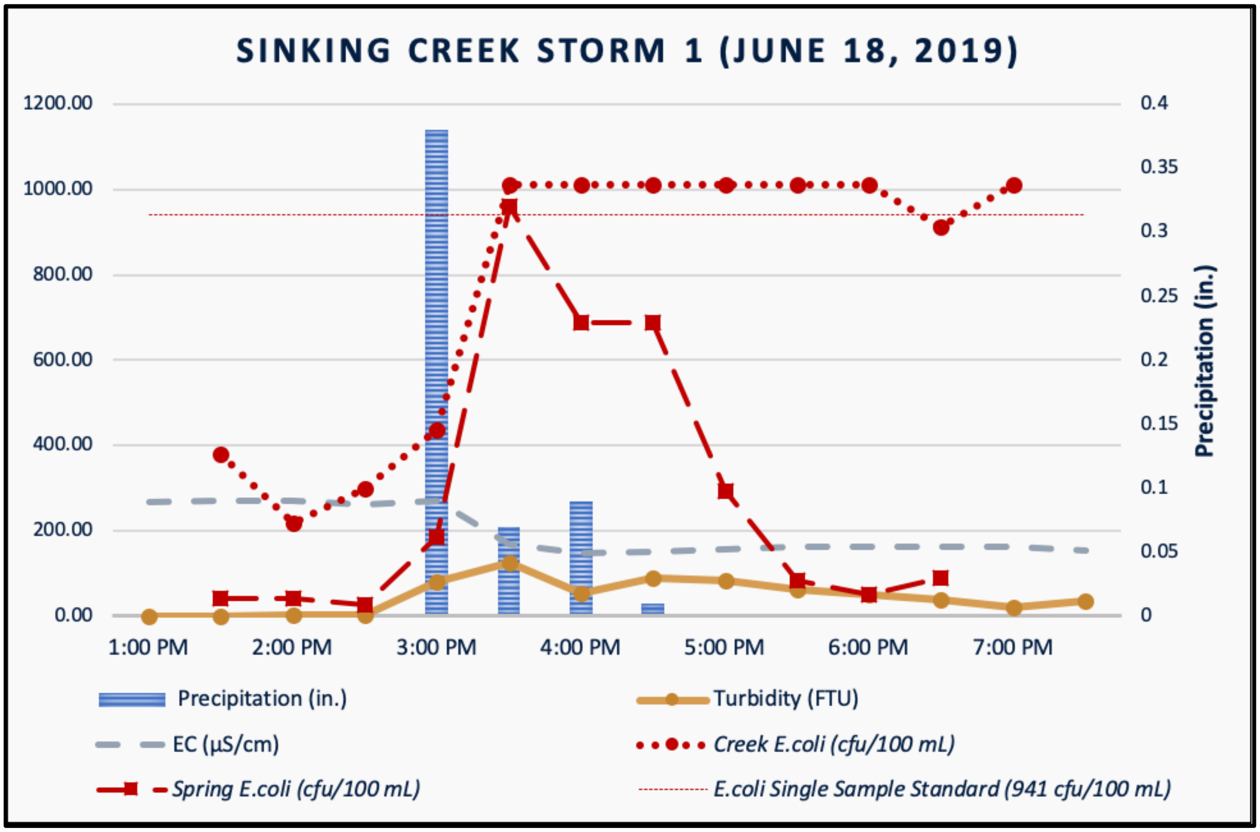

Figure 3. Sinking Creek Storm 1 – June 18, 2019. Precipitation begins around 2:45 pm and is measured on the secondary y-axis. Turbidity peaks ~1 hour after precipitation while EC begins decreasing 30 minutes after precipitation. *E. coli* concentrations at the creek and spring peak 30 minutes following inclement weather.

Storm sampling for storm 2 (Figure 4) began at 12:00 pm on July 17, 2019, and precipitation started at 12:30 pm. Turbidity concentrations remained between 2 to 5 FTU throughout the storm. Similar to turbidity, electrical conductivity was constant at ~ 320 µS/cm. *E. coli* at the creek initially had values near ~180 cfu/100 mL and began increasing at the onset of precipitation. Within 2.5 hours of the storm, *E. coli* at the creek peaked at 870 cfu/100 mL and fluctuated throughout the remainder of the storm. *E. coli* at the spring peaked at 137 cfu/100 mL as the storm began. Thirty minutes after precipitation began, the concentrations decreased and remained <25 cfu/100 mL for the duration of the storm. *E. coli* at both the creek and spring remained below the single sample standard throughout the storm.

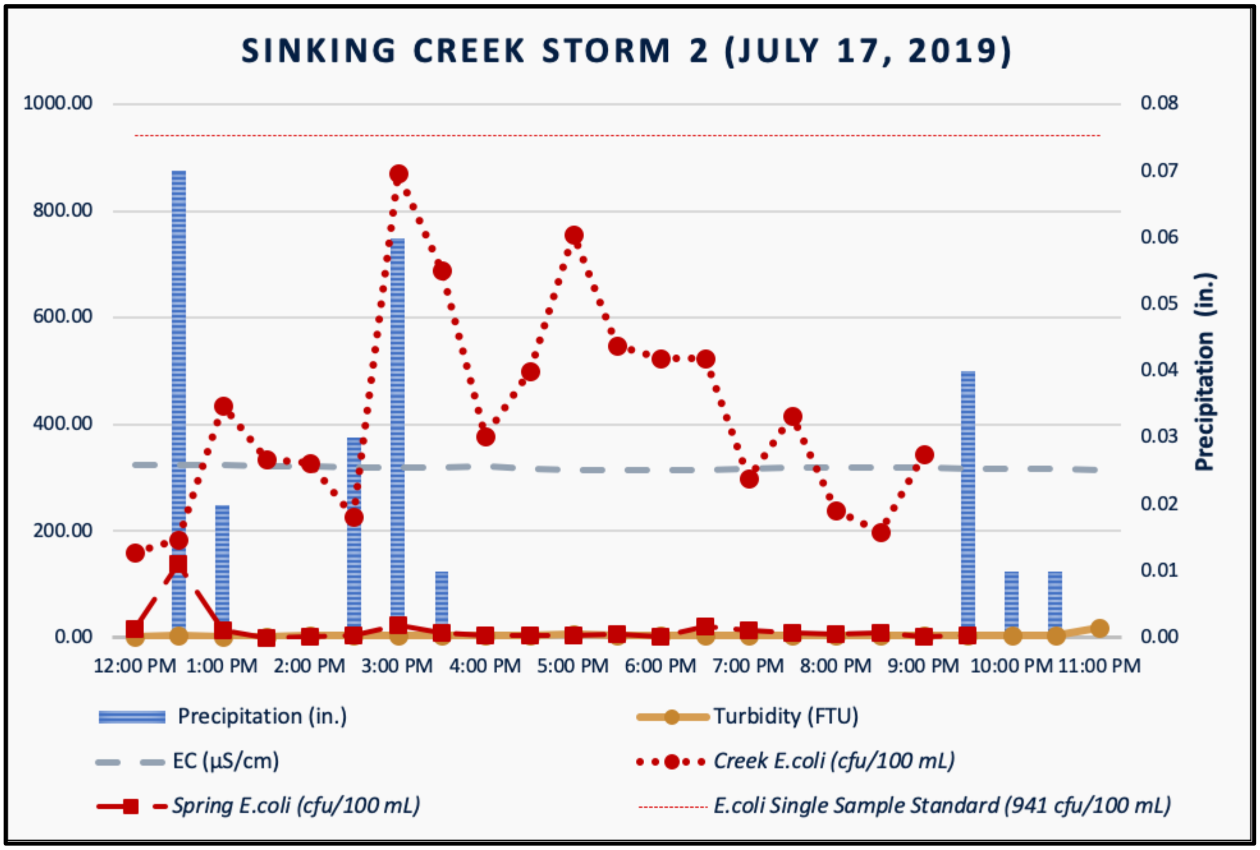

Figure 4. Sinking Creek Storm 2 – July 17, 2019. Precipitation began around 12:30 pm and is measured on the secondary y-axis. Turbidity and EC remained at consistent concentrations. *E. coli* concentrations at the creek fluctuated throughout the inclement weather and peaked 3 hours after rainfall initially began. *E. coli* at the spring peaked approximately the same time precipitation began.

Precipitation for Storm 3 (Figure 5) began at 3:30 pm on August 2, 2019. Storm sampling began at 3:00 pm. Turbidity peaked 30 minutes later at 45 FTU before decreasing and remaining below <10 FTU for the remainder of the storm. EC began at ~ 320 µS/cm and after one hour of rainfall, decreased to 217 µS/cm. As the precipitation ended, conductivity concentrations began returning to baseline. *E. coli* at the creek peaked at 1011 cfu/100 mL one hour after the storm began. *E. coli* concentrations at the spring became slightly elevated at 48 cfu/100 mL and then decreased to <10 cfu/100 mL. *E. coli* at the creek was above the single sample standard for the majority of the storm event, while concentrations at the spring remained below the standard.

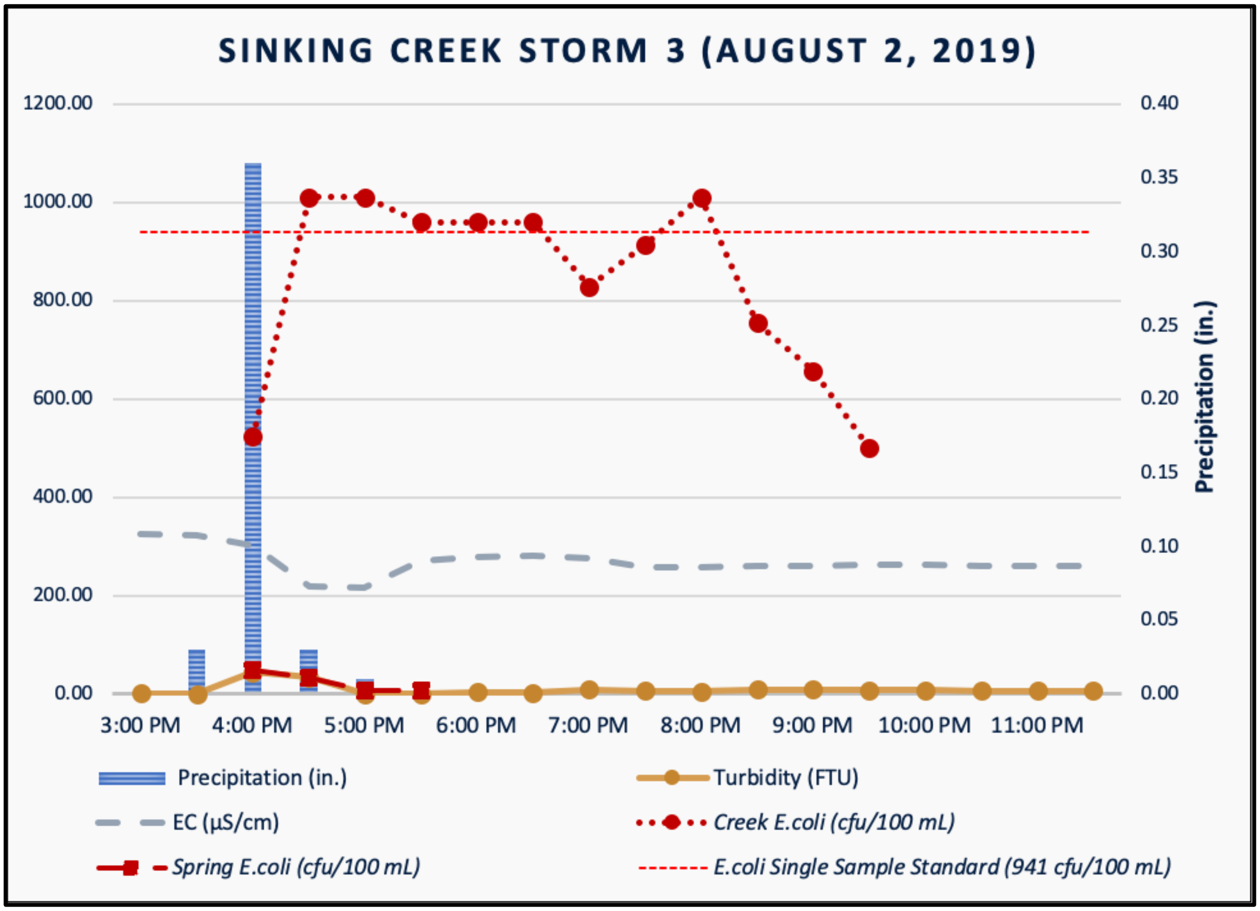

Figure 5. Sinking Creek Storm 3 – August 2, 2019. Precipitation began at 3:30 pm and is measured on the secondary y-axis. Turbidity peaked 30 minutes after precipitation began. EC began decreasing 30 minutes after precipitation. *E. coli* concentrations at the creek peaked 1 hour after rainfall began and concentrations at the spring peaked 30 minutes after rainfall.

On August 13, 2019 rainfall and storm sampling began at 2:00 pm for storm 4 (Figure 6). Turbidity concentrations increased to 70 FTU within 30 minutes of precipitation and then decreased to <10 FTU for the remainder of the storm. EC began at ~320 µS/cm and decreased to 217 µS/cm, 1.5 hours after precipitation began. *E. coli* at the creek began increasing directly after precipitation began. It peaked at 1011 cfu/100 mL one hour after precipitation started and concentrations remained at 1011 cfu/100 mL three hours before decreasing. *E. coli* at the spring peaked at a concentration of 173 cfu/100 mL, 30 minutes after the beginning of the storm, decreasing to low values near 1 cfu/100 mL for the duration of storm sampling. *E. coli* at the creek were above the single sample standard for about 2.5 hours after the storm while concentrations at the spring were below the standard throughout the storm.

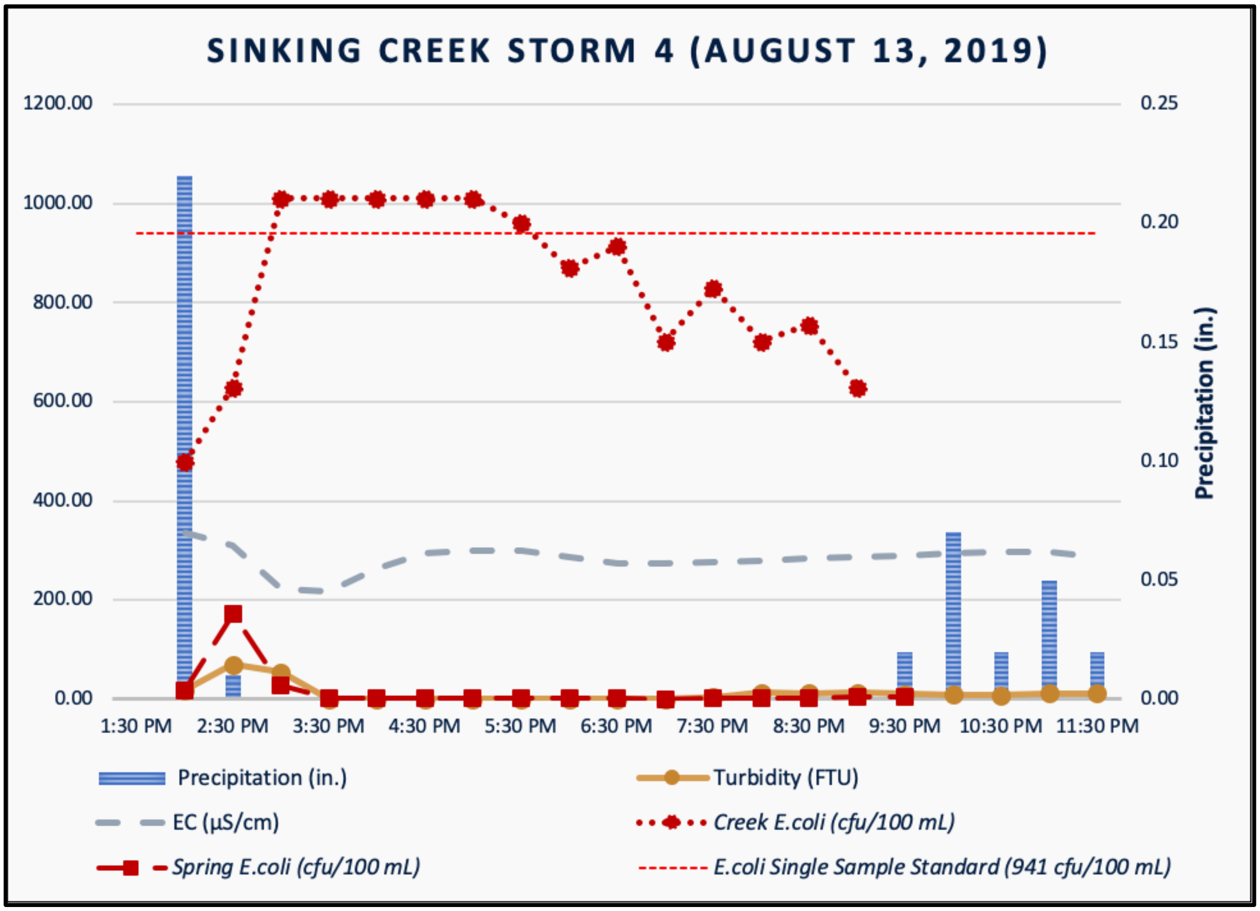

Figure 6. Sinking Creek Storm 4 – August 13, 2019. Precipitation began at 2:00 pm and is measured on the secondary y-axis. Turbidity peaked 30 minutes after precipitation began. EC began decreasing less than 30 minutes after precipitation. *E. coli* concentrations at the creek peak 1 hour after rainfall began and concentrations at the spring peaked 30 minutes after rainfall.

Precipitation began at 12:00 pm for storm 5 (Figure 7) on August 22, 2019. Storm sampling began at 10:00 am. Turbidity was low throughout the duration of the storm and only marginally responded to rainfall by increasing to 17 FTU. EC baseline was ~325 µS/cm prior to the storm, and within 1.5 hours of precipitation it dropped to 250 µS/cm. After precipitation ended, conductivity began to rise to baseline. *E. coli* at the creek peaked at 1011 cfu/100 mL one hour after the storm began. *E. coli* remained at 1011 cfu/100 mL for 1.5 hours after rainfall subsided, before decreasing. *E. coli* concentrations at the spring became elevated at 1011 cfu/100 mL as rainfall began. At the spring, the *E. coli* steadily decreased within 1.5 hours of the storm; eventually settling near 25 cfu/100 mL. Initial *E. coli* concentrations at the spring were above the single sample standard while the creek had concentrations over the standard for about 1.5 hours during the beginning of the storm.

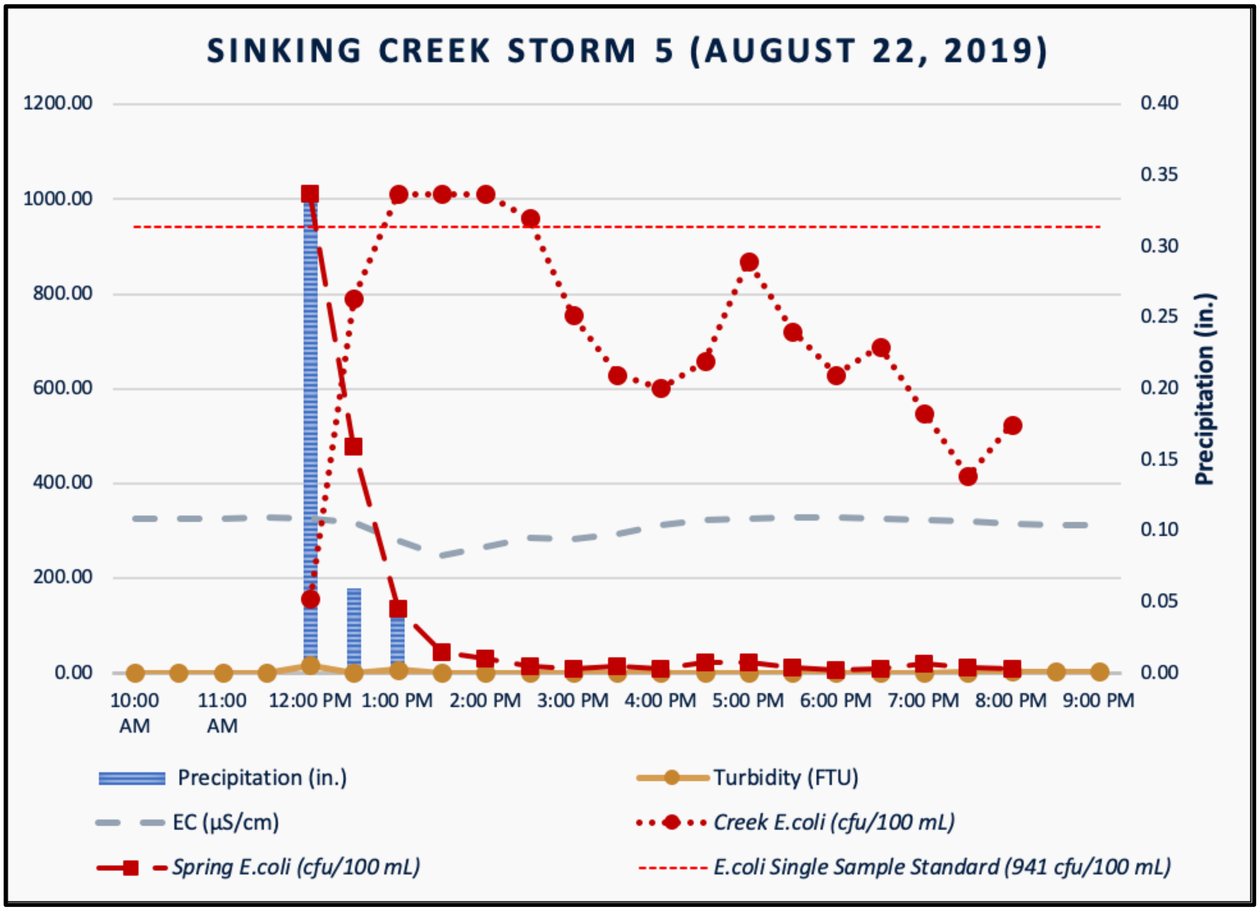

Figure 7. Sinking Creek Storm 5 – August 22, 2019. Precipitation began at 12:00 pm and is measured on the secondary y-axis. Turbidity remained consistent throughout the storm.EC slightly decreased 1 hour after precipitation started and concentrations quickly returned to baseline. *E. coli* concentrations at the creek peaked 1 hour after rainfall began and concentrations at the spring were highest at the beginning of the rainfall.

On September 26, 2019, precipitation for Storm 6 (Figure 8) began at 6:00 pm and sampling began at 4:30 pm. No rainfall was recorded at the ETSU Geoscience weather station; however, precipitation was recorded at the Garland Acres weather station (Figure 2, https://www.wunderground.com/dashboard/pws/KTNJOHNS41), which is within the Sinking Creek watershed (~2 miles from the creek). Electrical conductivity remained at consistent concentrations near 340 µS/cm throughout the storm. *E. coli* at the creek had a baseline range of 100 to 130 cfu/100 mL. *E. coli* quickly spiked to 755 cfu/100 mL ~30 minutes before rainfall began and fluctuated as storm sampling continued and peaked at 913 cfu/100 mL. During the beginning of the storm, *E. coli* at the spring were elevated at 436 cfu/100 mL, before decreasing to 79 cfu/100 mL 30 minutes before rainfall. Thirty minutes after precipitation began, *E. coli* spiked to 1011 cfu/100 mL before decreasing. During this storm *E. coli* concentrations at the creek were below the single sample standard, while only one sample from the spring peaked above the concentration.

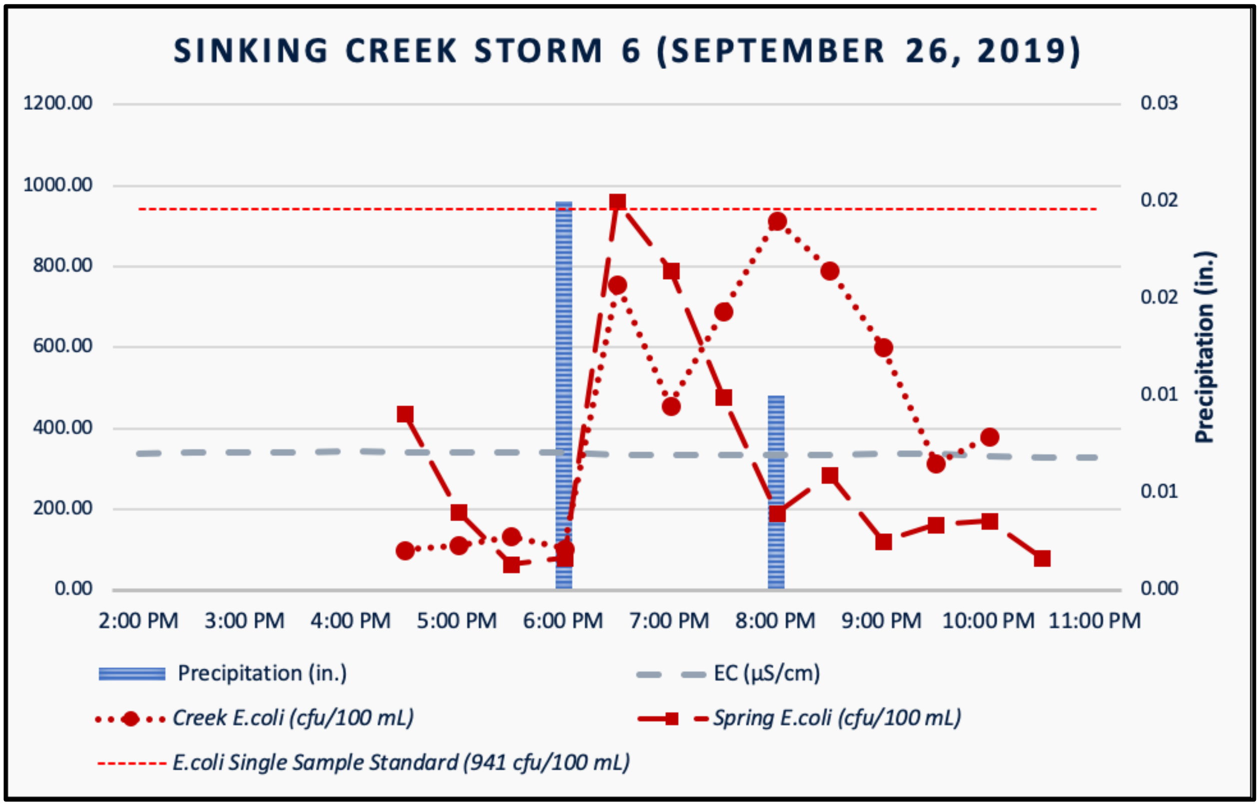

Figure 8. Sinking Creek Storm 6 – September 26, 2019. Precipitation began at 6:00 pm and is measured on the secondary y-axis. EC concentrations were consistent throughout the inclement weather event. *E. coli* concentrations at the creek peaked at 6:30 pm, and at the spring it peaked at 8:30 pm.

On October 7, 2019, storm 7 (Figure 9) was recorded and precipitation began at 4:00 pm. Turbidity concentrations were initially very low at concentrations <10 FTU and then increased 30 minutes after rainfall began. Turbidity peaked at 222 FTU, 1.5 hours after rainfall began before decreasing. The onset of precipitation at 8:00 pm resulted in a turbidity spike to 106 FTU. EC values during the first two hours of the storm were low near 10 µS/cm before increasing and peaking at 283 µS/cm, one hour after precipitation. Two hours after precipitation began, conductivity dropped to 198 µS/cm. EC remained near~285 µS/cm during intermittent rainfall that occurred over the next two hours before settling near 230 µS/cm for the remainder of the storm. *E. coli* at the creek began at <100 cfu/100 mL. *E. coli* at the creek peaked at 1011 cfu/100 mL, 30 minutes after the onset of precipitation. The concentrations were elevated for 2.5 hours of the storm. *E. coli* at the spring fluctuated prior to precipitation and peaked at 1011 cfu/100 mL 30 minutes before rainfall. Concentrations were elevated for ~5 hours before decreasing after precipitation ended. *E. coli* concentrations at the creek and spring were elevated above the single sample standard for the majority of the storm.

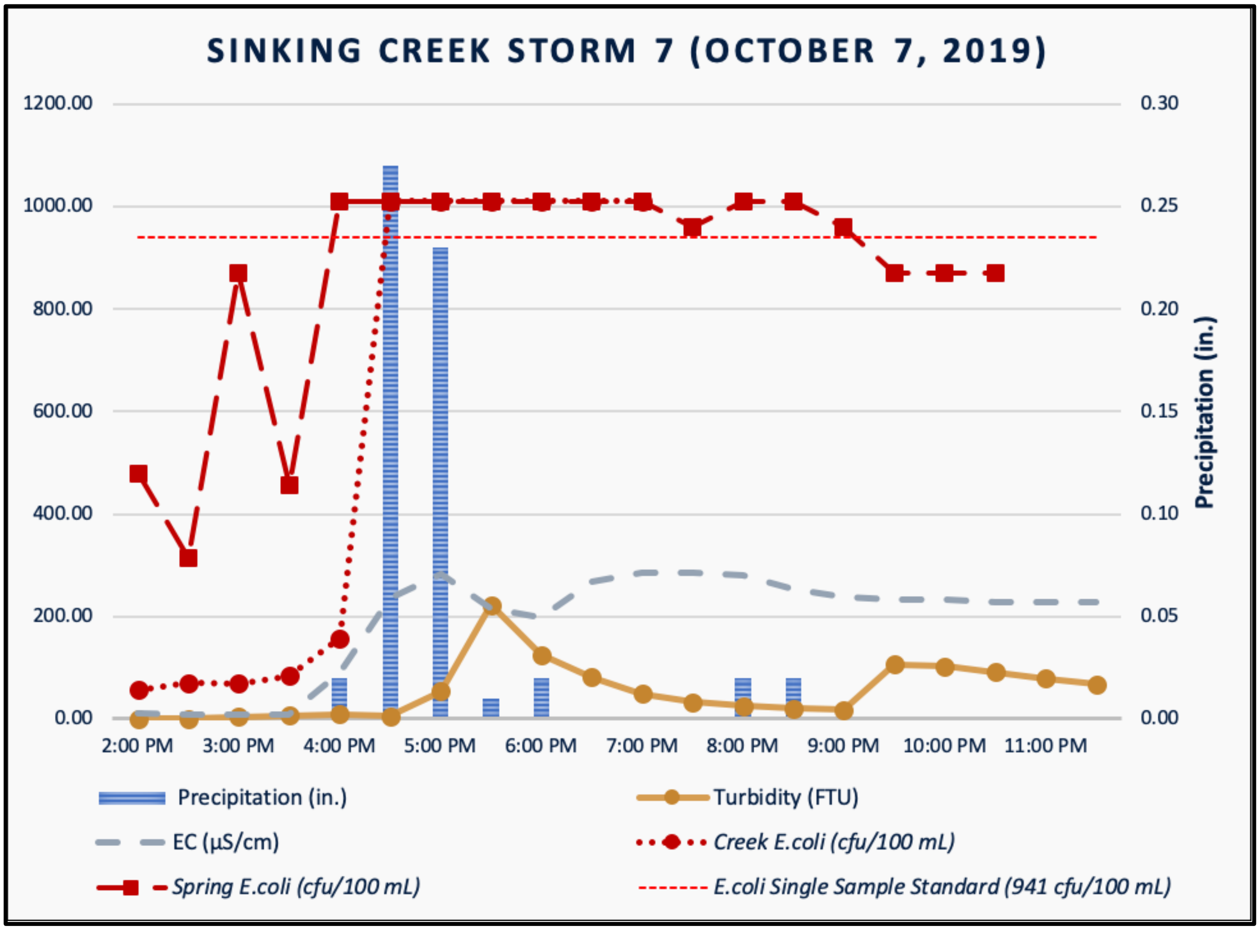

Figure 9. Sinking Creek Storm 7 – October 7, 2019. Precipitation began at 4:00 pm and is measured on the secondary y-axis. Turbidity peaked 1.5 hours after rainfall.EC decreased 1 hour after precipitation began. *E. coli* at the creek peaked as soon as precipitation began and concentrations at the spring increased 30 minutes after precipitation.

Storm 8 (Figure 10) occurred on October 16, 2019, and storm sampling began at 1:00 am. EC concentrations were ~300 µS/cm and decreased to 233 µS/cm after 3 hours of rainfall. EC continuously fluctuated as rainfall started and stopped throughout the storm; the concentrations dropped to their lowest concentration of 134 µS/cm after intense rainfall occurred between 8:00 and 10:00 am. *E. coli* at the creek began low with values near 140 cfu/100 mL during the first hour of rainfall. Three hours after the storm began, *E. coli* peaked at 1011 cfu/100 mL before steeply dropping to ~210 cfu/100 mL after rainfall accumulations decreased and as rainfall continued intermittently, *E. coli* at the creek continued to fluctuate. *E. coli* at the spring began at 460 cfu/100 mL and, 1.5 hours after precipitation began, it decreased to 68 cfu/100 mL. The concentrations fluctuated for the next few hours during the storm before peaking at 689 cfu/ 100 mL following a significant accumulation of rainfall. *E. coli* concentrations at the creek peaked above the single sample standard for the later duration of the storm while concentrations at the spring remained below the standard for the entire storm.

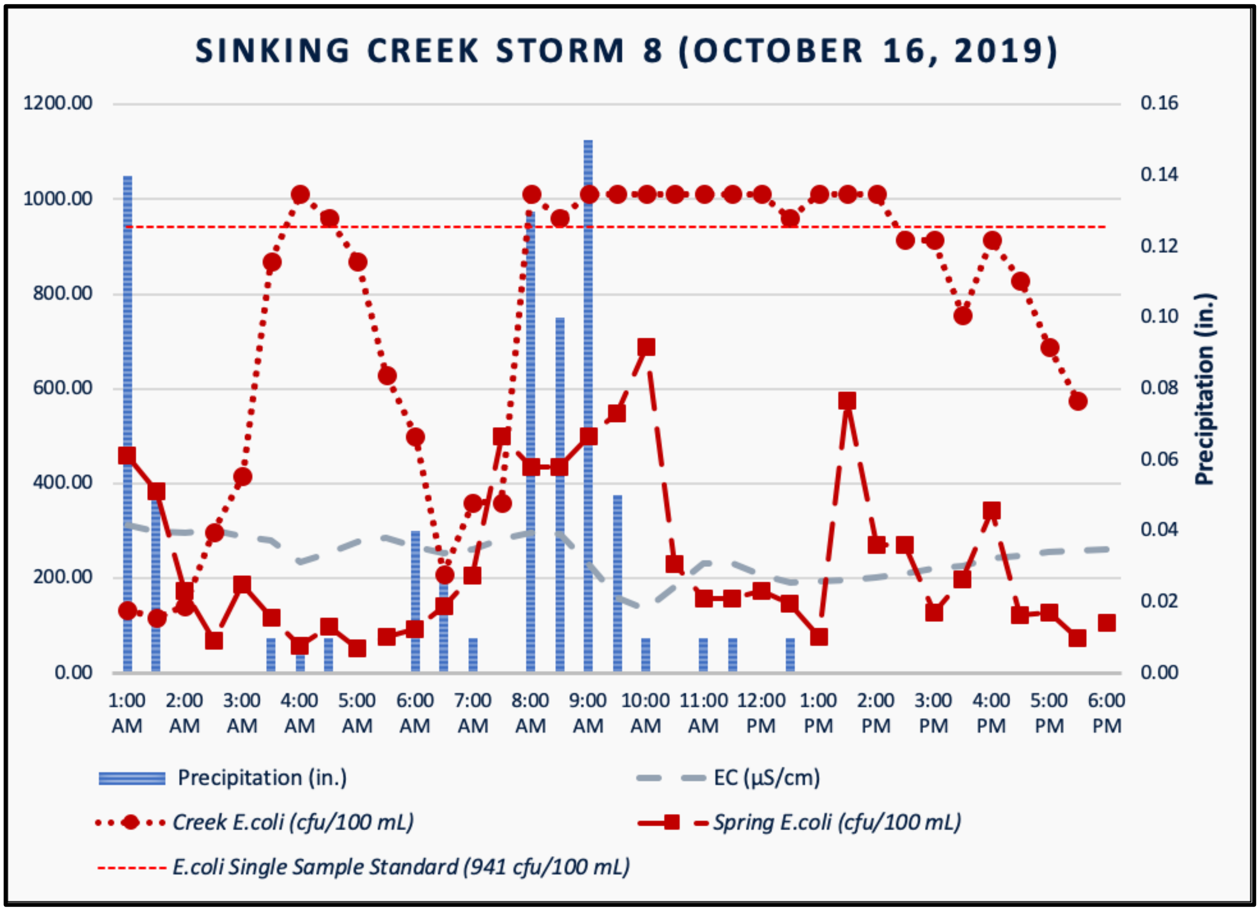

Figure 10. Sinking Creek Storm 8 – October 16, 2019. Precipitation began at 1:00 am and is measured on the secondary y-axis. EC decreased 3 hours after precipitation started and continued to fluctuate during intermittent rainfall. *E. coli* at the creek peaked 3 hours after precipitation began. *E. coli* at the spring began high and then fluctuated throughout the storm.

There were several trends that occurred during the storm sampling events. *E. coli* at the spring during all storms apart from storm 6, began with a peak before decreasing and remaining low until there was another onset of precipitation. *E. coli* at the creek began low and upon precipitation it increased with concentrations remaining above the single sample standard for several hours of the storm. During storm 6, even when there was very little precipitation, *E. coli* at both the creek and spring continuously fluctuated. Turbidity was usually observed to increase within 1.5 hours of precipitation and during storms with low rainfall accumulations. EC decreased within 1.5 hours or rainfall and, like turbidity, when rainfall accumulations were low.

Analytical Results: Creek vs. Spring

E. coli at the spring increased more quickly than the creek in all storms. At the spring, *E. coli* concentrations began elevated and then decreased as the storm progressed, noticeably this occurred in storm 5 (Figure 7), storm 7 (Figure 9), and storm 8 (Figure 10). In storm 2 (Figure 4) and storm 4 (Figure 6) *E. coli* at the spring peaked within 30 minutes of precipitation before decreasing. Concentrations at the spring were below the single sample standard for most storms, however, during more intense storm events concentrations exceeded the standard (Figure 3, Figure 7, Figure 9). At the creek, *E. coli* began at low base concentrations before increasing within 30 minutes of onset precipitation (Figure 5). The concentrations remained elevated for several hours of the storm before decreasing as evidenced in storms 3 and 4. *E. coli* at the creek increased with low rainfall accumulations (<0.25 inches) (Figure 4), while at the spring concentrations remained low. Mann-Whitney U-Test for *E. coli* at the creek and spring revealed that the concentration of *E. coli* at the creek ($\bar{x}$ = 676.5 cfu/100 mL) was significantly higher than at the spring ($\bar{x}$ = 252.3 cfu/100 mL). Similarly, the Mann-Whitney U-Test for DO revealed that DO was significantly higher at the creek ($\bar{x}$ = 8.05 mg/L) than at the spring ($\bar{x}$ = 6.77 mg/L).

OB analysis for the two storms revealed a moderately positive peak as the spring location during one of the storms (Table 1). Land cover for the watershed upstream of Jacob's Nature Park (Figure 11) was downloaded. All developed land was aggregated, forest land was aggregated, and shrubland, herbaceous, and pasture was combined. Analysis of the watershed indicates that majority of the watershed consists of developed, forested, and pasture (agricultural) areas (Table 2).

Table 1. Optical Brightener Results

				Optical Brighteners	
Station	**Date/Time Placed**	**Date/Time Collected**	**Peak (nm)**	**Height of peak on 16X ordinate scale**	**Results**
Creek	11/11/19 1700	11/18/19 1100			-
Spring	**11/11/19 1700**	**11/18/19 1100**	**417.0**	**42**	**Moderately Positive**
Creek	12/9/19 1400	12/15/19 1200			-
Spring	12/9/19 1400	12/15/19 1200			-
Laboratory control cotton blank					

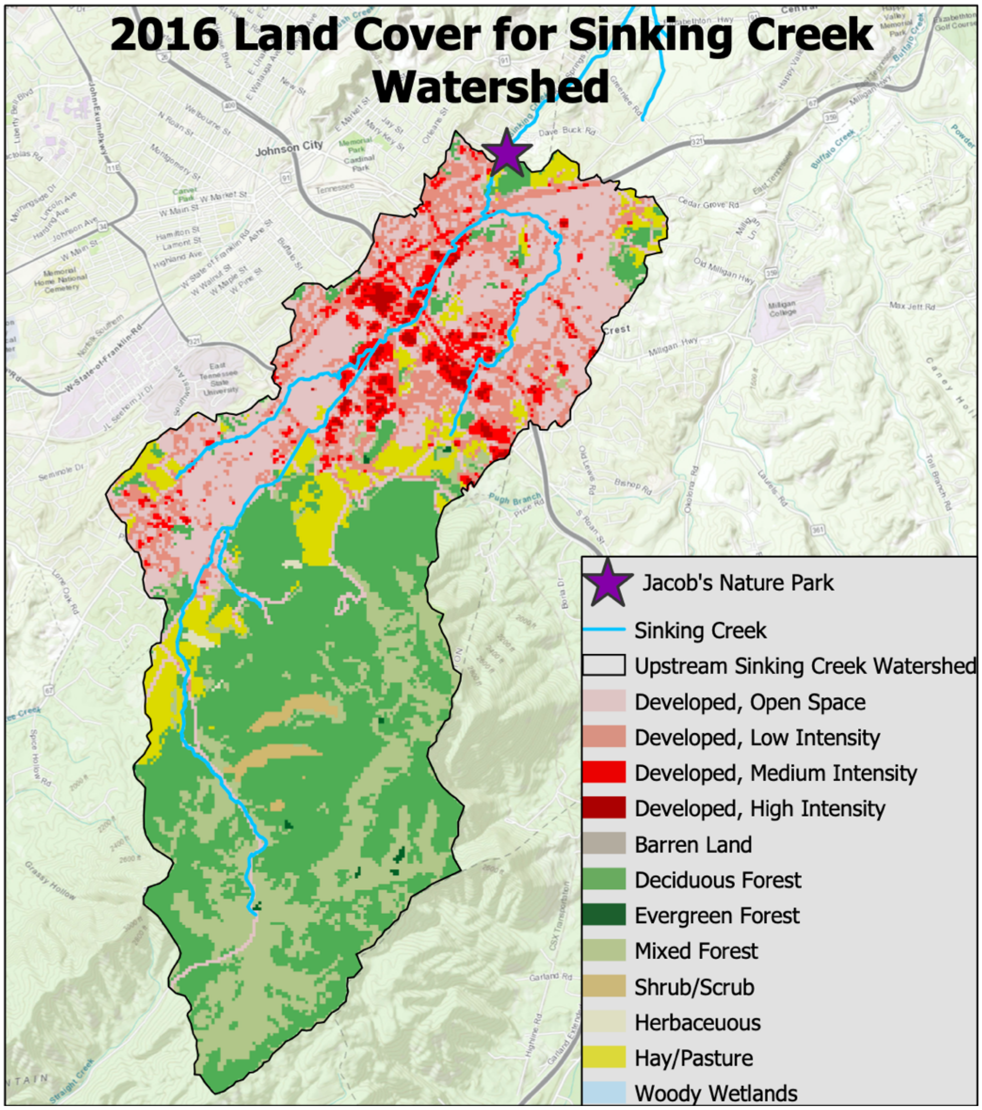

Figure 11. Sinking Creek land cover upstream of Jacob's Nature Park

Table 2. Sinking Creek Upstream Watershed Land Cover

Landcover	**Percentages**
Developed	38.30
Barren	0.02
Forest	53.22
Pasture	8.46
Wetlands	0.01
Total	100.00

Analytical Results: Precipitation vs. Water Quality

Antecedent precipitation at lags of 30-minute increments (Precip-1 to Precip-12) was correlated to water quality parameters (Table 3). *E. coli* at the creek was negatively correlated to EC (r = -0.352), while the *E. coli* at the spring was positively correlated with turbidity (r = 0.520) and negativity correlated to EC (r = -0.211). Antecedent precipitation affected water quality at both the creek and spring. *E. coli* at the creek was cross-correlated to Precips-2 through Precip-7, with the highest cross-correlation occurring at Precip-5 (r = .391) (Figure 12). This positive cross-correlation indicated that as precipitation increased at the creek, the amount of *E. coli* bacteria increased as well. *E. coli* at the spring was positively cross-correlated to Precip-1 through Precip-3, and its highest cross-correlation occurred at Precip-2 (r = .355) (Figure 12). Turbidity was cross-correlated to Precip-2 (r = .126). *E. coli* at the creek were more significantly correlated to antecedent precipitation than any of the other water quality parameters.

Table 3. Correlations and Cross – Correlation: Storms 1-8

Variables	***E. coli* Creek**	***E. coli* Spring**	**Turbidity**	**EC**
	Correlation Coefficient	**Correlation Coefficient**	**Correlation Coefficient**	**Correlation Coefficient**
E. coli Creek	-	-	-	$-.352^{**}$
E. coli Spring	-	-	$.520^{**}$	$-.211^{*}$
Turbidity	-	$.520^{**}$	-	-
EC	$-.352^{**}$	$-.211^{*}$	-	-
Precip-1	-	$.338^{**}$	-	-
Precip-2	$.303^{**}$	$.355^{**}$	$.126^{*}$	-
Precip-3	$.365^{**}$	$.245^{**}$	-	-
Precip-4	$.371^{**}$	-	-	-
Precip-5	$.391^{**}$	-	-	-
Precip-6	$.357^{**}$	-	-	-
Precip-7	$.229^{**}$	-	-	-
Precip-8	-	-	-	-
Precip-9	-	-	-	-
Precip-10	-	-	-	-
Precip-11	-	-	-	-
Precip-12	-	-	-	-
Precip-13	-	-	-	-

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

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

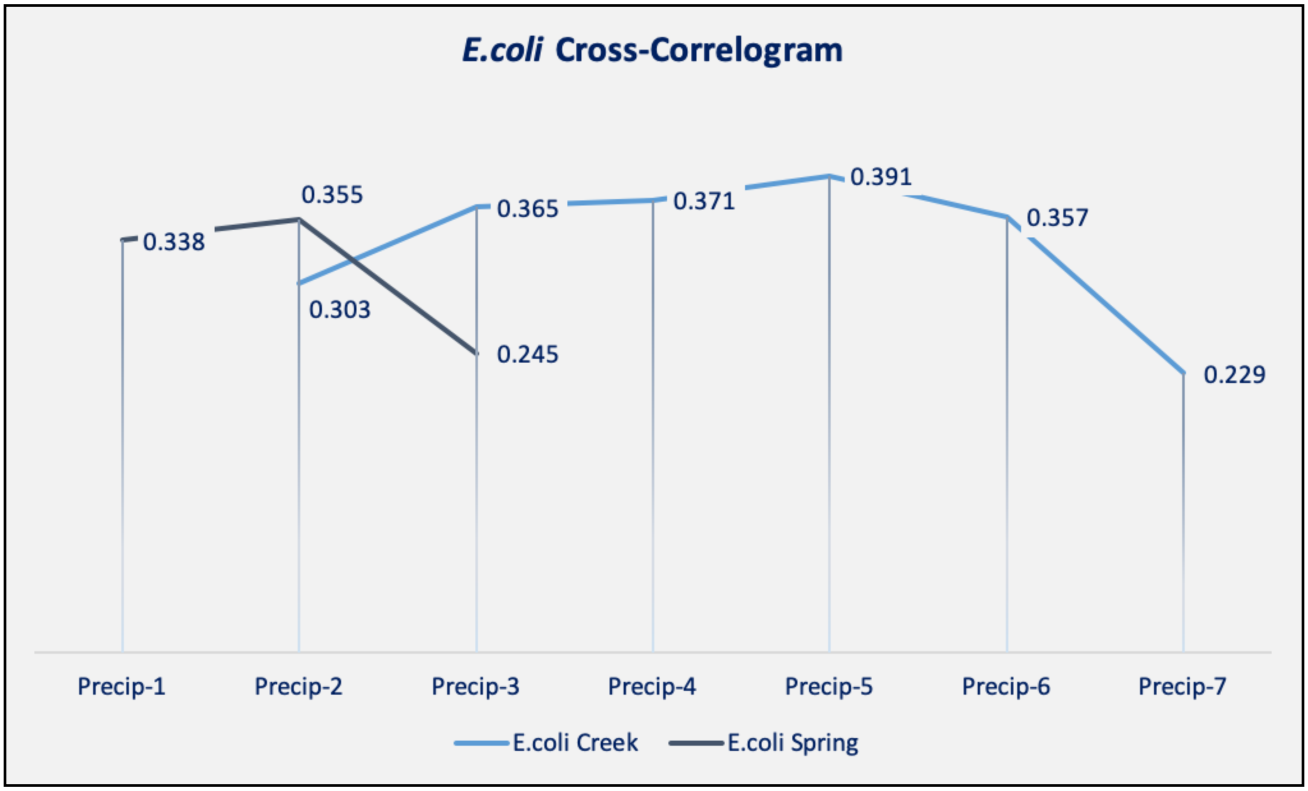

Figure 12. Cross-Correlogram of Storms 1-8

CHAPTER 4. DISCUSSION

Water Quality Parameters

The negative correlation between *E. coli* and EC at the creek may be related to storm discharge. Before the inclement weather events, EC was relatively stable, remaining near baselines of ~320 µS/cm. However, EC decreased when inclement weather occurred, which is attributed to lower amounts of dissolved ions in the water due to dilution from increased runoff (Shrestha and Kazama 2007). Turbidity increased via increased sediment erosion. The positive correlation between *E. coli* and turbidity at the spring suggests that as precipitation increases, *E. coli* concentrations and turbidity levels increase as well. The positive correlation between *E. coli* and turbidity at the spring may be indicating that when precipitation occurs bacteria is being adsorbed on to soil particles. The *E. coli* bacteria may be carried into the stream by sediment from runoff originating from nearby agriculture or residential areas. Fecal coliforms have been found to have significant positive relationships with agricultural and residential locations (Tong and Chen 2002), with strong correlations occurring within 24 hours of precipitation (Mallin et al. 2001), which is within the range of antecedent precipitation used in this study.

A possible explanation for the increase in *E. coli* concentrations during low rainfall events could be attributed to possible naturalized *E. coli* in the stream soil/sediment. *E. coli* bacteria are capable of thriving in environments with limited sunlight, without interaction from humans or wildlife (Myers 2006). Because *E. coli* is an indicator of water quality, more recent studies have investigated how *E. coli* may be resident in certain soils and is not completely indicative of runoff from agricultural or residential areas. This could explain conditions during storm 6 in which there was very little rainfall (0.03 in.), but *E. coli* concentrations in both the creek and spring fluctuated. Fine sediment along the channel bottom could be adsorbing *E. coli*, indicating that some bacteria may already be present in the water and/or soil and not completely caused by agricultural or residential runoff during inclement weather events. A soil's sorption and ability to transport fecal bacteria relies on factors such as the pH and pore size (Hall et al. 2014). Prior research by Hall et al. (2014) indicates that the sandy soil and organic material in the Sinking Creek watershed do influence *E. coli* concentrations at the stream. Finer soils adsorb more microorganisms, therefore low percentages of clay and silt in the top-soil layers have aided in *E. coli* concentrations at the creek (Hall et al. 2014).

These storm sampling events took place from June to October, extending through both summer and fall months. Higher *E. coli* concentrations have been linked with summer months, and warmer temperatures (Chen and Chang 2014)High fecal coliforms have been found during winter and spring months (Tong and Chen 2002), however, in a study in karst regions, *E. coli* increased during all storm events and was not controlled by seasonal variations (Knierim et al. 2015). *E. coli* concentrations during the summer months may also be influenced by land-use practices in the surrounding communities (Chen and Chang 2014). Storm 1 (Figure 3) was conducted in June, and the warm season in east Tennessee begins in May and extends to September. *E. coli* concentrations at the creek were elevated for the entire storm, these results may be attributed to the warmer temperatures during the summer, however, elevated *E. coli* concentrations were also apparent during storm 3 (Figure 5), storm 4 (Figure 6), storm 7 (Figure 9), and storm 8 (Figure 10) which was the only storm sampled during the fall season. This lack of observed change might have been due to only sampling during one month of the fall; concentrations may have been different during November and December.

Creek vs. Spring

Storm sampling at Sinking Creek and a tributary spring shows that *E. coli* concentration at both sites is correlated to antecedent precipitation. The spring responded more quickly than the creek: a short peak at onset or within 30 minutes of precipitation for the spring versus an elongated period of high *E. coli* concentrations beginning within one hour of onset and continuing throughout the storm for the creek. DO at the creek was higher, indicating that turbulence of the stream created more dissolved oxygen as opposed to low turbulence at the spring.

The 30-minute lag time between precipitation and increased *E. coli* concentrations at the spring is likely related to the karst topography of the area (Knierim et al. 2015) and suggest a connection between the *E. coli* source and the spring outlet. Moreover, the positive OB sample at the spring coupled with the initial high concentration of *E. coli* at the spring implicate residential wastewaters as a source of *E. coli* at this site. Failing septic systems along Sinking Creek have been proposed as one source of *E. coli* in Sinking Creek (Dulaney 2003) and a study by Knierim et al. (2015) investigating *E. coli* and precipitation at a spring in Arkansas found a similar result. Epikarst in the area is a possible explanation for the peak observed at the spring during most storms. Epikarst systems occur in carbonate rocks that are extensively weathered near the surface; it represents an area of the vadose zone in which groundwater or contaminants may be stored in a separate aquifer before infiltrating the carbonate rocks below (Klimchouk 2004). Effluent from failing septic systems may be "ponding" in this epikarst area (Knierim et al. 2015), and during storm events the effluent may be washed into the underlying fractured carbonate rocks, likely causing the immediate spike that is apparent at the spring.

Prolonged high concentrations of *E. coli* at the creek and the later arrival of peak concentrations relative to the onset of precipitation indicate that upstream agricultural areas may be responsible for high concentrations in the creek (Floresguerra 2003). Dye tracing may be a suitable method to discern water flowpaths or origins, and it has been utilized in several groundwater flow studies in northeast Tennessee (Wilson et al. 2016; Doyka 2017). Most dye tracing procedures work by injecting fluorescent dye into a water source and observing possible downgradient locations to determine where the dye will emerge. The method may be suitable, along with geologic mapping and geospatial analysis similar to the methods of Burnham et al. (2016), to identify the water source for the spring at Sinking Creek.

Precipitation vs. Water Quality

The effects of precipitation on water quality can be linked to storm intensity, duration, and location in the watershed. During several sampling events, turbidity did not respond to low rainfall accumulation (< 0.25 in.), however, when storm intensity was higher with accumulations >0.25 in., turbidity increased within 30 minutes. This increase in turbidity during higher rainfall intensities is likely a result of increased sediment erosion rates. Hamilton and Luffman (2009), also found positive correlation between precipitation and turbidity. EC decreased within 1.5 hours of precipitation, and like turbidity, EC also exhibited only slight responses to low rainfall amounts.

Peak concentrations for *E. coli* at the creek usually occurred within the first 2.5 hours of the storms, indicating that this is the first flush for *E. coli*. Turbidity at the creek began increasing at the onset of precipitation and, like *E. coli,* it too peaked within the first 2.5 hours. Observing EC values during the storms showed that as turbidity began increasing, EC would begin to decrease; both parameters begin responding at the same time. EC can be expected to decrease to its minimum within the first 2.5 hours.

Peak concentrations for *E. coli* at the spring occurred prior to the storm sampling event or during the onset of precipitation. Unlike the creek location in which *E. coli* concentrations were highly variable depending on precipitation, at the spring the concentrations only have slight responses to continued precipitation during the storm apart from storm 7 (Figure 9) and storm 8 (Figure 10). From August 22, 2019 through October 7, 2019, there were very few rainfall events; and when precipitation did occur (storm 6), accumulations were very low (<0.10 in.). *E. coli* concentrations at the spring were elevated for several hours of storm 7 (Figure 9), the first substantial rainfall event after the period of dry weather. The elevated *E. coli* concentrations at the spring during storm 7 may be indicative of septic tank leaks, with the contaminated groundwater remaining in the epikarst boundary of the subsurface. A possibly contaminated vadose zone along with the prolonged period of low rainfall (August 22, 2019 through October 7, 2019) could have resulted in such high concentrations in the spring due to insufficient rain to flush and dilute the system. The spring location produced positive results for optical brighteners during one of the storms, reiterating that anthropogenic sources, possibly failing septic systems, could be a source of contamination at the spring.

Limitations

Data from this research provided information about surface water and groundwater quality at the stream and spring, but there were limitations that may have impacted the results. There were only 8 storm events that were sampled and although similar trends were observed throughout the storms, variations in location of the watershed and storm intensities may have resulted in several different storm outputs. Another limitation was the location of the weather station; the ETSU weather stations is 3.3 miles from Sinking Creek and is not located in the watershed, therefore some rain estimates may have been slightly different from the values in the watershed. Since the ETSU weather station is maintained by faculty in the Dept. of Geoscience, it was deemed a reliable source to use. Research grant funding was important in determining how many storm events could be sampled as well as the duration of sampling, materials were allocated to allow storm sampling for approximately 10 storms depending on sampling duration. From analyzing the storms captured, it became apparent that at the spring, there was a response prior to sampling that was not fully captured, as this trend was not expected storm sampling was unable to capture these values. The prior response at the spring may have been due to influences from precipitation occurring at different locations of the watershed and were therefore not captured at the ETSU weather station.

CHAPTER 5. CONCLUSION

Sinking Creek of the Watauga watershed is impaired for *E. coli* (TDEC 2018). During June 2019 to October 2019 eight storm sampling events were conducted at Sinking Creek to understand how precipitation affects *E. coli* at both the creek and a feeder spring, along with turbidity and EC. Other objectives were to understand when the first flush occurred at both the creek and spring and to understand how DO concentrations may have varied at the creek and spring location. OB analysis was conducted to determine the origin of the *E. coli. E. coli* at the creek, turbidity, and EC respond to precipitation within 30 minutes and the first flush for the parameters occurs within 2.5 hours of precipitation. Variations in water quality are dependent on storm intensity as well as rainfall accumulations. DO at the creek was higher than at the spring due to increased turbulence and circulation of oxygen at the creek. OB analysis revealed that the origin of *E. coli* at the spring could be due to anthropogenic sources. This conclusion provided helpful answers to better understand water quality in Sinking Creek, but more storm events should be sampled, and more OB analysis should be performed before mitigation can begin in the area.

Future Recommendations (Inclement Weather)

- Decreasing sampling collection to 15 minutes and increasing the number of sampling events to have more robust understanding of the water quality.
- Place multiple weather stations in the watershed to provide more accurate data.

Future Recommendations (Identifying Sources)

- Compare *E. coli* present at the creek and spring to *E. coli* that would be found from either humans or wildlife (Perchec-Merien and Lewis 2013) through the process of Microbial Source Tracking (MST) or DNA-ribo typing.

- Test for OB during more storm events.
- Dye tracing in the Sinking Creek watershed may help determine where the spring water originates.

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APPENDIX: Correlation and Cross-Correlation Tables

A1. Storm 1. Correlation and Cross-Correlation

Variables	***E. coli* Creek** **Correlation Coefficient**	***E. coli* Spring** **Correlation Coefficient**	**Turbidity** **Correlation Coefficient**	**EC** **Correlation Coefficient**
E. coli Creek	-	.720*	.671*	-.834**
E. coli Spring	.720*	-	.884**	-
Turbidity	.671*	.884**	-	-
EC	-.834**	-	-	-
Precip-1	-	.707*	.543**	-
Precip-2	-	.860**	.561**	-
Precip-3	-	-	.514**	-
Precip-4	-	-	.509**	-
Precip-5	-	-	.444*	-
Precip-6	-	-	-	-
Precip-7	-	-	-	-
Precip-8	-	-	-	-
Precip-9	-	-	-	-
Precip-10	-	-	-	-
Precip-11	-	-	-	-
Precip-12	-	-	-	-
Precip-13	-	-	-	-

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

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

A 2. Storm 2. Correlations and Cross-Correlations

Variables	***E. coli* Creek**	***E. coli* Spring**	**Turbidity**	**EC**
	Correlation Coefficient	**Correlation Coefficient**	**Correlation Coefficient**	**Correlation Coefficient**
E. coli Creek	-	-	.589**	-.548*
E. coli Spring	-	-	-	-
Turbidity	.589**	-	-	-.837**
EC	-.548*	-	-.837**	-
Precip-1	-	-	-	-
Precip-2	-	-	-	-
Precip-3	-	-.450*	-	-
Precip-4	-	-	-	-
Precip-5	-	-	-	-
Precip-6	.760**	-	-	-
Precip-7	.495*	-	-	-
Precip-8	-	-	-	-
Precip-9	-	-	-	-
Precip-10	-	-	-	-
Precip-11	-	-	-	-
Precip-12	-	-	-	-
Precip-13	-	-	-	-

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

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

A 3. Storm 3. Correlations and Cross-Correlations

Variables	***E. coli* Creek**	***E. coli* Spring**	**Turbidity**	**EC**
	Correlation Coefficient	**Correlation Coefficient**	**Correlation Coefficient**	**Correlation Coefficient**
E. coli Creek	-	-	-	-
E. coli Spring	-	-	-	-
Turbidity	-	-	-	-
EC	-	-	-	-
Precip-1	-	-	-	-
Precip-2	-	-	-	-
Precip-3	-	-	-	-
Precip-4	-	-	-.491*	-
Precip-5	-	-	-	-
Precip-6	-	-	-	-
Precip-7	-.640*	-	-	-
Precip-8	-.600*	-	-	-
Precip-9	-	-	-	-.463*
Precip-10	-	-	-	-
Precip-11	-	-	.411*	-
Precip-12	-	-	-	-
Precip-13	-	-	-	-

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

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

A 4. Storm 4. Correlations and Cross-Correlations

Variables	***E. coli* Creek**	***E. coli* Spring**	**Turbidity**	**EC**
	Correlation Coefficient	**Correlation Coefficient**	**Correlation Coefficient**	**Correlation Coefficient**
E. coli Creek	-	-	-.582*	-
E. coli Spring	-	-	.836**	-
Turbidity	-.582*	.836**	-	-
EC	-	-	-	-
Precip-1	-.582*	.595*	-	.397*
Precip-2	-	.592*	.387*	-
Precip-3	-	-	-	-
Precip-4	-	-	-	-.429*
Precip-5	-	-	-	-
Precip-6	-	-	-	-
Precip-7	-	-	-	-
Precip-8	-	-	-	-
Precip-9	-	-	-	-
Precip-10	-	-	-	-
Precip-11	-	-	-	-
Precip-12	-	-	-	-
Precip-13	-	-	-	-

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

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

A 5. Storm 5 Correlation and Cross-Correlations

Variables	***E. coli* Creek**	***E. coli* Spring**	**Turbidity**	**EC**
	Correlation Coefficient	**Correlation Coefficient**	**Correlation Coefficient**	**Correlation Coefficient**
E. coli Creek	-	-	-	$-.494^{*}$
E. coli Spring	-	-	-	-
Turbidity	-	-	-	$-.468^{**}$
EC	$-.494^{*}$	-	$-.468^{**}$	-
Precip-1	-	$.665^{**}$	$.358^{*}$	-
Precip-2	$.519^{*}$	$.571^{*}$	-	$-.370^{*}$
Precip-3	$.660^{**}$	-	-	$-.647^{**}$
Precip-4	$.604^{*}$	-	-	$-.639^{**}$
Precip-5	-	-	-	$-.526^{**}$
Precip-6	-	-	-	$-.375^{*}$
Precip-7	-	-	-	-
Precip-8	-	-	$-.358^{*}$	-
Precip-9	-	-	$-.358^{*}$	-
Precip-10	-	-	$-.358^{*}$	$.434^{*}$
Precip-11	-	-	$-.358^{*}$	$.498^{**}$
Precip-12	-	$-.549^{*}$	$-.358^{*}$	$.513^{**}$
Precip-13	-	-	-	$.454^{*}$

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

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

A 6. Storm 6 Correlations and Cross-Correlation

Variables	***E. coli* Creek**	***E. coli* Spring**	**Turbidity**	**EC**
	Correlation Coefficient	**Correlation Coefficient**	**Correlation Coefficient**	**Correlation Coefficient**
E. coli Creek	-	-	-	-
E. coli Spring	-	-	-	.630*
Turbidity	-	-	-	-.776**
EC	-	.630*	-.776**	-
Precip-1	-	-	.389*	-
Precip-2	-	-	.406*	-
Precip-3	-	-	-	-
Precip-4	-	-	-	-
Precip-5	-	-	-	-
Precip-6	-	-	-	-
Precip-7	-	-	-	-
Precip-8	-	-	-	-
Precip-9	-	-	-	-
Precip-10	-	-	-	-
Precip-11	-	-	-	-
Precip-12	-	-	-	-
Precip-13	-	-	-	-

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

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

A 7. Storm 7 Correlations and Cross-Correlations

Variables	***E. coli* Creek**	***E. coli* Spring**	**Turbidity**	**EC**
	Correlation Coefficient	**Correlation Coefficient**	**Correlation Coefficient**	**Correlation Coefficient**
E. coli Creek	-	.850**	.820**	.828**
E. coli Spring	.850**	-	-	.584*
Turbidity	.820**	-	-	.376*
EC	.828**	.584*	.376*	-
Precip-1	.556	.721**	-	.395*
Precip-2	.745**	.602**	-	.466**
Precip-3	.745**	-	.490**	.452*
Precip-4	.632*	-	.613**	.451*
Precip-5	-	-	.447*	.541**
Precip-6	-	-	-	.610**
Precip-7	-	-	-	.586**
Precip-8	-	-	-	.504**
Precip-9	-	-	-	.378*
Precip-10	-	-	-	-
Precip-11	-	-	.366*	-
Precip-12	-	-	.481**	-
Precip-13	-	-	.395*	-

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

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

A 8. Storm 8 Correlations and Cross-Correlation

Variables	***E. coli* Creek**	***E. coli* Spring**	**EC**
	Correlation Coefficient	**Correlation Coefficient**	**Correlation Coefficient**
E. coli Creek	-	-	-.719**
E. coli Spring	-	-	-
EC	-.719**	-	-
Precip-1	-	-	-
Precip-2	-	-	-
Precip-3	-	-	-
Precip-4	-	-	-
Precip-5	.404*	-	-
Precip-6	.497**	-	-.478**
Precip-7	.500**	-	-.623**
Precip-8	.475**	-	-.552**
Precip-9	.432*	-	-.411**
Precip-10	.422*	-	-.411**
Precip-11	.353*	-	-.536**
Precip-12	-	-	-.651**
Precip-13	-	-	-.597**

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

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

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