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Application of Multivariate Statistical Methodology to Model Factors Influencing Fate and Transport of Fecal Pollution in Surface Waters

Kimberlee K. Hall
East Tennessee State University

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by

Kimberlee K Hall

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Philip Scheuerman, Ph.D., Chair

Kurt Maier, Ph.D.

Ranjan Chakraborty, Ph.D.

Eric Mustain, Ph.D.

Nathan Fethke, Ph.D.

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ABSTRACT

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Degraded surface water quality is a growing public health concern. While indicator organisms are frequently used as a surrogate measure of pathogen contamination, poor correlation is often observed between indicators and pathogens. Because of adverse health effects associated with poor water quality, an assessment of the factors influencing the fate and transport of fecal pollution is necessary to identify sources and effectively design and implement Best Management Practices (BMPs) to protect and restore surface water quality. Sinking Creek is listed on the State of Tennessee's 303D list as impaired due to pathogen contamination. The need to address the listing of this and other water bodies on the 303D list through the Total Maximum Daily Load (TMDL) process has resulted in increased research to find methods that effectively and universally identify sources of fecal pollution. The main objective of this research is to better understand how microbial, chemical, and physical factors influence pathogen fate and transport in Sinking Creek. This increased understanding can be used to improve source identification and remediation. To accomplish this objective, physical, chemical, and microbial water quality parameters were measured and the data were analyzed using multivariate statistical methods to identify those parameters influencing pathogen fate and transport. Physical, chemical, and microbial water and soil properties were also characterized along Sinking Creek to determine their influences on the introduction of fecal pollution to surface water. Results indicate that the 30-day geometric mean of

fecal indicator organisms is not representative of true watershed dynamics and that their presence does not correlate with the presence of bacterial, protozoan, or viral pathogens in Sinking Creek. The use of multivariate statistical analyses coupled with a targeted water quality-monitoring program has demonstrated that nonpoint sources of fecal pollution vary spatially and temporally and are related to land use patterns. It is suggested that this data analysis approach can be used to effectively identify nonpoint sources of fecal pollution in surface water.

DEDICATION

This dissertation is dedicated to my parents. Their support throughout this journey has been immeasurable.

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CHAPTER 1

INTRODUCTION AND RESEARCH OBJECTIVES

Waterborne disease through recreational contact remains an important public health threat. The Centers for Disease Control and Prevention (CDC) reported that there were 134 recreational water-associated outbreaks in 38 states and Puerto Rico in a recent report on outbreaks during 2007−2008 (Hlavsa et al. 2011). Acute gastrointestinal illness (AGI) accounted for 60.4% of the outbreaks and 89.3% (12,477) of the total cases. The remaining outbreaks were dermatologic (17.9%) and acute respiratory illness (12.4%). The etiology of the AGI cases reported were 74.1% *Cryptosporidium sp.,* 6.2% Norovirus, 4.9% *Shigella* sp., 3.7% *E. coli* O157:H7, and 3.7% *Giardia intestinalis*.

In the United States, 41,288 impaired surface waters are listed on impaired waters (303d) lists. Of these impaired waters, 10,722 are impaired due to pathogen contamination and 236 pathogen-impaired waters are located in Tennessee (USEPA, 2010). The increased listings of surface waters on 303d lists and subsequent development of Total Maximum Daily Loads (TMDL) have resulted in methods that effectively and universally identify sources of fecal pollution to avoid adverse human health outcomes associated with fecal contamination of surface waters such as the outbreak of *Cryptosporidium* in Milwaukee in 1993 (Mackenzie et al. 1993).

A fundamental requirement of methods that identify sources of fecal pollution to prevent waterborne disease outbreaks is understanding the processes that influence fate and transport of fecal indicators and pathogens from the various sources to the

receiving waters. Variability in land use patterns, the types and nature of pollutants, climatic conditions, and watershed characteristics add to the difficulty of modeling fate and transport of fecal pollution. In addition, the interactions between chemical and microbial processes in the water add to the complexity of understanding pathogen loading and transport in the watershed. The need to address impaired waters through the TMDL process has led to an urgent need to develop methods that successfully identify the types and sources of fecal pollution. Pathogen TMDL development is currently based on a 30-day geometric mean that does not take into consideration seasonal effects, variability in land use patterns, or the influence of runoff events on water quality. Examining the influence of chemical, physical and microbial factors on the fate and transport of fecal pollution and pathogens can improve our understanding of these influences on water quality and help identify sources of fecal pollution to aid in effective TMDL development to protect surface water resources and human health.

The listing of pathogen-impaired waters on 303d lists is based on the use of indicator organisms as a surrogate measure of pathogen presence. Indicators of fecal pollution are frequently used to assess the extent of fecal pollution because it is not feasible to monitor surface waters for every pathogen. A successful fecal indicator should be associated with the source of the pathogen, be easily detectable, and respond to environmental conditions in a manner similar to that of the pathogen to help effectively protect human health. Total and fecal coliform bacteria, *E. coli* and Enterococci are commonly used indicators because of their association with fecal material and ease of monitoring. These indicators may be associated and correlated with fecal pollution (Schaffter and Parriaux, 2002; Gersberg et al. 2006), but they may

also be contributed to surface waters by sources other than fecal material and may not respond to environmental conditions in the same manner as the pathogen. There is often a lack of correlation between fecal indicator bacteria and pathogen presence, that puts public health at risk (Harwood et al. 2005; Wu et al. 2011). The lack of correlation between fecal indicator bacteria and pathogens may be due to differences in excretion densities (Davenport et al. 1976) and regrowth and survival (Lemarchand et al. 2003) of fecal indicators in the environment. *Bifidobacterium* and *Bacteriodes* have been suggested as potential indicators of fecal pollution but do not survive as long as *E. coli* in the environment, thus indicating only recent fecal pollution events (Carillo et al. 1985; Kreader, 1998). *Clostridium* has also been proposed as a conservative estimator of protozoan contamination (Hörman et al. 2004), and f-RNA and somatic coliphages have been suggested as indicators of virus pollution (Sinton et al. 2002). Although these indicators have demonstrated some usefulness, no single indicator has been shown to effectively and universally identify the presence and source of fecal pollution.

Reliance on these indicators alone is not sufficient to protect surface water resources and human health and may hinder TMDL development and remediation efforts to remove impaired waters from 303d lists. The shortcomings of conventional indicators and methods identifying sources of fecal pollution have spawned a need to identify and employ alternative methods of water quality monitoring program design, methods, and data analysis to better protect human health. Examining the relationships between indicator organisms and pathogen prevalence and the influences of chemical and microbial processes in surface water can improve our understanding of their influences on water quality. A better understanding of those factors that influence

pathogen loading can help identify sources of fecal pollution to aid in effective TMDL development and the protection of human health.

A Total Maximum Daily Load (TMDL) for *E. coli* was approved by the U.S. Environmental Protection Agency (USEPA) for Sinking Creek in 1998, a tributary of the Watauga River in Northeast Tennessee which has remained on the 303d list for continued failure to meet surface water quality standards for pathogens, thus impairing recreational use (TDEC, 2010). While it is known that Sinking Creek is not meeting surface water quality standards based on the monitoring of fecal indicator bacteria, sources of contamination and the factors that may be influencing pathogen loading remain unknown. To accurately determine the risk presented by contact with contaminated surface waters such as Sinking Creek an understanding of the bacterial, protozoan, and viral pathogens present is necessary.

Bacterial Pathogens Associated with Waterborne Outbreaks

Escherichia coli is a gram-negative bacillus in the family *Enterobacteriaceae* and is a common inhabitant of the gastrointestinal tract of warm-blooded animals. While most strains of *E. coli* are not pathogenic, some strains, such as O157:H7, are opportunistic pathogens. Enterohemorrhagic strains such as O157:H7 are capable of causing hemorrhagic colitis and hemolytic uremic syndrome in humans. Originating from cattle hosts, *E. coli* O157:H7 was first recognized in 1982 as an emerging pathogen (Riley and Remis, 1982) and is transmitted through fecal-oral contact. In Tennessee in 2008, 54 cases of *E. coli* O157:H7 were reported as a result of waterborne or foodborne outbreaks (Cooper et al. 2008). The infectious dose of *E. coli*

O157:H7 has been reported to be as low as 100 CFU in both humans and cattle (Hancock et al. 1997). Sources of infection include ingestion of fecally contaminated foods and water, particularly meats and unpasteurized foods. Incubation following ingestion ranges from 10 – 72 hours.

E. coli O157:H7 has created a niche in the mucoual layer of the human colon, where it adheres to the intestinal epitheilium using fimbriae. The bacteria are able to exploit the host's ability to use gluconate more efficiently than resident species of the intestinal tract, as they inhibit protein synthesis by the production of verotoxins *stx1* and *stx2*. These verotoxins interfere with cellular respiration repair mechanisms and result in red blood cell damage. These virulence factors are recognized by the host immune response following insertion of the verotoxins into the host cell using the type III secretion system. Once the toxin is internalized, it is carried to the endoplasmic reticulum of the cell via the Golgi apparatus, ultimately arriving in the cytoplasm.

Symptoms of infection include diarrhea, vomiting, and dehydration for 3 – 5 days and may result in death for young, elderly, and immunocompromised populations. In vitro studies have demonstrated that antibiotics can induce transcription of *stx2* genes (Kimmitt et al. 2000). As a result, antibiotic treatment is not recommended for *E. coli* O157:H7 infection because of its association with an increased risk of hemolytic uremic syndrome (Wong et al. 2000). Only supportive therapy is used to treat symptoms of infection. Post-infection irritable bowel syndrome has been associated with *E. coli* O157:H7 infection (Marshall, 2009). In addition to enterohemorhagic strains, other groups of enterovirulent *E. coli* including enterotoxogenic, enteropathogenic, and

enteroinvasive groups are capable of causing symptoms similar to those of *E. coli* O157:H7 through different modes of infection (Table 1.1).

Table 1.1. Classificaiton of enterovirulent *E. coli* groups

***E. coli* Group**	**Mode of Infection**	**Examples**
Enterotoxigenic	Adherence to the intestinal epithelium and secretion of either heat-stable and/or heat-labile toxins	*E. coli* O6:H16 *E. coli* O15:H11
Enteropathogenic	Formation of lesions following adherence to the intestinal cell wall resulting in localized destruction and physical alteration of the intestinal epithelium	*E. coli* O44 *E. coli* O55
Enteroinvasive	Invasion and destruction of intestinal epithelium cells	*E. coli* O28 *E. coli* O112

Outside of its preferred niche, *E. coli* O157:H7 is able to tolerate extreme environmental conditions, including acidic and dry conditions (Glass et al. 1992; Arnold and Kasper, 1995) and temperature fluctuations (Wang and Doyle, 1998). It has been reported to survive for 109 days in surface water and 97 days in cattle feces (Scott et al. 2006). Survival of O157:H7 is also affected by soil moisture, the presence of other microbes (Jiang et al. 2002), nutrient and mineral concentrations (Artz and Killham, 2002; Ravva and Korn, 2007), and UV light exposure (Sommer et al. 2000). Detection

of the bacteria can be accomplished using several methodologies, including DNA, immunoassay and biochemical techniques.

S*higella sp.* is a gram-negative bacillus in the family *Enterobacteriaceae* and is a facultative anaerobic bacterium. *Shigella* is a pathogenic organism primarily found in the mucosal layer of the human colon, as it is capable of surviving exposure to proteases and acids in the digestive tract. The ability of the bacterium to invade non-phagocytic cells using a type III secretion system allows the bacterium to inject toxins directly into the host cell (Yee et al. 1957). Following ingestion, the bacterium transverses the intestinal epithelial barrier through M-cells and gain access to lymphoid follicles containing tissue macrophages. After phagocytosis, the bacteria destroy the phagosome membrane and are free within the host cytoplasm. Within the cytoplasm, the bacterium secretes IpaB that binds to capase-1 and induces macrophage apoptosis and the release of IL-8 and IL-18. The release of IL-8 and IL-18 results in acute colonic inflammation and tissue destruction. As with *E. coli* O157:H7, *Shigella* inhibits protein synthesis through the production of Shiga toxins and damages red blood cells.

Shigella is rarely found in animals other than man and the infectious dose ranges from $10^1 – 10^4$ organisms (Rowe and Gross, 1984). Infection occurs via the fecal-oral route and sources of infection include fecally contaminated foods and water. Incubation following ingestion ranges from 16 – 72 hours. Symptoms of infection include abdominal cramps, diarrhea, and fever and can last from 2 – 7 days. Antibiotic treatment is available for infection but is often not necessary as the disease is self-limiting. It has been reported that post-infection irritable bowel syndrome is associated with *Shigella* infection (Thabane et al. 2007).

Outside of its niche, *Shigella* can survive in groundwater for up to 24 days (Goldshmid, 1972) and the half-life in fresh water ranges from 22.4 – 26.8 hours at temperatures of 9.5 – 12.5^{o} C (McFeters et al. 1974). Within the environment, nutrient availability is a stronger limiting factor for virulence compared to temperature (Durand and Björk, 2009) and maximum invasion is achieved under anaerobic conditions (Mareyn et al. 2005). *Shigella* spp. are the second most common etiological agent associated with waterborne outbreaks of infectious disease and have been associated with both drinking water and recreational swimming (Hlavsa et al. 2011). During 2008, *E. coli* O157:H7 and *Shigella* infections were responsible for 3.7% and 4.9% of AGI illnesses in the United States, respectively (Hlavsa et al. 2011). In Tennessee in 2008, 968 cases of *Shigella sp*. and zero cases of *E. coli* O157:H7 infections were reported as a result of waterborne or foodborne outbreaks in Tennessee (Cooper et al. 2008).

Protozoan Pathogens Associated with Waterborne Outbreaks

Giardia sp. is a parasitic facultative anaerobic protozoan within the phylum *Sarcomastigophora* that infects the gastrointestinal tract of humans and other warm-blooded animals through fecal-oral contact. The organism forms oval shaped cysts approximately 8-12 μm in length and are transmitted via the fecal-oral route. Ingested cysts excyst in the intestinal tract and release 2 trophozoites that divide by binary fission. The organism attaches to the intestinal epithelium, where it interferes with the adsorption of fat-soluble vitamins within the gastrointestinal tract of the host organism due to the inability of *Giardia* trophozoites to synthesize their own lipids. Glucose is the only carbohydrate metabolized by the organism via the glycolytic pathway, resulting in

the production of ethanol, acetate and CO_2. Energy is produced using substrate level phosphorylation, due to the lack of cytochrome-mediated oxidative phosphorylation and a functional TCA cycle (Lindmark, 1980). In the presence of oxygen, *Giardia* respires using a flavin, iron-sulfur protein-mediated electron transport system (Jarroll et al. 1989). Within the colon, the trophozoites begin to encyst and are passed into the environment (Figure 1.1).

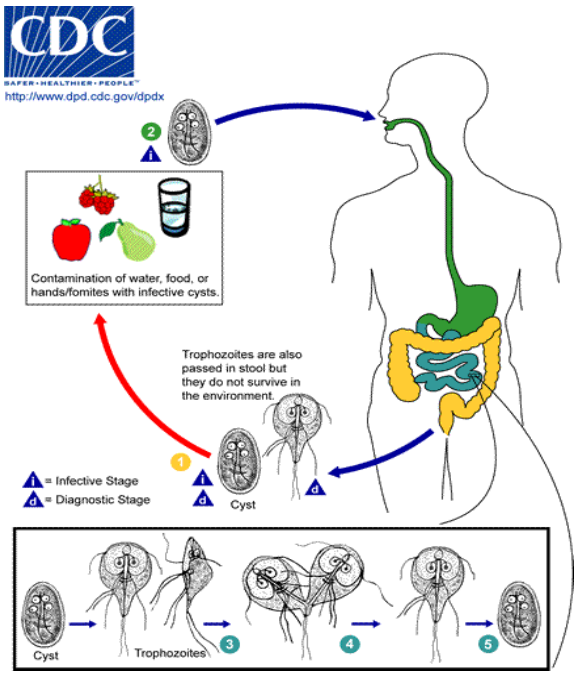

Figure 1.1. Lifecycle of *Giardia lamblia* (image courtesy of CDC)

Cysts are environmentally stable and able to withstand a variety of environmental conditions. Cysts have been reported to survive in surface waters for 28 days during warmer months and up to 56 days during winter months (deRegnier et al. 1989). Infection by *Giardia* occurs through fecal-oral contamination and can result in a disease state known as giardiasis or “backpackers disease” that is characterized by chronic diarrhea, fatigue, and weight loss. There is no treatment for infection, but supportive

therapy is commonly used to prevent dehydration. Giardia was responsible for 3.7% of AGI cases in the United States during 2008 (Hlavsa et al. 2011). Two hundred fourteen cases of waterborne *Giardia* were reported in Tennessee in 2008 (Cooper et al. 2008).

Cryptosporidium parvum is a parasitic facultative anaerobic protozoan that infects the gastrointestinal tract of humans and other warm-blooded animals through fecal-oral contact. *Cryptosporidium* is classified in the phylum *Apicomplexa.* Oocysts measure 2-6 μm in diameter and infection also occurs via the fecal-oral route. Once oocysts are ingested, they release sporozoites into epithelial cells of the intestinal tract. The sporozoites then undergo asexual reproduction to form merozoites, which then form micro and macrogametocytes. The micro and macrogametocytes then form a zygote, which becomes a new oocyst and is passed in the feces (Figure 1.2).

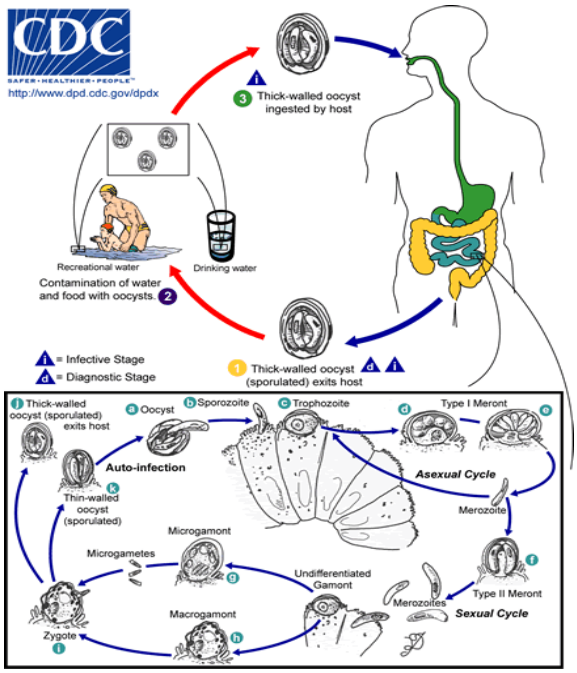

Figure 1.2. Lifecycle of *Cryptosporidium parvum* (image courtesy of CDC)

As with *Giardia, Cryptosporidium* interferes with the adsorption of fat-soluble vitamins within the gastrointestinal tract of the host organism, as it is unable to synthesize its own lipids and relies on a series of fatty acid metabolic enzymes to obtain the lipids necessary for biosynthesis (Xi et al. 2004). The organism also relies on amino acid uptake from its host using a series of amino acid transporters (Zhu, 2004). Its niche is within the intestinal epithelium, where it is contained within a host membrane-derived parasitophorous vacuole. Rather than become internalized within the host cell cytoplasm, the parasitophorous vacuole resides on the surface of the intestinal epithelial cell. This niche may provide some protection from the host's immune system but still take advantage of solute transport systems. *Cryptosporidium* relies on glycolysis for energy production, resulting in the production of lactate, acetate, and ethanol. Instead

of employing the TCA cycle and oxidative phosphorylation, pyruvate:$NADP^+$ oxidoreductase is used under anaerobic conditions, and an alternative oxidase system is used under aerobic conditions to economize ATP (Abrahamsen et al. 2004).

Although the organism's preferred niche is within the host intestinal epithelium, oocysts are environmentally stable and able to tolerate a range of environmental conditions similar to those of *Giardia*. Cryptosporidium is a common etiological agent associated with waterborne outbreaks and accounted for 74.1% of AGI cases in the United States in 2008 (Hlavsa et al. 2011), while 43 cases of waterborne *Cryptosporidium* were reported in Tennessee in 2008 (Cooper et al. 2008). Infection results in a disease state known as cryptosporidosis with symptoms similar to those of *Giardia*. Several treatments are available for infection with *Giardia* and *Cryptosporidium* including metronidazole, tinidazole, and nitazoxanide that may be used in non-immunosuppressed patients to prevent complications of infection (Gardner and Hill, 2001; Baily and Erramouspe, 2004).

Enteric Viruses Associated with Waterborne Outbreaks

Over 100 human enteric viruses can exist in surface waters as a result of fecal contamination. The major groups of enteric viruses that are associated with fecal pollution are shown in Table 2. Ranging in size from 20 – 70 nm, enteric viruses have icosahedral nucleocapsids and, depending on the group, can contain single or double stranded DNA, or single stranded RNA. Infection occurs through fecal oral contact and the viruses attack cells within the gastrointestinal tract resulting primarily in symptoms of gastroenteritis. As a result of infection within the gastrointestinal tract, virus particles

are shed in large numbers. For instance, Rotavirus is excreted in numbers ranging from 10^{10} – 10^{11} particles/g of stool (Shaw et al. 1995) and poliovirus is excreted in quantities of approximately 10^{10} particles/g of stool (Poyry et al. 1988). Vaccines have been developed for some enteric viruses to reduce the risk of disease outbreak. Vaccines including RotaTeq® and RotaRix® for rotavirus infection and inactivated polio vaccine are available and have been successful at reducing disease occurrence, but once infected, only supportive therapy is used to treat symptoms, as the disease is typically self limiting.

Table 1.2. Groups of human enteric viruses

Virus Group	Symptoms of Infection
Enteroviruses	
Poliovirus	Meningitis, poliomyelitis
Coxsackievirus	Malaise, rash, meningitis, encephalitis
Echovirus	Meningitis, diarrhea, fever
Enterovirus	Meningitis, encephalitis, respiratory disease, fever
Hepatitis A	Gastroenteritis, fever, malaise
Reovirus	Gastroenteritis
Rotavirus	Gastroenteritis, respiratory disease, conjunctivitis
Adenovirus	Gastroenteritis
Astrovirus	Gastroenteritis
Torovirus	Gastroenteritis
Caliciviruses	
Hepatitis E	Gastroenteritis
Norwalk virus	Gastroenteritis

Environmental Health Sciences Laboratory Water Quality Monitoring at East Tennessee State University

This work is part of a larger project involving the routine monitoring of 9 creeks within the Watauga River watershed to identify impaired surface waters. The project described in this dissertation focused on Sinking Creek because of its inclusion on the

State of Tennessee's 303d list and its land use characteristics that make it an excellent study site to better understand the relationship between fecal indictor bacteria and pathogen presence and the influence of physical, chemical, and microbial processes on pathogen fate and transport. The objectives of this research were to

1. Determine the ability of non-standardized methods to detect *E. coli* O157:H7, *Shigella sp., Giardia sp., Cryptosporidium sp., and* bacteriophages in seeded samples.
2. Assess the physical, chemical, and microbial water quality of Sinking Creek.
3. Survey the level of *E. coli* O157:H7, *Shigella sp., Giardia sp., Cryptosporidium sp., and* bacteriphages at 6 selected sites in Sinking Creek to assess the usefulness of fecal indicator bacteria as predictors of pathogen presence.
4. Characterize the physical, chemical, and microbial properties of soil along Sinking Creek to understand its role in physical, chemical, and microbial water quality in Sinking Creek.
5. Evaluate the use of multivariate statistical methodology to
    a. understand the water and soil characteristics influencing the fate and transport of fecal pollution, and
    b. identify nonpoint sources of fecal pollution as they relate to land use patterns in Sinking Creek.

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CHAPTER 2

LABORATORY PERCENT RECOVERY STUDIES AND METHOD OPTIMIZATION FOR THE DETECTION OF BACTERIAL, VIRAL AND PROTOZOAN PATHOGENS IN SURFACE WATER

K.K. Hall and P.R. Scheuerman

Abstract

Indicators of fecal pollution are frequently used to assess the extent of fecal pollution because it is not feasible to monitor surface waters for every pathogen. A successful fecal indicator should be associated with the source of the pathogen, be easily detectable, and respond to environmental conditions in a manner similar to that of the pathogen to help effectively protect human health. The inclusion of Sinking Creek on the State of Tennessee's 303d list due to pathogen contamination is based on the monitoring of fecal coliform bacteria, but it is not known what specific pathogens may be present and there has been no direct monitoring of specific pathogens to assess the ability of fecal indicator bacteria to predict the presence of pathogens. It may be necessary to monitor directly for pathogens, but it is difficult to accurately determine pathogen concentrations in surface waters due to a lack of standard methods and variability in pathogen recovery of published methods. In order to determine the ability of the pathogen detection methods, percent recovery (PR) analyses were performed using published methods for the detection of *E. coli* O157:H7, *Shigella sp., Giardia sp., Cryptosporidium sp.,* and MS2 bacteriophage. Observed detection limits for the *E. coli* O157:H7 and Shigella sp. differed from published detection limits, while detection limits

for *Giardia sp., Cryptosporidium sp.,* and MS2 bacteriophage were within reported ranges.

Introduction

Fecal coliform bacteria and *E. coli* are commonly used as indicators of fecal pollution and pathogen prevalence in part because they are easy to detect in environmental samples using standardized methods. Total and fecal coliform bacteria and *E. coli* can easily be detected in surface waters using the membrane filtration and Colilert™ methods described in Standard Methods for the Examination of Water and Wastewater (APHA, 1992). These standardized methods have been demonstrated to reliably detect fecal pollution indicators in surface water and can provide results within 24 hours.

The inclusion of Sinking Creek on the State of Tennessee's 303d list by the Tennessee Department of Environment and Conservation (TDEC) due to pathogen contamination is based on the monitoring of *E. coli* as an indicator of fecal pollution (TDEC, 2010). Although some studies have demonstrated the ability to predict pathogen presence using fecal indicator bacteria (Schaffter and Parriaux, 2002; Gersberg et al. 2006), it is not known if fecal indicator bacteria in Sinking Creek are successfully predicting the presence of pathogens.

Direct monitoring of pathogens in surface water is complicated by the difficulty and expense of monitoring for the vast number of pathogens associated with fecal pollution and, in some cases, lack of standardized methods. Various non-molecular and molecular methodologies have been developed and used in an effort to quickly identify

and quantify pathogens in surface waters. One of the main obstacles of method development is the inability to routinely and accurately detect pathogens between methods and between the types of sample analyzed.

Culture and biochemical methods are commonly used for the identification of bacterial pathogens including *E. coli* O157:H7 and *Shigella.* Detection of *E. coli* O157:H7 can be accomplished using Sorbitol-MacConkey (SMAC) medium (March and Ratnam 1986; Nataro and Kaper, 1998). This agar replaces lactose with sorbitol and exploits the inability of *E. coli* O157:H7 to ferment sorbitol unlike other *E. coli* strains. As a result, *E. coli* O157:H7 colonies appear colorless while other colonies of *E. coli* appear red. Although SMAC medium relies on biochemical properties to identify *E. coli* O157:H7, false positives have been observed in part due to the limited selectivity of SMAC medium (Schets et al. 2005). A standard method for the culturing of *Shigella sp.* has been described using Xylose Lysine Deoxycholate (XLD) medium and Triple Sugar Iron (TSI) slant test (APHA, 1992). Colonies appearing red on XLD agar are considered to be *Shigella sp.* or *Salmonella sp*. Red colonies are tested using the TSI slant test, and samples positive for *Shigella sp.* will have a red slant indicating a lack of lactose and sucrose fermentation and a yellow butt indicating glucose fermentation and acid production.

Biochemical testing using API® strips has been used to confirm the presence of *E. coli* O157:H7 and *Shigella sp.* in environmental samples based on the biochemical profiles of the organisms (Faith et al. 1996, Shere et al. 2002; Hsu et al. 2010). These methods have proven successful in identifying various pathogenic bacteria in environmental samples and can be quickly and inexpensively performed. However,

their application to impaired waters may delay or impede the protection of public health due to need for sample incubation (usually 24 hours) and inability to detect viable but non-culturable (VBNC) organisms (Roszak and Colwell 1987; Byrd et al. 1991; Wang and Doyle 1998).

Culture methods for the detection and quantification of bacteriophages have also been described (USEPA, 2001a; USEPA, 2001b) and are commonly used as a surrogate measure of virus pollution (Wentsel et al. 1982; Stetler, 1984; Havelaar et al.1993). Using an *E. coli* host strain, bacteriophages are enumerated using either a single or double agar layer procedure. Bacteriphages will infect and lyse the host cells, resulting in the formation of plaques that are enumerated following 24 hours of incubation. These methods are relatively quick (24h) and easy to perform compared to virus cell culture methods (up to 3 weeks), and are considered to represent suitable indicators of enteric virus pollution.

Immunological methods for the detection of *E. coli* O157:H7, *Shigella sp.*, *Giardia,* and *Cryptosporidium* have been proposed to overcome the challenges presented by culturing and biochemical methods. Enzyme-linked immunosorbent assays (ELISA) and immunomagnetic separation methods have been developed to identify bacterial pathogens including *E. coli* O157:H7 and *Shigella sp.* in environmental samples and rely on the reactivity of specific antibodies with the sample. Both ELISA and immunomagnetic separation methods have been shown to more accurately and quickly identify the presence of *E. coli* O157:H7 and *Shigella sp.* in human and environmental samples compared to culture methods (Islam et al. 1993b; Dylla et al. 1995; Park et al. 1996; Fratamico and Strobaugh, 1998; Zhu et al. 2005). In addition to

their use for the detection of bacterial pathogens, immunomagnetic separation and immunofluorescent methods have been applied to protozoan pathogen detection including *Giardia and Cryptosporidium* (USEPA 2005). Immunomagnetic separation and immunofluorescent methods have been shown to be insensitive to environmental interferences including highly turbid surface waters (LeChevallier et al. 1995; Bukhari et al. 1998; Rochelle et al. 1999; McCuin et al. 2001) but are subject to recovery losses during filtration, elution, and centrifugation of the sample (LeChevallier et al. 1995; Hu et al. 2004). Immunological methods provide relatively quick results (24 hours), can be easily performed, but may be subject to cross-reactivity of antibodies resulting in false positive results (Sauch 1985; Rice et al. 1992; Islam et al. 1993a; Koompapong et al. 2009).

Molecular methods including polymerase chain reaction (PCR) are widely used for the detection of a variety of pathogens including *E. coli* O157:H7, *Shigella sp. Giardia sp., Crytposporidium sp.,* and bacteriophages in environmental samples. Based on the replication of a particular gene sequence specific to the pathogen of interest, PCR methods have become popular for their ability to provide quicker identification and confirmation of pathogen presence beyond traditional culture or biochemical methods. The speed of analysis, typically a few hours, combined with method sensitivity and ability to detect VBNC organisms make PCR methods appealing for the identification of pathogens in surface water (Josephson et al.1993; Abd-El-Haleem et al. 2003). Numerous qualitative and quantitative PCR methods have been used either on their own or in combination with culture or immunological methods for the identification of bacterial pathogens, pathogenic protozoa, and bacteriophage in surface waters based

on DNA primers, annealing temperatures and reaction components (Bej et al. 1991; Mahbubani et al. 1992; Johnson et al. 1995; Rose et al. 1997; Puig et al. 2000; Campbell et al. 2001; Guy et al. 2003; Ibekwe and Grieve, 2003). Although PCR methods for the identification of pathogens can be rapidly completed and highly sensitive, they are often difficult to standardize and apply to environmental samples due to inhibiting substances in the soil and water matrix such as humic acids (Tebbe and Vahjenm 1993; Campbell et al. 2001; Bhagwat, 2003). Environmental stress has also been shown to affect the stability of the target gene further complicating the sensitivity of the method (Cooley et al. 2010).

There are inherent positive and negative aspects associated with each of the various methodologies available for the detection of pathogens in surface water. To overcome the issues of selectivity and VBNC bacteria, published PCR methods were selected for the analysis of *E. coli* O157:H7 and *Shigella sp.* in this study (Bej et al. 1991; Theron et al. 2003). Standardized methods were selected for the detection of *Giardia, Cryptosporidium,* and bacteriophages (USEPA, 2001a; USEPA, 2001b; USEPA, 2005). The recovery efficiencies of each method may vary from the published detection limits based on the type of sample and the particular analytical laboratory. To address these issues, each method was subjected to PR analyses to determine the sensitivity of each method prior to the collection and analysis of field samples.

Materials and Methods

Bacterial Analysis

Stock culture of *E. coli* O157:H7 (ATCC® Number 43895™) and *Shigella flexneriI* (ATCC® Number 12022™) were obtained from the American Type Culture Collection (ATCC®). *E. coli* O157:H7 was cultured using tryptic soy agar (TSA) and *Shigella flexneri* was cultured using nutrient agar. A known number of colony forming units (CFUs) of each bacterial strain were seeded into 100ml samples of tap water dechlorinated with sodium thiosulfate. For *E. coli* O157:H7, water samples were seeded with 10, 25, and 50 CFU/100ml and filtered. For detection limit determination of *Shigella flexneri*, water samples were seeded with 10, 25, and 50CFU/100ml and filtered. Following filtration, the samples were eluted with either tryptic soy broth (TSB) or 1% Tween solution to assess the bacterial elution using each solution. The filter was then washed with 10ml of a 1% Tween 80 solution and centrifuged for 10 minutes to create a cell pellet. The supernatant was removed and the cell pellet was washed twice with 10ml phosphate buffered saline. Fifty microliters of diethylpyrocarbonate solution was added to the final cell pellet and subjected to 6 freeze-thaw cycles at -20^{o}C and 100^{o}C, respectively.

PCR amplification for *E. coli* O157:H7 was performed as described by Kimura et al. (2000) using primers EC-1 (GGCAGCCAGCATTTTTTA) and EC-2 (CACCCAACAGAGAAGCCA) for the *chuA* gene. The final 50µl PCR mixture contained 2.5X PCR buffer (mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl), 0.8 mM of each deoxynucleoside triphosphate (dATP, dCTP, dGTP, and dTTP), 4 µM concentrations of each primer, 5 U *Taq* DNA polymerase (Fisher Scientific, Pittsburg, PA) and 5µl of the

resuspended cell pellet. The PCR mixture was subjected to an initial denaturation step at 95^{o}C for 5 minutes, followed by 35 cycles of 1 minute denaturation at 94°C, 2 minutes of annealing at 42°C, and 5 minutes of primer extension at 72°C. A final extension step was performed at 72^{o}C for 10 minutes using a BioRad Thermocycler PCR Machine (BioRad, Hurcules, CA). PCR products were resolved on a 2% agarose gel for 1.5h at 80V and subjected to ethidium bromide staining to visualize DNA base pair bands. The presence of a 901 base pair band indicated a sample positive for *E. coli* O157:H7.

PCR amplification for *Shigella sp.* was performed as described by Theron et al. (2001). Thirty cycles of a seminested PCR reaction were performed using primers H8 (GTTCCTTGACCGCCTTTCCGATAC) and H15 (GCCGGTCAGCCACCCTC) for the *ipaH* gene (Islam, et al. 1993a) in the first round of PCR. The 50µl reaction volume contained 1X PCR buffer (mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl), 0.1mM of each deoxynucleoside triphosphate (dATP, dCTP, dGTP, and dTTP), 24pmol of H8 primer, 34pmol of H15 primer, 1U *Taq* DNA polymerase (Fisher Scientific, Pittsburg, PA), and 10µl of resuspended cell pellet. The PCR mixture was subjected to an initial denaturation step at 94^{o}C for 3 minutes, followed by 10 cycles of 1 minute denaturation at 94°C, 1 minute of annealing at 60°C, and 1 minute of primer extension at 72°C. One microliter of PCR product from the first PCR round was added to a reaction tube containing the reagents described above, with the addition of 31pmol of H10 primer (CATTTCCTTCACGGCAGTGGA) described by Hartman et al. (1990). An initial denaturation step was performed at 94^{o}C for 3 minutes, followed by 20 cycles of 1 minute denaturation at 94°C, 1 minute of annealing at 60°C, and 1 minute of primer extension at 72°C. A final extension step was performed at 72^{o}C for 7 minutes using a

BioRad Thermocycler PCR Machine (BioRad, Hurcules, CA). PCR products were resolved on a 2% agarose gel for 1.5h at 80V and subjected to ethidium bromide staining to visualize DNA base pair bands. The presence of both a 401 and 620 base pair band indicated a sample positive for *Shigella sp*.

Protozoan Analysis

PR analyses for *Giardia* and *Cryptosporidium* were performed using a stock concentration of *Giardia lamblia* cycts (Human Isolate H-3, Waterborne Inc.). A stock solution of 12,500 *Giardia lamblia* cysts was seeded into a carboy containing 20L of tap water dechlorinated with sodium thiosulfate. A filtration apparatus was assembled (Figure 2.1) and the entire 20L sample filtered though an Envirochek™ sampling filter (Pall Corporation, Ann Arbor, MI) powered by an electric water pump and Badger™flow meter at a flow rate of 2.5L per minute.

Filters were initially washed by adding 120ml of elution buffer to the filter capsule and placing on a wrist action shaker for 30 minutes. The elution buffer was removed and the filter capsule broken open and the filter cut out using a sterile razor blade and hand washed using 120ml of elution buffer. The buffer was then added to a sterile 250ml centrifuge tube containing the elution buffer from the initial wash on the wrist action shaker. The samples were centrifuged at 2,300 x g for 30min and the supernatant removed. The concentrated pellet collected was subjected to an immunofluorescent assay using the Waterborne Aqua-Glo™ G/C Direct FL antibody stain (Waterborne, Inc. New Orleans, LA) as described by the manufacturer. The

prepared slides were examined at 200X using the Olympus BH2 epifluorescent microscope (Olympus, New Hyde Park, NY).

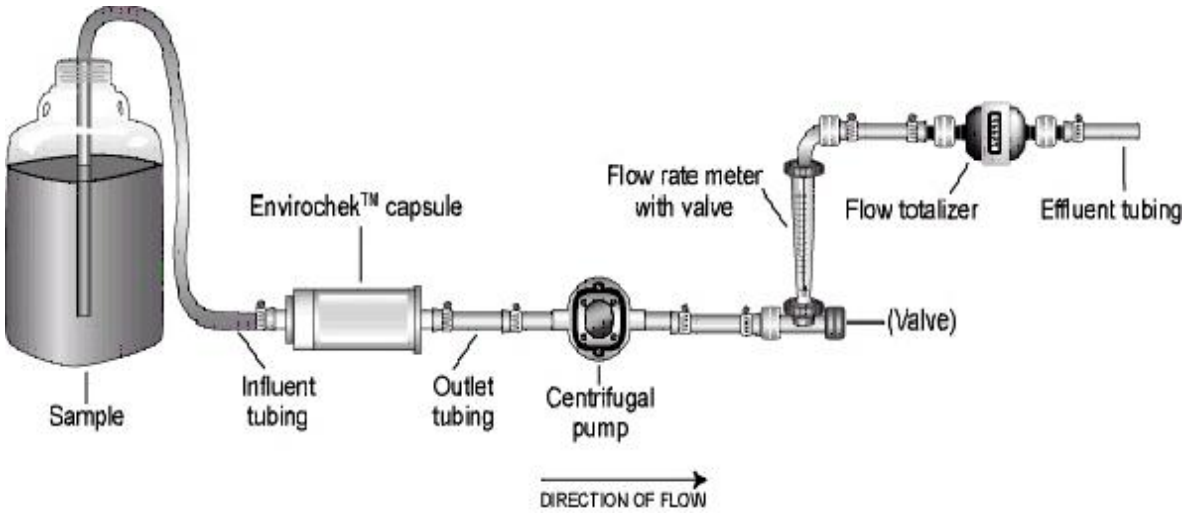

Figure 2.1. Filtration apparatus used to sample Giardia and Cryptosporidium in laboratory seeded samples (USEPA, 2005)

Fluorescently-labeled carboxylate modified polystyrene latex beads with a mean particle size of 2µm (Sigma-Aldrich) were used in PR analyses as a substitute for *Cryptosporidium* oocysts because of similarity in size. The seeding and recovery procedures for the latex beads were performed using the methods described for *Giardia lamblia* seeding samples. The prepared IFA slides and recovered pellets were enumerated microscopically at 200X on a hemacytometer using a using the Olympus BH2 epifluorescent microscope (Olympus, New Hyde Park, NY) to determine the percent of beads recovered.

Bacteriophage Analysis

PR analyses for bacteriphage were performed using MS2 bacteriophage (ATCC® Number 15597-1B™) and *E.* coli C3000 (ATCC® Number 15597™) as a host strain. The host strain was cultured using ATCC 271 broth (10g/L tryptone, 1g/L yeast extract, 8g NaCl, 10ml/L of 10% glucose solution, 2ml/L of 1M $CaCl_2$, 1ml/L of 10mg/ml thiamine) at 37^{o}C. An overnight culture of the host strain was prepared the day before analysis by inoculating a 30ml ATCC broth culture with the host strain. On the day of analysis, 100µl of the prepared overnight culture of the host strain was inoculated into a 30ml of fresh ATCC 271 broth and incubated at 37^{o}C until log phase was reached (~4h). This culture was used to propogate the MS2 bacteriophage for PR analyses.

Five hundred microliters of each MS2 dilution was added to a test tube containing 5ml of 0.7% ATCC® 271 agar (ATCC® 271 broth with 1.4g/L agar) and 100µl of host bacteria. The tubes were gently mixed and poured onto a plate containing 1.5% ATCC 271 agar (ATCC® 271 broth with 18g/L agar). Plates were allowed to solidify prior to incubation at 37^{o}C for 24h and plaque forming units (PFUs) were enumerated. Following bactriophage enumeration of the culture, a known number of PFUs were seeded into 10ml tap water samples with sodium thiosulfate to remove any chlorine residual and analyzed in using USEPA method 1062 to determine the percent of bacteriophages recovered and the method detection limit.

Results and Discussion

Bacterial Analysis

The results of PCR and gel electrophoresis are shown in Figure 2.2. Both the TSB and 1% Tween solution were successful in eluting bacteria from the filters containing 25 and 50 CFUs but not the filter containing 10CFUs. The intensity of the target 901 base pair bands for the samples eluted with 1% Tween suggest that it more successful at eluting bacteria from the filter than TSB because of its surfactant properties.

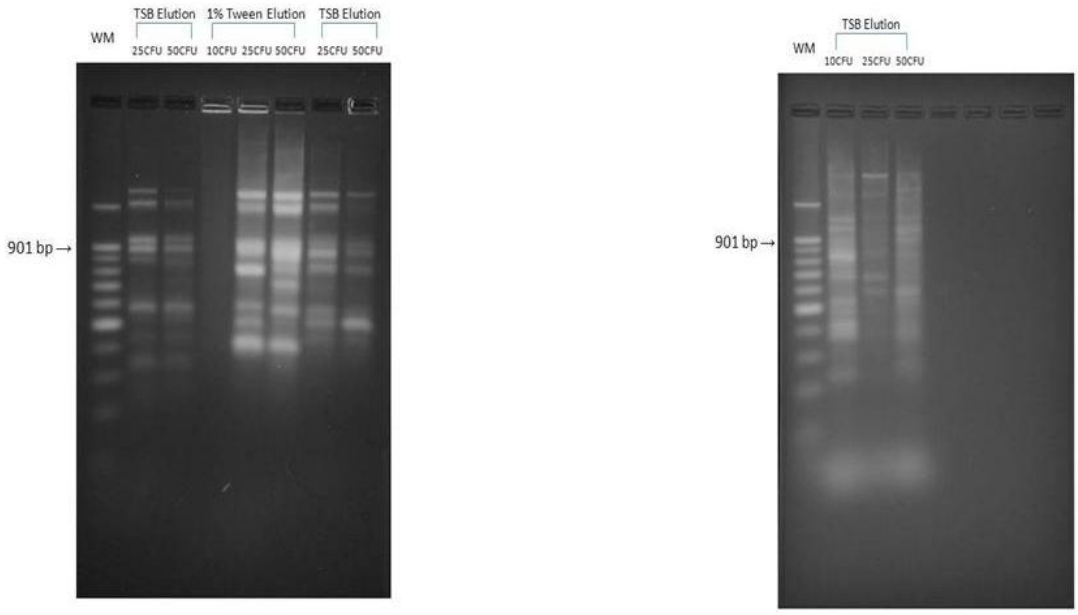

Figure 2.2. Gel electrophoresis of PCR products to determine the detection limit of E. coli O157:H7 using TSB and 1% Tween as elution buffers

The results of PCR and gel electrophoresis are shown in Figure 2.3. In this instance, *Shigella* was not recovered in samples eluted with TSB but the target 620 and 401 base pair bands were detected for all seeded concentrations. As with *E. coli* O157:H7, the 1% Tween solution may be more successful eluting bacteria from the filter because of its surfactant properties.

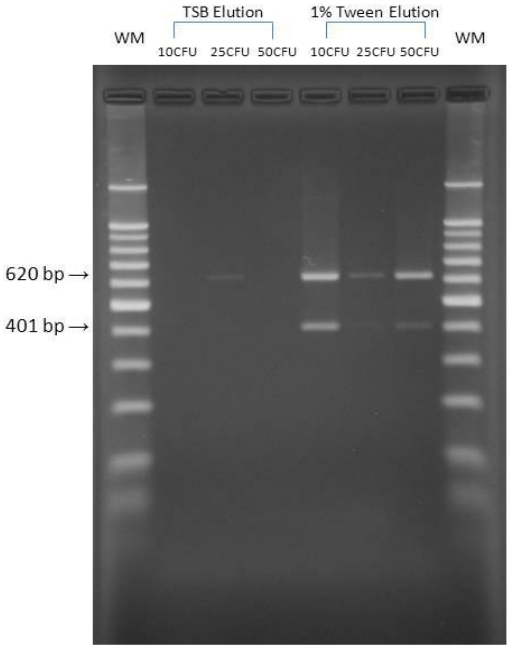

Figure 2.3. Gel electrophoresis of PCR products to determine the detection limit of Shigella flexneri using TSB and 1% Tween as elution buffers

The use of PCR methods for the analysis of *E. coli* O157:H7 and *Shigella sp.* in surface water samples were selected for their greater speed and selectivity than the traditional plating methods and their ability to detect VBNC organisms. The detection limits determined in this study for both *E. coli* O157:H7 and *Shigella sp.* vary greatly

compared to published detection limits in environmental samples and clinical isolates (Table 2.1). PCR analyses for the detection of *E. coli* O157:H7 and *Shigella sp.* varied based on the type of sample, but wastewater and surface water generally display the highest detection limits (Ibekwe et al. 2002; Ibekwe et al. 2003; Barak et al. 2005; Hsu et al. 2007). Higher detection limits in these types of samples are most likely due to the presence of PCR inhibitors such as humic acids that may be present during isolation and purification of the sample (Tebbe and Vahjen, 1992).

Table 2.1. Published detection limits of Polymerase Chain Reaction (PCR) methods for the detection of *E. coli* O157:H7 and *Shigella* sp.

Organism	Sample Type	Type of PCR Method	Detection Limit	Reference
Shigella sonnei	Surface water	PCR	1.7 – 24.7 CFU/50ml	Hsu et al. (2007)
Shigella dysenteriae	Surface water	PCR	270 – 8000 CFU/50ml	Hsu et al. (2007)
Shigella flexneri	Sea water	Multiplex PCR	10 – 100 CFU	Kong et al. (2002)
Shigella spp.	Surface water	Semi-nested PCR	14 CFU/ml	Theron et al. (2002)
Shigella spp.	Surface water	Enrichment/real time PCR	1.8 CFU/100ml	Maheux et al. (2011)
Shigella flenxeri	Stool	Multiplex PCR	300 cells/g	Oyofo et al. (1996)
Shigella dysenteriae	Surface water	PCR	27.5 CFU/100ml	Liu et al. (2009)
E. coli O157:H7	Irrigation water	Real time PCR	10 – 1000 CFU/reaction	Barak et al. (2005)
E. coli O157:H7	Drinking water/soil	Multiplex PCR	1 CFU/ml , 2 CFU/g	Campbell et al. (2001)
E. coli O157:H7	Wastewater	Multiplex fluorogenic RT-PCR	6,400 CFU/ml	Ibekwe et al. (2002)
E. coli O157:H7	Surface water/soil	Real time PCR	3,500 CFU/ml, 26,000 CFU/g	Ibekwe et al. (2003)
E. coli O157:H7	Surface water	Reverse transcriptase PCR	7 CFU/L	Liu et al. (2008)
E. coli O157:H7	Surface water	RT-PCR	1.8 CFU/100ml	Maheux et al. (2011)
E. coli O157:H7	Clinical isolates	RT-PCR	30 cells	Morin et al. (2004)
E. coli O157:H7	Drinking water	Culture/q-PCR	500 cells	Sen et al. (2011)
E. coli O157:H7	Drinking water	PCR	1 cell/ml	Bej et al. (1991)
E. coli O157:H7	Water	Enrichment/PCR	3 CFU/L	Bonetta et al. (2011)

Protozoan Analysis

Four water samples were seeded with *Giardia lamblia* cysts and analyzed for PR determination. Two seeding concentrations (625 cysts/L and 2,500 cysts/L) were analyzed to assess the recovery efficiency of different protozoan concentrations. The average percent recovery of the seeded water samples was 35.7% and the concentration of cysts in the sample does not seem to improve recovery efficiencies (Table 2.2). Three water samples were seeded with latex beads to assess the ability of the analytical methods to recover *Cryptosporidium* oocysts. The average percent recovery of the seeded water samples was 35.3% (Table 2.3). According to the USEPA, method 1623 recoveries range from 11 – 100% for *Giardia* and 14 – 100% for *Cryptosporidium* and are considered acceptable (USEPA, 2005). The results of the PR analyses are within the USEPA established acceptable detection range.

Table 2.2. IPR results for filtered water sampled seeded *Giardia lamblia* cysts

Number of Seeded *Giardia* Cysts	**Number of *Giardia* Cysts Recovered**	**Percent Recovery**
12,500	3800	30.4%
12,500	6750	54.0%
12,500	5625	45%
50,000	6745	13.5%
	Average	35.7%

Table 2.3. IPR results for filtered water samples seeded latex beads as a surrogate measure of *Cryptosporidium* oocysts

Number of Seeded Latex Beads	Number of Latex Beads Recovered	Percent Recovery
8.6×10^7	1.4×10^7	16.3%
8.6×10^7	2.1×10^7	24.4%
8.6×10^7	5.6×10^7	65.1%
	Average	35.3%

Much variability has been reported in *Giardia* cyst and *Cryptosporidium* oocyst recovery using USEPA method 1623 (Table 2.4). Most loss of cyst and oocyst is reported to occur during the elution and concentration steps, and the smaller size of *Cryptosporidium* oocysts (2-6µm) is responsible for the lower recovery efficiencies compared to *Giardia* cysts (8-12µm) (LeChevallier et al. 1995; Hu et al. 2004). It has also been reported that the presence of organic and inorganic particles in surface waters resulting in increased turbidity may impede *Giardia* and *Cryptosporidium* recovery (Nieeminski et al. 1995; DiGiorgio et al. 2002; Krometis et al. 2009). The presence of organic material may interfere with adsorption and absorption of cysts and oocysts to the filter and influence recovery during the elution procedure. To address these potential interferences, hand washing of the filter was performed following elution for 30 minutes using a wrist action shaker to improve elution efficiency.

Table 2.4. Published detection limits of USEPA method 1623 for the detection of *Giardia* and *Cryptosporidium* in water

Average Giardia Recovery	Average Cryptosporidium Recovery	Reference
11-100%	14-100%	EPA (2005)
22% [Range 3-45%]	17% [Range 0-074%]	Krometis et al. (2009)
Site 1: 61 ± 0.06% SE Site 2: 0.83 ± 0.01% SE	Site 1: 43 ± 0.01% SE Site 2: 37 ± 0.05% SE	DiGiorgio et al. (2002)
51.4 ± 12.6% SD	40.4 ± 17.8% SD	McCuin et al. (2003)
9.1%	2.8%	Clancy et al. (1994)
48%	42%	Nieminski et al. (1995)

Bacteriophage Analysis

MS2 bacteriophage were isolated and enumerated from a secondary effluent sample collected at Knob Creek Wastewater Treatment Facility using the double agar layer method. Three tap water samples treated with sodium thiosulfate to neutralize chlorine residual were seeded with a known concentration of bacteriophage PFUs/ml and subjected to the described isolation procedures in triplicate. Analysis of the seeded samples resulted in complete recovery of the seeded bacteriophage PFUs (Table 2.5). The ability of this method to detect 1PFU/ml is reliant on the filtration of the raw water sample to remove any bacteria that may inhibit the growth of the host bacteria and the use of a pure host bacterial culture (*E. coli* C3000). It should be noted that the bacteriophage detected in this assay and the recovery of bacteriophage in

environmental samples are somewhat limited because of specificity of the *E. coli* host strain used.

Table 2.5. IPR results for water samples seeded with a known concentration of bacteriophage PFUs

Concentration of Seeded Bacteriophage	Concentration of Recovered Bacteriophage	Percent Recovery
1 PFU/ml	1 PFU/ml	100%
5 PFU/ml	5 PFU/ml	100%
10 PFU/ml	10 PFU/ml	100%

Conclusions

The observed PR tests and detection limits determined in these experiments demonstrate variability when compared to the recovery efficiencies of the published methods. The detection limits of *E. coli* O157:H7 and *Shigella sp.* using PCR methods were determined to be 25 and 10 CFUs, respectively. Percent recoveries for Giardia (35.7%) and Cryptosporidium (35.3%) are within acceptable guidelines described in USEPA method 1623, but it may be difficult to compare these recoveries to those of environmental samples based on the influences of organic and inorganic materials in surface waters. The PR test of bacteriophage samples demonstrated 100% recovery of samples seeded with 1PFU/ml of MS2 bacteriophage.

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CHAPTER 3

PHYSICAL, CHEMICAL, AND MICROBIAL WATER QUALITY TRENDS IN SINKING CREEK, JANUARY – DECEMBER 2011

K.K. Hall and P.R. Scheuerman

Abstract

A Total Maximum Daily Load (TMDL) was approved by the U.S. Environmental Protection Agency (USEPA) for Sinking Creek, a tributary of the Watauga River in Northeast Tennessee, in 1998. Sinking Creek has since remained on the State of Tennessee's 303d list for continued failure to meet surface water quality standards for pathogens, thus impairing recreational use. While Sinking Creek is not meeting surface water quality standards, the factors influencing pathogen loading are unknown. The inclusion of Sinking Creek on the state of Tennessee's 303d list due to pathogen contamination is based on the monitoring of fecal indicator bacteria, but it is not known what specific pathogens may be present. The objectives of this experiment was to 1) assess the physical, chemical, and microbial water quality in Sinking Creek, and 2) to determine the usefulness of fecal indicator bacteria as predictors of *E. coli* O157:H7, *Shigella sp., Giardia sp., Cryptosporidium sp.,* and bacteriophage. Elevated concentrations of fecal indicator bacteria suggest that Sinking Creek is impaired by fecal pollution but fecal indicator bacteria concentrations do not correlate with pathogen presence, suggesting that fecal indicator bacteria do not accurately predict pathogen presence.

Introduction

In 2002, Dulaney and co-workers initially selected 14 sites in Sinking Creek for fecal coliform monitoring based on their proximity to livestock and human populations, which may serve as sources of fecal pollution (Dulaney et al. 2003). The physical, chemical, and microbial water quality of Sinking Creek have since been monitored using this targeted sampling approach following its inclusion on the State of Tennessee's 303d list for pathogen impairment based on the monitoring of fecal indicator bacteria. Fecal coliform bacteria are commonly used as a surrogate measure of pathogen contamination in surface waters because they are easy to detect using inexpensive methods compared to methods for the monitoring of every pathogen. Some studies have observed a correlation between indicator organisms and pathogens (Payment and Franco 1993; Schaffter and Parriaux, 2002; Gersberg et al. 2006). Despite the advantages of monitoring fecal indicator bacteria and their occasional correlation with pathogen presence, a lack of correlation between the presence of fecal indicator bacteria and pathogens is more often observed (Goyal et al. 1977; Carrillo et al. 1985; Havelaar et al. 1993; Harwood et al. 2005). The lack of correlation observed between fecal coliform bacteria and pathogens may be due to differences in excretion densities and transport behaviors of pathogens and indicators (Lemarchand and Lebaron, 2003), regrowth of fecal indicators (Howell et al. 1996), survival of fecal coliforms compared to pathogens (McFeters et al. 1974; Scott et al. 2006) and physiochemical water and soil parameters (Burton et al. 1987; Gantzer et al. 2001).

Sources, Fate, and Transport of Fecal Coliforms and Pathogens

Fate and transport of fecal coliforms and pathogens are dependent on several physical, chemical, and microbial processes in water. The transport of the pathogen from the source to water, transport following entry into the water, and pathogen survival in the water influence pathogen fate and transport in surface waters. Fecal coliform concentrations in Sinking Creek have been consistently above regulatory limits and display seasonal variation (Hall et al. 2011). Seasonal variability of fecal coliform concentrations in water is often influenced by water chemistry (McFeters and Stuart, 1972) temperature (Hunter et al. 1999), rainfall and discharge (Lipp et al. 2001), dissolved oxygen (Hanes et al. 1964), UV light exposure (McCambridge and McMeekin, 1981; Davies and Evison, 1991), organic matter concentrations (Orlab 1956), predators (McCambridge and McMeekin, 1981) and heavy metals (Jana and Chattacharya, 1988). Partitioning of fecal coliforms into the gas-water interface (Powelson and Mills, 2001), and deposition into sediment and subsequent resuspension can influence fecal coliform concentrations in water (Sherer et al. 1992; Crabill et al. 1999).

In addition to seasonal variability, land use patterns significantly influence fecal coliform concentrations in Sinking Creek (Hall et al. 2011). Sinking Creek undergoes a rapid transition from forest to urban and agricultural land use. Agricultural activity is a common contributor to increased fecal coliform and nutrient concentrations in surface waters (Lenat and Crawford 1994; Whiles et al. 2000; Tong and Chen 2002). Spatial patterns (Hunsaker and Levine; 1995), agricultural densities (Harding et al. 1999), ecological patterns (Buck and Townsend, 2004), surface runoff, rainfall, and stream characteristics (Sheshane et al. 2005) influence agricultural contribution to fecal

pollution. Urban runoff also influences water quality primarily due to impervious surfaces and residential activity. Additional pollution sources that contribute to fecal and nutrient pollution include septic systems, storm sewers, and fertilizer application (Olyphant et al. 2003; Ning et al. 2006; Zeilhofer et al. 2006). Six sites on Sinking Creek were monitored monthly from January 2011 through December 2011 to assess physical, chemical, and microbial water quality in relation to land use and to better understand the influences of these parameters on surface water quality. In addition, the presence and concentrations of *E. coli* O157:H7, *Shigella sp., Giardia sp., Cryptosporidium sp.,* and bacteriophages were determined to assess the usefulness of fecal coliform bacteria as indicators of pathogen pollution.

Materials and Methods

Sinking Creek Location and Water Quality Monitoring

The Sinking Creek sub-watershed (06010103130) is one of 13 sub-watersheds that belong to the Watauga River watershed (TDEC, 2000a). Sinking Creek is a 9.8 mile long tributary of the Watauga River partially located in Washington and Carter Counties in Tennessee. The headwaters of Sinking Creek are located on Buffalo Mountain and it enters the Watauga River at mile 19.9. The main land uses within the 13.1 square mile drainage basin of the Sinking Creek watershed include: forest (65.5%), urban (25.3%), and agricultural areas (9.0%) (TDEC 2000b). There are 19.8 impaired stream miles in the Sinking Creek watershed including tributaries (TDEC, 2000b).

Upstream locations on Buffalo Mountain are forested, and land use transitions to urban, followed by agricultural land use at downstream sites. Fourteen sites were initially selected for routine water quality monitoring in 2002 and are described in Table 3.1 and Figure 3.1. From these 14 sampling locations, 2 sites were randomly selected from each land use classification and sampled monthly for the physical, chemical, and microbial parameters described in Table 3.2. The sites selected for representation of agricultural land use were sites 2 and 4, sites selected to represent urban land use were sites 7 and 10, and sites 13 and 14 represented forested land use.

Table 3.1. Sampling locations on Sinking Creek sampled during this study

Site Number	Site Location	Predominant Land Use	Physical Description	Habitat Assessment Score (%)	Latitude/Longitude Coordinates and Elevation
2	Upstream of Bob Peoples bridge on Sinking Creek Road	Agriculture	Moderately eroded banks with little vegetation buffer or riparian zone. Creek bed predominantly cobble and gravel	52%	19.837' N, 18.254' W 1530 ft
4	Upstream of crossing on Joe Carr Road	Agriculture	Moderately eroded banks with poor bank stability and little vegetative buffer or riparian zone. Creek bed predominantly boulders, cobble and gravel	43%	19.594' N, 18.579' W 1552 ft
7	Upstream of bridge on Miami Drive, King Springs Baptist Church	Urban	Heavily eroded left bank, concrete bank on right with no vegetative buffer or riparian zone. Creek bed predominantly cobble	53%	18.772' N, 19.685' W 1583 ft
10	Upstream of bridge crossing Sinking Creek at Hickory Springs Road	Urban	Heavily eroded banks with no vegetative buffer. Creek bed predominantly boulders and cobble	57%	17.431' N, 21.397' W 1720 ft
13	Upstream of road crossing on Jim McNeese Road	Forest	No visible bank erosion with moderate riparian zone. Creek bed predominantly boulders and cobble	71%	16.035' N, 22.163' W 2048 ft
14	Downstream of path crossing at Dry Springs Road	Forest	No visible bank erosion with optimal riparian zone and vegetative buffer. Creek bed predominantly boulders, cobble and gravel	83%	14.800' N, 22.033' W 2148 ft

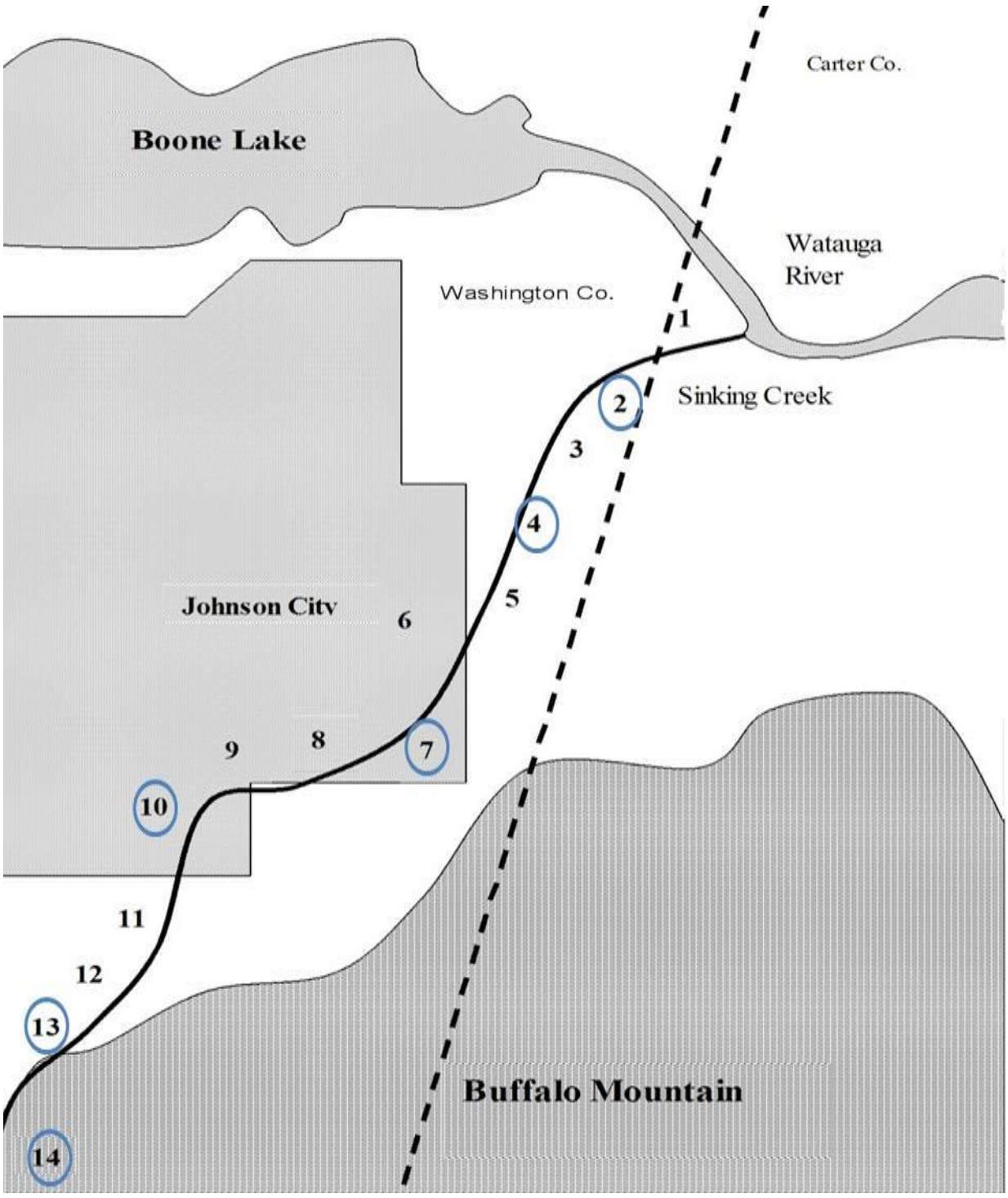


Figure 3.1. Map of Sinking Creek sampling locations (sites sampled in this study are circled).

Table 3.2. Physical, chemical, and microbial water quality parameters measured

Parameter	Abbreviation	Units	Holding Time
pH	pH	pH	Field measurement
Water temperature	WT	^{o}C	Field measurement
Air temperature	AT	^{o}C	Field measurement
Dissolved oxygen	DO	mg/l as O_2	Field measurement
Conductivity	Cond	µmohs	Field measurement
Fecal coliform in water	FCW	CFU/100ml	6h
Total coliform in water	TCW	CFU/100ml	6h
Fecal coliform in sediment	FCS	CFU/100ml	6h
Total coliform in sediment	TCS	CFU/100ml	6h
Colilert	Colilert	CFU/100ml	6h
Standard plate count	SPC	CFU/ml	6h
Acridine orange direct counts	AODC	cells/g sediment	6h
Acid phosphatase	AcidP	µg/g sediment	24h
Alkaline phosphatase	AlkP	µg/g sediment	24h
Dehydrogenase	DHA	µg/g sediment	24h
Galactosidase	Gal	µg/g sediment	24h
Glucosidase	Glu	µg/g sediment	24h
Nitrates	NO_3	mg/l	24h
Phosphates	PO_4^{2-}	mg/l	24h
Ammonia	NH_3^{+}	mg/l	24h
Biochemical oxygen demand	BOD_5	mg/l	24h
Hardness	Hard	mg/l	48h
Alkalinity	Alk	mg/l	24h
E. coli O157:H7	O157:H7	CFU/100ml	24h
Shigella sp.	*Shigella*	CFU/100ml	24h
Giardia sp.	*Giardia*	Cysts/l	72h
Cryptosporidium sp.	*Crypto*	Oocysts/l	72h
F+ - specific bacteriophage	bacteriophage	PFU/ml	48h

Sample Collection

Water samples were collected monthly from 6 pre-selected sites on Sinking Creek from January 2011 through December 2011 and were analyzed for the variables described in Table 2. Water samples for total and fecal coliform bacteria (TC/FC), standard plate counts (SPC), *E. coli* 057:H7, *Shigella sp.*, and bacteriophage analyses were collected and analyzed in triplicate (SPC samples analyzed in duplicate) in sterile,

1-L Nalgene™ bottles. Water samples for Colilert® analyses were collected in sterile 100ml plastic bottles (IDEXX Laboratories, Westbrook, Maine). Water samples for nitrates (NO_3^-), phosphates (PO_4^-), ammonia (NH_3^+), 5-day biochemical oxygen demand (BOD_5), alkalinity, and hardness were collected and analyzed in triplicate in sterile 2-L Nalgene™ bottles. Sediment samples for TC/FC in water, microbial enzyme activity (MEA), and acridine orange direct counts (AODC) were collected in 2oz sterile Whirl-Pak™ bags. All samples were transported to the laboratory on ice and analyzed within the holding times described in Table 3.2. Field measurements for pH, air and water temperature, dissolved oxygen, and conductivity were also collected at each site.

Quality assurance and quality control (QA/QC) practices included the analysis of chemical parameters consisted of one trip blank, one field blank, a negative control, one replicate, one spiked sample, and one quality control standard. QA/QC practices included in the analysis of microbial parameters included the analysis of one trip blank, one field blank, a negative control, and a positive control. A secondary wastewater effluent sample was used as the positive control for TC/FC, Colilert®, SPC, and bacteriophage analyses. Laboratory strains of *E. coli* O157:H7 and *Shigella flexneri* (ATCC® Number 43895™ and ATCC® 12022™, respectively) were used to seed water samples that served as a positive control for PCR analysis.

Microbial Analyses

TC/FC analyses for water samples were conducted according to Standard Methods for Examination of Water and Wastewater (APHA, 1992). Briefly, 0.5ml of water were filtered through a 0.45µm membrane filter (EMD Millipore, Billerica, MA) and

the filter placed in a petri dish containing an absorbent pad (EMD Millipore, Billerica, MA) with 2ml of m-Endo media for total coliform analysis or m-FC media for fecal coliform analysis. All plates were inverted and enumerated following 24h incubation at 37^{o}C and 44.5^{o}C for total coliform and fecal coliforms, respectively. For TC/FC sediment analyses, 0.5g of sediment was added to 25ml of sterile water + 1% Tween 80. The samples were vortexed and allowed to settle for 30 minutes, and 0.5ml of the buffer suspension was filtered according to Standard Methods for Examination of Water and Wastewater as described above (APHA, 1992).

SPC were conducted according to Standard Methods for Examination of Water and Wastewater (APHA, 1992) using R2A agar. One milliliter of water was placed in the center of a sterile petri dish (Fisher Scientific, Pittsburgh, PA) and 10ml of R2A agar was added to the dish. The plate was swirled in a figure eight motion to allow the sample to disperse in the media and cover the plate. Plates were allowed to solidify and were enumerated following incubation at 25^{o}C for 48h. *Escherichia coli* concentrations were determined using the Colilert® Quanti-Tray method (APHA, 1995). To each 100ml water sample, a packet of Defined Substrate Technology® (DST®) reagent (IDEXX Laboratories, Westbrook, Maine) was added and mixed. The sample was then poured into a Quanti-Tray®, sealed using the Quanti-Tray® sealer, and incubated for 24h at 37^{o}C. *E. coli* were then enumerated using the Standard Method most probable number (MPN) procedure. Samples for water TC/FC were processed in triplicate and samples for sediment TC/FC were processed in duplicate. SPC were processed in duplicate and one Colilert® sample was processed for each site.

MEA analyses were conducted and included acid and alkaline phosphatases, glucosidase, galactosidase, and dehydrogenase activities. For each enzyme analyzed, 1g of sediment was added to a test tube containing a specific buffer and enzyme. Sediment samples for acid phosphatase were mixed with 4ml of 1M TRIS buffer (pH 4.8) and 4ml of 1M TRIS buffer (pH) 8.4 for alkaline phosphatase. For both acid and alkaline phosphatase, 1ml of 1M TRIS buffer with 0.1% phosphatase substrate (pH 7.6) was added to each tube (Sayler et al. 1979). Sediment samples for galactosidase and glucosidase activities were mixed with 4ml of 0.1M phosphate buffer (pH 9.0). Galactosidase activity was measured by adding 1ml of 0.01M phosphate buffer with 0.15% p-nitrophenyl-β-D-galactopyranoside as an indicator of galactosidase activity. One milliliter of 0.01M phosphate buffer with 0.15% 4-nitrophenyl-β-D-glucopyranoside was used as an indicator to assess glucosidase activities (Morrison et al. 1977). Following addition of buffers and indicators, all tubes were vortexted and incubated at 25^{o}C for 24h. Acid and alkaline phosphatase, galactosidase, and glucosidase activities were determined using a spectrophotometer at an absorbance of 418nm.

For dehydrogenase (DHA) activity, 1g of sediment was added to a test tube containing 2ml of 0.1M phosphate buffer (pH 7.6) and 1ml of 0.5% iodonitrotetrazolium chloride (INT) salt solution. The samples were vortexed and incubated in the dark at 25^{o}C for 45 minutes. One milliliter of the sample was filtered through a 0.22µm porosity cellulose membrane (GE Water and Process Technologies, Trevose, PA) and allowed to dry at room temperature. The membrane, was then added to a test tube containing 5ml of dimethyl sulfoxide, vortexted to dissolve the membrane, and incubated in the

dark at 25°C for 24h. Dehydrogenase activity was then determined using a spectrophotometer at an absorbance of 460nm.

AODC analysis was performed as described by Ghiorse and Balkwill (1983). Three hundred milligrams of sediment was added to 30ml of sterile PBS+Tween 80, vortexed for 60s, and allowed to settle for 3h. Two hundred fifty microliters of the suspension was mixed with 5ml sterile water + 500µl acridine orange stain, and samples were vortexed for 30s. Samples were filtered using 25mm, 0.2µm pore polycarbonate nucleopore filters (Osmonics, Inc., Minnetonka, MN), and the filters were mounted and fixed on slides for enumeration at 1000X using the Olympus BH2 epifluorescent microscope (Olympus, New Hyde Park, NY). One sediment sample was processed per site and 3 microscopic fields were enumerated on each slide.

Chemical Analyses

NO_3^-, PO_4^-, NH_3^+, alkalinity, and hardness analyses were performed in triplicate using colorimetric HACH™ methods and HACH™ reagents as described by the manufacturer (HACH Company, Loveland, CO). Briefly, NO_3^-, PO_4^-, NH_3^+ analyses were conducted by adding 10ml of water to a vial containing the appropriate reagent packet; NitraVer5, PhosVer3 and salicylate/ammonia cyanurate reagents, respectively. The vials were shaken to dissolve the reagent and samples were analyzed using pocket colorimeters specific to the nutrient of interest. Alkalinity and hardness analyses were conducted using 100ml sample volumes and a digital titrator. For alkalinity determination, 1 packet of phenolthalein indicator and bromcresol green-methyl red indicator were added to the sample and mixed. The sample was then titrated with 1.6N

sulfuric acid to a grey-green endpoint. For hardness determination, 1 packet of ManVer2 reagent and 2ml of hardness buffer (pH 10) were added to the 100ml sample and mixed. The sample was then titrated with 0.8N Ethylenediaminetetraacetic acid (EDTA) to a blue endpoint. BOD_5 analyses were conducted according to Standard Methods for Examination of Water and Wastewater (APHA, 1992). Wheaton BOD bottles (Wheaton Science Products, Millville, NJ) were completely filled with sample water and capped with glass stoppers to ensure no air bubbles were present. Initial (Day 0) and final (Day 5) dissolved oxygen concentrations were measured using the YSI Model 5000 dissolved oxygen meter (YSI Inc., Yellow Springs, OH).

Pathogenic Bacteria Analyses

Samples for *E. coli* O157:H7 and *Shigella sp.* were analyzed in triplicate. The method used for the filtration and isolation of the bacteria is described by Bej et al. (1991). One hundred milliliters of water was collected and filtered through a 0.22µm membrane filter. The filter was then washed with 10ml of a 1% Tween 80 solution and centrifuged for 10 minutes to create a cell pellet. The supernatant was removed and the cell pellet was washed twice with 10ml phosphate buffered saline. Fifty microliters of diethylpyrocarbonate solution was added to the final cell pellet and subjected to 6 freeze-thaw cycles at -20^{o}C and 100^{o}C, respectively.

PCR amplification for *E. coli* O157:H7 was performed as described by Kimura et al. (2000) using primers EC-1 (GGCAGCCAGCATTTTTTA) and EC-2 (CACCCAACAGAGAAGCCA) for the *chuA* gene. The final 50µl PCR mixture contained 2.5X PCR buffer (mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl), 0.8 mM of each

deoxynucleoside triphosphate (dATP, dCTP, dGTP, and dTTP), 4 µM concentrations of each primer, 5 U *Taq* DNA polymerase (Fisher Scientific, Pittsburg, PA) and 5µl of the resuspended cell pellet. The PCR mixture was subjected to an initial denaturation step at 95^{o}C for 5 minutes, followed by 35 cycles of 1 minute denaturation at 94°C, 2 minutes of annealing at 42°C, and 5 minutes of primer extension at 72°C. A final extension step was performed at 72^{o}C for 10 minutes using a BioRad Thermocycler PCR Machine (BioRad, Hurcules, CA). PCR products were resolved on a 2% agarose gel for 1.5h at 80V and subjected to ethidium bromide staining to visualize DNA base pair bands. The presence of a 901 base pair band indicated a sample positive for *E. coli* O157:H7.

PCR amplification for *Shigella sp.* was performed as described by Theron et al. (2001). Thirty cycles of a seminested PCR reaction were performed using primers H8 (GTTCCTTGACCGCCTTTCCGATAC) and H15 (GCCGGTCAGCCACCCTC) for the *ipaH* gene (Islam, et al. 1993a) in the first round of PCR. The 50µl reaction volume contained 1X PCR buffer (mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl), 0.1mM of each deoxynucleoside triphosphate (dATP, dCTP, dGTP, and dTTP), 24pmol of H8 primer, 34pmol of H15 primer, 1U *Taq* DNA polymerase (Fisher Scientific, Pittsburg, PA), and 10µl of resuspended cell pellet. The PCR mixture was subjected to an initial denaturation step at 94^{o}C for 3 minutes, followed by 10 cycles of 1 minute denaturation at 94°C, 1 minute of annealing at 60°C, and 1 minute of primer extension at 72°C. One microliter of PCR product from the first PCR round was added to a reaction tube containing the reagents described above, with the addition of 31pmol of H10 primer (CATTTCCTTCACGGCAGTGGA) described by Hartman et al. (1990). An initial denaturation step was performed at 94^{o}C for 3 minutes, followed by 20 cycles of 1

minute denaturation at 94°C, 1 minute of annealing at 60°C, and 1 minute of primer extension at 72°C. A final extension step was performed at 72°C for 7 minutes using a BioRad Thermocycler PCR Machine (BioRad, Hurcules, CA). PCR products were resolved on a 2% agarose gel for 1.5h at 80V and subjected to ethidium bromide staining to visualize DNA base pair bands. The presence of both a 401 and 620 base pair band indicated a sample positive for *Shigella sp*.

Protozoan Analysis

One water sample was collected and analyzed monthly at each site for the analysis of *Giardia* and *Cryptosporidium*. Samples were collected as described by USEPA method 1623 for water filtration (USEPA, 2005). One hundred liters of water were filtered at each site though Envirochek™ sampling filters (Pall Corporation, Ann Arbor, MI) using a gas powered water pump and Badger™ flow meter at a rate of 2.5L/minute. The filtration apparatus was assembled as shown in Figure 3.

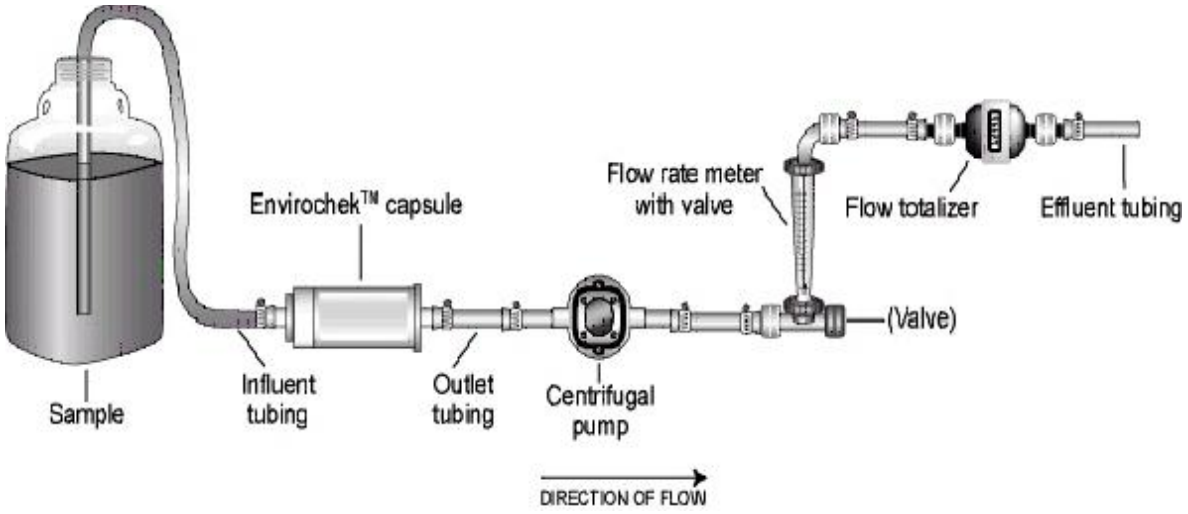


Figure 3.2. Filtration apparatus used to sample Giardia and Cryptosporidium in laboratory seeded samples (USEPA, 2005)

The filters were transported to the lab on ice and analyzed within 72h of collection. Filters were initially washed by adding 120ml of elution buffer to the filter capsule and placing on a wrist action shaker for 30 minutes. The elution buffer was removed and the filter capsule broken open and the filter cut out using a sterile razor blade and hand washed using 120ml of elution buffer. The buffer was then added to a sterile 250ml centrifuge tube containing the elution buffer from the initial wash on the wrist action shaker. The samples were centrifuged at 2,300 x g for 30min and the supernatant removed. The concentrated pellet collected was subjected to an immunofluorescent assay using the Waterborne Aqua-Glo™ G/C Direct FL antibody stain (Waterborne, Inc. New Orleans, LA) as described by the manufacturer. The prepared slides were examined at 200X using the Olympus BH2 epifluorescent microscope (Olympus, New Hyde Park, NY).

Bacteriophage Analyses

Samples for bacteriophage analysis were collected and analyzed in triplicate using the double-layer agar procedure described in USEPA method 1601 (USEPA, 2001a) using *E. coli* C3000 as the host strain (ATCC® Number 15597™). The host strain was cultured using ATCC 271 broth (10g/L tryptone, 1g/L yeast extract, 8g NaCl, 10ml/L of 10% glucose solution, 2ml/L of 1M $CaCl_2$, 1ml/L of 10mg/ml thiamine) at 37^{o}C. An overnight culture of the host strain was prepared the day before analysis by inoculating a 30ml ATCC broth culture with the host strain. On the day of analysis, 100µl of the prepared overnight culture of the host strain was inoculated into a 30ml of fresh ATCC 271 broth and incubated at 37^{o}C until log phase was reached (~4h).

Ten milliliters of collected sample water were filtered through a syringe filter fitted with a 0.22µm membrane filter to remove bacteria and the filtrate was serially diluted in phosphate buffered water for analysis. Five hundred microliters of each dilution were added to a test tube containing 5ml of 0.7% ATCC® 271 agar (ATCC® 271 broth with 1.4g/L agar) and 100µl of host bacteria. The tubes were gently mixed and poured onto a plate containing 1.5% ATCC 271 agar (ATCC® 271 broth with 18g/L agar). Plates were allowed to solidify prior to incubation at 37^{o}C for 24h and plaques were enumerated.

Data Analysis of Fecal Coliform Data and Pathogen Prevalence

Statistical analyses were performed using SAS/STAT software version 9.2 (SAS Institute, Cary, NC). Fecal coliform data were log transformed to achieve normality, and concentrations were compared by site, season, and land use pattern using the Analysis

of Variance (ANOVA) procedure to assess the influences of spatial and temporal variability on fecal coliform concentrations. Linear regression analyses were also performed using the REG procedure to estimate the correlation between fecal indicator organisms (fecal coliform bacteria and *E. coli*) and pathogens to assess their usefulness as indicators of pathogen presence in Sinking Creek.

Results and Discussion

General Trends of Water Quality Parameters

pH values (Figure 3.3) were within the acceptable range of 6.0 – 9.0 for recreational activity throughout the duration of the study (TDEC, 2008). Fall air and water temperatures were lower than the winter, spring, and summer months (Figures 3.4 and 3.5). All water temperatures were within the acceptable range for recreational waters throughout the duration of the study, as the water temperature was not observed to exceed 30.5^{o}C at any point and did not have a water change greater than 3^{o}C between any 2 sampling locations (TDEC, 2008). Dissolved oxygen concentrations were higher during the winter months compared to other seasons (Figure 3.6) because cold water is able to hold more dissolved oxygen than warm water.

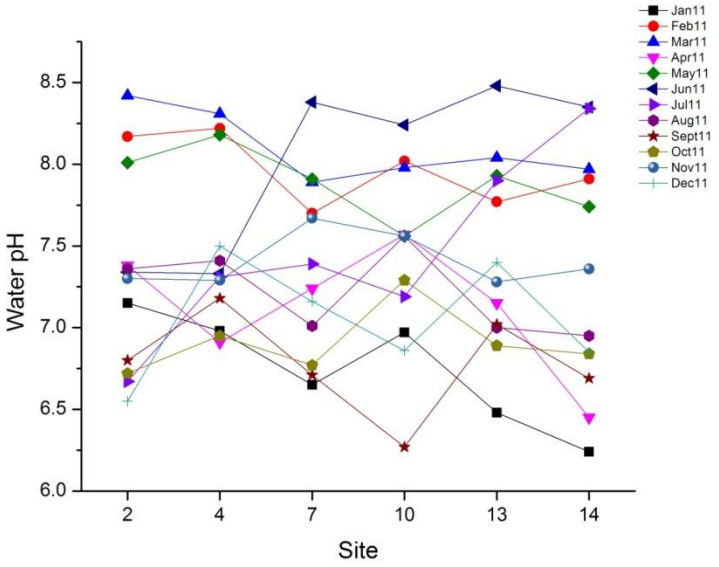


Figure 3.3. pH in the water of Sinking Creek by site and month

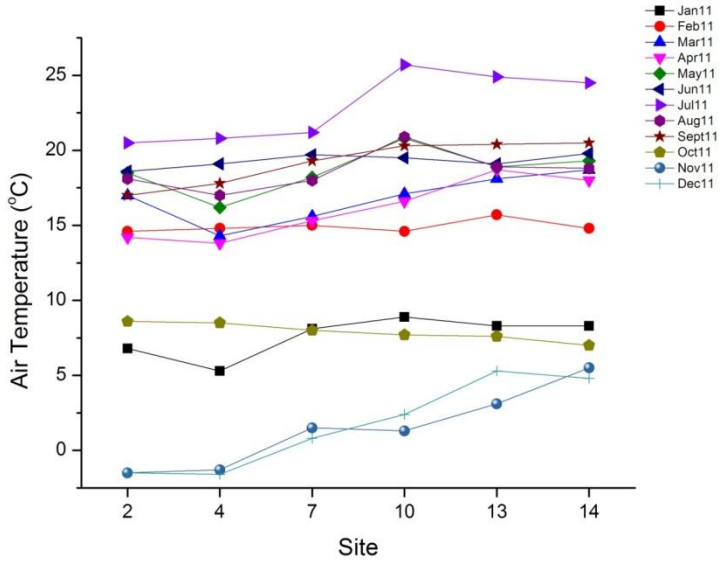


Figure 3.4. Air temperature along Sinking Creek by site and month

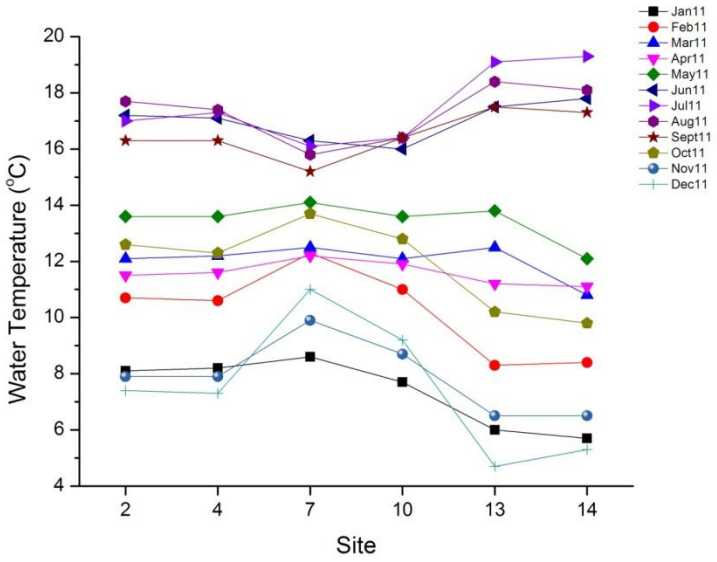


Figure 3.5. Water temperature of Sinking Creek by site and month

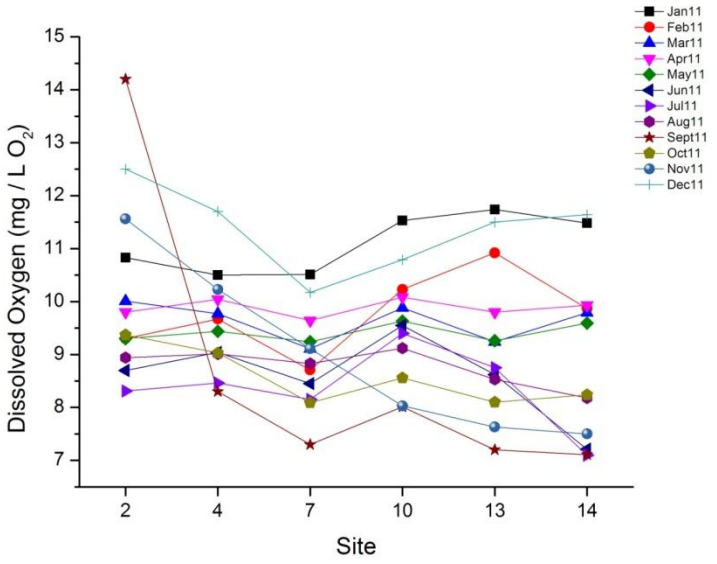


Figure 3.6. Dissolved oxygen of Sinking Creek by site and month

Conductivity is affected by the presence of ions and was shown to increase between upstream and downstream sites (Figure 3.7) in a similar pattern to that of

alkalinity and hardness (Figures 3.8 and 3.9). This relationship is expected, as the cations contributing to alkalinity and hardness are introduced through runoff or organic matter addition, thus resulting in an increase of conductivity with distance downstream. Discharge also increased with distance downstream (Figure 3.10) due to increases in water depth and flow and creek width that also suggests that observed alkalinity and hardness concentrations are related to runoff and organic matter conditions.

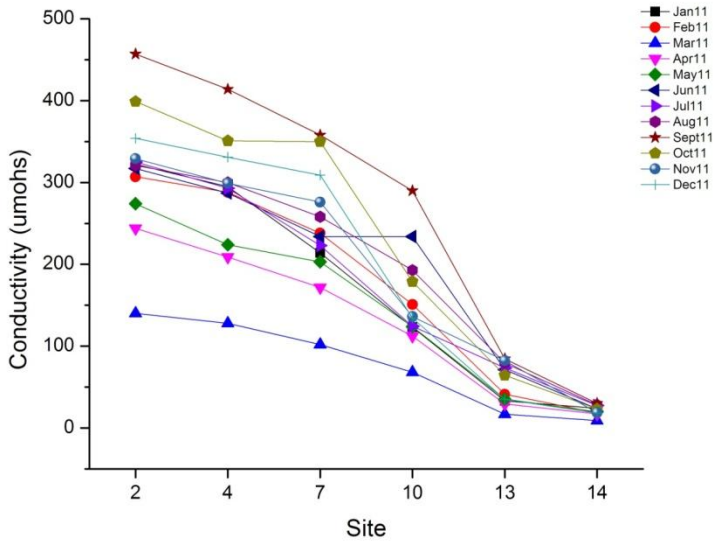


Figure 3.7. Conductivity of Sinking Creek by site and month

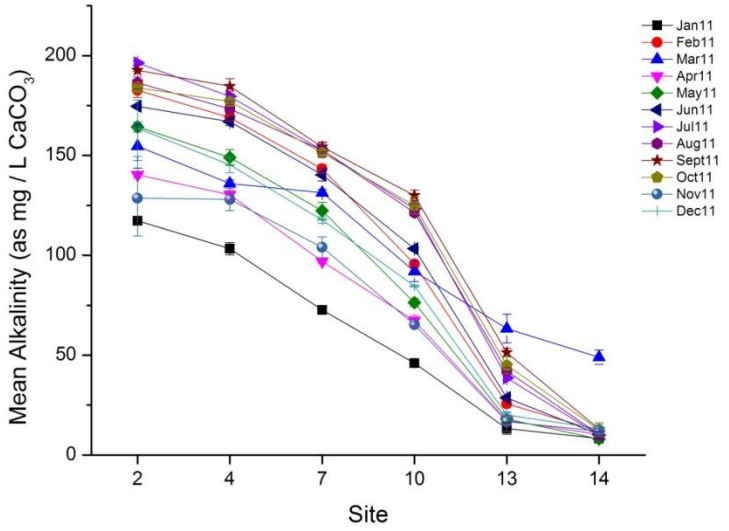


Figure 3.8. Alkalinity of Sinking Creek by site and month

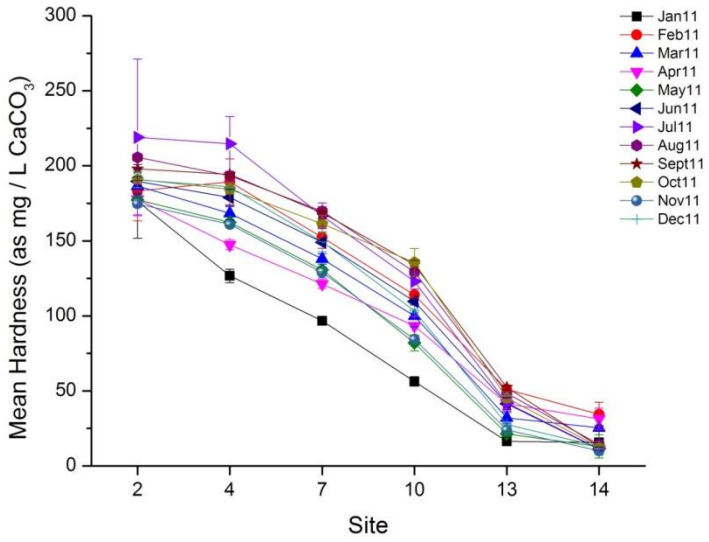


Figure 3.9. Hardness of Sinking Creek by site and month

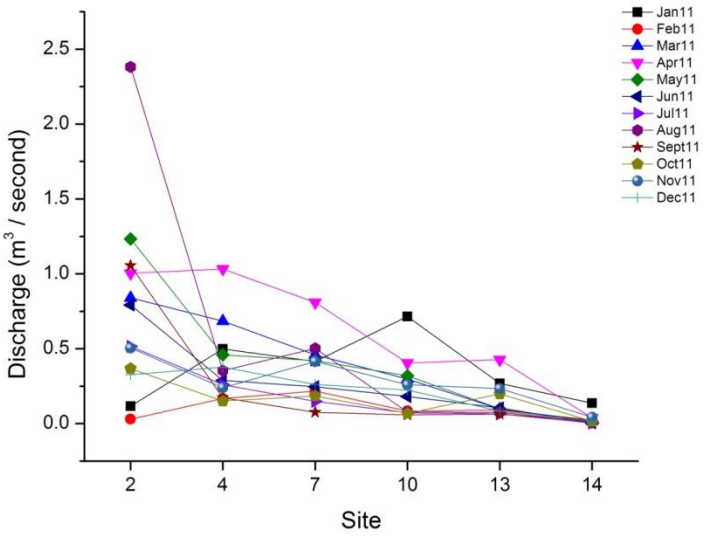


Figure 3.10. Discharge of Sinking Creek by site and month

Visual observation of total and fecal coliform concentrations in water demonstrates that the highest concentrations are at the downstream (agricultural) sites (Figures 3.11 and 3.12). Total and fecal coliform concentrations in sediment (Figure 3.13 and 3.14) are lower and show more fluctuation between sites compared to water concentrations. The lack of a total and fecal coliform trend based on site is likely a function of creek discharge because total and fecal coliform concentrations at those sites with lower discharges are more likely to partition into sediment (Jamison et al. 2003; Whitman and Nevers, 2003). *E. coli* concentrations and standard plate counts (Figures 3.15 and 3.16) also vary by season and site, as concentrations tend to be higher during the spring and summer months and increase with increasing distance downstream.

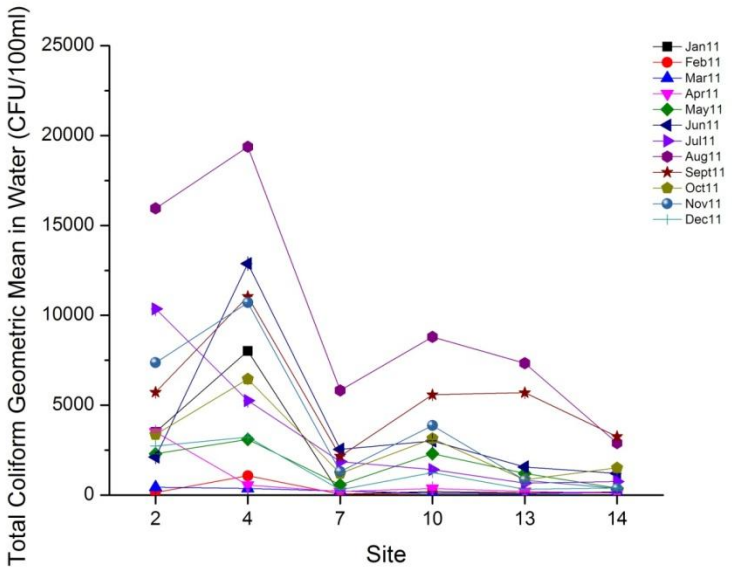


Figure 3.11. Geometric mean of total coliform concentrations in water in Sinking Creek by site and month

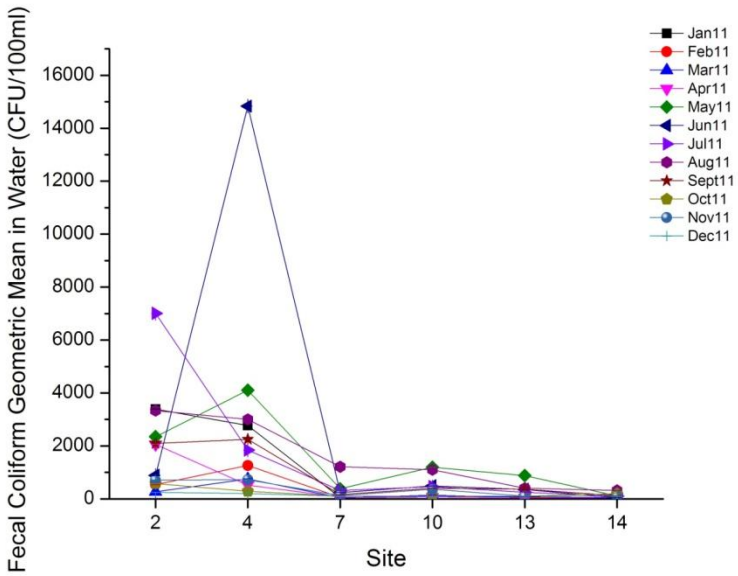


Figure 3.12. Geometric mean of fecal coliform concentrations in water in Sinking Creek by site and month

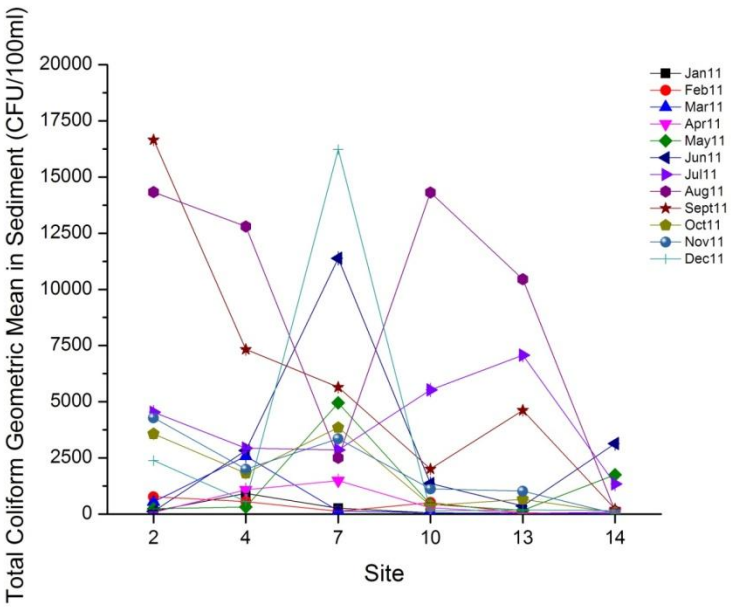


Figure 3.13. Geometric mean of total coliform concentrations in sediment in Sinking Creek by site and month

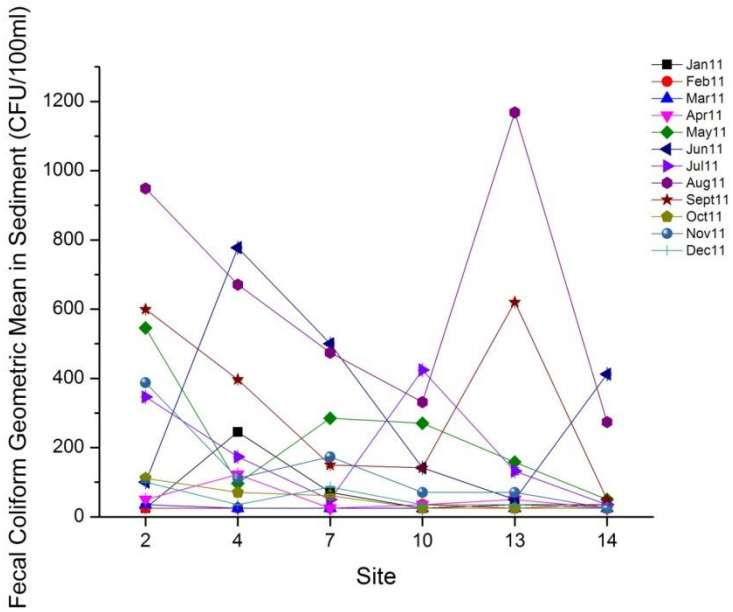


Figure 3.14. Geometric mean of fecal coliform concentrations in sediment in Sinking Creek by site and month

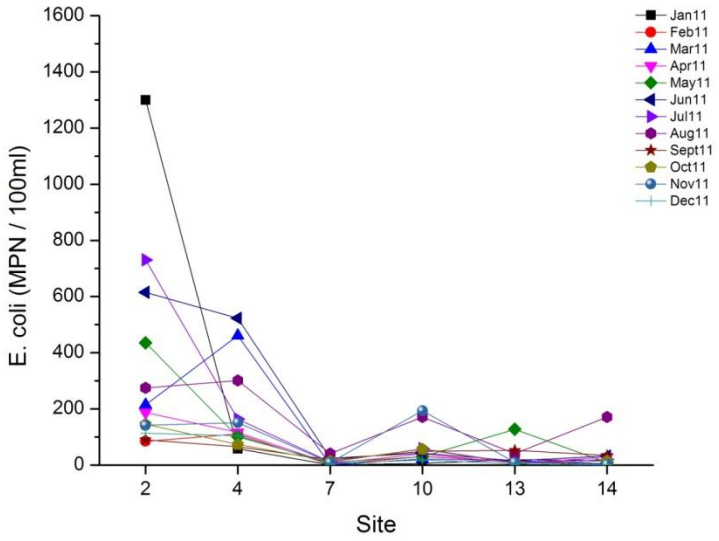


Figure 3.15. *E. coli* concentrations in Sinking Creek by site and month

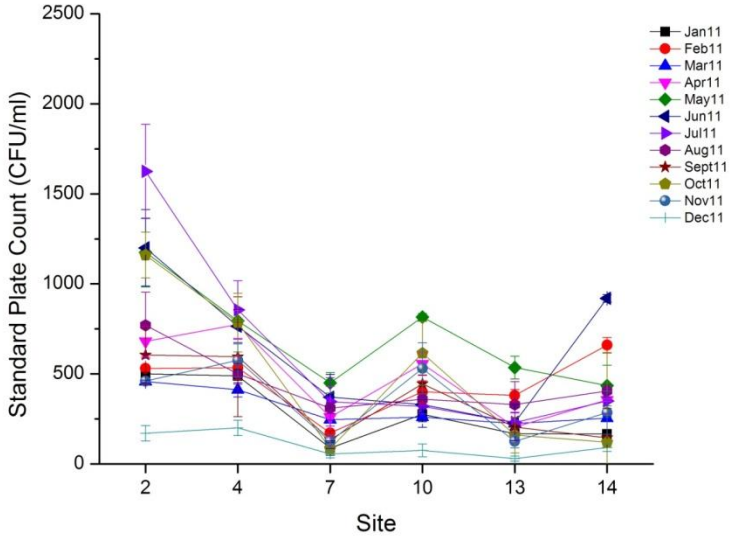


Figure 3.16. Mean heterotrophic bacteria as determined by standard plate counts in Sinking Creek by site and month

Acridine orange direct counts, acid phosphatase, alkaline phosphatase, dehydrogenase, galactosidase, and glucosidase activities display seasonal and spatial variability of microbial activity in sediment (Figures 3.17 – 3.22). ANOVA demonstrated significant seasonal differences for all MEAs except for dehydrogenase activity (Figure 3.23 a – d). The significantly higher galactosidase and glucosidase concentrations during the fall and winter months indicates the ability of the microbial communities to use organic matter inputs, most likely in the form of leaf litter. The higher phosphatase concentrations observed during the fall also suggests the processing of organic matter by the microbial communities.

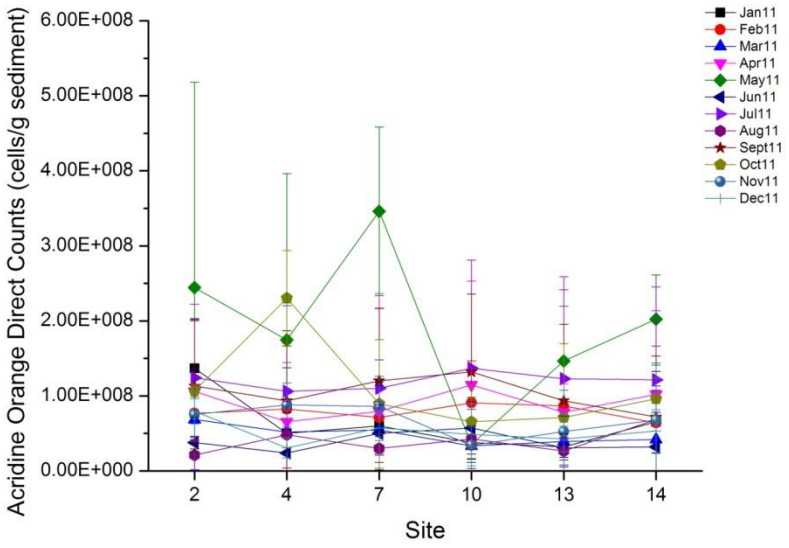


Figure 3.17. Mean acridine orange direct counts in Sinking Creek by site and month

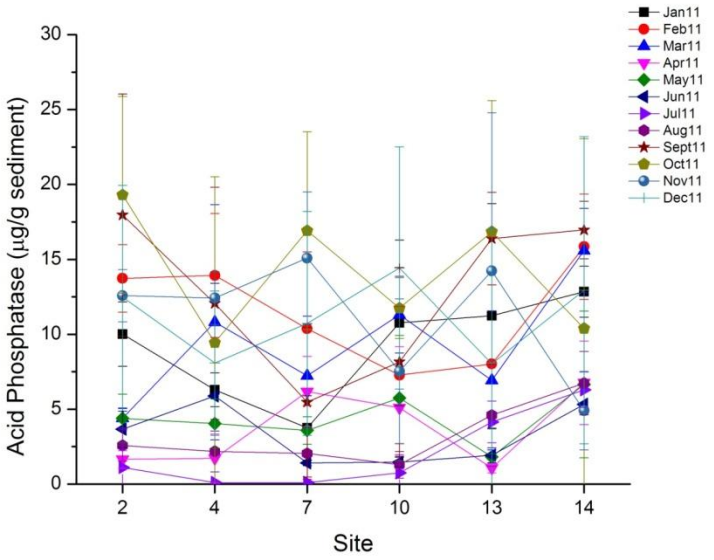


Figure 3.18. Mean acid phosphatase concentrations in Sinking Creek by site and month

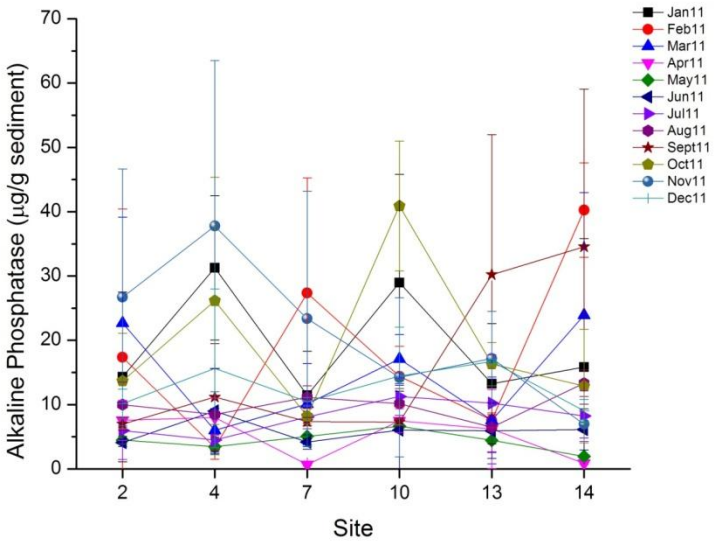


Figure 3.19. Mean alkaline phosphatase concentrations in Sinking Creek by site and month

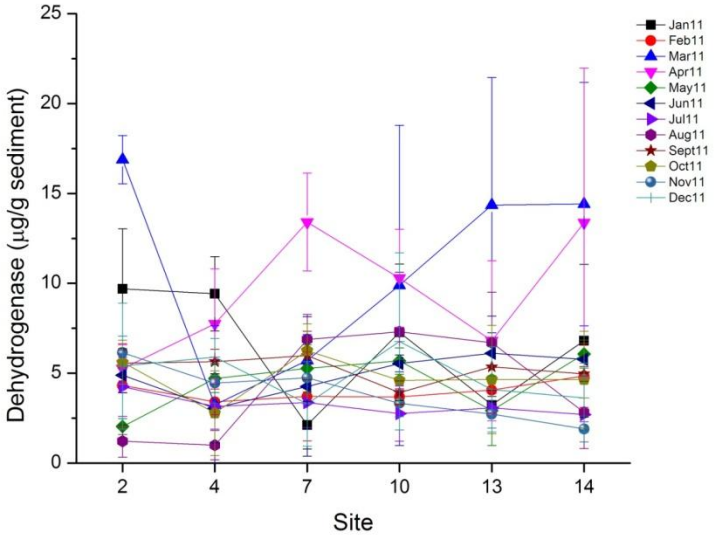


Figure 3.20. Mean dehydrogenase concentrations in Sinking Creek by site and month

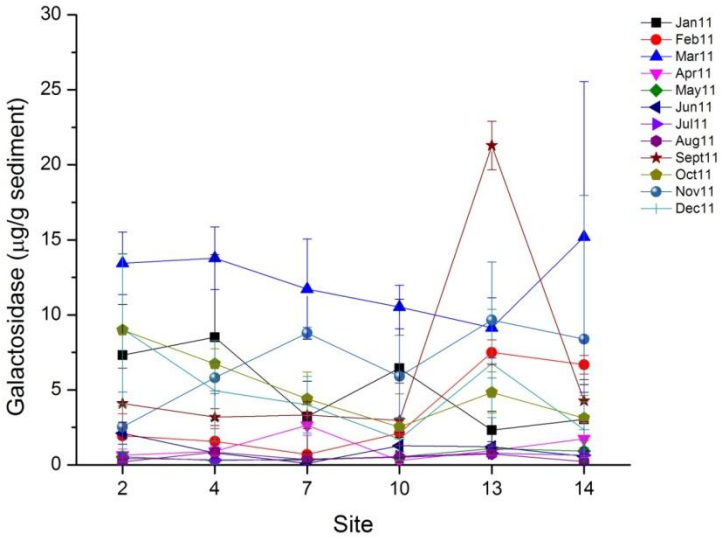


Figure 3.21. Mean galactosidase concentrations in Sinking Creek by site and month

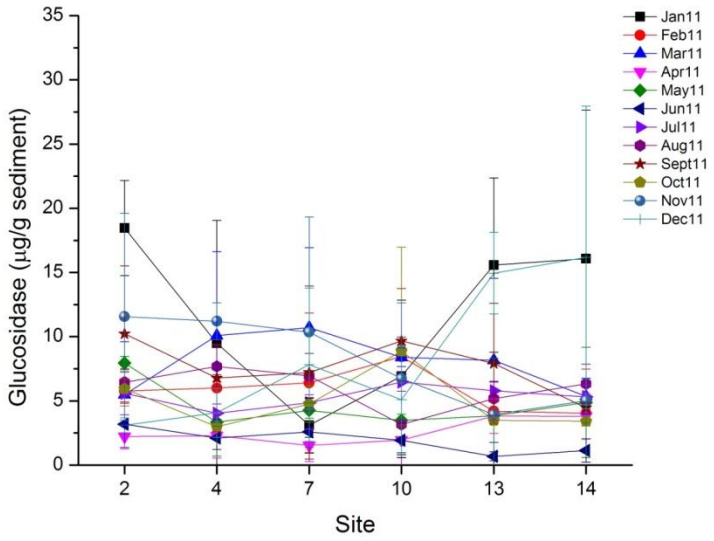


Figure 3.22. Mean glucosidase concentrations in Sinking Creek by site and month

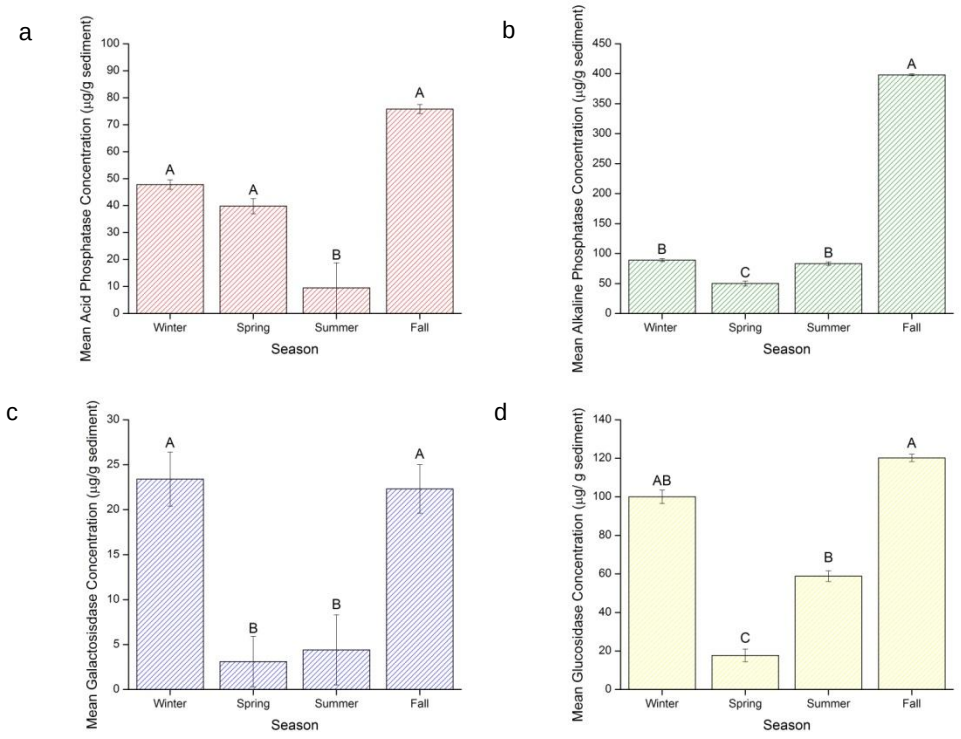


Figure 3.23. Mean acid phosphatase (a), alkaline phosphatase (b), galactosidase (c), and glucosidase (d) concentrations in Sinking Creek by season (significant differences are indicated by different letters)

E. coli O157:H7 was not detected in any field sample, and *Shigella sp.* was only detected at upstream sites in April 2011. *Giardia sp.*, *Cryptosporidium sp.*, and bacteriophage were detected at all sites and demonstrated spatial and temporal variability (Figures 3.24 – 3.26). *Giardia sp*. and *Cryptosporidium sp*. were detected in 87.3% and 88.7% of samples, respectively, and bacteriophages were detected in 10.2% of samples.

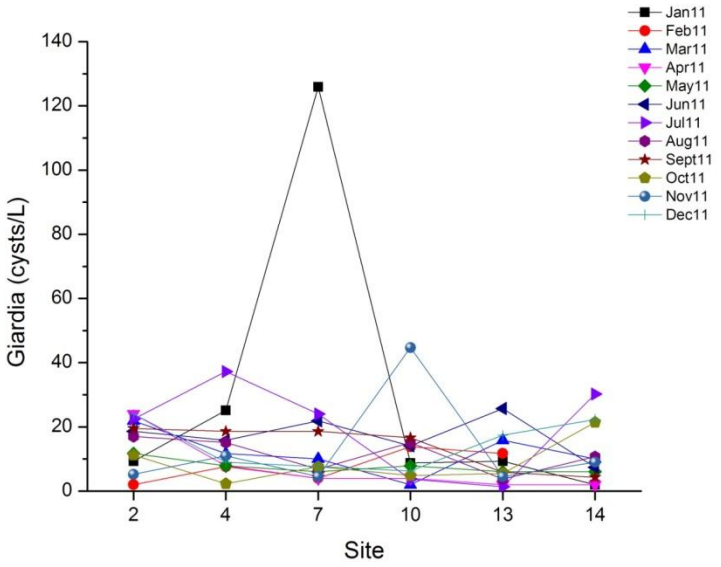


Figure 3.24. *Giardia* sp. concentrations in Sinking Creek by site and month

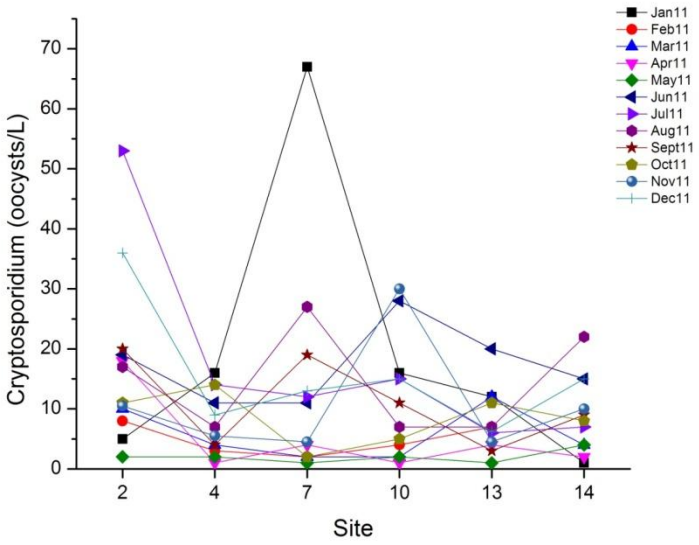


Figure 3.25. *Cryptosporidium* sp. concentrations in Sinking Creek by site and month

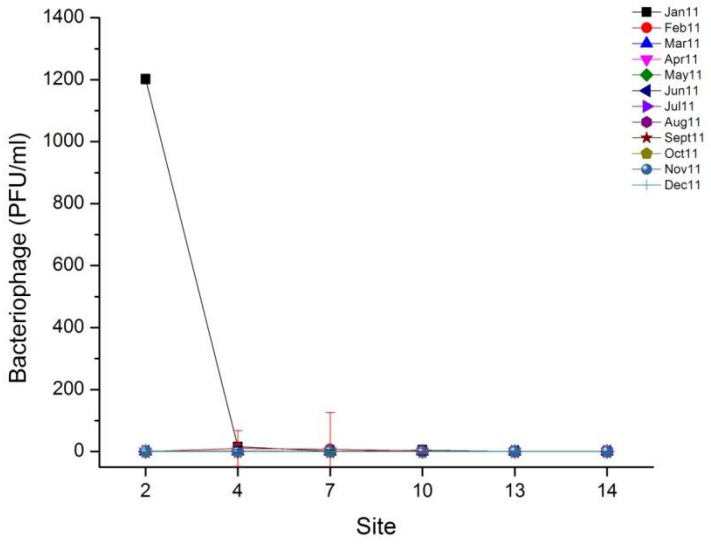


Figure 3.26. Mean bacteriophage concentrations in Sinking Creek by site and month

Seasonal and spatial variability in nutrient concentrations was observed (Figures 3.27 – 3.29). Nitrate, phosphate, and ammonia concentrations tended to increase with increasing distance downstream, suggesting the influence of surface runoff at urban and agricultural land use sites, yet these concentrations did not appear to contribute to aquatic plant or algal growth. Biochemical oxygen demand demonstrated temporal variability (Figure 3.30) with the highest concentrations of oxygen observed during the winter and spring months. The elevated BOD_5 values during these months compared to other months may be influenced by the presence of organic matter and leaf litter introduced into Sinking Creek.

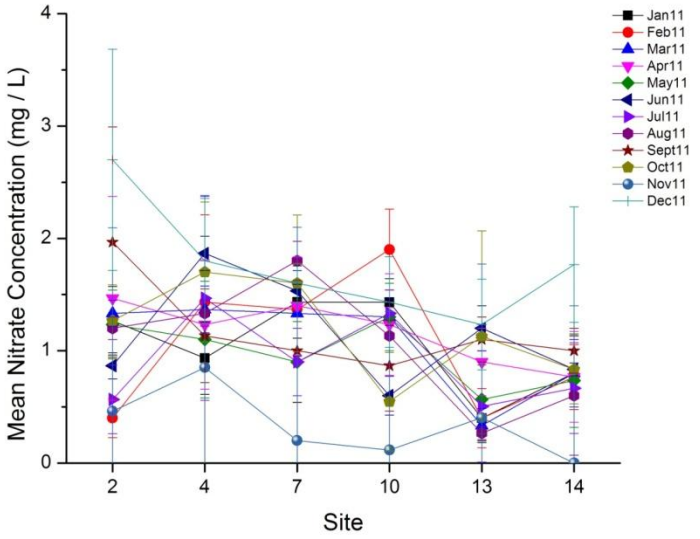


Figure 3.27. Mean nitrate concentrations in Sinking Creek by site and month

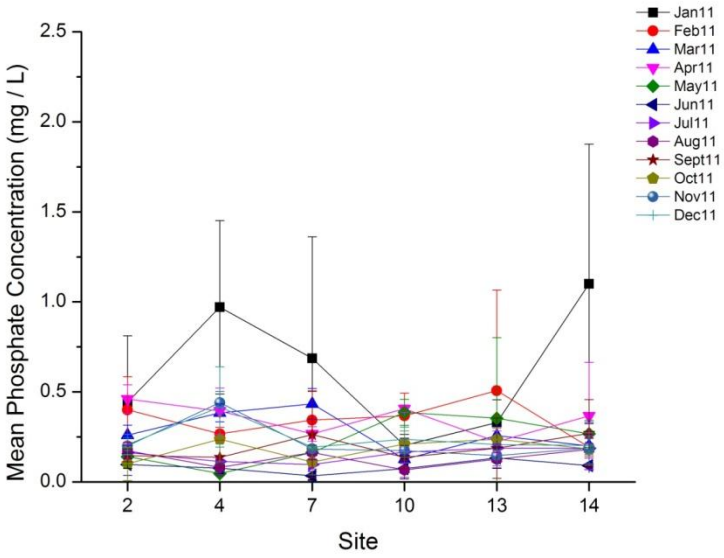


Figure 3.28. Mean phosphate concentrations in Sinking Creek by site and month

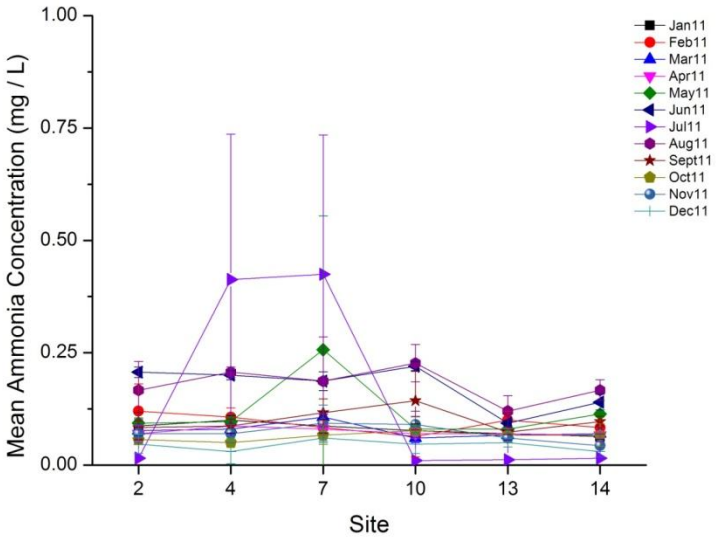


Figure 3.29. Mean ammonia concentrations in Sinking Creek by site and month

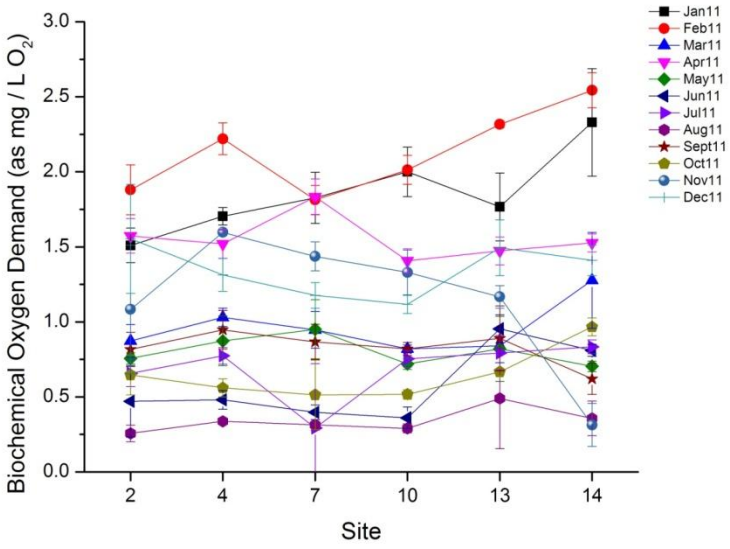


Figure 3.30. Mean biochemical oxygen demand in Sinking Creek by site and month

Comparison of Fecal Coliform Concentrations

ANOVA for fecal coliform concentrations was performed by season, land use pattern, and site. Temporal (seasonal) variability was observed for fecal coliform concentrations (Figure 3.31). The winter and fall months had significantly lower fecal coliform concentrations compared to the spring and summer months. The significant differences observed between seasons indicate variation in climatic conditions, including rainfall, runoff events, and water temperature. Runoff events, warm temperatures, and the addition of organic matter contribute to the higher fecal coliform concentrations observed during the spring and summer months (Hunter et al. 1999; Hyland et al. 2003).

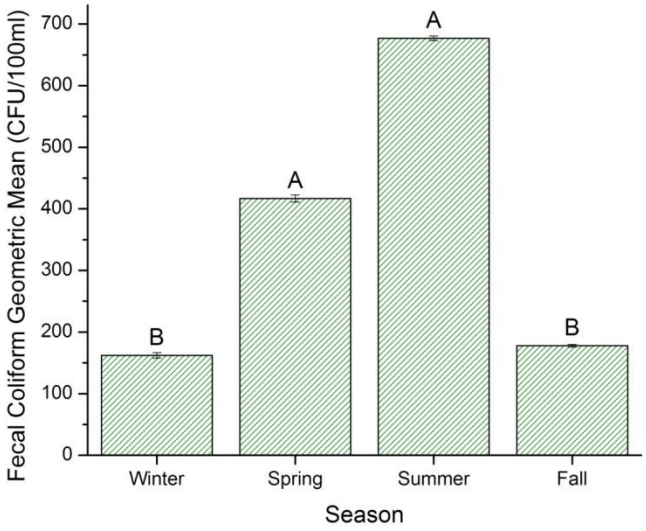


Figure 3.31. Geometric mean of fecal coliform concentrations in Sinking Creek by season (significant differences are indicated by different letters)

Low flow conditions during spring and summer months and subsequent partitioning of fecal coliforms into the sediment could also account for the higher fecal coliform concentrations compared to the winter and fall months (Malan et al. 2003). Lower fecal coliform concentrations during the winter and fall may be the result of colder temperatures and fewer runoff events that contribute to fecal coliform loading during the fall and winter months. Seasonal variation in fecal coliform bacteria is commonly observed in surface water, as colder temperatures can reduce the survival of fecal coliform bacteria (Malan et al. 2003; Hörman et al. 2004). However, colder water temperatures during these months may also promote the survival of fecal coliform bacteria that were introduced during the spring and summer months (Smith et al. 1994, Maajel et al. 2003).

ANOVA of fecal coliform data by land use pattern demonstrated that significant differences for fecal coliform concentrations existed between the 3 land use patterns (Figure 3.32). The lowest fecal coliform concentrations were observed at forest land use sites (sites 13 and 14) and are just below the regulatory limit of 126CFU/100ml for recreational water use at 117CFU/100ml. These sites are located on Buffalo Mountain at the headwaters of Sinking Creek. Although there are hiking and recreational trails, and some residential establishment in proximity to the headwaters of Sinking Creek, it is likely that fecal pollution at these sites is associated primarily with wildlife activity.

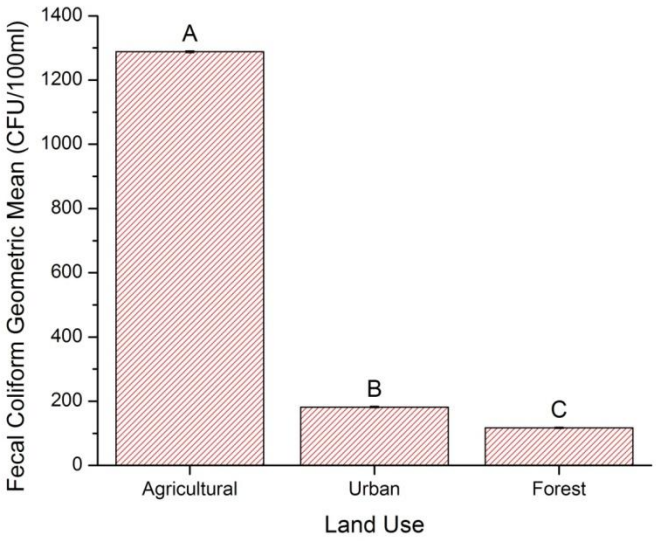


Figure 3.32. Geometric mean of fecal coliform concentrations in Sinking Creek by land use (significant differences are indicated by different letters)

Fecal coliform concentrations at urban sites are significantly higher than the concentrations at forested sites, suggesting that the addition of fecal pollution occurs with increasing distance downstream and is possibly influenced by impervious surfaces and runoff events (Kistemann et al. 2002). In addition to an influx of fecal coliform bacteria from surface runoff, resuspension from sediment (Goyal et al. 1977) can also result in higher fecal coliform concentrations following rainfall events. Sources contributing to fecal pollution in urban settings may include sources such as septic systems, storm sewers, and household pets (Weiskel et al. 1996; Olyphant et al. 2003; Ning et al. 2006; Zeilhofer et al. 2006). The deposition of fecal coliform bacteria at these sites may also occur by sedimentation, bank erosion, or the attachment of bacteria to particles (Vega et al. 1998; Lemarchand and Lebaron 2003).

The highest fecal coliform concentrations were observed at agricultural land use sites. Agricultural activity is a common contributor to fecal coliform concentrations in surface water (Lenat and Crawford, 1994; Whiles et al. 2000; Tong and Chen, 2002). Direct deposition of fecal material into surface water and/or the continued release of fecal coliform bacteria from manure deposited on pastureland may be contributing to the observed fecal coliform concentrations at these sites (Thelin and Gifford, 1983). Once in the water, warmer water temperatures may also promote replication and survival of fecal coliform bacteria in water and sediment (Byappanahalli et al. 2003). In addition to these sources of fecal pollution at agricultural sites, spatial patterns (Hunsaker and Levine, 1995), agricultural densities (Harding et al. 1999), ecological patterns (Buck and Townsend, 2004), rainfall and subsequent surface runoff, and stream characteristics (Sheshane et al. 2005) can all influence fecal coliform loading into surface waters.

Fecal coliform concentrations were also analyzed by site over the entire sampling period to determine the extent of fecal pollution at each site to help identify areas of potential fecal pollution introduction. As previously seen with ANOVA by land use, spatial variability was observed (Figure 3.33). Agricultural sites had significantly higher fecal coliform concentrations compared to urban and forest land use sites, with peak fecal coliform concentrations at site 4. Comparing fecal coliform concentrations by site and season assessed the combined effects of spatial and temporal variability on fecal coliform concentrations.

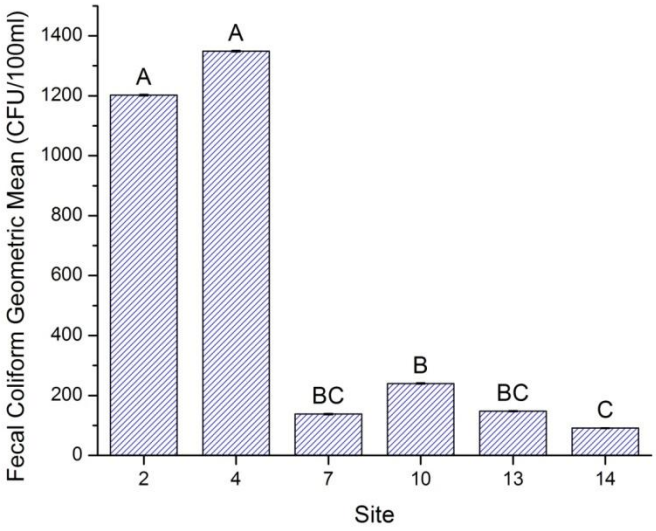


Figure 3.33. Geometric mean of fecal coliform concentrations in Sinking Creek by site (significant differences are indicated by different letters)

Fecal coliform concentrations at the agricultural sites were significantly higher during all seasons (Figure 3.34 a – d). Significant increases in fecal coliform concentrations were observed between sites 4 and 7 for all seasons, suggesting that the bulk of fecal pollution occurred between these sites. During the winter and spring months, fecal coliform concentrations decreased between sites 2 and 4. This suggests that there may be something inhibiting fecal coliform survival or transport such as colder temperatures (Hörman et al. 2004), settling into sediment (Gannon et al. 1983) or predation (Korhonen and Martikainen, 1991). In contrast, fecal coliform concentrations increase between sites 2 and 4 during the summer and fall months. This may be due to the continued introduction of fecal coliform bacteria downstream through runoff events and agricultural activities, or the influence of warmer water temperatures during the

summer and fall months, and the addition of organic matter that promote survival of fecal coliform bacteria (Hunter et al. 1999; Hyland et al. 2003)

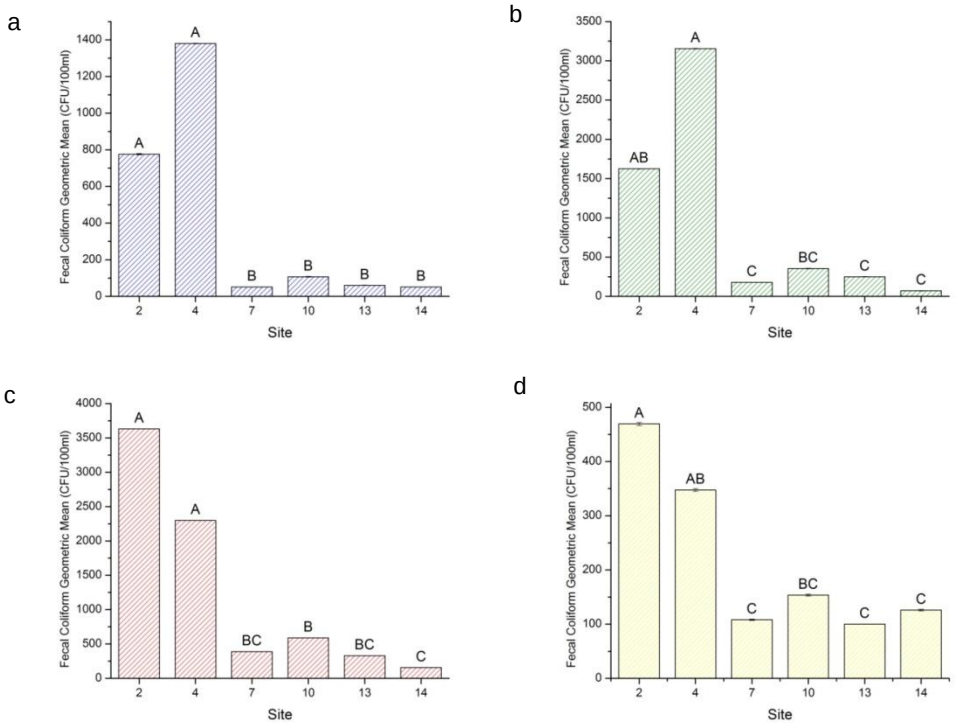


Figure 3.34. Geometric mean of fecal coliform concentrations in Sinking Creek for winter (a), spring (b), summer (c), and fall (d) by site (significant differences are indicated by different letters)

Correlation Between Fecal Coliform Bacteria, E. coli, and Pathogens

Regression analysis was performed to determine the ability of fecal coliform bacteria and *E. coli* to predict the presence of *E. coli* O157:H7, *Shigella sp., Giardia sp., Cryptosporidium sp.,* and male specific (F^+) bacteriophage. A complete lack of correlation was observed between *E. coli* or fecal coliform bacteria and the pathogenic bacteria of interest due to the failure to detect either organism in the field samples. The

linear regression statistics for *Giardia sp., Cryptosporidium sp.,* and male specific (F^+) bacteriophage, and the indicator organisms are displayed in Table 3.3.

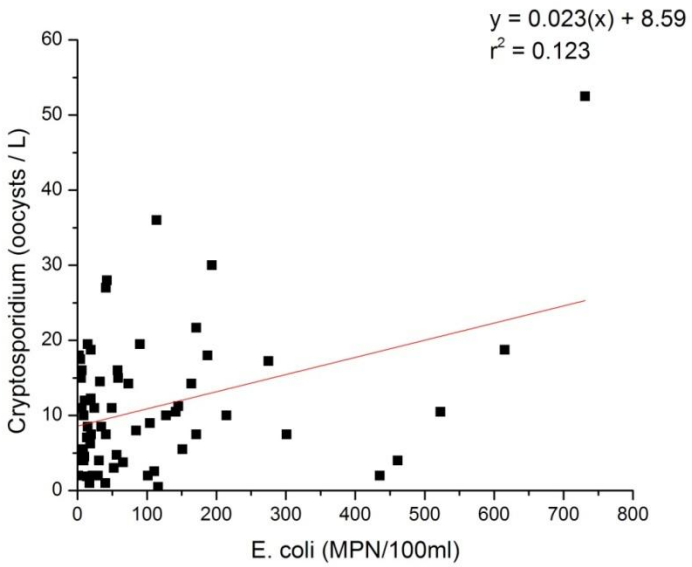
Table 3.3. Regression statistics for pathogens vs. indicators

Pathogen vs. Indicator	**r^2 value**	**p – value**
Giardia sp. vs. E. coli	0.053	p > 0.03
Giardia sp. vs. fecal coliforms	0.046	p > 0.04
Cryptosporidium sp. vs. E. coli	0.123	p > 0.002
Cryptosporidium sp. vs. fecal coliforms	0.116	p > 0.002
Bacteriophage vs. E. coli	-0.009	p > 0.54
Bacteriophage vs. fecal coliforms	-0.009	p > 0.56

These results suggest that neither *E. coli* nor fecal coliform bacteria are sufficient indicators of presence of pathogenic bacteria. The failure to detect *E. coli* O157:H7 or *Shigella sp.* may be due to the use of PCR methods in the absence of standardized methods. The speed of analysis, typically a few hours, combined with method sensitivity and ability to detect VBNC organisms make molecular methods such as PCR appealing for the identification of pathogens in surface water (Josephson et al.1993; Abd-El-Haleem et al. 2003). Although PCR methods for the identification of pathogens can be rapidly completed and highly sensitive, they are often difficult to standardize and apply to environmental samples due to inhibiting substances in the soil and water matrix such as humic acids (Tebbe and Vahjen, 1993; Campbell et al. 2001; Bhagwat, 2003). Environmental stress has also been shown to affect the stability of the target gene further complicating the sensitivity of the method (Cooley et al. 2010). These factors may have inhibited detection of *E. coli* O157:H7 and *Shigella sp.* in Sinking Creek.

Figures 3.35 (a and b) and 3.36 (a and b) display the linear regression plots for *E. coli* or fecal coliform bacteria vs. *Cryptosporidium sp.* and *Giardia sp.* with their associated r^2 values. All 4 of the regression models are statistically significant ($p < 0.05$), yet have low r^2 values. Indicating that little variability in protozoan concentrations is explained by either fecal coliform bacteria or *E. coli* concentrations. This indicates that fecal coliform bacteria and *E. coli* are poor predictors of protozoan contamination in Sinking Creek. Correlation between fecal coliform bacteria and *Giardia sp.* and *Cryptosporidium sp.* has been reported (LeChevallier et al. 1991; Touron et al. 2007), but the vast majority of studies do not show a correlation between fecal indicator organisms and protozoan pathogens (Rose et al. 1988; Lemarchand and Lebaron, 2003; Harwood et al. 2005). It may be possible that the observed differences in the literature are due in part to the types of water sampled. As reported by LeChevallier et al. (1991), water samples with higher fecal coliform concentrations have an increased probability that the pathogens will be present. It may also be possible that the protozoans isolated were associated with sediment that was filtered while the fecal indicator organisms were suspended in the water.

a



b

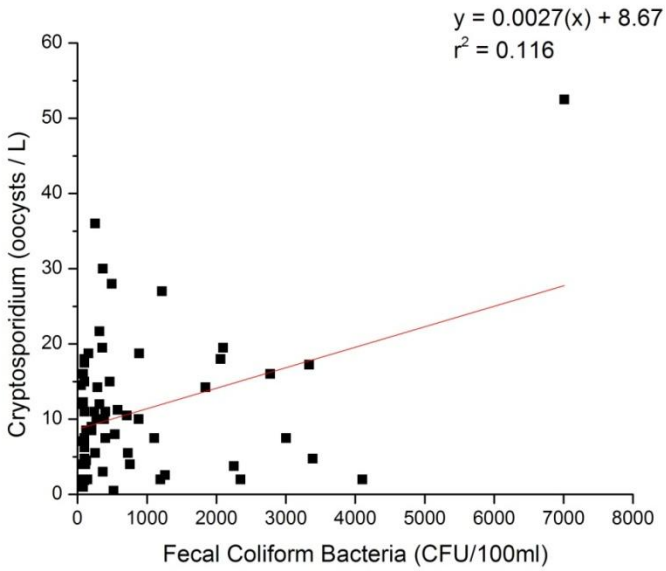
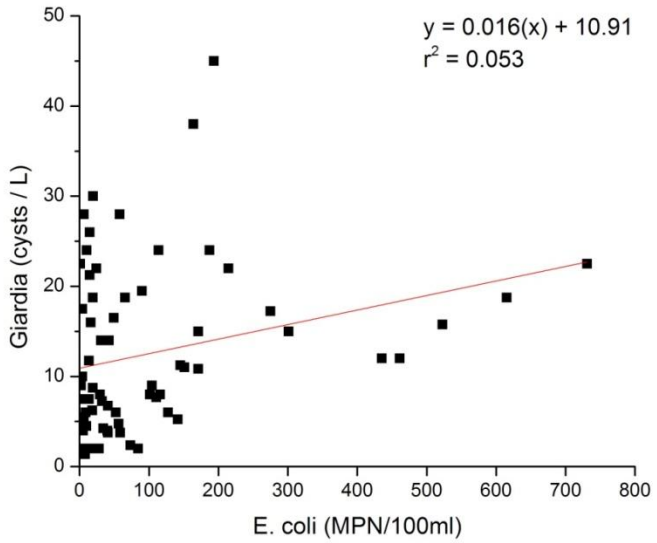


Figure 3.35. Linear regression of *Cryptosporidium* sp. and *E. coli* (a) and fecal coliform bacteria (b)

a



b

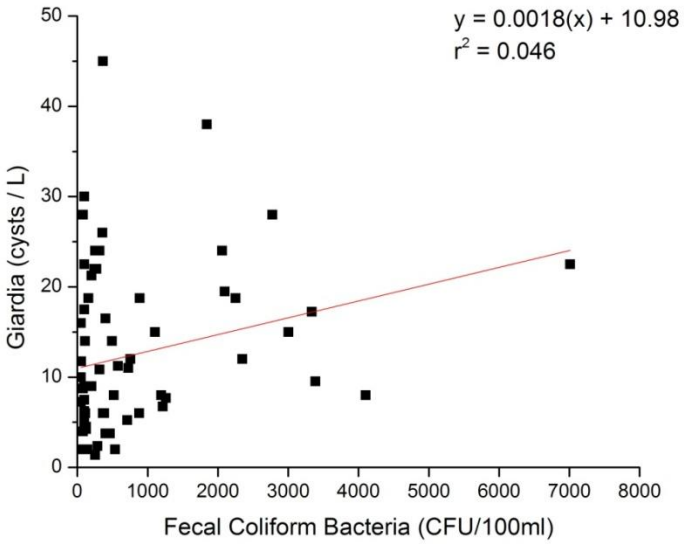


Figure 3.36. Linear regression of *Giardia* sp. and *E. coli* (a) and fecal coliform bacteria (b)

Figure 3.37 (a and b) display the linear regression plots for *E. coli* and fecal coliform bacteria and male-specific bacteriophage with their associated r^2 values. A lack of correlation is often observed between enteric viruses and fecal indicator organisms (Gerba et al. 1979; Noble and Fuhrman 2001). As a result, alternative indicators of enteric viruses such as bacteriophages have been used as successful indicators of enteric virus pollution in surface waters (Wentsel et al. 1982; Stetler, 1984; Havelaar et al. 1993) and have been shown to correlate with fecal coliform concentrations (Kenard and Valentine 1974; Borrego et al. 1987). In this study, the regression models are not statistically significant, indicating that neither *E. coli* nor fecal coliform bacteria are sufficient indicators of the presence of bacteriophage prevalence. Assuming that bacteriophages are successful indicators of enteric virus pollution in surface waters, these results also suggest that indicator organisms are not sufficient predictors of enteric virus pollution in Sinking Creek.

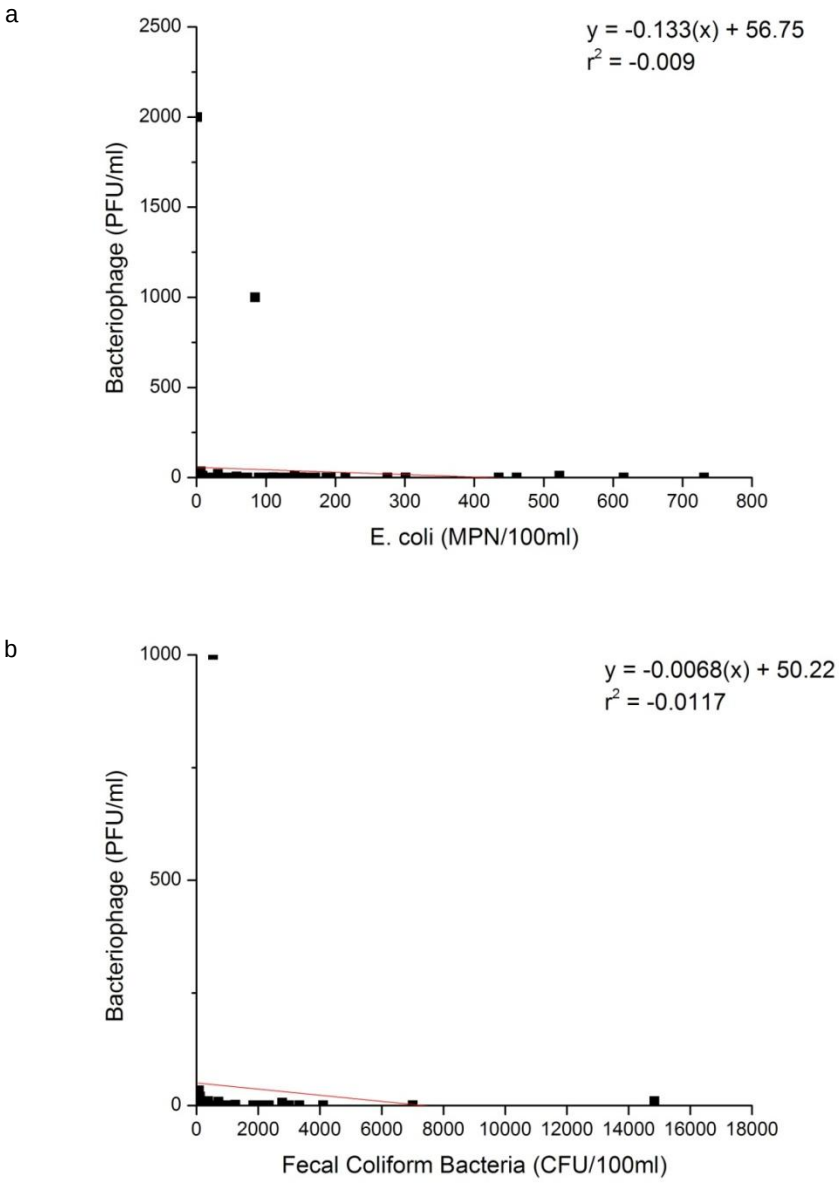


Figure 3.37. Linear regression male-specific bacteriophage with E. coli (a) and fecal coliform bacteria (b)

Conclusion

Physical, chemical, and microbial parameters were monitored monthly for one year to assess the water quality of Sinking Creek and display temporal and spatial variability. Fecal coliform data indicate that Sinking Creek is impaired, particularly at agricultural and urban land use sites. Linear regression analyses using *E. coli* and fecal coliform bacteria were performed to assess their usefulness as indicators of pathogen prevalence. Only regression analyses for fecal indicator organisms and protozoan pathogens were statistically significant, suggesting that the use of fecal indicators may overestimate the risk of pathogen exposure in Sinking Creek.

Acknowledgements

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CHAPTER 4

MULTIVARIATE STATISTICAL ANALYSES OF SINKING CREEK WATER QUALITY DATA TO IDENTIFY SOURCES OF FECAL POLLUTION IN RELATION TO LAND USE PATTERN

K.K. Hall and P.R. Scheuerman

Abstract

In the United States the increased listing of surface waters on impaired waters (303d) lists for pathogen impairment and the requirement to address these through the Total Maximum Daily Load (TMDL) process has resulted in increased need to develop methods that effectively and universally identify sources of fecal pollution. Pathogen TMDL development is currently based on a 30-day geometric mean, which does not take into consideration seasonal effects, variability in land use patterns, or the influence of runoff events on water quality. To account for these sources of variability, alternative water quality monitoring program design, methods, and data analysis may be necessary. This experiment used canonical correlation and canonical discriminant analyses to identify nonpoint sources of impairment in Sinking Creek. Results of these multivariate statistical analyses demonstrate that Sinking Creek is impacted by multiple nonpoint sources of impairment and souces of impairment are related to land use patterns.

Introduction

Rapid growth and urbanization in many previously rural and agricultural regions is a significant factor influencing deterioration of surface water quality. The addition of surface water bodies to impaired waters (303d) lists for pathogen impairment and the

need to address these through the Total Maximum Daily Load (TMDL) process has resulted in increased research to find methods that effectively and universally identify fecal pollution sources. A fundamental requirement to identify such methods is understanding the microbial and chemical processes that influence fate and transport of fecal indicators from various sources to receiving streams. Variability in land use patterns, the types and nature of pollutants, climatic conditions, and watershed characteristics add to the difficulty of modeling fate and transport of fecal pollution. In addition, the interactions between chemical and microbial processes in the water further add to the complexity of understanding pathogen loading and transport in the watershed.

In addition to the use of fecal indicator bacteria to predict pathogen prevalence, molecular methods such as ribotyping and pulsed-field gel electrophoresis have been suggested to address source identification of fecal pollution. Ribotyping and pulsed-field gel electrophoresis allow for the discrimination between human and nonhuman sources of fecal pollution but rely on large geographically specific genetic databases to correctly classify sources (Tynkkynen et al. 1999; Carson et al. 2001). While the use of these molecular methods may help identify more pathogens, their application still doesn't make it feasible to monitor for all pathogens. Non-molecular methods including antibiotic resistance analysis also allow for the classification of fecal pollution sources based on antibiotic resistance of bacteria from human and animal sources. As with ribotyping and pulsed-field gel electrophoresis, antibiotic resistance analysis requires a large database that may be geographically specific (Wiggins et al. 1999). Monitoring for fecal pollution using optical brighteners and caffeine indicate human sources of pollution

but are sensitive to regional environmental conditions (Kramer et al. 1996; Buerge et al. 2003). Although these methods may be regionally successful at identifying sources of fecal pollution, they cannot be universally applied to all bodies of water to effectively identify and remediate fecal pollution to protect surface waters and public health.

Fecal pollution detection and source identification methods do not influence the correlations between indicators and pathogens, and they do not provide any additional information regarding fate and transport mechanisms of the fecal pollution from source to receiving waters. Reliance on these indicators alone is not sufficient to protect surface water resources and human health and may hinder TMDL development and remediation efforts to remove impaired waters from 303d lists. The United States Environmental Protection Agency (USEPA) recommends the use of a 30-day geometric mean of *E. coli* for the assessment of bacteriological water quality in recreational waters (USEPA, 1986). Several states, including Tennessee, rely on the 30-day geometric mean of fecal indicator bacteria to assess pathogen contamination and develop TMDLs that can prevent further pathogen pollution. However, the use of the 30-day geometric mean does not take into consideration seasonal effects, variability in land use patterns, or the influence of runoff events on water quality. TMDLs developed using this method do not provide sufficient data to identify the presence of pathogens or sources of fecal pollution based on a small sample size, and long-term monitoring may be necessary to fully assess the potential degree of pathogen contamination.

The shortcomings of conventional indicators and source identification methods of fecal pollution have spawned a need to identify and employ alternative methods of water quality monitoring program design, methods, and data analysis to better protect

human health. Examining the influence of physical, chemical, and microbial water quality parameters on the fate and transport of fecal pollution using multivariate statistical approaches can improve our understanding of these influences on water quality, help identify sources of fecal pollution, and aid in effective TMDL development. To examine these relationships, multivariate statistical methods can be applied to water quality data to quantify the influence of nonpoint sources of pollution and to model the fate and transport of microbial and chemical pollutants.

Multivariate statistical methods including principal component analyses (PCA) can be applied to water quality data to quantify the influence of nonpoint sources of pollution and to model the fate and transport of microbial and chemical pollutants. Several studies have applied these techniques to better understand the microbial, physical, and chemical factors that influence water quality (Christophersen and Hooper, 1992; Vega et al. 1998; Bernard et al. 2004). However, PCA is used as a data reduction technique and is often applied to small environmental data sets. Rather than reduce the data set to identify the common factors influencing water quality, canonical correlation analyses (CCA) can be applied to large complex environmental data sets. Based on the linear relationships within and between data sets determined by CCA, a measure of the strength of association between the data sets can be determined (Johnson and Wichern, 1992). The application of separate regression analyses for each criterion measure defeats the purpose of having multiple criterion measures and doesn't take into consideration interrelationship among the criterion variables.

Canonical Correlation Analysis

CCA is a multivariate statistical technique that can be used to better understand response measures that cannot be described using a single criterion. While multiple regression analysis involves finding a linear combination of predictor variables that best explain the variation in the criterion, canonical correlation analysis allows for the simultaneous analysis of several predictor and explanatory variables by determining the largest correlations within each data set and between the 2 data sets. Canonical correlation analysis first examines the linear combinations of the variables within the predictor and explanatory data sets (canonical variables) and then determines the largest correlation between the 2 data sets (canonical correlations). These calculated canonical correlations are a measure of the strength of association between the 2 data sets and help explain how chemical parameters influence fate and transport of fecal pollution (Hair et al. 1998).

The first step in canonical correlation analysis is the definition of variance-covariance matrices, where X' is the dimensional vector of predictor variables, Y' is the dimensional vector of the criterion measures, and μ_x and μ_y denote the respective mean vectors associated with the variables X and Y:

$$\Sigma_{xx} = E \{ (X - \mu_x) (X - \mu_x)' \} \quad \text{(Eq. 4.1)}$$

$$\Sigma_{yy} = E \{ (X - \mu_y) (X - \mu_y)' \} \quad \text{(Eq. 4.2)}$$

$$\Sigma_{xy} = E \{ (X - \mu_x) (X - \mu_y)' \} \quad \text{(Eq. 4.3)}$$

The objective of canonical correlation analysis is to find the linear combination of predictor variables that maximally correlates with the linear combination of explanatory

variables using the dimensional vectors determined from the variance-covariance matrices, denoted as:

$$X^* = a'x = a_1x_1 + a_2x_2 + \ldots + a_mx_m \quad \text{(Eq. 4.4)}$$

$$Y^* = b'y = b_1y_1 + b_2y_2 + \ldots + b_mx_m \quad \text{(Eq. 4.5)}$$

The correlation between X* and Y* is then determined by:

$$\rho(a, b) = (a'\Sigma_{xy}b) / \{(a'\Sigma_{xx}a)(b'\Sigma_{yy}b)\}^{1/2} \quad \text{(Eq. 4.6)}$$

where ρ represents the correlation coefficient. The correlation coefficient represents the maximum correlation between the canonical variates and the strength of the overall relationship between the predictor and explanatory data sets. The set of linear combinations that maximizes the correlation $\rho(a, b)$ is determined using the following equations where *I* is the identity matrix and λ is the largest eigenvalue of the product matrix:

$$(\Sigma_{xx}^{-1}\Sigma_{xy}\Sigma_{yy}^{-1}\Sigma_{yx} - \lambda I)\, a = 0 \quad \text{(Eq. 4.7)}$$

$$(\Sigma_{yy}^{-1}\Sigma_{yx}\Sigma_{xx}^{-1}\Sigma_{xy} - \lambda I)\, b = 0 \quad \text{(Eq. 4.8)}$$

The eigenvalue (squared canonical correlation coefficient) is an estimate of the amount of shared variance between the weighted canonical variates of the predictive and explanatory variables. The largest eigenvalue is the result of the nonzero eigenvector being multiplied by the matrix (*I*). The eigenvalue is determined for the 2 sets of eigenvectors ($\Sigma_{xx}^{-1}\Sigma_{xy}\Sigma_{yy}^{-1}\Sigma_{yx}$ and $\Sigma_{yy}^{-1}\Sigma_{yx}\Sigma_{xx}^{-1}\Sigma_{xy}$) and is used to scale the eigenvector. The eigenvectors associated with the eigenvalue will become the vector of coefficients for a and b. Thus:

$$a = (\Sigma_{xx}^{-1}\Sigma_{xy}b) / \sqrt{\lambda} \quad \text{(Eq. 4.9)}$$

$$b = (\Sigma_{yy}^{-1}\Sigma_{yx}a) / \sqrt{\lambda} \quad \text{(Eq. 4.10)}$$

Therefore, the canonical weights a_1 and b_1 are the corresponding nonzero eigenvectors associated with the largest eigenvalue (λ_1), and a_1x and b_1y are the first canonical variate pair. The process results in the successive extraction of canonical variates so the second pair is the second most highly correlated pair out of all possible linear combinations that are uncorrelated with the first canonical variate pair, resulting in the generation of pairs of canonical variates. Canonical loadings can also be used to interpret the overall canonical structure by assessing the contribution of each variable to the overall canonical structure. Canonical loadings measure the correlation between the original variables and the sets of canonical variates determined using equations 5.9 and 5.10. These loadings reflect the variance that the original variable shares with the canonical variate.

The application of canonical correlation analyses to water quality data to examine the influences and interactions between microbial, chemical, and physical water quality parameters has been used to identify pollution sources and coordinate remediation efforts (Gotz et al. 1998; Bonadonna et al. 2002; Zeng and Rasmussen, 2005). In this study, CCA can also be used to determine the relationship between chemical and microbial water quality parameters to assess their influence in the fate and transport of fecal indicator organisms and pathogens in Sinking Creek.

In addition to canonical correlation analysis, canonical discriminant analysis (CDA) can be used to better understand the factors that influence surface water quality

and their relationship to land use patterns. CDA can be used to reveal patterns of pollution types based on sources and land use patterns. This technique identifies the canonical variables that find the maximum amount of separation to discriminate between groups based on the strength of the linear associations (i.e., site, season). Each linear combination of variables is a canonical variable. In this case, the variables are measured water quality parameters and the groups are land use patterns. A plot of the first 2 canonical variables will display the degree of discrimination between each group. By applying CDA to water quality data, it may be possible to identify common pollution sources based on the key discriminatory variables and associate them with specific land use patterns along Sinking Creek.

Physical, chemical, and microbial water quality data were collected from Sinking Creek to examine the usefulness of this methodology and identify nonpoint sources of pollution. In a previous study using regression analyses conducted on data collected from Sinking Creek, we demonstrated that chemical parameters (nitrates, phosphates, biochemical oxygen demand) did not individually correlate with fecal coliform concentrations (Hall et al. 2006). This lack of correlation suggests either no interaction or more complex interactions between water chemistry and pathogen fate and transport. If interaction is more complex then multivariate statistical techniques may be a better tool to understand the complex interactions and effectively identify the parameters that most influence watershed dynamics.

Using a targeted sampling program and statistical modeling to identify pollution sources is potentially a cost-effective method for water quality monitoring and assessment (Johnson and Wichern, 1992). While the statistical methodology is useful

to identify pollution sources and can be applied to other large environmental data sets, the developed models may be specific to the individual water bodies or watersheds for which they are developed and may under-represent true watershed dynamics (Callies, 2005). However, we suggest that this data analysis approach can be successfully applied to other watersheds to better understand the influence of seasonal effects, variability in land use patterns, and runoff events on water quality. The objective of this group of experiments was to better understand the factors influencing the fate and transport of fecal pollution and identify nonpoint sources of fecal pollution as they relate to land use patterns in Sinking Creek using multivariate statistical analyses.

Materials and Methods

Sinking Creek Location and Water Quality Monitoring

The Sinking Creek sub-watershed (06010103130) is one of 13 sub-watersheds that belong to the Watauga River watershed (TDEC, 2000a). Sinking Creek is a 9.8 mile long tributary of the Watauga River partially located in Washington and Carter Counties in Tennessee. The headwaters of Sinking Creek are located on Buffalo Mountain and it enters the Watauga River at mile 19.9. The main land uses within the 13.1 square mile drainage basin of the Sinking Creek watershed include: forest (65.5%), urban (25.3%), and agricultural areas (9.0%) (TDEC 2000b). There are 19.8 impaired stream miles in the Sinking Creek watershed including tributaries (TDEC, 2000b).

Upstream locations on Buffalo Mountain are forested, and land use transitions to urban, followed by agricultural land use at downstream sites. Fourteen sites were initially selected for routine water quality monitoring in 2002 and are described in Table

4.1 and Figure 4.1. From these 14 sampling locations, 2 sites were randomly selected from each land use classification and sampled monthly for the physical, chemical, and microbial parameters described in Table 4.2. The sites selected for representation of agricultural land use were sites 2 and 4, sites selected to represent urban land use were sites 7 and 10, and sites 13 and 14 represented forested land use.

Table 4.1. Sampling locations on Sinking Creek sampled during this study

Site Number	Site Location	Predominant Land Use	Physical Description	Habitat Assessment Score (%)	Latitude/Longitude Coordinates and Elevation
2	Upstream of Bob Peoples bridge on Sinking Creek Road	Agriculture	Moderately eroded banks with little vegetation buffer or riparian zone. Creek bed predominantly cobble and gravel	52%	19.837' N, 18.254' W 1530 ft
4	Upstream of crossing on Joe Carr Road	Agriculture	Moderately eroded banks with poor bank stability and little vegetative buffer or riparian zone. Creek bed predominantly boulders, cobble and gravel	43%	19.594' N, 18.579' W 1552 ft
7	Upstream of bridge on Miami Drive, King Springs Baptist Church	Urban	Heavily eroded left bank, concrete bank on right with no vegetative buffer or riparian zone. Creek bed predominantly cobble	53%	18.772' N, 19.685' W 1583 ft
10	Upstream of bridge crossing Sinking Creek at Hickory Springs Road	Urban	Heavily eroded banks with no vegetative buffer. Creek bed predominantly boulders and cobble	57%	17.431' N, 21.397' W 1720 ft
13	Upstream of road crossing on Jim McNeese Road	Forest	No visible bank erosion with moderate riparian zone. Creek bed predominantly boulders and cobble	71%	16.035' N, 22.163' W 2048 ft
14	Downstream of path crossing at Dry Springs Road	Forest	No visible bank erosion with optimal riparian zone and vegetative buffer. Creek bed predominantly boulders, cobble and gravel	83%	14.800' N, 22.033' W 2148 ft

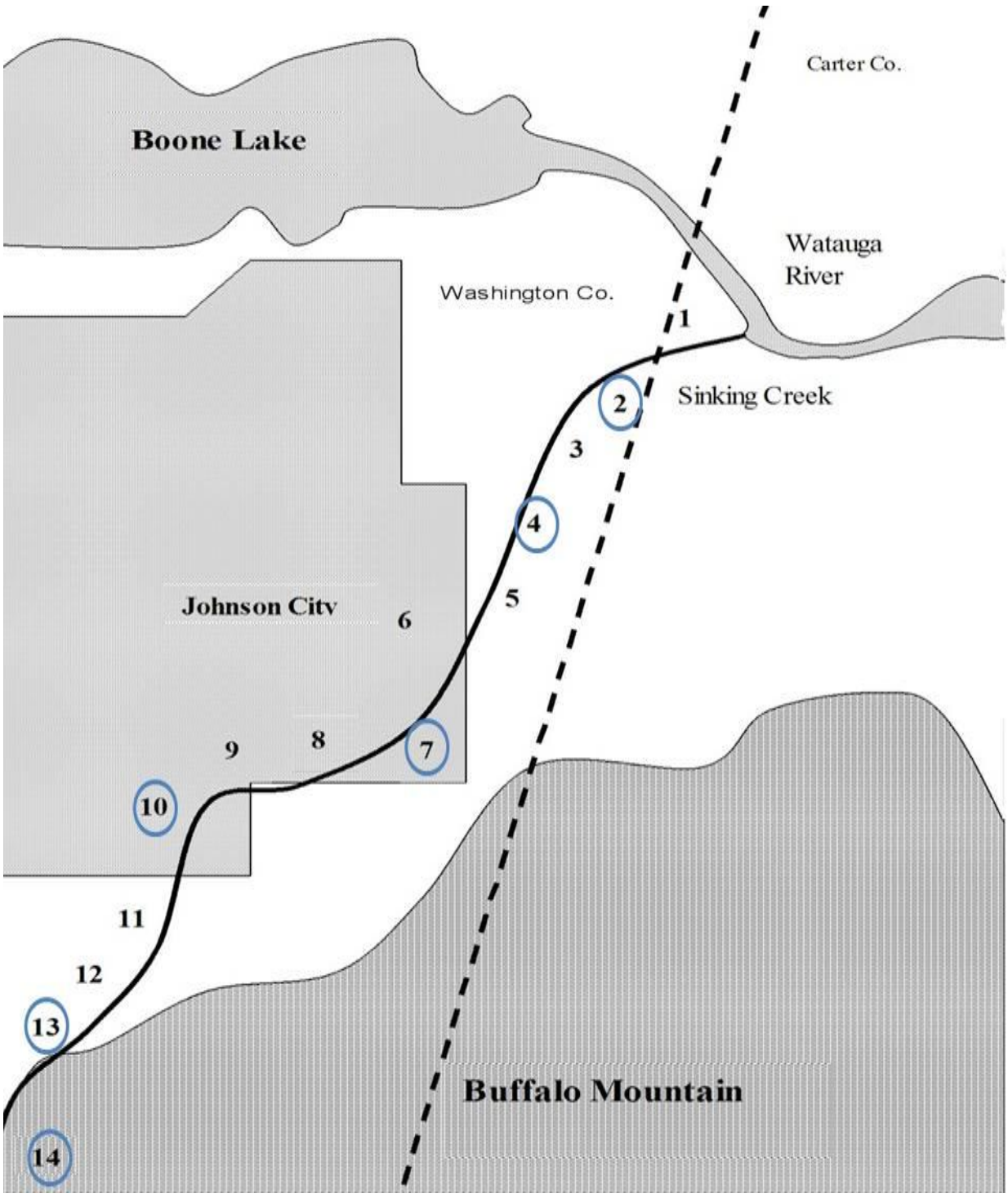

Figure 4.1. Map of Sinking Creek sampling locations (sites sampled in this study are circled).

Table 4.2. Physical, chemical, and microbial water quality parameters measured

Parameter	Abbreviation	Units	Holding Time
pH	pH	pH	Field measurement
Water temperature	WT	^{o}C	Field measurement
Air temperature	AT	^{o}C	Field measurement
Dissolved oxygen	DO	mg/l as O_2	Field measurement
Conductivity	Cond	µmohs	Field measurement
Fecal coliform in water	FCW	CFU/100ml	6h
Total coliform in water	TCW	CFU/100ml	6h
Fecal coliform in sediment	FCS	CFU/100ml	6h
Total coliform in sediment	TCS	CFU/100ml	6h
Colilert	Colilert	CFU/100ml	6h
Standard plate count	SPC	CFU/ml	6h
Acridine orange direct counts	AODC	cells/g sediment	6h
Acid phosphatase	AcidP	µg/g sediment	24h
Alkaline phosphatase	AlkP	µg/g sediment	24h
Dehydrogenase	DHA	µg/g sediment	24h
Galactosidase	Gal	µg/g sediment	24h
Glucosidase	Glu	µg/g sediment	24h
Nitrates	NO_3	mg/l	24h
Phosphates	PO_4^{2-}	mg/l	24h
Ammonia	NH_3^{+}	mg/l	24h
Biochemical oxygen demand	BOD_5	mg/l	24h
Hardness	Hard	mg/l	48h
Alkalinity	Alk	mg/l	24h
E. coli O157:H7	O157:H7	CFU/100ml	24h
Shigella sp.	*Shigella*	CFU/100ml	24h
Giardia sp.	*Giardia*	Cysts/l	72h
Cryptosporidium sp.	*Crypto*	Oocysts/l	72h
F+ - specific bacteriophage	bacteriophage	PFU/ml	48h

Sample Collection

Water samples were collected monthly from 6 pre-selected sites on Sinking Creek from January 2011 through December 2011 and were analyzed for the variables described in Table 4.2. Water samples for total and fecal coliform bacteria (TC/FC), standard plate counts (SPC), *E. coli* 057:H7, *Shigella sp.*, and bacteriophage analyses were collected and analyzed in triplicate (SPC samples analyzed in duplicate) in sterile,

1-L Nalgene™ bottles. Water samples for Colilert® analyses were collected in sterile 100ml plastic bottles (IDEXX Laboratories, Westbrook, Maine). Water samples for nitrates (NO_3^-), phosphates (PO_4^-), ammonia (NH_3^+), 5-day biochemical oxygen demand (BOD_5), alkalinity, and hardness were collected and analyzed in triplicate in sterile 2-L Nalgene™ bottles. Sediment samples for TC/FC in water, microbial enzyme activity (MEA), and acridine orange direct counts (AODC) were collected in 2oz sterile Whirl-Pak™ bags. All samples were transported to the laboratory on ice and analyzed within the holding times described in Table 3. Field measurements for pH, air and water temperature, dissolved oxygen, and conductivity were also collected at each site.

Quality assurance and quality control (QA/QC) practices included the analysis of chemical parameters consisted of one trip blank, one field blank, a negative control, one replicate, one spiked sample, and one quality control standard. QA/QC practices included in the analysis of microbial parameters included the analysis of one trip blank, one field blank, a negative control, and a positive control. A secondary wastewater effluent sample was used as the positive control for TC/FC, Colilert®, SPC, and bacteriophage analyses. Laboratory strains of *E. coli* O157:H7 and *Shigella flexneri* (ATCC® Number 43895™ and ATCC® 12022™, respectively) were used to seed water samples that served as a positive control for PCR analysis.

Microbial Analyses

TC/FC analyses for water samples were conducted according to Standard Methods for Examination of Water and Wastewater (APHA, 1992). Briefly, 0.5ml of water were filtered through a 0.45µm membrane filter (EMD Millipore, Billerica, MA) and

the filter placed in a petri dish containing an absorbent pad (EMD Millipore, Billerica, MA) with 2ml of m-Endo media for total coliform analysis or m-FC media for fecal coliform analysis. All plates were inverted and enumerated following 24h incubation at 37^{o}C and 44.5^{o}C for total coliform and fecal coliforms, respectively. For TC/FC sediment analyses, 0.5g of sediment was added to 25ml of sterile water + 1% Tween 80. The samples were vortexed and allowed to settle for 30 minutes, and 0.5ml of the buffer suspension was filtered according to Standard Methods for Examination of Water and Wastewater as described above (APHA, 1992).

SPC were conducted according to Standard Methods for Examination of Water and Wastewater (APHA, 1992) using R2A agar. One milliliter of water was placed in the center of a sterile petri dish (Fisher Scientific, Pittsburgh, PA) and 10ml of R2A agar was added to the dish. The plate was swirled in a figure eight motion to allow the sample to disperse in the media and cover the plate. Plates were allowed to solidify and were enumerated following incubation at 25^{o}C for 48h. *Escherichia coli* concentrations were determined using the Colilert® Quanti-Tray method (APHA, 1995). To each 100ml water sample, a packet of Defined Substrate Technology® (DST®) reagent (IDEXX Laboratories, Westbrook, Maine) was added and mixed. The sample was then poured into a Quanti-Tray®, sealed using the Quanti-Tray® sealer, and incubated for 24h at 37^{o}C. *E. coli* were then enumerated using the Standard Method most probable number (MPN) procedure. Samples for water TC/FC were processed in triplicate and samples for sediment TC/FC were processed in duplicate. SPC were processed in duplicate and one Colilert® sample was processed for each site.

MEA analyses were conducted and included acid and alkaline phosphatases, glucosidase, galactosidase, and dehydrogenase activities. For each enzyme analyzed, 1g of sediment was added to a test tube containing a specific buffer and enzyme. Sediment samples for acid phosphatase were mixed with 4ml of 1M TRIS buffer (pH 4.8) and 4ml of 1M TRIS buffer (pH) 8.4 for alkaline phosphatase. For both acid and alkaline phosphatase, 1ml of 1M TRIS buffer with 0.1% phosphatase substrate (pH 7.6) was added to each tube (Sayler et al. 1979). Sediment samples for galactosidase and glucosidase activities were mixed with 4ml of 0.1M phosphate buffer (pH 9.0). Galactosidase activity was measured by adding 1ml of 0.01M phosphate buffer with 0.15% p-nitrophenyl-β-D-galactopyranoside as an indicator of galactosidase activity. One milliliter of 0.01M phosphate buffer with 0.15% 4-nitrophenyl-β-D-glucopyranoside was used as an indicator to assess glucosidase activities (Morrison et al. 1977). Following addition of buffers and indicators, all tubes were vortexted and incubated at 25^{o}C for 24h. Acid and alkaline phosphatase, galactosidase, and glucosidase activities were determined using a spectrophotometer at an absorbance of 418nm.

For dehydrogenase (DHA) activity, 1g of sediment was added to a test tube containing 2ml of 0.1M phosphate buffer (pH 7.6) and 1ml of 0.5% iodonitrotetrazolium chloride (INT) salt solution. The samples were vortexed and incubated in the dark at 25^{o}C for 45 minutes. One milliliter of the sample was filtered through a 0.22µm porosity cellulose membrane (GE Water and Process Technologies, Trevose, PA) and allowed to dry at room temperature. The membrane, was then added to a test tube containing 5ml of dimethyl sulfoxide, vortexted to dissolve the membrane, and incubated in the

dark at 25°C for 24h. Dehydrogenase activity was then determined using a spectrophotometer at an absorbance of 460nm.

AODC analysis was performed as described by Ghiorse and Balkwill (1983). Three hundred milligrams of sediment was added to 30ml of sterile PBS+Tween 80, vortexed for 60s, and allowed to settle for 3h. Two hundred fifty microliters of the suspension was mixed with 5ml sterile water + 500µl acridine orange stain, and samples were vortexed for 30s. Samples were filtered using 25mm, 0.2µm pore polycarbonate nucleopore filters (Osmonics, Inc., Minnetonka, MN), and the filters were mounted and fixed on slides for enumeration at 1000X using the Olympus BH2 epifluorescent microscope (Olympus, New Hyde Park, NY). One sediment sample was processed per site and 3 microscopic fields were enumerated on each slide.

Chemical Analyses

NO_3^-, PO_4^-, NH_3^+, alkalinity, and hardness analyses were performed in triplicate using colorimetric HACH™ methods and HACH™ reagents as described by the manufacturer (HACH Company, Loveland, CO). Briefly, NO_3^-, PO_4^-, NH_3^+ analyses were conducted by adding 10ml of water to a vial containing the appropriate reagent packet; NitraVer5, PhosVer3 and salicylate/ammonia cyanurate reagents, respectively. The vials were shaken to dissolve the reagent and samples were analyzed using pocket colorimeters specific to the nutrient of interest. Alkalinity and hardness analyses were conducted using 100ml sample volumes and a digital titrator. For alkalinity determination, 1 packet of phenolthalein indicator and bromcresol green-methyl red indicator were added to the sample and mixed. The sample was then titrated with 1.6N

sulfuric acid to a grey-green endpoint. For hardness determination, 1 packet of ManVer2 reagent and 2ml of hardness buffer (pH 10) were added to the 100ml sample and mixed. The sample was then titrated with 0.8N Ethylenediaminetetraacetic acid (EDTA) to a blue endpoint. BOD_5 analyses were conducted according to Standard Methods for Examination of Water and Wastewater (APHA, 1992). Wheaton BOD bottles (Wheaton Science Products, Millville, NJ) were completely filled with sample water and capped with glass stoppers to ensure no air bubbles were present. Initial (Day 0) and final (Day 5) dissolved oxygen concentrations were measured using the YSI Model 5000 dissolved oxygen meter (YSI Inc., Yellow Springs, OH).

Pathogenic Bacteria Analyses

Samples for *E. coli* O157:H7 and *Shigella sp.* were analyzed in triplicate. The method used for the filtration and isolation of the bacteria is described by Bej et al. (1991). One hundred milliliters of water was collected and filtered through a 0.22µm membrane filter. The filter was then washed with 10ml of a 1% Tween 80 solution and centrifuged for 10 minutes to create a cell pellet. The supernatant was removed and the cell pellet was washed twice with 10ml phosphate buffered saline. Fifty microliters of diethylpyrocarbonate solution was added to the final cell pellet and subjected to 6 freeze-thaw cycles at -20^{o}C and 100^{o}C, respectively.

PCR amplification for *E. coli* O157:H7 was performed as described by Kimura et al. (2000) using primers EC-1 (GGCAGCCAGCATTTTTTA) and EC-2 (CACCCAACAGAGAAGCCA) for the *chuA* gene. The final 50µl PCR mixture contained 2.5X PCR buffer (mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl), 0.8 mM of each

deoxynucleoside triphosphate (dATP, dCTP, dGTP, and dTTP), 4 µM concentrations of each primer, 5 U *Taq* DNA polymerase (Fisher Scientific, Pittsburg, PA) and 5µl of the resuspended cell pellet. The PCR mixture was subjected to an initial denaturation step at 95^{o}C for 5 minutes, followed by 35 cycles of 1 minute denaturation at 94°C, 2 minutes of annealing at 42°C, and 5 minutes of primer extension at 72°C. A final extension step was performed at 72^{o}C for 10 minutes using a BioRad Thermocycler PCR Machine (BioRad, Hurcules, CA). PCR products were resolved on a 2% agarose gel for 1.5h at 80V and subjected to ethidium bromide staining to visualize DNA base pair bands. The presence of a 901 base pair band indicated a sample positive for *E. coli* O157:H7.

PCR amplification for *Shigella sp.* was performed as described by Theron et al. (2001). Thirty cycles of a seminested PCR reaction were performed using primers H8 (GTTCCTTGACCGCCTTTCCGATAC) and H15 (GCCGGTCAGCCACCCTC) for the *ipaH* gene (Islam, et al. 1993a) in the first round of PCR. The 50µl reaction volume contained 1X PCR buffer (mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl), 0.1mM of each deoxynucleoside triphosphate (dATP, dCTP, dGTP, and dTTP), 24pmol of H8 primer, 34pmol of H15 primer, 1U *Taq* DNA polymerase (Fisher Scientific, Pittsburg, PA), and 10µl of resuspended cell pellet. The PCR mixture was subjected to an initial denaturation step at 94^{o}C for 3 minutes, followed by 10 cycles of 1 minute denaturation at 94°C, 1 minute of annealing at 60°C, and 1 minute of primer extension at 72°C. One microliter of PCR product from the first PCR round was added to a reaction tube containing the reagents described above, with the addition of 31pmol of H10 primer (CATTTCCTTCACGGCAGTGGA) described by Hartman et al. (1990). An initial denaturation step was performed at 94^{o}C for 3 minutes, followed by 20 cycles of 1

minute denaturation at 94°C, 1 minute of annealing at 60°C, and 1 minute of primer extension at 72°C. A final extension step was performed at 72°C for 7 minutes using a BioRad Thermocycler PCR Machine (BioRad, Hurcules, CA). PCR products were resolved on a 2% agarose gel for 1.5h at 80V and subjected to ethidium bromide staining to visualize DNA base pair bands. The presence of both a 401 and 620 base pair band indicated a sample positive for *Shigella sp*.

Protozoan Analysis

One water sample was collected and analyzed monthly at each site for the analysis of *Giardia* and *Cryptosporidium*. Samples were collected as described by USEPA method 1623 for water filtration (USEPA, 2005). One hundred liters of water were filtered at each site though Envirochek™ sampling filters (Pall Corporation, Ann Arbor, MI) using a gas powered water pump and Badger™ flow meter at a rate of 2.5L/minute. The filtration apparatus was assembled as shown in Figure 4.2.

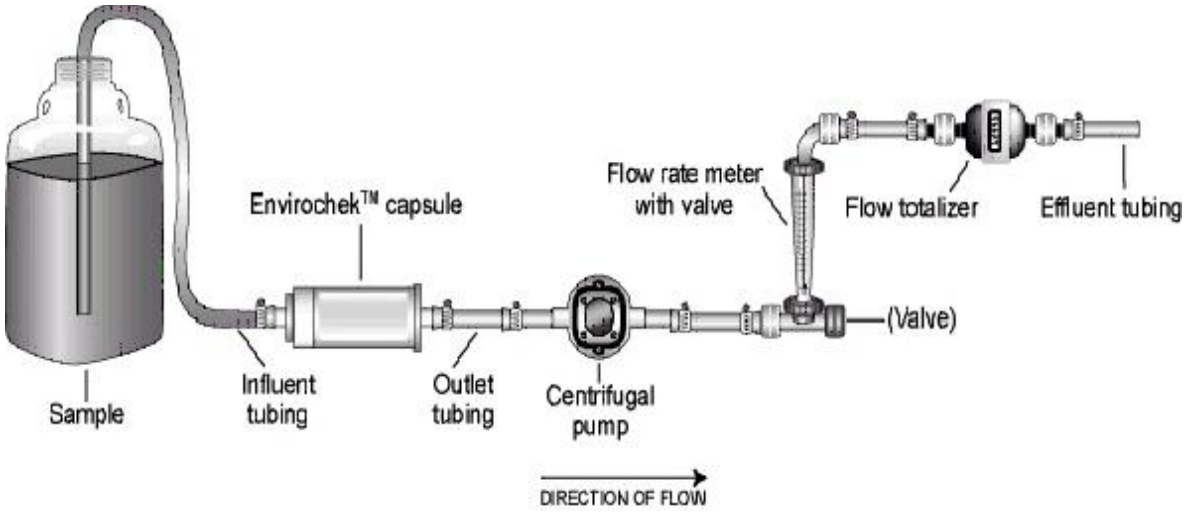

Figure 4.2. Filtration apparatus used to sample *Giardia* and *Cryptosporidium* in laboratory seeded samples (USEPA, 2005)

The filters were transported to the lab on ice and analyzed within 72h of collection. Filters were initially washed by adding 120ml of elution buffer to the filter capsule and placing on a wrist action shaker for 30 minutes. The elution buffer was removed and the filter capsule broken open and the filter cut out using a sterile razor blade and hand washed using 120ml of elution buffer. The buffer was then added to a sterile 250ml centrifuge tube containing the elution buffer from the initial wash on the wrist action shaker. The samples were centrifuged at 2,300 x g for 30min and the supernatant removed. The concentrated pellet collected was subjected to an immunofluorescent assay using the Waterborne Aqua-Glo™ G/C Direct FL antibody stain (Waterborne, Inc. New Orleans, LA) as described by the manufacturer. The prepared slides were examined at 200X using the Olympus BH2 epifluorescent microscope (Olympus, New Hyde Park, NY).

Bacteriophage Analyses

Samples for bacteriophage analysis were collected and analyzed in triplicate using the double-layer agar procedure described in USEPA method 1601 (USEPA, 2001a) using *E. coli* C3000 as the host strain (ATCC® Number 15597™). The host strain was cultured using ATCC 271 broth (10g/L tryptone, 1g/L yeast extract, 8g NaCl, 10ml/L of 10% glucose solution, 2ml/L of 1M $CaCl_2$, 1ml/L of 10mg/ml thiamine) at 37^{o}C. An overnight culture of the host strain was prepared the day before analysis by inoculating a 30ml ATCC broth culture with the host strain. On the day of analysis, 100µl of the prepared overnight culture of the host strain was inoculated into a 30ml of fresh ATCC 271 broth and incubated at 37^{o}C until log phase was reached (~4h).

Ten milliliters of collected sample water were filtered through a syringe filter fitted with a 0.22µm membrane filter to remove bacteria and the filtrate was serially diluted in phosphate buffered water for analysis. Five hundred microliters of each dilution were added to a test tube containing 5ml of 0.7% ATCC® 271 agar (ATCC® 271 broth with 1.4g/L agar) and 100µl of host bacteria. The tubes were gently mixed and poured onto a plate containing 1.5% ATCC 271 agar (ATCC® 271 broth with 18g/L agar). Plates were allowed to solidify prior to incubation at 37^{o}C for 24h and plaques were enumerated.

Statistical Analysis

Canonical correlation analysis was conducted to describe the relationship between the microbial and chemical data sets using SAS/STAT statistical software

(SAS Institute, Cary, NC). In this study the canonical correlations are a measure of the strength of association between the chemical and microbial data sets and help explain how chemical parameters influence microbial fate and transport and how these interactions influence fecal coliform loading in the creek (Johnson and Wichern, 1992). Only canonical coefficients greater than 0.30 were considered to be important, as this is the value at which about 10% of the variance is explained by a given canonical coefficient (Hair et al. 1998).

Data were initially analyzed using the CANCORR procedure for the entire creek in an effort to determine the extent and types of pollution impacting Sinking Creek. The variables in the microbial data set for this CANCORR analysis included total and fecal coliform counts for water and sediment, Colilert, standard plate counts, acridine orange direct counts, acid phosphatase, alkaline phosphatase, galactosidase, glucosidase, *E. coli* O157:H7, *Shigella sp., Giardia sp., Cryptosporidium sp.,* and bacteriophages. The variables in the chemical data set included total nitrates, total phosphates, ammonia, biochemical oxygen demand, alkalinity, and hardness. Additional CANCORR procedures were also conducted by the season and land use types in an attempt to identify common patterns associating spatial and temporal variability to sources of fecal pollution. The variables included in the chemical data set remained the same. Variables in the microbial data set included those mentioned above but without *E. coli* O157:H7, *Shigella sp., Giardia sp., Cryptosporidium sp.,* and bacteriophages. Only the protozoans and bacteriophages were detected in the collected samples and their infrequent detection did not significantly correlate with fecal coliform bacteria or *E. coli* concentrations. The observed lack of correlation between the pathogens and

indicators lead to their exclusion from the canonical correlation analysis, as they did not allow for the detection of significant correlations at the season, site, or land use level.

Discriminant analysis was performed using the CANDISC procedure to identify the canonical variables that allow for the maximum amount of separation to discriminate between groups based on the strength of the linear associations. CANDISC procedures the chemical and microbial parameters described in Table 4.2 and were performed at the season and land use levels.

Results and Discussion

Canonical Correlation Analysis Interpretation

The first canonical correlation analysis was performed at the creek level (Figure 4.3) and the process for interpretation of the canonical correlation analysis is discussed using this analysis.

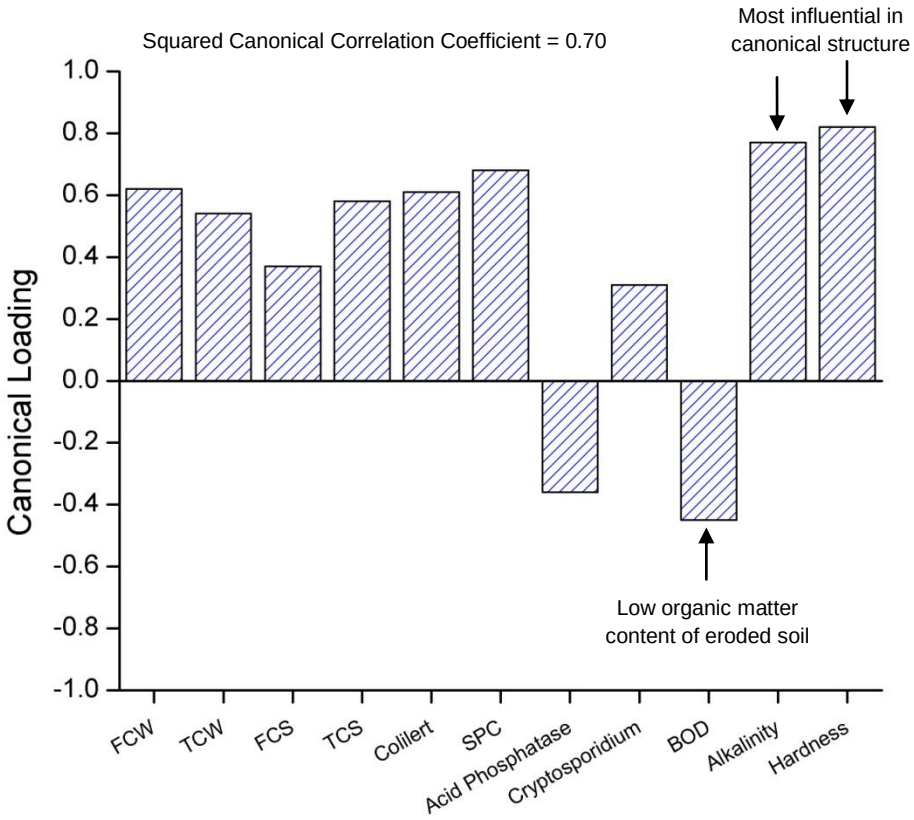

Figure 4.3. Sinking Creek canonical loadings relating chemical water quality parameters to microbial water quality parameters to identify sources of fecal pollution

The initial calculations determine the canonical correlation based on the variance-covariance matrices calculated using equations 4.1 – 4.3. The adjusted canonical correlation, approximate standard error, and the squared canonical correlations for each pair of canonical variables are also determined. The first canonical correlation determined using equations 4.4 and 4.5 is 0.78, which represents the highest correlation between any linear combination of microbial variables and any linear combination of chemical variables. The likelihood ratio and associated statistics are also provided for testing the hypothesis that the canonical correlations are zero. The first p-value is small (0.003), forcing the rejection of the null hypothesis at the 0.05 level.

The null hypothesis states that there is no correlation between the data sets and its rejection is confirmed by four separate multivariate statistics and F approximations for the null hypothesis. Because the first set canonical variables are significant, only they need to be identified.

The raw canonical coefficients for the microbial and chemical variables are determined using equation 5.6 and are then standardized to account for the absence of equal variances. The standardized canonical coefficients show that the first pair of canonical variates in the microbial data set (determined using equations 5.7 – 5.10) are the weighted sum of the variables for sediment total coliforms (0.34), standard plate counts (0.48), glucosidase activity, *Giardia* (-0.32), and *Cryptosporidium* (0.43). The standardized canonical coefficients show that the first pair of canonical variables in the chemical data set are the weighted sum of the variable for nitrates (-0.38), biochemical oxygen demand (-0.41), alkalinity (-1.01), and hardness (1.96).

The standardized canonical coefficients are then used to determine the correlation between the canonical variables and the original variables. These values are referred to as canonical loadings and are useful to assess the contribution of that variable to the overall canonical function but do not indicate how the original variables contribute jointly to the canonical analysis. In our example, the canonical loadings of the microbial canonical variables show that total and fecal coliforms in water and sediment, *E. coli*, standard plate counts, acid phosphatase activity, and *Cryptosporidium* are significant, with standard plate counts and fecal coliforms in water being the most influential (0.68 and 0.62, respectively). The first pair of chemical canonical variables show that BOD_5, alkalinity, and hardness are significant, with alkalinity and hardness

being the most influential (0.77 and 0.82, respectively). The significance of the chemical and microbial variables indicates that these data sets are related. The extent of these relationships can be used to help identify the source(s) of fecal pollution.

Alkalinity and hardness are the chemical variables most contributing to the canonical structure. The ions that contribute to alkalinity and hardness concentrations in water may be introduced by the erosion of soil and geologic formations such as shale, sandstone, siltstone, and limestone. These metasedimentary rock formations are common in Northeast Tennessee, which is characterized by karst topography (NRCS, 2010a – c) and are likely contributing to the observed alkalinity and hardness concentrations. The organic matter fraction of the eroded soil is likely contributing to the observed biochemical oxygen demand and is influencing the correlation between this chemical parameter and the microbial parameters. The correlation of alkalinity, hardness, and BOD_5 with the microbial variables suggests that surface runoff containing eroded soil is a contributing factor to fecal pollution and heterotrophic activity in Sinking Creek.

Canonical Correlation Analysis by Season

The factors influencing the fate and transport of fecal indicator organisms demonstrated temporal variation. Canonical correlation analysis for the winter months indicate that fecal coliform bacteria are introduced by a combination of organic matter and soil erosion (Figure 4.4) based on the significant canonical loadings for phosphates, ammonia, BOD_5, alkalinity, hardness, fecal coliform, and heterotrophic bacteria concentrations in water.

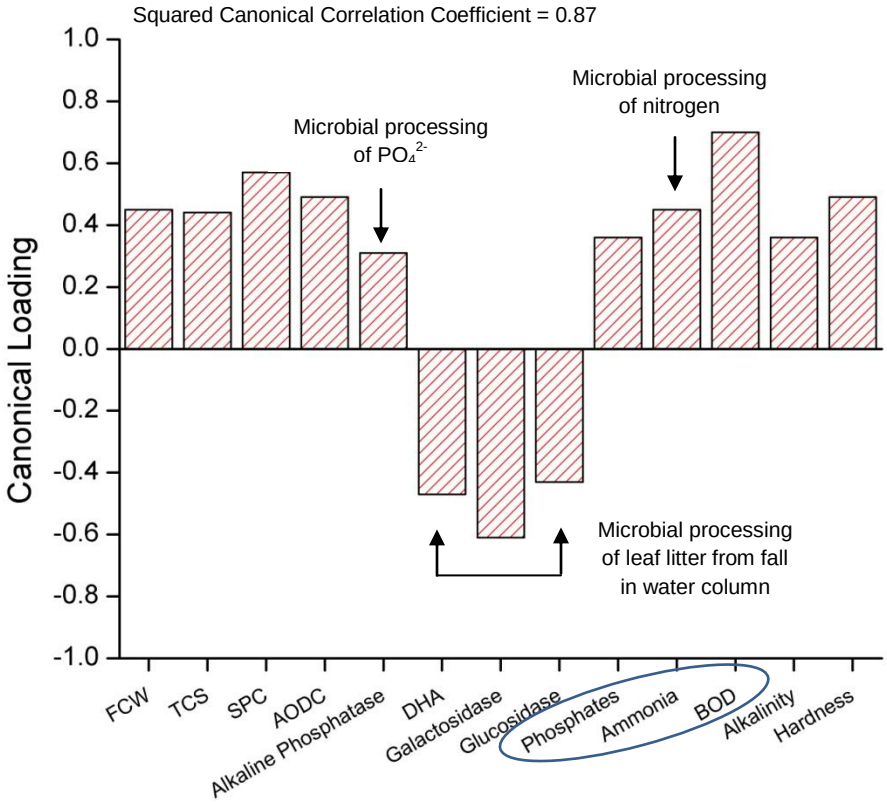

Figure 4.4. Sinking Creek canonical loadings observed during the winter months to relate chemical water quality parameters to microbial water quality parameters to identify sources of fecal pollution

The significant canonical loadings for alkalinity and hardness are likely the result of eroded soil containing fecal coliforms as described above. The high canonical loading for BOD_5 suggests the influence of microbial activity on organic matter introduced with soil erosion. Ammonia concentrations may be the result of transformation of the nitrogen component of the organic matter, resulting in its conversion to inorganic nitrogen through ammonification. It may also be possible that the significance of BOD_5, ammonia, and phosphates are the result of organic matter introduced from wastewater. Brasfield (1972) has demonstrated that phosphate

concentrations in surface water positively correlate with total and fecal coliforms and may indicate the input of sewage into the receiving surface water body. It is likely that the addition of leaf litter during the fall account for the observed microbial activities in water and sediment, as the MEA variables also contribute to the overall canonical structure. Alkaline phosphatase activity is positively correlated with the chemical parameters, suggesting the ability of the sediment microbial community to process the added phosphates. The negative correlations of dehydrogenase, galactosidase, and glucosidase with the chemical parameters indicate that processing of the carbon content of the organic matter is being carried out by heterotrophic communities suspended in the water.

Canonical correlation analysis for the spring months demonstrate that fecal pollution is greatly associated with alkalinity, hardness, nitrates, and BOD_5 (Figure 4.5). The significant loadings for these variables suggest that fecal pollution in water and sediment is the result of nutrient additions associated with the introduction of eroded soil thorough runoff events. In addition to the introduction of fecal coliforms in water through runoff events, the cations contributing to alkalinity and hardness concentrations may be influencing total and fecal coliform concentrations in sediment. As cation concentrations increase in surface water, they may adhere to the bacteria and organic matter, flocculate and settle out of the water column (Ayoub et al. 1999). This occurrence would likely account for the inverse relationship between fecal coliforms and BOD_5, as alkalinity and hardness concentrations resulted in flocculation of bacteria and organic matter associated with the eroded soil, resulting in their partitioning into the sediment.

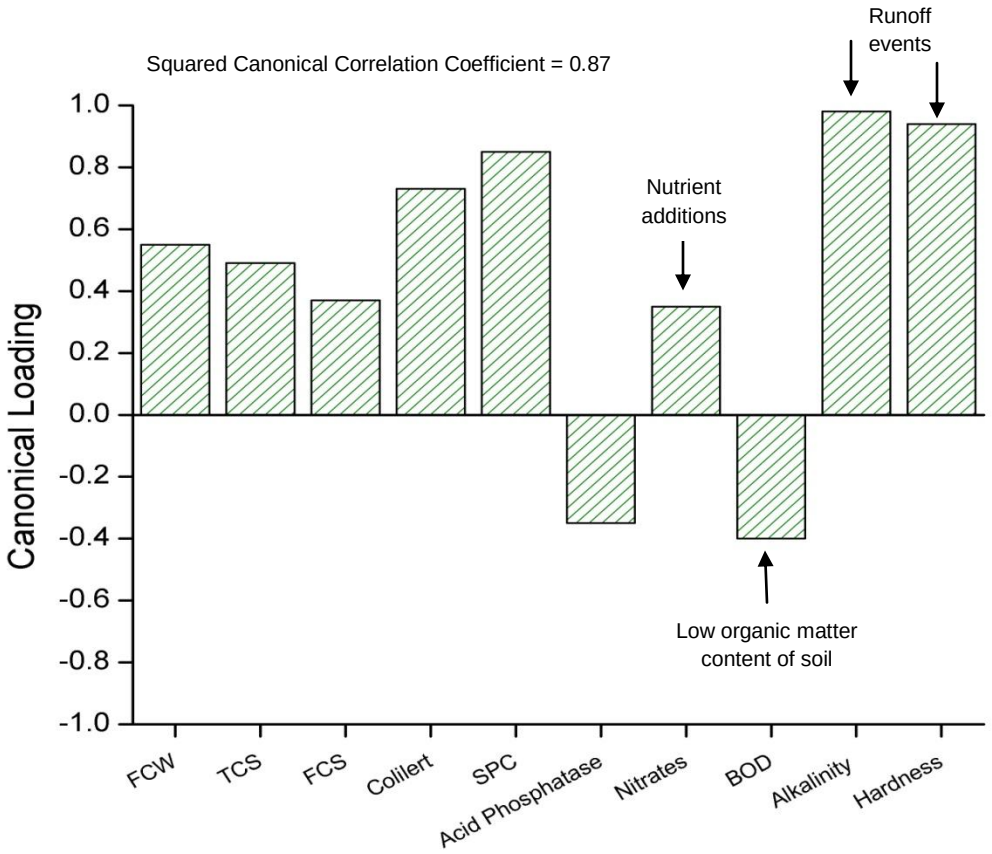

Figure 4.5. Sinking Creek canonical loadings observed during the spring months to relate chemical water quality parameters to microbial water quality parameters to identify sources of fecal pollution

The canonical structure for the summer months is similar to that of the spring months in that it suggests the influence of soil erosion and organic matter inputs on fecal pollution (Figure 4.6). In the first canonical structure, alkalinity and hardness are major contributors to the overall canonical structure, and total and fecal coliforms are likely introduced through runoff containing eroded soil. As described above, the introduced cations can adhere to bacteria and organic matter, causing flocculation and partitioning into the sediment. The influences of sediment partioning are evidenced by the second canonical structure. In addition to organic matter processing in the water

column, organic matter is also being processed by sediment heterotrophic communities as evidenced by the significant canonical loadings for acid and alkaline phosphatases, galactosidase, and glucosidase. The significant loading for nitrates in the second canonical structure demonstrates a relationship between the 2 canonical structures. In the presence of organic matter, microbial populations can reduce nitrates resulting in increasing cation concentrations, which may result in bacterial settling into sediment (Ayoub et al. 1999), as is suggested by the first canonical structure. This relationship is further supported by the negative correlation of phosphates in the first canonical structure and the influence of BOD_5 in the second canonical structure, which both suggest that fecal pollution is associated with a combination of organic matter and soil erosion containing nutrients.

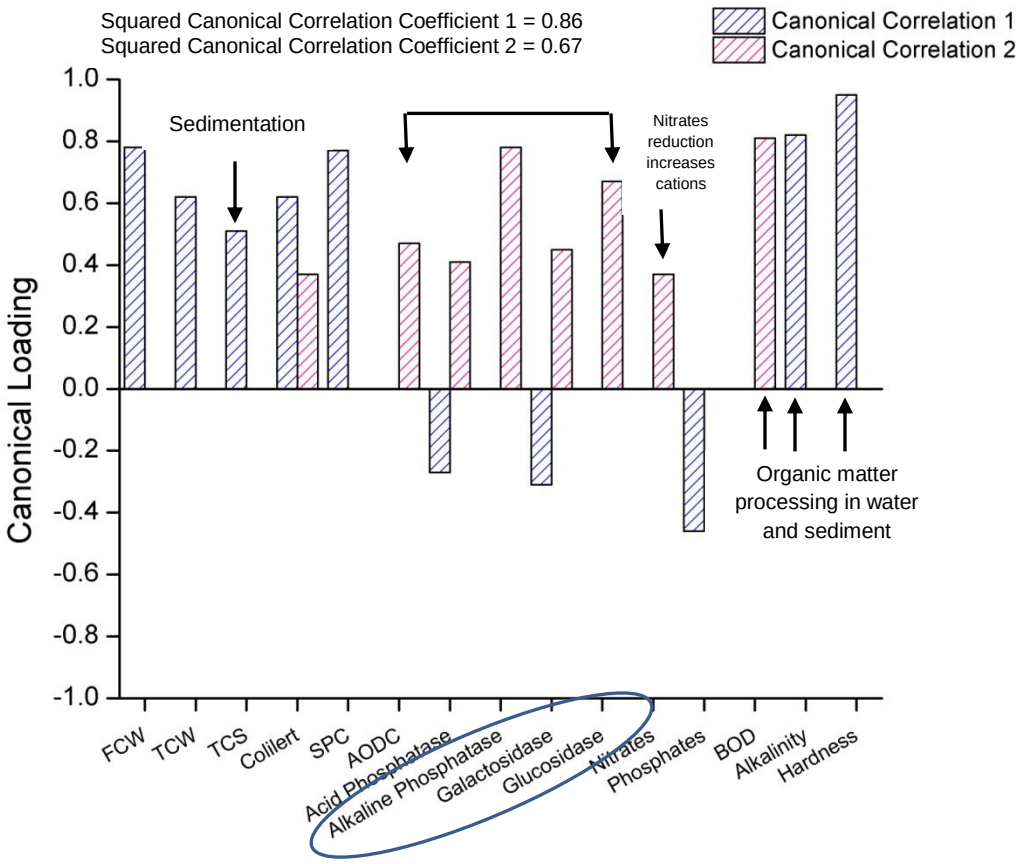

Figure 4.6. Sinking Creek canonical loadings observed during the summer months to relate chemical water quality parameters to microbial water quality parameters to identify sources of fecal pollution

Fecal pollution during the fall months is likely associated with surface runoff containing eroded soil and organic matter (Figure 4.7). In the first canonical structure, fecal indicators in both water and sediment are highly associated with nitrates, ammonia, BOD_5, alkalinity, and hardness. The strong influence of BOD_5, alkalinity, and hardness indicate the influence of eroded soil containing organic matter on the introduction of fecal indicator bacteria. High alkalinity and hardness concentrations can result from the input of humic acids and organic matter, which is supported by the

significant correlation of total and fecal coliform concentrations with BOD_5. The positive loadings for nitrates and ammonia in the first canonical variable also demonstrate the processing of the nitrogen content of the organic matter by heterotrophic communities via ammonification and nitrification in the water column. The loadings for these variables could also suggest that the introduction of fecal pollution is associated with sewage or septic effluent. The influence of organic matter processing in sediment is also evidenced by the second canonical structure. The negative correlation of BOD_5 and the positive canonical loadings for glucosidase, total coliforms, and acridine orange direct counts suggests that there is also organic matter processing occurring in the sediment. Microbial populations can reduce nitrates in the presence of organic matter, thus increasing cation concentrations and contribute to elevated hardness and alkalinity concentrations. These elevated cation concentrations can result in flocculation of organic matter and heterotrophic bacteria (Ayoub et al. 1999).

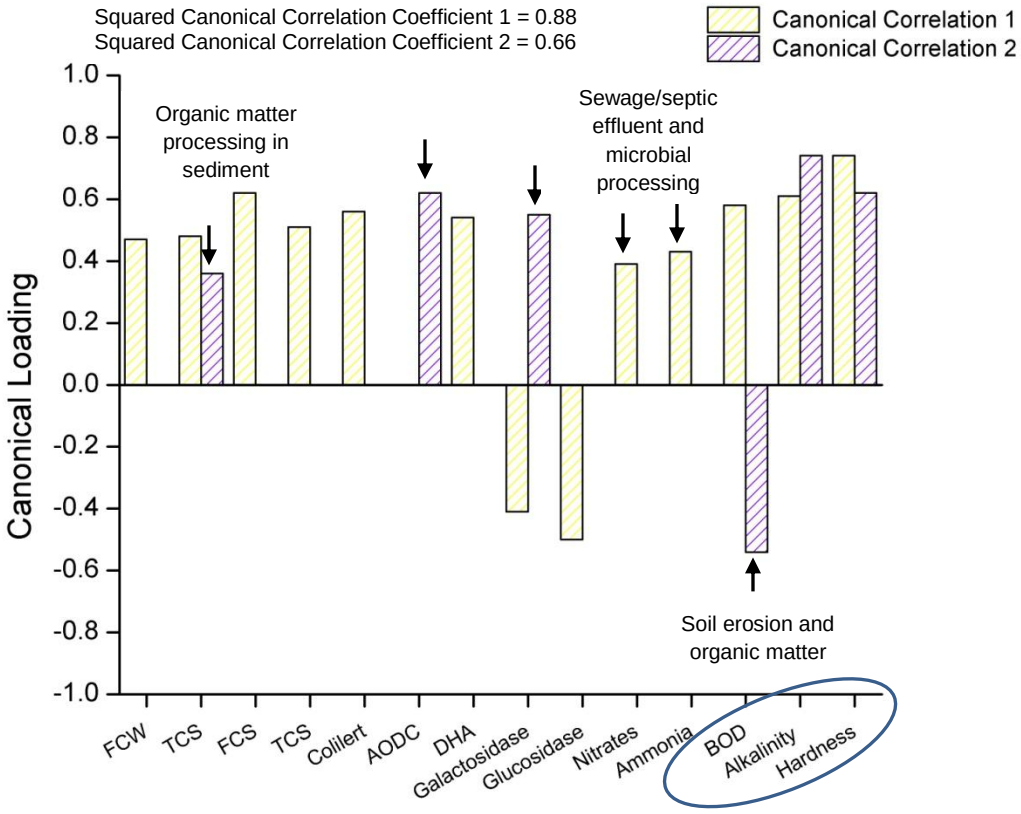

Figure 4.7. Sinking Creek canonical loadings observed during the fall months to relate chemical water quality parameters to microbial water quality parameters to identify sources of fecal pollution

Canonical Correlation Analysis by Land Use

Canonical correlation analyses were also conducted at the land use level to assess spatial variation. This approach was selected because canonical correlation analysis by site did not produce significant correlations for the individual urban land use sites and because of the low cumulative percentages of explained variance compared to those from grouping similar land use sites. The canonical structure for agricultural

sites demonstrates that fecal pollution is associated with soil erosion and bacterial sedimentation (Figure 4.8).

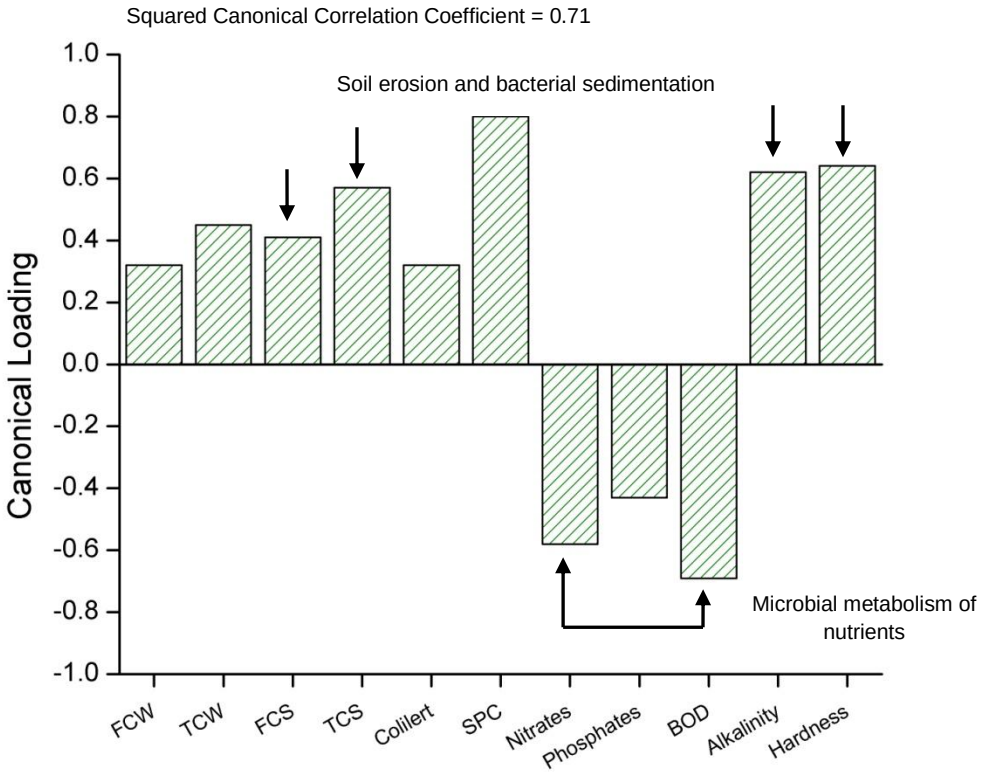

Figure 4.8. Sinking Creek canonical loadings observed at agricultural land use sites to relate chemical water quality parameters to microbial water quality parameters to identify sources of fecal pollution

The positive loadings for total and fecal coliforms in water and sediment and alkalinity and hardness suggest that eroded soil introduced through runoff events contribute to the observed heterotrophic bacteria concentrations. The negative loadings for nitrates, phosphate, and BOD_5 suggest that these microbial populations are using available nutrients and organic matter. Fecal coliform survival is enhanced in water with

moderate amounts of organic matter pollution (Whitman et al. 2006). The observed inverse relationship between fecal indicator bacteria and nutrient availability due to surface runoff and organic matter pollution may promote heterotrophic activity and fecal indicator organism survival or replication, resulting in an inaccurate indication of fecal pollution and pathogen prevalence.

Fecal pollution at urban land use sites is associated with runoff of eroded soil, as suggested by the correlation between fecal indicators in water and sediment and alkalinity (Figure 4.9). The inverse correlation between fecal indicators in water and sediment and BOD_5 concentrations suggests that the introduction of organic matter may have an inhibitory effect on fecal coliform and heterotrophic bacteria concentrations. The processing of the organic matter by heterotrophic populations results in increased oxygen demand. The concentration of dissolved oxygen that is required to process the organic matter may not be available to the microbial population, resulting in the decrease of heterotrophic bacteria with increasing organic matter loads.

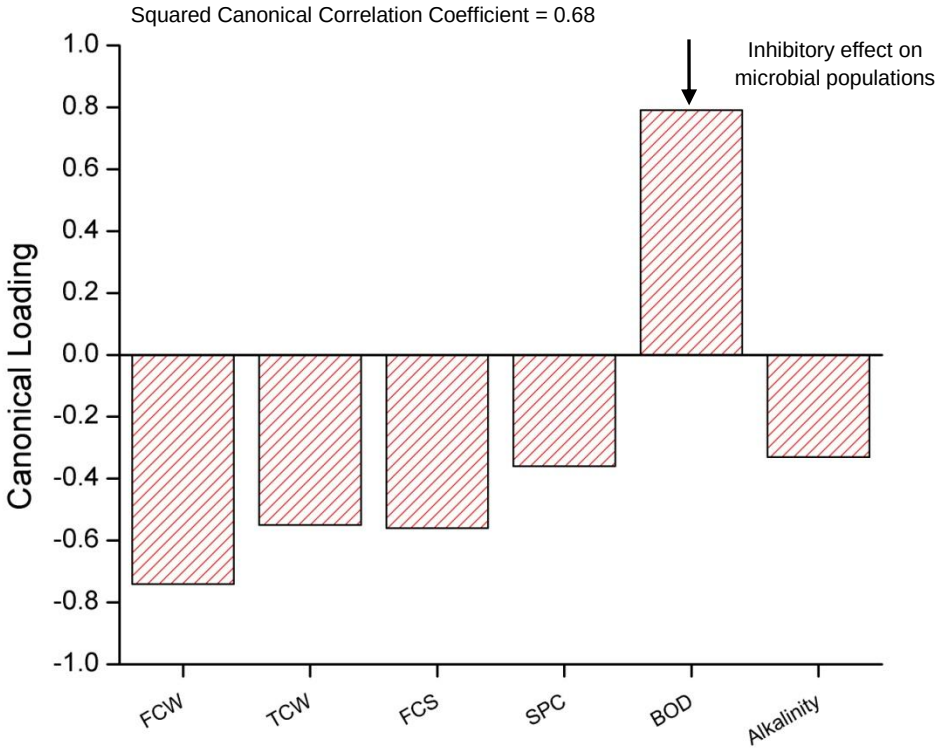

Figure 4.9. Sinking Creek canonical loading observed at urban land use sites to relate chemical water quality parameters to microbial water quality parameters to identify sources of fecal pollution

Similar to agricultural sites, fecal pollution at forested sites is associated with soil erosion and sedimentation (Table 4.10). The positive loadings for total and fecal coliforms in sediment with alkalinity and hardness suggest soil erosion introduces heterotrophic bacteria and that flocculation occurs as a result of increased cation concentrations from alkalinity and hardness.

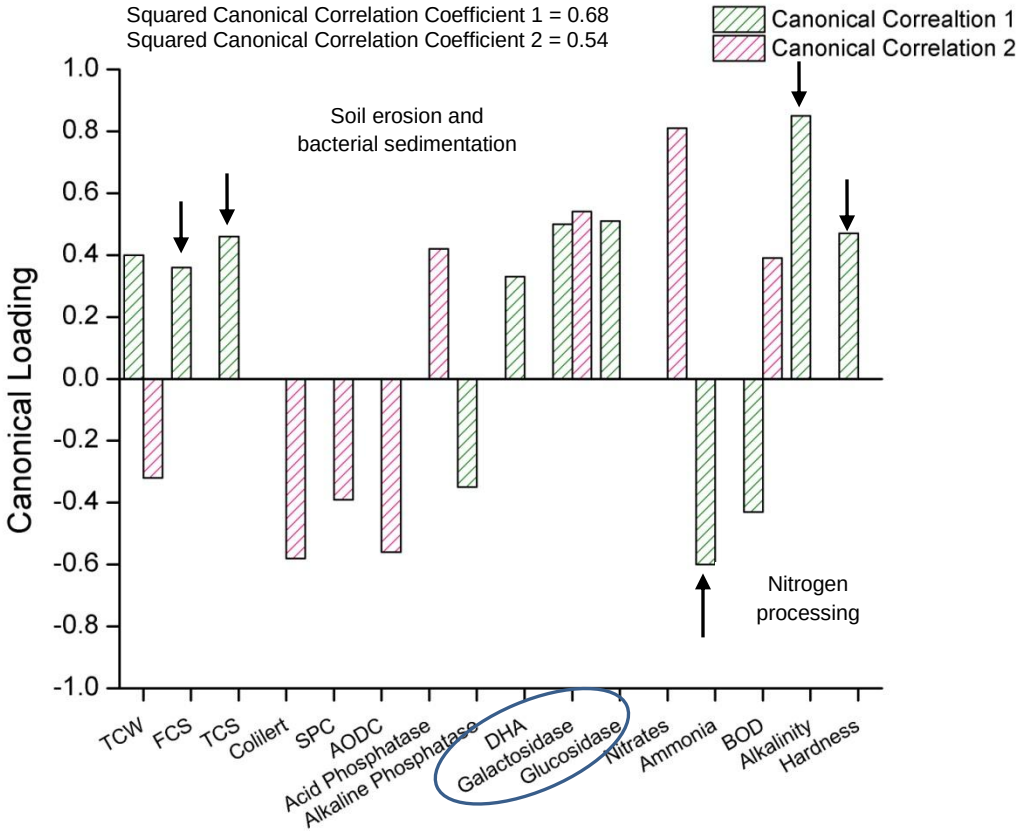

Figure 4.10. Sinking Creek canonical loadings observed at forest land use sites to relate chemical water quality parameters to microbial water quality parameters to identify sources of fecal pollution

As discussed previously, the cations associated with alkalinity and hardness may allow bacterial flocculation and settling into the sediment. The sedimentation of total and fecal coliforms is further suggested by their correlation with dehydrogenase, galactosidase, and glucosidase activities in sediment, indicating that heterotrophic bacteria in the sediment are actively processing the introduced organic matter associated with the eroded soil. The negative canonical loading for BOD_5 and its inverse relationship with the microbial parameters also indicate that the processing of

the organic matter is occurring in the sediments following organic matter settling. The negative canonical loading for ammonia also indicates the influence of organic matter processing by the microbial community in sediment. Ammonia is formed during the process of ammonification and is then readily converted to nitrite and nitrate through the process of nitrification. The inverse relationship between ammonia and the microbial variables suggests that the microbial community in the sediment is processing the nitrogen content of the organic matter following ammonification. The inverse relationship between fecal indicator and heterotrophic bacteria and nutrient availability and BOD_5 in the second canonical structure suggests that the introduction of organic matter may not be the sole source of fecal pollution. The relationship between these variables suggests that there is the addition of organic matter, but that these microbial populations are using the available materials in a way that may promote replication of fecal indicators in the sediment. The combination of the first and second canonical structures suggests that in addition to organic matter contributing to fecal pollution, introduced fecal indicator organisms may be replicating in the environment providing an inaccurate indication of fecal pollution and pathogen prevalence at these sites.

Indicators of fecal pollution in Sinking Creek exhibit spatial and temporal variability both in the extent and sources of fecal pollution. Overall, the relatively high canonical communality coefficients for the chemical parameters observed for canonical correlation analyses by season and land use indicate that the chemical parameters are good predictors of fecal pollution in Sinking Creek. The canonical communality coefficients describe the proportion of each variable that is explained by the entire canonical structure and identify the variables that most contribute to the overall

canonical structure. The squared canonical coefficients also suggests the ability of the chemical parameters to predict fecal pollution in Sinking Creek, as it indicates the variance in the microbial parameters that is explained by the chemical parameters.

Canonical Discriminant Analysis

Canonical discriminant analyses were conducted to further identify common patterns associating fecal indicator organisms to pollution sources in the in Sinking Creek by season, site, and land use. The variables listed in Table 4.3 were included in the CANDISC analysis. Canonical discriminant analysis finds the linear combinations of variables that allow for the maximum separation between classes and determines the distance between class means (the mean of canonical variables for each data set).

Table 4.3. Chemical and microbial variables included in canonical discriminant analysis (CANDISC procedure)

Variable	Units
Fecal coliform in water	CFU/100ml
Total coliform in water	CFU/100ml
Fecal coliform in sediment	CFU/100ml
Total coliform in sediment	CFU/100ml
Colilert	CFU/100ml
Standard plate count	CFU/ml
Acridine orange direct counts	cells/g sediment
Acid phosphatase	μg/g sediment
Alkaline phosphatase	μg/g sediment
Dehydrogenase	μg/g sediment
Galactosidase	μg/g sediment
Glucosidase	μg/g sediment
Nitrates	mg/l
Phosphates	mg/L
Ammonia	mg/l
Biochemical oxygen demand	mg/l
Hardness	mg/l
Alkalinity	mg/l

The plot of canonical means by season is shown in Figure 4.11. The first canonical variable separates the spring and summer seasons by their increased total and fecal coliform concentrations in sediment, heterotrophic activity in water and the lowest galactosidase and phosphates and BOD_5 concentrations (Table 4.4). The grouping of spring and summer suggest that these months are characterized by the setting of fecal pollution in sediment in relation to decreasing creek discharge (Table 1, Appendix A)

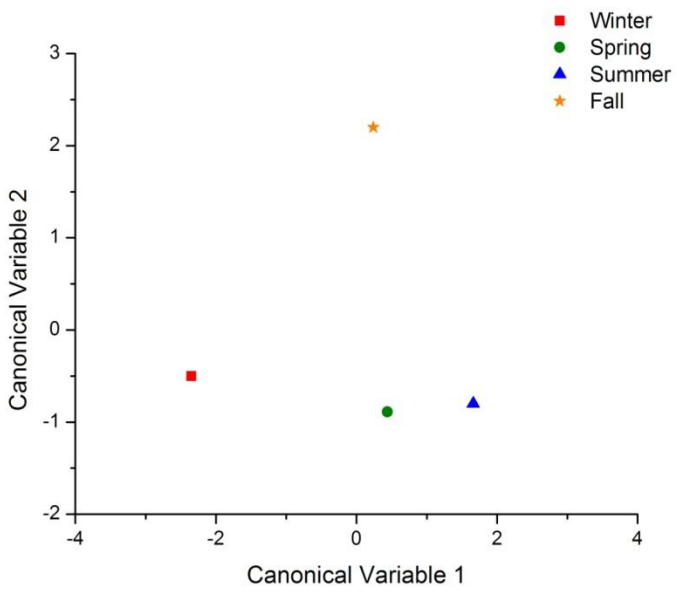

Figure 4.11. Plot of canonical means determined using canonical discriminant analysis for Sinking Creek by season

Table 4.4. Description of canonical structure as determined using canonical discriminant analysis for Sinking Creek by season

Canonical Variable	Water Quality Variables Describing the Canonical Structure
Canonical Variable 1	Fecal coliforms in sediment (0.55) Total coliforms in water (0.55) Total coliforms in sediment (0.50) Galactosidase (-0.41) Phosphates (-0.48) BOD (-0.77)
Canonical Variable 2	Acid Phosphatase (0.86) Nitrates (0.40) Galactosidase (0.32) Fecal coliforms in sediment (-0.34)

The fall months are characterized by less settling of fecal coliforms in sediment and more organic matter introduction and processing by heterotrophic bacteria in both water and sediment. The second canonical variable separates the fall months from the other seasons by increased acid phosphatase, nitrate, and galactosidase concentrations and decreased fecal coliform concentrations in sediment. This separation suggests the greater influence of soil erosion on nutrient introductions and organic matter processing and less settling of fecal pollution in sediment during the fall compared to other seasons. During the winter months total and fecal coliform concentrations in water and sediment are at their lowest and heterotrophic communities in water and sediment are actively processing introduced organic matter. Winter

months are characterized by less heterotrophic activity compared to the spring, summer, and fall months. However, there is more introduction and processing of organic matter introduced from soil erosion during this time as suggested by the influence of BOD_5, phosphates and galactosidase on the canonical structure.

The canonical plot of means by land use is shown in Figure 4.12. The strong separation of all land use groups suggests the influence of land use type on fecal pollution in Sinking Creek. The first canonical separates the agricultural sites by increased alkalinity and hardness, *E. coli*, total and fecal coliform, standard plate count, and nitrate concentrations (Table 4.5).

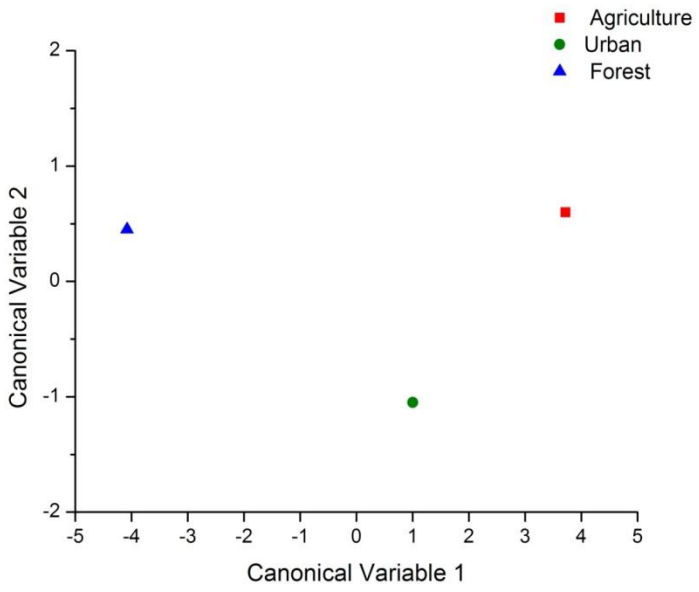

Figure 4.12. Plot of canonical means determined using canonical discriminant analysis for Sinking Creek by land use pattern

Table 4.5. Description of canonical structure as determined using canonical discriminant analysis for Sinking Creek by land use pattern

Canonical Variable	Water Quality Variables Describing the Canonical Structure
Canonical Variable 1	Hardness (0.98) Alkalinity (0.95) *E. coli* (0.50) Fecal coliforms in water (0.47) Total coliforms in water (0.46) Standard plate count (0.45) Nitrates (0.39)
Canonical Variable 2	*E. coli* (0.51) Standard plate count (0.45) Fecal coliforms in water (0.45) Total coliforms in water (0.33) Nitrates (-0.32)

Alkalinity and hardness concentrations have the strongest influence on the first canonical variable, which suggests the influence of soil erosion on fecal pollution based on land use. Fecal pollution at agricultural sites is most influenced by runoff of eroded soil, followed by urban and forested land use sites. The likelihood of separation of land use sites by the first canonical variable is enhanced by the significantly different fecal coliform concentrations observed between land use classification and the highest fecal coliform concentrations observed at agricultural land use sites. The second canonical variable separates agricultural and forested from urban land use sites based on *E. coli*,

total and fecal coliform, standard plate count, and nitrate concentrations. The negative influence of nitrates on the second canonical variable suggests that fecal pollution at these sites is associated with the processing of organic matter through nitrification. In contrast, fecal pollution at urban sites is likely due to the influence of nutrients with eroded soil and the processing of organic matter by heterotrophic bacteria. The similarities between agricultural and forested land use sites based on the second canonical variable is supported by similarities in their canonical structures (Figures 4.8 and 4.10, respectively) both of which suggest that fecal pollution and organic matter is associated primarily with runoff of eroded soil.

Conclusions

Using the Sinking Creek as a model, it has been demonstrated that the combined application of a targeted water quality monitoring program and multivariate statistical analyses are a useful tool to learn more about the responses of surface waters to anthropogenic stresses. Because the amounts and types of pollution, including fecal indicator bacteria, vary spatially and temporally, TMDL development may require multi-year data at multiple sampling points rather than the limited 30-day geometric mean that is currently used to more accurately reflect pollution loadings and patterns. The application of multivariate statistics to water quality data has been demonstrated to help improve our understanding of the interactions of physical, chemical, and microbial water quality parameters and their combined influences on water quality. A better understanding of loading patterns, temporal distribution, and spatial distribution should lead to the correct identification and quantification of nonpoint sources of fecal pollution,

and subsequently better and faster BMP selection and implementation. It is suggested that this data analysis approach can be applied to other watersheds to identify common patterns associating pollution types to various sources and to effectively develop and implement BMPs to prevent and remediate the effects of rapid urbanization.

Acknowledgements

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CHAPTER 5

CLASSIFICATION OF PHYICAL, CHEMICAL, AND MICROBIAL SOIL PROPERTIES AND THEIR INFLUENCE ON PATHOGEN FATE AND TRANSPORT

K.K. Hall and P.R. Scheuerman

Abstract

Interactions between physical, chemical, and microbial processes in soil add to the complexity of understanding pathogen fate and transport within a watershed. The purpose of this experiment was to characterize the soil within the Sinking Creek watershed based on physical and chemical properties. An understanding of the physical and chemical soil structure and microbial activities within the watershed can lead to a better understanding of pathogen loading into Sinking Creek and aid in the design and implementation of effective best management practices (BMPs). Results are not reflective of the full pedon structure and may not represent the heterogeneous nature of the soil, as samples were collected within 6 – 8 inches of the soil surface. However, the physical, chemical, and microbial properties of the soil can help to understand soil structure and dynamics in the Sinking Creek watershed and its role in pathogen loading into receiving waters.

Introduction

Soil formation is influenced by several factors including the nature of the parent material, climate, topography of the area, the presence and activity of organisms, and the length of time that the parent material is exposed to soil forming conditions. The variation in soil formation factors and activities is partially responsible for the

heterogeneity of soil. The Sinking Creek watershed encompasses Washington and Carter Counties in Northeast Tennessee and the predominant soil orders are alfisols, inceptisols, and ultisols (Figure 5.1). Northeast Tennessee is characterized by karst topography, and soil formation is primarily due to alluvial and colluvial movement of metasedimentary rocks such as shale, sandstone, siltstone, and limestone (NRCS, 2010a).

Soil ratings and classifications as described by the Natural Resources Conservation Service (NRCS) for the 14 monitored sites on Sinking Creek are given in Table 5.1 (NRCS, 2010b). Alfisols are characterized as well developed, moderately leached forest soils with high fertility. Ultisols are heavily leached forest soils that exhibit intense weathering and leaching of calcium, magnesium, and potassium. Soils within this order are commonly thought of as “red clays” that are characteristic of the Southeast United States. Inceptisols are often found on steep slopes and on resistant parent material and lack a strongly defined set of characteristics as compared to other soil orders (NRCS, 1999).

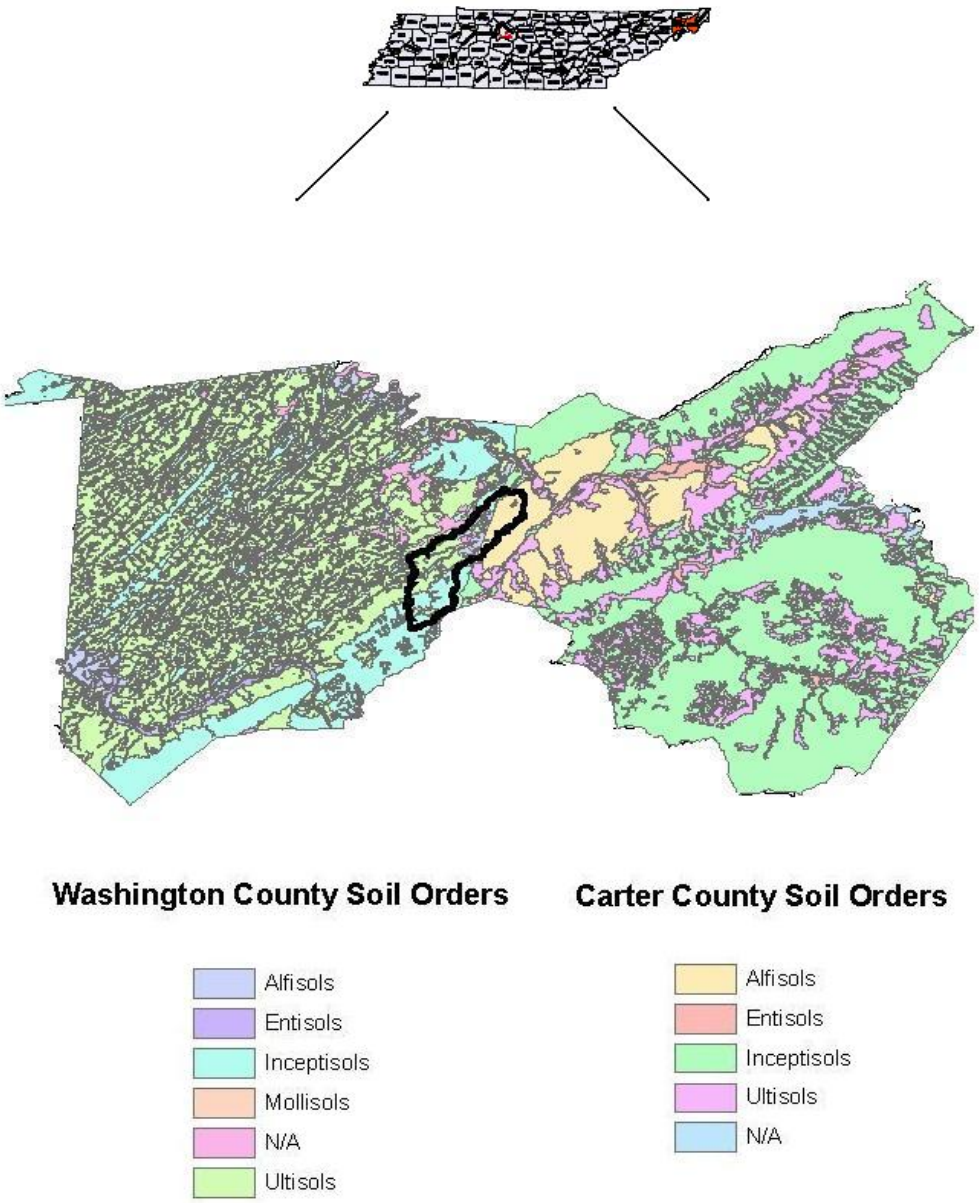

Figure 5.1. Soil orders and the Watauga River watershed in Washington and Carter Counties, Tennessee (Reference for data used to generate figure, NRCS, 2010a-c)

Table 5.1. Soil classification of monitored sites on Sinking Creek

Site	Land Use	Rating	Classification	Textural Classification	pH	CEC (meq/100cm^3)	Available Water (cm/cm)	Organic Matter (%)	Bulk Density (g/cm^3)
1	Agricultural	Fine, mixed, thermic, Typic Paleudalfs	Braxton-talbott-rock outcrop complex, 12-20% slopes, eroded	Silt loam	5.6	18.4	0.18	1.08	1.35-1.50
2	Agricultural	Fine, mixed, thermic, Typic Paleudalfs	Braxton-talbott-rock outcrop complex, 12-20% slopes, eroded	Silt loam	5.6	18.4	0.18	1.08	1.35-1.50
3	Agricultural	Fine, mixed, thermic, Typic Paleudalfs	Baxton silt loam, 2-5% slopes	Silt loam	5.6	14.6	0.20	1.50	1.35-1.50
4	Agricultural	Fine, mixed, thermic, Typic Paleudalfs	Braxton silt loam, 2-5% slopes	Silt loam	5.6	14.6	0.20	1.50	1.35-1.50
5	Agricultural	Fine, mixed, active, nonacidic, mesic Fluvaquentic Endoaquepts	Melvin silt loam, 0-2% slopes, occasionally flooded	Silt loam	6.7	7.9	0.21	1.75	1.20-1.60
6	Urban	Fine, kaolinitic, thermic Typic Paleudults	Dewey-Udorthents-Urban land complexes, 5-20% slopes	Silt loam	5.5	8.2	0.20	1.17	1.30-1.45
7	Urban	Fine, kaolinitic, thermic Typic Paleudults	Dewey-Udorthents-Urban land complexes, 5-20% slopes	Silt loam	5.5	8.2	0.20	1.17	1.30-1.45
8	Urban	Not available	Urban land-Udorthents complex	NA	NA	NA	NA	NA	NA
9	Urban	Fine-loamy, siliceous, semiactiv e, thermic Fluvaquentic Eutrudepts	Hamblen loam, 0-3% slopes, occasionally flooded	Loam	6.2	7.3	0.19	2.0	1.30-1.45
10	Urban	Fine-loamy, siliceous, semiactiv e, thermic Fluvaquentic Eutrudepts	Hamblen loam, 03-% slopes, occasionally flooded	Loam	6.2	7.3	0.19	2.0	1.30-1.45
11	Urban	Fine-loamy, siliceous, semiactiv e, thermic Fluvaquentic Eutrudepts	Hamblen loam, 03-% slopes, occasionally flooded	Loam	6.2	7.3	0.19	2.0	1.30-1.45
12	Urban	Fine-loamy, siliceous, semiactiv e, thermic Fluvaquentic Eutrudepts	Hamblen loam, 03-% slopes, occasionally flooded	Loam	6.2	7.3	0.19	2.0	1.30-1.45
13	Forest	Fine-loamy, siliceous, semiactiv e, mesic typic Hapludults	Keener loam, 5-12% slopes	Loam	4.8	NA	0.16	1.5	0.8-4.7
14	Forest	Fine-loamy, mixed, active mesic Typic Hapludults	Shelocta silt loam, 35-50% slopes	Silt loam	5.0	2.2-8.3	0.18	2.25	1.15-1.30

The classifications of soil from the 14 monitored sites on Sinking Creek include a combination of alluvial (sites 1-12) and colluvial soils (sites 13 and 14). Soil at the agricultural and urban land use sites are classified as being formed in alluvium and residuum weathered from limestone, and soil from the forest land use sites are classified as being formed in colluvium from shale, siltstone, and sandstone (NRCS, 1954; NRCS, 2004; NRCS, 2010c). Changes in land use have been shown to affect soil development and structure in agricultural and forested landscapes (Vacca, 2000; Greenwood and McKenzie, 2001; Li et al. 2007). Determination of soil structure and dynamics within the watershed as they pertain to land use patterns can help better understand the influence of soil in pathogen fate and transport and help identify and remediate sources of fecal pollution in Sinking Creek.

It has been demonstrated that fecal pollution in Sinking Creek is associated with runoff, primarily from agricultural land use sites (Dulaney et al. 2003; Hall et al. 2008; Hall et al. 2011), so it is crucial to understand the role of physiochemical soil parameters to better understand the fate and transport of these organisms from their sources to receiving streams. Pathogen fate and transport through the soil matrix is dependent on several physical, chemical, and microbial processes. The transport of the pathogen from the source to water, transport following entry into the water, and pathogen survival in the water require consideration (Bishop et al. 2005). Physiochemical soil properties such as particle and pore size, pH, organic matter content, cation exchange capacity (CEC), and matric potential can influence sorption processes and pathogen transport. Bacterial cells will adsorb more to finer textured soils than to coarser textured soils (Abu-Ashour et al. 1998; Hijnen et al. 2005) and microbial retention increases as soil

adhesion and sorption increase (Hörman et al. 2004). Microbial cell surface properties also influence their transport throughout the soil matrix (Pirszel et al. 1995), and cell characteristics such as length, surface charge, appendages, and the production of extracellular polysaccharides have been shown to impact bacterial movement throughout soil (Greenwood and McKenzie, 2001).

Physiochemical soil properties such as particle size, bulk density, water holding capacity, and cation exchange capacity contribute to soil saturation and can result in greater transport of bacteria because they inhibit filtration processes or prevent interaction between the microorganism and the soil matrix (Van Donsel et al. 1967; Yeager and O'Brian, 1979; Gagliardi and Karns, 2000). Differences in these soil properties as they relate to different land use patterns have been shown to influence runoff and drainage mechanisms (Kurz et al. 2006; Bormann et al. 2007). Physical characteristics including sunlight exposure, temperature (Hurst et al. 1980; Kemp et al. 1992), nutrient availability, extreme pH values (Huysman and Verstraete, 1993), and the presence of other microorganisms may also affect pathogen transport through soil (Wong and Griffin, 1976).

The microbial ecology of soil must be considered in addition to the physiochemical properties. Microbial properties of soil are some of the more difficult properties to characterize based on the great deal of diversity at the ecosystem, population and genetic levels. As with physiochemical soil parameters, the microbial properties can represent a dynamic system where the types and numbers of microorganisms may be altered with temporal and spatial changes (Anderson and Domsch, 1990; Yao et al. 2000). To better understand the microbial ecology of soils

and characterize heterotrophic communities, methods such as Biolog® plates have been used (Biolog Inc., Hayward, CA).

These 96 well plates contain 95 different carbon substrates and rely on the ability of inoculated microbial populations to reduce tetrazolium violet. Originally used for the identification of bacterial isolates for strain identification, Biolog® plates have since been used with environmental samples to determine the microbial community structure (Garland and Mills, 1991; Winding, 1993; Zak et al. 1994). When applied to soil samples, the use of the different carbon sources by the inoculum generates a pattern that provides an indication of carbon use to understand how the microbial community contributes to organic matter processing in the environment to distinguish between soil types (Zak et al. 1994; Bossio and Scow, 1995). The community level approach to examining soil microbial ecology allows for a more sensitive measure of heterotrophic community structure (Garland and Mills, 1991).

To differentiate microbial communities based on spatial and temporal variability, statistical methods such as principle component analysis (PCA) can be used (Garland and Mills, 1991; Winding, 1993; Zak et al. 1994). PCA is a multivariate statistical procedure that computes principle components for every numerical variable (i.e., Biolog® substrates), each of which is a linear combination of the variables that account for the most variance explained by the fewest number of variables. Data are reduced based on the establishment of dimensions in the data with the first principle component accounting for the most variability and the second principle component accounting for the remaining variability not accounted for in the first principle component (Dillon and Goldstein, 1984).

One of the main drawbacks associated with the use of Biolog® plates to understand microbial ecology includes the influence of inoculum density on the rate of color development, as it is unknown if all members of the microbial community contribute to the observed color development (Garland and Mills, 1991). Data can be transformed based on the average well color development (AWCD) to help reduce the influence of inoculum density on color development (Garland and Mills 1991). It should also be noted that the observed patterns of Biolog® analyses are a measurement of ability of the inoculum to use a carbon source and that the carbon sources used in the analysis may not be present in the environment.

The objective of this group of experiments was to determine physical, chemical, and microbial parameters of soil collected from the Sinking Creek watershed. Characterization of the soil within the watershed may help to better understand the interactions between physiochemical soil properties and microbial populations and the influence of these properties on pathogen fate and transport. Understanding these interactions can help to develop appropriate and successful best management practices to remediate fecal pollution and prevent future pollution events.

Methods

Sinking Creek Location and Water Quality Monitoring

The Sinking Creek sub-watershed (06010103130) is one of 13 sub-watersheds that belong to the Watauga River watershed (TDEC, 2000a). Sinking Creek is a 9.8 mile long tributary of the Watauga River partially located in Washington and Carter

Counties in Tennessee. The headwaters of Sinking Creek are located on Buffalo Mountain and it enters the Watauga River at mile 19.9. The main land uses within the 13.1 square mile drainage basin of the Sinking Creek watershed include: forest (65.5%), urban (25.3%), and agricultural areas (9.0%) (TDEC 2000b). There are 19.8 impaired stream miles in the Sinking Creek watershed including tributaries (TDEC, 2000b).

Upstream locations on Buffalo Mountain are forested, and land use transitions to urban, followed by agricultural land use at downstream sites. Fourteen sites were initially selected for routine water quality monitoring in 2002 and are described in Table 5.1 and Figure 5.2. From these 14 sampling locations, 2 sites were randomly selected from each land use classification and sampled monthly for the physical, chemical, and microbial parameters described Table 5.2. The sites selected for representation of agricultural land use were sites 2 and 4, sites selected to represent urban land use were sites 7 and 10, and sites 13 and 14 represented forested land use.

Table 5.2. Sampling locations on Sinking Creek sampled during this study for water quality analysis

Site Number	Site Location	Predominant Land Use	Physical Description	Habitat Assessment Score (%)	Latitude/Longitude Coordinates and Elevation
2	Upstream of Bob Peoples bridge on Sinking Creek Road	Agriculture	Moderately eroded banks with little vegetation buffer or riparian zone. Creek bed predominantly cobble and gravel	52%	19.837' N, 18.254' W 1530 ft
4	Upstream of crossing on Joe Carr Road	Agriculture	Moderately eroded banks with poor bank stability and little vegetative buffer or riparian zone. Creek bed predominantly boulders, cobble and gravel	43%	19.594' N, 18.579' W 1552 ft
7	Upstream of bridge on Miami Drive, King Springs Baptist Church	Urban	Heavily eroded left bank, concrete bank on right with no vegetative buffer or riparian zone. Creek bed predominantly cobble	53%	18.772' N, 19.685' W 1583 ft
10	Upstream of bridge crossing Sinking Creek at Hickory Springs Road	Urban	Heavily eroded banks with no vegetative buffer. Creek bed predominantly boulders and cobble	57%	17.431' N, 21.397' W 1720 ft
13	Upstream of road crossing on Jim McNeese Road	Forest	No visible bank erosion with moderate riparian zone. Creek bed predominantly boulders and cobble	71%	16.035' N, 22.163' W 2048 ft
14	Downstream of path crossing at Dry Springs Road	Forest	No visible bank erosion with optimal riparian zone and vegetative buffer. Creek bed predominantly boulders, cobble and gravel	83%	14.800' N, 22.033' W 2148 ft

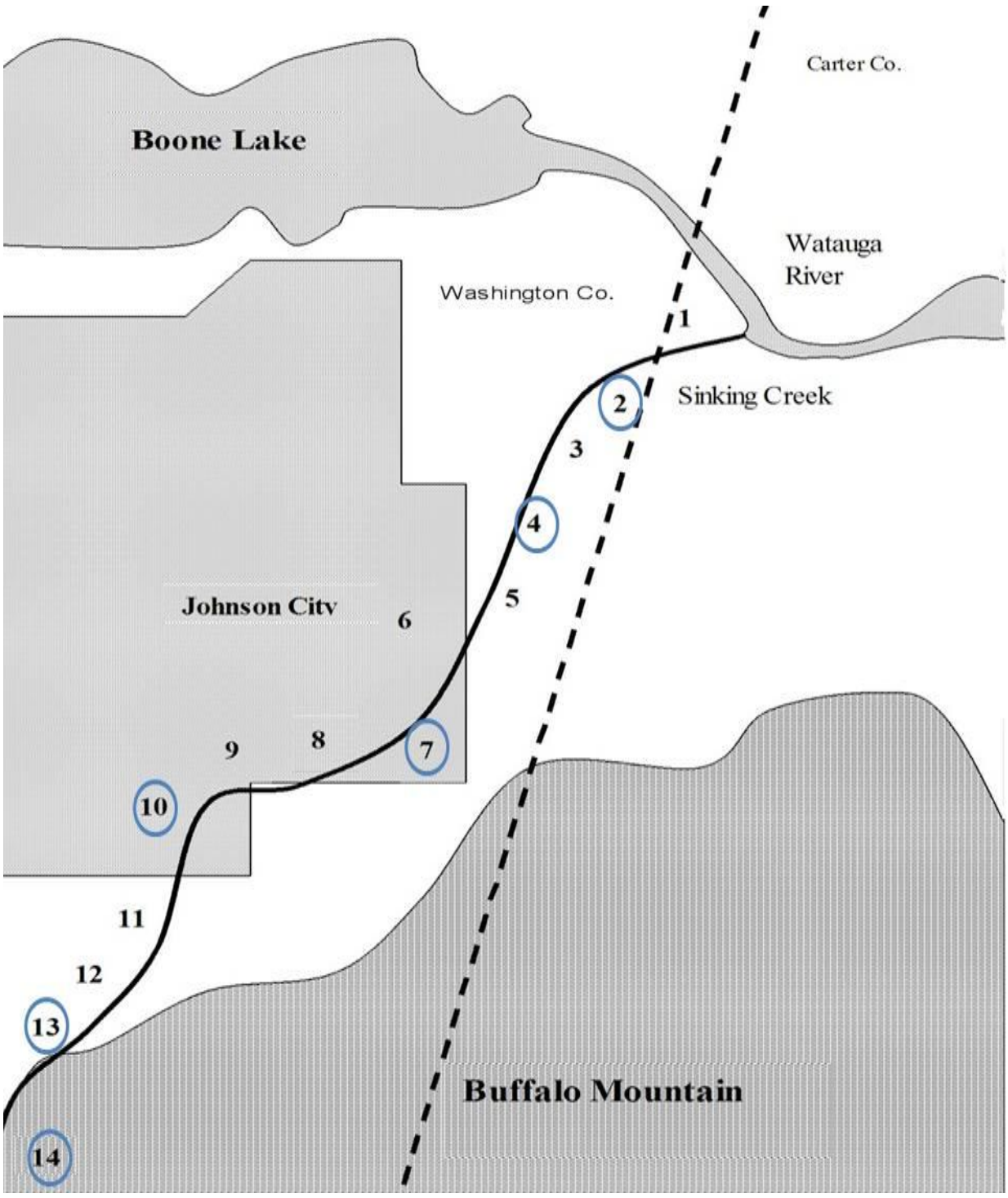

Figure 5.2. Map of Sinking Creek sampling locations (sites sampled for water quality analysis in this study are circled).

Water and Sediment Sample Collection

Water samples were collected monthly from 6 pre-selected sites on Sinking Creek from January 2011 through December 2011 and were analyzed for the variables described in Table 2. Water samples for total and fecal coliform bacteria (TC/FC), standard plate counts (SPC), *E. coli* 057:H7, *Shigella sp.*, and bacteriophage analyses were collected and analyzed in triplicate (SPC samples analyzed in duplicate) in sterile, 1-L Nalgene™ bottles. Water samples for Colilert® analyses were collected in sterile 100ml plastic bottles (IDEXX Laboratories, Westbrook, Maine). Water samples for nitrates (NO_3^-), phosphates (PO_4^-), ammonia (NH_3^+), 5-day biochemical oxygen demand (BOD_5), alkalinity, and hardness were collected and analyzed in triplicate in sterile 2-L Nalgene™ bottles. Sediment samples for TC/FC in water, microbial enzyme activity (MEA), and acridine orange direct counts (AODC) were collected in 2oz sterile Whirl-Pak™ bags. All samples were transported to the laboratory on ice and analyzed within the holding times described in Table 5.3. Field measurements for pH, air and water temperature, dissolved oxygen, and conductivity were also collected at each site.

Quality assurance and quality control (QA/QC) practices included the analysis of chemical parameters consisted of one trip blank, one field blank, a negative control, one replicate, one spiked sample, and one quality control standard. QA/QC practices included in the analysis of microbial parameters included the analysis of one trip blank, one field blank, a negative control, and a positive control. A secondary wastewater effluent sample was used as the positive control for TC/FC, Colilert®, SPC, and bacteriophage analyses. Laboratory strains of *E. coli* O157:H7 and *Shigella flexneri*

(ATCC® Number 43895™ and ATCC® 12022™, respectively) were used to seed water samples that served as a positive control for PCR analysis.

Table 5.3. Physical, chemical, and microbial water quality parameters measured

Parameter	Abbreviation	Units	Holding Time
pH	pH	pH	Field measurement
Water temperature	WT	^{o}C	Field measurement
Air temperature	AT	^{o}C	Field measurement
Dissolved oxygen	DO	mg/l as O_2	Field measurement
Conductivity	Cond	µmohs	Field measurement
Fecal coliform in water	FCW	CFU/100ml	6h
Total coliform in water	TCW	CFU/100ml	6h
Fecal coliform in sediment	FCS	CFU/100ml	6h
Total coliform in sediment	TCS	CFU/100ml	6h
Colilert	Colilert	CFU/100ml	6h
Standard plate count	SPC	CFU/ml	6h
Acridine orange direct counts	AODC	cells/g sediment	6h
Acid phosphatase	AcidP	µg/g sediment	24h
Alkaline phosphatase	AlkP	µg/g sediment	24h
Dehydrogenase	DHA	µg/g sediment	24h
Galactosidase	Gal	µg/g sediment	24h
Glucosidase	Glu	µg/g sediment	24h
Nitrates	NO_3	mg/l	24h
Phosphates	PO_4^{2-}	mg/l	24h
Ammonia	NH_3^{+}	mg/l	24h
Biochemical oxygen demand	BOD_5	mg/l	24h
Hardness	Hard	mg/l	48h
Alkalinity	Alk	mg/l	24h
E. coli O157:H7	O157:H7	CFU/100ml	24h
Shigella sp.	*Shigella*	CFU/100ml	24h
Giardia sp.	*Giardia*	Cysts/l	72h
Cryptosporidium sp.	*Crypto*	Oocysts/l	72h
F+ - specific bacteriophage	bacteriophage	PFU/ml	48h

Water Microbial Analyses

TC/FC analyses for water samples were conducted according to Standard Methods for Examination of Water and Wastewater (APHA, 1992). Briefly, 0.5ml of

water were filtered through a 0.45µm membrane filter (EMD Millipore, Billerica, MA) and the filter placed in a petri dish containing an absorbent pad (EMD Millipore, Billerica, MA) with 2ml of m-Endo media for total coliform analysis or m-FC media for fecal coliform analysis. All plates were inverted and enumerated following 24h incubation at 37^{o}C and 44.5^{o}C for total coliform and fecal coliforms, respectively. For TC/FC sediment analyses, 0.5g of sediment was added to 25ml of sterile water + 1% Tween 80. The samples were vortexed and allowed to settle for 30 minutes, and 0.5ml of the buffer suspension was filtered according to Standard Methods for Examination of Water and Wastewater as described above (APHA, 1992).

SPC were conducted according to Standard Methods for Examination of Water and Wastewater (APHA, 1992) using R2A agar. One milliliter of water was placed in the center of a sterile petri dish (Fisher Scientific, Pittsburgh, PA) and 10ml of R2A agar was added to the dish. The plate was swirled in a figure eight motion to allow the sample to disperse in the media and cover the plate. Plates were allowed to solidify and were enumerated following incubation at 25^{o}C for 48h. *Escherichia coli* concentrations were determined using the Colilert® Quanti-Tray method (APHA, 1995). To each 100ml water sample, a packet of Defined Substrate Technology® (DST®) reagent (IDEXX Laboratories, Westbrook, Maine) was added and mixed. The sample was then poured into a Quanti-Tray®, sealed using the Quanti-Tray® sealer, and incubated for 24h at 37^{o}C. *E. coli* were then enumerated using the Standard Method most probable number (MPN) procedure. Samples for water TC/FC were processed in triplicate and samples for sediment TC/FC were processed in duplicate. SPC were processed in duplicate and one Colilert® sample was processed for each site.

MEA analyses were conducted and included acid and alkaline phosphatases, glucosidase, galactosidase, and dehydrogenase activities. For each enzyme analyzed, 1g of sediment was added to a test tube containing a specific buffer and enzyme. Sediment samples for acid phosphatase were mixed with 4ml of 1M TRIS buffer (pH 4.8) and 4ml of 1M TRIS buffer (pH) 8.4 for alkaline phosphatase. For both acid and alkaline phosphatase, 1ml of 1M TRIS buffer with 0.1% phosphatase substrate (pH 7.6) was added to each tube (Sayler et al. 1979). Sediment samples for galactosidase and glucosidase activities were mixed with 4ml of 0.1M phosphate buffer (pH 9.0). Galactosidase activity was measured by adding 1ml of 0.01M phosphate buffer with 0.15% p-nitrophenyl-β-D-galactopyranoside as an indicator of galactosidase activity. One milliliter of 0.01M phosphate buffer with 0.15% 4-nitrophenyl-β-D-glucopyranoside was used as an indicator to assess glucosidase activities (Morrison et al. 1977). Following addition of buffers and indicators, all tubes were vortexted and incubated at 25^{o}C for 24h. Acid and alkaline phosphatase, galactosidase, and glucosidase activities were determined using a spectrophotometer at an absorbance of 418nm.

For dehydrogenase (DHA) activity, 1g of sediment was added to a test tube containing 2ml of 0.1M phosphate buffer (pH 7.6) and 1ml of 0.5% iodonitrotetrazolium chloride (INT) salt solution. The samples were vortexed and incubated in the dark at 25^{o}C for 45 minutes. One milliliter of the sample was filtered through a 0.22µm porosity cellulose membrane (GE Water and Process Technologies, Trevose, PA) and allowed to dry at room temperature. The membrane, was then added to a test tube containing 5ml of dimethyl sulfoxide, vortexted to dissolve the membrane, and incubated in the

dark at 25°C for 24h. Dehydrogenase activity was then determined using a spectrophotometer at an absorbance of 460nm.

AODC analysis was performed as described by Ghiorse and Balkwill (1983). Three hundred milligrams of sediment was added to 30ml of sterile PBS+Tween 80, vortexed for 60s, and allowed to settle for 3h. Two hundred fifty microliters of the suspension was mixed with 5ml sterile water + 500µl acridine orange stain, and samples were vortexed for 30s. Samples were filtered using 25mm, 0.2µm pore polycarbonate nucleopore filters (Osmonics, Inc., Minnetonka, MN), and the filters were mounted and fixed on slides for enumeration at 1000X using the Olympus BH2 epifluorescent microscope (Olympus, New Hyde Park, NY). One sediment sample was processed per site and 3 microscopic fields were enumerated on each slide.

Water Chemical Analyses

NO_3^-, PO_4^-, NH_3^+, alkalinity, and hardness analyses were performed in triplicate using colorimetric HACH™ methods and HACH™ reagents as described by the manufacturer (HACH Company, Loveland, CO). Briefly, NO_3^-, PO_4^-, NH_3^+ analyses were conducted by adding 10ml of water to a vial containing the appropriate reagent packet; NitraVer5, PhosVer3 and salicylate/ammonia cyanurate reagents, respectively. The vials were shaken to dissolve the reagent and samples were analyzed using pocket colorimeters specific to the nutrient of interest. Alkalinity and hardness analyses were conducted using 100ml sample volumes and a digital titrator. For alkalinity determination, 1 packet of phenolthalein indicator and bromcresol green-methyl red indicator were added to the sample and mixed. The sample was then titrated with 1.6N

sulfuric acid to a grey-green endpoint. For hardness determination, 1 packet of ManVer2 reagent and 2ml of hardness buffer (pH 10) were added to the 100ml sample and mixed. The sample was then titrated with 0.8N Ethylenediaminetetraacetic acid (EDTA) to a blue endpoint. BOD_5 analyses were conducted according to Standard Methods for Examination of Water and Wastewater (APHA, 1992). Wheaton BOD bottles (Wheaton Science Products, Millville, NJ) were completely filled with sample water and capped with glass stoppers to ensure no air bubbles were present. Initial (Day 0) and final (Day 5) dissolved oxygen concentrations were measured using the YSI Model 5000 dissolved oxygen meter (YSI Inc., Yellow Springs, OH).

Soil Sample Collection

For analysis of chemical and physical soil parameters, samples were collected at the 14 established sites assigned by the Environmental Health Sciences Laboratory. Fifteen samples were collected from each site (n = 210). This sample size was based on previously collected physical and chemical soil data on Sinking Creek at $\alpha = 0.05$ (Hall, 2006a). Soil was collected using a soil auger within 6 to 8 inches of the soil surface and placed into a sterile sampling bag. Samples were transported to the laboratory, spread in a 3cm thick layer on drying trays, and allowed to dry. Clods were broken with a rolling pin and the samples were passed through a 2mm sieve to remove the gravel fraction. The <2mm fraction was transferred back to the sampling bag until further use and analyzed for the paramters listed in Table 5.4.

Table 5.4. Analyzed soil parameters

Physical Parameters	Particle Size (% sand, silt and clay)
	Total Organic Carbon (% organic carbon)
	Water Holding Capacity (%)
	Bulk Density (g/cm^3)
Chemical Parameters	pH
	Cation Exchange Capacity (meq/100g soil)

Samples for microbial analysis were collected quarterly from the 6 selected sites described in Table 5.2. Fifteen samples were collected from each site per quarter (n = 360). Soil was collected using a soil auger within 6 inches of the soil surface and placed in a sterile sampling bag. Samples were transported to the laboratory and processed within 48h of arrival.

Particle Size Analysis

Particle size analysis was performed as described by The Soil Science Society of America and The American Society of Agronomy (Klute 1996). Twenty to 40 grams were weighed and placed into a 250ml centrifuge tube containing 100ml DI water and 10ml of 1.0M sodium acetate (pH 5.0). Tubes were mixed for 1 min., centrifuged for 10 min., and the supernatant discarded. Samples were washed twice with DI water. To remove organic matter, 25ml of DI water + 5ml of H_2O_2 were added to the soil sample. Samples were allowed to cool following frothing and this step was repeated until there was no further frothing activity. Samples were then heated to 90^{o}C until a bleached

color was reached and frothing ceased. To remove iron oxides, 150ml of 0.3M sodium citrate/sodium bicarbonate solution was added. Samples were shaken for 30 min and placed in an 80^{o}C water bath for 20 min. with intermittent agitation. Samples were removed from the water bath and 10ml of 10% NaCl solution was added. The samples were shaken for 1 minute and centrifuged for 10 minutes. The samples were washed twice with DI water and shaken overnight in 100ml of sodium hexametaphosphate (HMP). Samples were then quantitatively transferred to 1-L graduated cylinders and the volume adjusted to 1L with DI water. The graduated cylinders were inverted several times to mix the sample and hydrometer measurements were taken at 30s, 60s, 1.5h, and 24h. The hydrometer was rinsed and dried between each sample and reading, and a blank solution was measured for every 15 samples.

pH

Soil pH analyses were performed as described by The Soil Science Society of America and The American Society of Agronomy (Sparks, 1996). Ten grams of soil was added to 10ml DI water, and the slurry was stirred for 30s and allowed to settle for 10 minutes. Using a calibrated pH meter (Fisher Accumet Model 230A), pH was determined for each sample by lowering the electrode into the slurry at the soil-water interface. The pH was read to the nearest tenth of a unit while the slurry was slowly stirred. The probe was rinsed between each sample and reading and was standardized every 15 samples.

Cation Exchange Capacity

Cation exchange capacity (CEC) was determined using an ammonium acetate adapted method from Chapman (Chapman 1965). Twenty-five grams of soil were mixed with 125ml of 1M NH_4OAc, shaken, and allowed to stand for 16h. A Buchner funnel apparatus was assembled. Ashless, quantitative Whatman® filter papers (Florham Park, New Jersey) were placed in the funnel, moistened with DI water, and the soil was filtered. The filtrate was refiltered through the soil until it was clear. The soil was then washed 4 times with 25ml of 1M NH_4OAc and the leachate discarded. The soil was then washed 8 times with 10ml of 95% ethanol and the leachate discarded. To obtain the adsorbed NH_4^+, the soil was leached 8 times with 25ml of 1M KCl. The leachate was collected in a 250ml volumetric flask and brought to volume using 1M KCl. Colorimetric detection of NH_4^+-N^+ in the KCl extract was determined using a Nessler Method adapted from Standard Methods for the Examination of Water and Wastewater 4500-NH_3 B and C. To 25ml of DI water, 250µl of sample was added and mixed. Three drops each of mineral stabilizer and polyvinyl alcohol dispersing agent were added to the sample and mixed. Nessler reagent was then added (1ml) to each sample, mixed, and allowed to sit for 1 min. Ten milliliters of sample were then read using the HACH™ DR5000 Spectrophotometer (Loveland, CO). CEC (meq/100g) was determined using the equation:

CEC (meq/100g) = NH_4^+-N^+ (mg/L as N) / 14(NH_4^+-N^+ in extract - NH_4^+-N^+ in blank) (Eq. 5.1)

Total Organic Carbon

Total organic carbon (TOC) was determined using a method described by The Soil Science Society of America and The American Society of Agronomy (Sparks, 1996). Two milliliters of concentrated HCl was added to 1g of soil in glass vials. The samples were allowed to sit until frothing ceased and were then placed in a 103^{o} C oven to dry. After drying, 30mg was weighed, placed in a quartz crucible, and analyzed using the Elementar™ LiquiTOC Analyzer (Elementar Analysensysteme, Hanau, Germany). A standard curve was constructed for every site using sodium bicarbonate standards, and this curve was used to determine the total organic carbon present in the sample.

Water Holding Capacity

Water holding capacity (WHC) was determined as described by Whilke (2005). Thirty grams of soil were weighed and placed in a cylinder with a plugged base. To the cylinders, 30ml of DI water was added and the samples were allowed to sit for 1h. Following this time, the plug was removed from the cylinder and the excess water was allowed to drain. The moist soil was placed in a dry pre-weighed beaker and weighed. The beaker of moist soil was then dried overnight in a 105^{0} C oven and weighed after it was cooled. WHC was determined using the following equation:

$$WHC_{max}\ (\%\ \text{dry mass}) = (m_s - m_t \times 100) / (m_t - m_b) \quad \text{(Eq. 5.2)}$$

where:

m_s mass of beaker containing the water saturated soil (g)

m_t mass of beaker containing the oven dried soil (g)

m_b mass of beaker (g)

Bulk Density

Bulk density was determined in the field using the excavation method as described by Whilke (2005). The soil surface was leveled off using a straight metal blade, and a hole was dug to avoid compaction of the sides. The excavated soil was placed in a heavy paper-lined soil sampling bag (Fisher Scientific Inc., Pittsburgh, PA) and transported to the laboratory for further analysis. The hole was lined with plastic film and filled with sand. The surface was leveled and care was taken not to compact the sand. The sand was then excavated and the volume determined using a graduated cylinder. In the laboratory, the mass of the excavated soil was determined, and stones and gravel were separated from the fine soil using a 2mm sieve. The dry stones and gravel were then weighed, dried in a 105^0 C oven, and reweighed after cooling. The water content of the fine soil was determined by weighing 5g of the sample in a 105^0 C oven and reweighing after cooling. Bulk density was determined using the following equations:

$$\varphi_b = \frac{m_x - m_{tp}}{V} \quad \text{(Eq. 5.3)}$$

$$m_{tp} = m_{pw} - m_{xw} - m_w \quad \text{(Eq. 5.4)}$$

$$m_w = m_{pw} \times m_{tw} \quad \text{(Eq. 5.5)}$$

$$m_{tw} = m_{pw} - m_{xw} \quad \text{(Eq. 5.6)}$$

where:

φ_b bulk density (g/cm^3)

m_x mass of stones and dry gravel (g)

m_{tp} mass of dry fine soil (g)

V volume of the hole (cm^3)

m_{pw} mass of excavated moist soil (g)

m_w mass of the water excavated from the fine soil (g)

w water content of the excavated moist fine soil (g/g oven-dried soil)

m_{tw} mass of the moist fine soil (g)

m_{xw} mass of the moist gravel and stones (g)

Data Analysis of Chemical and Physical Parameters

All data analyses were performed using SAS/STAT statistical software (SAS Institute, Cary, NC). Data were tested for normality using the Shapiro-Wilk test. WHC,

CEC, and particle sizes were normally distributed. Bulk density, pH and TOC displayed a lognormal distribution and were log transformed to achieve normality. Parameter comparisons between land uses were performed using ANOVA tests and significant differences between sites were detected using Tukey's test. Linear regressions were performed using the normally distributed data set for each parameter.

Microbial Soil Analysis/Carbon Use

Fifteen soil samples were collected and assayed for each of the 6 sites to examine carbon use patterns of the microbial community on a quarterly basis. One gram of collected soil was added to 20ml of sterile phosphate buffered saline and vortexed to disperse soil particles. One hundred fifty microliters of the soil solution was pipetted into each of the 96 wells on a Biolog® GN2 plates as described by the manufacturer (Biolog Inc., Hayward, CA) for the identification of gram negative bacteria. Each well contained a different carbon substrate, and use of that carbon substrate by the microbial population resulted in the reduction of tetrazolium violet resulting in development of a purple color. Plates were incubated at 28^{o}C for 24h and read using the Multiskan MMC 5111340 microplate reader (Fisher Scientific, Pittsburgh, PA) at an optical density of 570nm.

The measured absorbances for each well were standardized by subtracting the blank absorbance to determine the raw differences. Wells were considered positive if the raw difference was greater than or equal to the average absorbance of all 95 wells. The average well color development (AWCD) value for each well was determined using the following equation to express overall color development:

$$AWCD = [\Sigma(C - R)]/95 \quad \text{(Eq. 5.7)}$$

where:

C = absorbance of the control well

R = absorbance of the response well

Data were transformed for analysis using the AWCD for each plate determined by the following equation:

$$\text{Transformed AWCD} = (C - R) / \{[\Sigma(C - R)]/95\} \quad \text{(Eq. 5.8)}$$

Transformed AWCD values were analyzed at the land use level using the PRINCOMP procedure in SAS/STAT statistical software (SAS Institute, Cary, NC) to determine carbon use patterns based on land use type.

Results and Discussion

Physiochemical Soil Parameters

Summary statistics for the measured soil parameters are provided in Table 5.5. Results are not reflective of the full pedon structure and may not represent the heterogeneous nature of the soil, as samples were collected from the O and A horizons within 6 – 8 inches of the soil surface. These surface layers were analyzed as they are thought to be the most responsible for the transport of microorganisms into surface waters. The results of particle size analyses from the collected samples are shown in Figure 5.3. Significant differences in particle sizes were observed between all land use

groups, and these results demonstrate that the soil analyzed from every site can be classified as sandy soil.

Table 5.5. Mean, standard deviation, and range of measured physical and chemical soil parameters

Site	Land Use	Textural Classification	pH	CEC (meq/100g)	Water Holding Capacity (%)	Total Organic Carbon (%)	Bulk Density (g/cm^3)
1	Agricultural	Sand	6.95 (0.44) [6.0 – 7.3]	0.03 (0.01) [0.015 – 0.05]	17.90 (11.78) [3.76 – 44.85]	2.38 (1.93) [0.31 - 7.53]	1.02 (0.18) [0.47 – 2.29]
2	Agricultural	Sand	7.08 (0.23) [6.7 – 7.4]	0.05 (0.02) [0.008 – 0.08]	54.36 (12.68) [33.31 – 84.20]	4.12 (2.92) [0.76 – 11.89]	0.61 (0.38) [0.14 – 1.34]
3	Agricultural	Sand	7.71 (0.24) [6.7 – 7.6]	0.06 (0.02) [0.02 – 0.09]	65.10 (16.44) [41.28 – 86.85]	10.94 (7.20) [0.98 – 22.53]	0.64 (0.28) [0.21 – 1.01]
4	Agricultural	Sand	6.98 (0.44) [5.6 – 7.4]	0.04 (0.04) [0.01 – 0.19]	51.71 (17.15) [31.37 – 96.10]	4.70 (8.06) [0.63 – 33.06]	0.45 (0.23) [0.18 – 0.81]
5	Agricultural	Sand	6.84 (0.27) [6.5 – 7.2]	0.04 (0.01) [0.02 – 0.05]	57.69 (7.65) [43.77 – 70.95]	9.61 (6.69) [1.51 – 25.13]	0.35 (0.18) [0.10 – 0.60]
6	Urban	Sand	6.78 (0.54) [5.8 – 7.5]	0.04 (0.1) [0.01 – 0.05]	53.28 (8.85) [30.87 – 60.52]	5.67 (3.14) [0.99 – 10.41]	1.15 (0.66) [0.21 – 2.77]
7	Urban	Sand	7.18 (0.15) [6.9 – 7.4]	0.05 (0.03) [0.02 – 0.09]	62.14 (17.02) [31.65 – 88.15]	6.38 (5.35) [1.33 – 19.28]	0.32 (0.31) [0.03 – 1.13]
8	Urban	Sand	7.21 (0.10) [7.0 – 7.4]	0.07(0.07) [0.04 – 0.08]	66.31 (6.48) [55.20 – 81.54]	6.12 (3.40) [0.59 – 12.49]	1.28 (0.78) [0.13 – 2.84]
9	Urban	Sand	6.93 (0.20) [6.7 – 7.1]	0.06 (0.02) [0.02 – 0.08]	57.42 (12.58) [31.55 – 77.12]	7.15 (5.65) [1.36 – 21.98]	0.44 (0.30) [0.09 – 1.09]
10	Urban	Sand	6.68 (0.30) [5.7 – 7.0]	0.05 (0.01) 0.03 – 0.07]	63.38 (8.72) [52.17 – 76.12]	10.52 (5.25) [4.90 – 23.60]	0.56 (0.39) [0.21 – 1.49]
11	Urban	Sand	6.79 (0.35) [5.9 – 7.1]	0.06 (0.01) [0.03 – 0.08]	63.29 (7.57) [48.18 – 75.78]	5.71 (2.45) [3.69 – 10.66]	0.41 (0.44) [0.04 – 1.47]
12	Urban	Sand	7.21 (0.10) [7.1 – 7.4]	0.07 (0.07) [0.05 – 0.14)	66.78 (6.66) [52.24 – 78.13]	21.13 (6.73) [9.13 – 35.57]	1.22 (0.83) [0.16 – 2.71]
13	Forest	Sand	6.20 (0.66) [4.7 – 7.1]	0.05 (0.03) [0.01 – 0.11]	59.57 (18.00) [33.08 – 88.92]	24.04 (24.17) [1.83 – 65.47]	1.32(0.94) [0.20 – 2.99]
14	Forest	Sand	5.43 (0.25) [5.1 – 6.0]	0.05 (0.02) [0.02 – 0.08]	63.27 (13.91) [36.04 – 85.28]	31.23 (27.47) [0.76 – 84.24]	0.62 (0.53) [0.07 – 1.81]

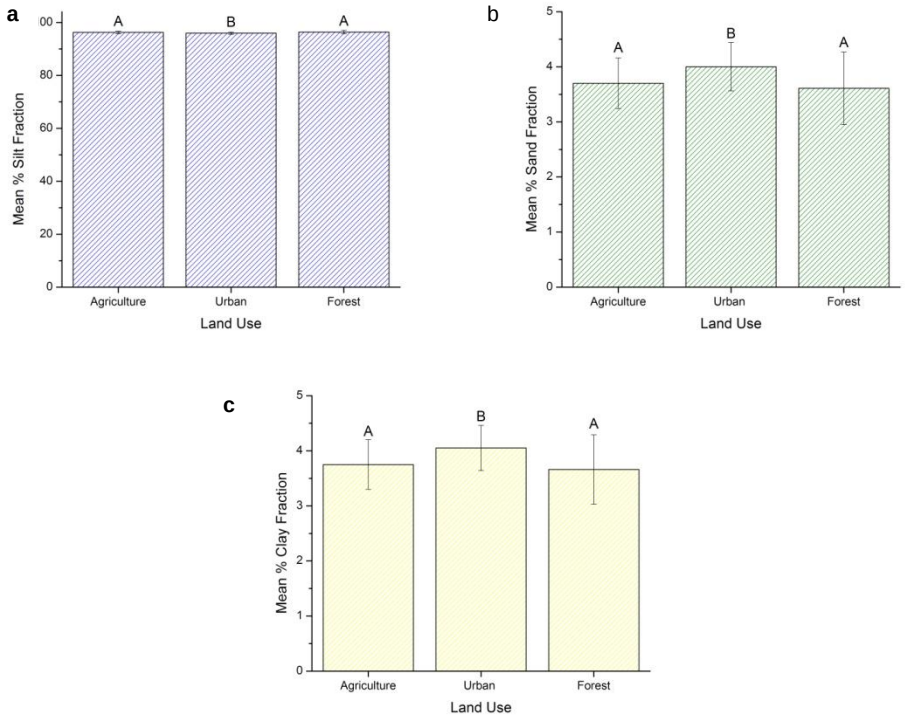

Figure 5.3. Mean texture composition values for silt (a), sand (b) and clay (c) (significant differences are indicated by different letters)

Statistically significant differences in organic matter content were observed between all land use groups (Figure 5.4). The increased percentages of total organic carbon at urban and forest land use sites is expected based on the presence of leaf litter and plant matter in the upper soil surface. These findings can also be explained by the observed particle sizes, as more carbon is typically found in coarse soils than in clays (Peinemann et al. 2000). The presence of organic matter can improve overall soil quality through nutrient cycling and can increase soil acidity through the release of CO_2 (Zhang et al. 2008), yet decomposition rates are also slowed by low pH values (Motavalli, 1995).

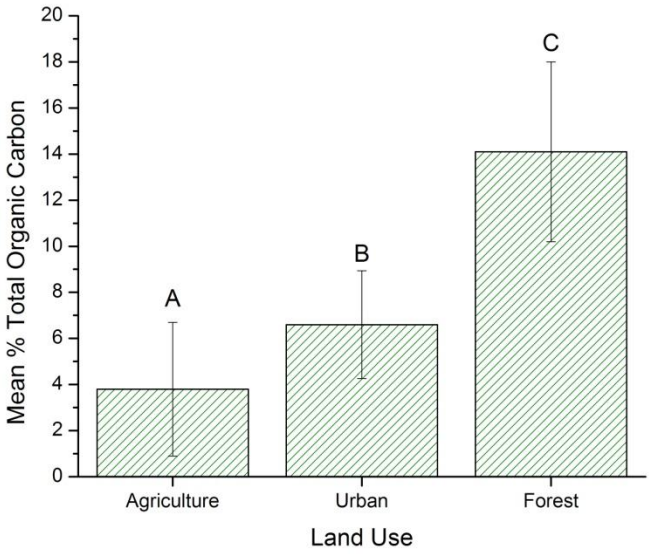

Figure 5.4. Mean total organic carbon values (significant differences are indicated by different letters)

The relatively low CEC activity can likely be attributed to the high fraction of sand in the samples and the lack of negatively charged binding sites on sand particles. The presence of charged carboxyl groups on organic matter and low percent fractions of clay in the upper soil horizons likely contributed to the observed low CEC activity (Parfitt et al. 1995, Schjønning, 1999). CEC activity of the collected soil may be due to the presence of organic matter which can block available binding sites for CEC activity (Peinemann et al. 2000), as demonstrated by the significant correlation between the organic matter and CEC (Figure 5.5). Though the presence of clay particles can also influence CEC, organic matter has a greater influence on CEC activity compared to particle size (Peinemann et al. 2000). Figure 5.6 suggests that clay content also influences CEC but not as strongly as organic matter content. Soil pH results are

displayed in Figure 5.7 and the lowest pH values were observed at the forest sites. Acidic soils have been shown to slow decomposition rates (Motavalli, 1995). The low pH values at forested sites along with the presence of leaf litter help explain the higher organic matter contents observed at the forested sites.

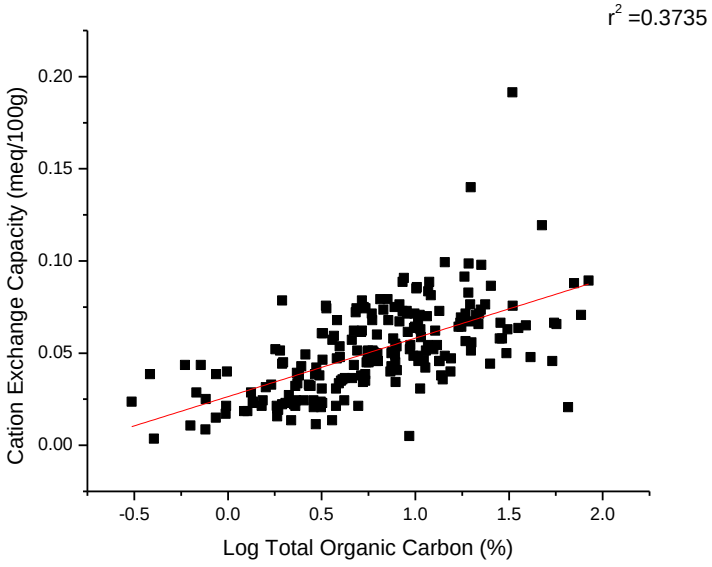

Figure 5.5. Linear relationship between TOC and CEC

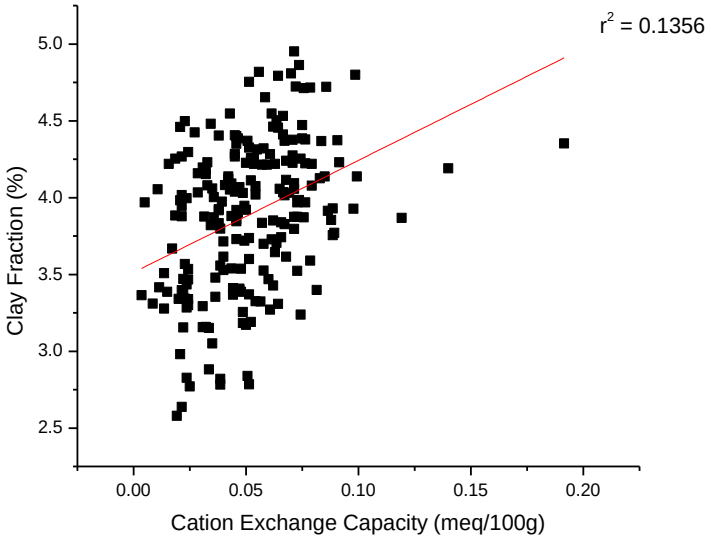

Figure 5.6. Linear relationship between CEC and clay fraction

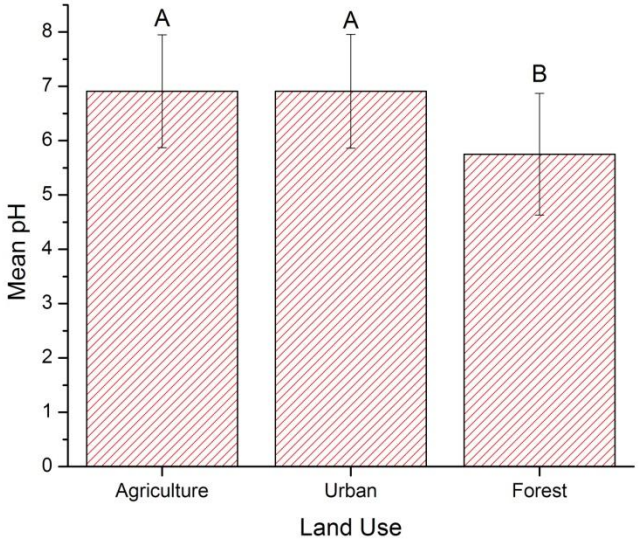

Figure 5.7. Mean pH values by land use (significant differences are indicated by different letters)

Observed water holding capacity percentages are displayed in Figure 5.8. Agricultural sites have a significantly lower water holding capacity compared to urban and forest land use sites. Agricultural sites along Sinking Creek had been affected by drought conditions at the time of soil sampling and site 1 the only site that had a dry creek bed at the time of sampling. The lack of water at this site may have influenced sedimentation of silt and clay particles, resulting in the observed low water holding capacity compared to other sites along Sinking Creek (Jenny, 1980). An expected, significant correlation was observed between CEC and WHC (Figure 5.9). Low CEC values are the result of a lack of negatively charged binding sites from either clay particles or the presence of organic matter. CEC values increase as binding sites become available and these binding sites can also retain moisture. A significant correlation is also observed between organic matter content and water holding capacity (Figure 5.10). This observation is also expected, as water content and holding capacity are related to organic matter content (Kemmitt et al. 2006; Li et al. 2007). This finding also supports the previous suggestion that the presence of organic matter is more influential than clay particles in CEC activity of these soils.

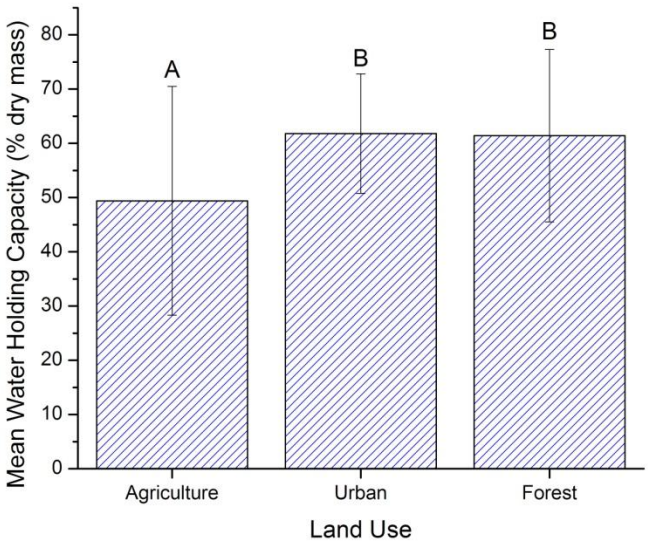

Figure 5.8. Mean water holding capacity values (significant differences are indicated by different letters)

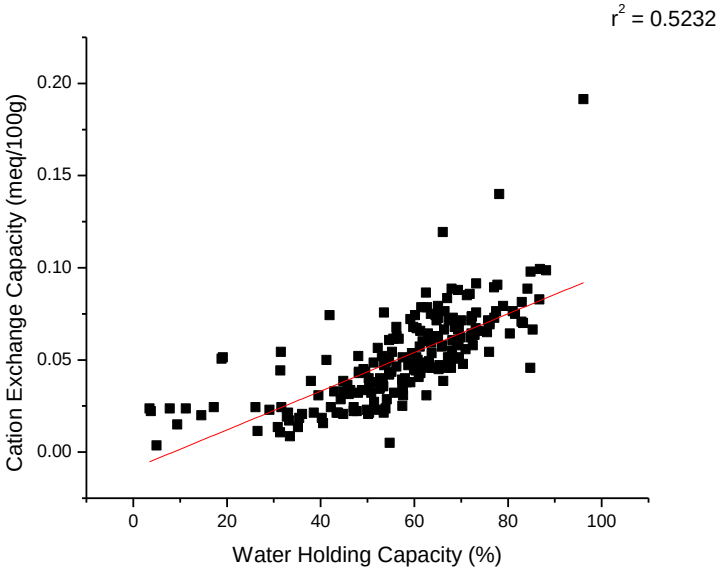

Figure 5.9. Linear relationship between WHC and CEC

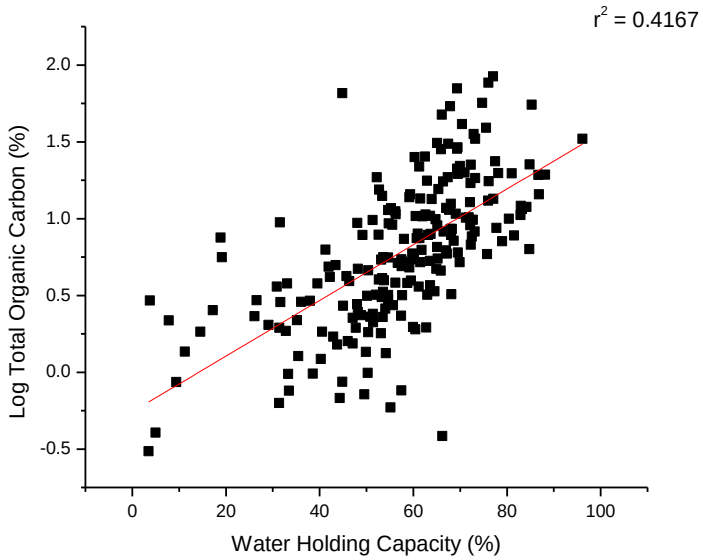

Figure 5.10. Linear relationship between WHC and TOC

Bulk density observations did not vary between land use groups (Figure 5.11) as was expected due to the coarse texture of the soils. Bulk density is a function of organic matter and an inverse relationship exists between these parameters (Li et al. 2007). Results from these soils indicate an almost absent correlation between bulk density and organic matter. An inverse relationship between WHC and bulk density should also be observed, as an increase in bulk density should result in a decrease of soil porosity and, consequently, WHC (Li et al. 2007). Similar to the observed relationship between bulk density and organic matter, there is almost no correlation between the 2 parameters.

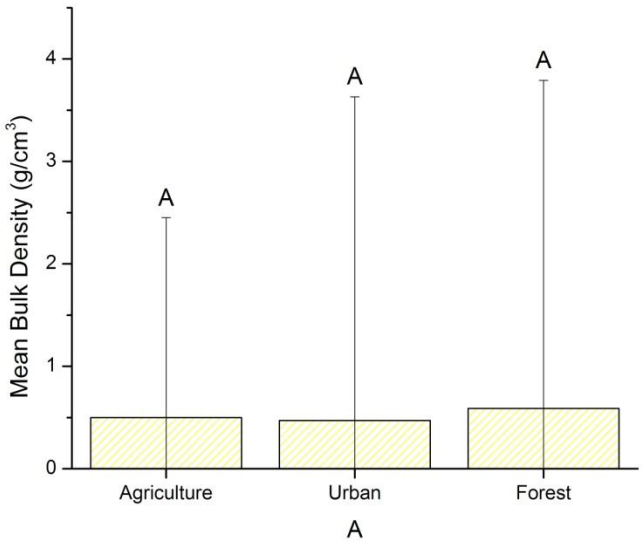

Figure 5.11. Mean bulk density values (significant differences are indicated by different letters)

Canonical discriminant analysis based on the measured physiochemical soil parameters demonstrates variability based on land use (Figure 5.12, Table 5.6). Agriculture and urban land use sites are nearly identical along the first canonical variable, which is defined by higher pH values and low TOC concentrations compared to forest land use sites. Separation is seen between all land use groups along the second canonical variable. This canonical variable is defined by particle size, CEC, and WHC.

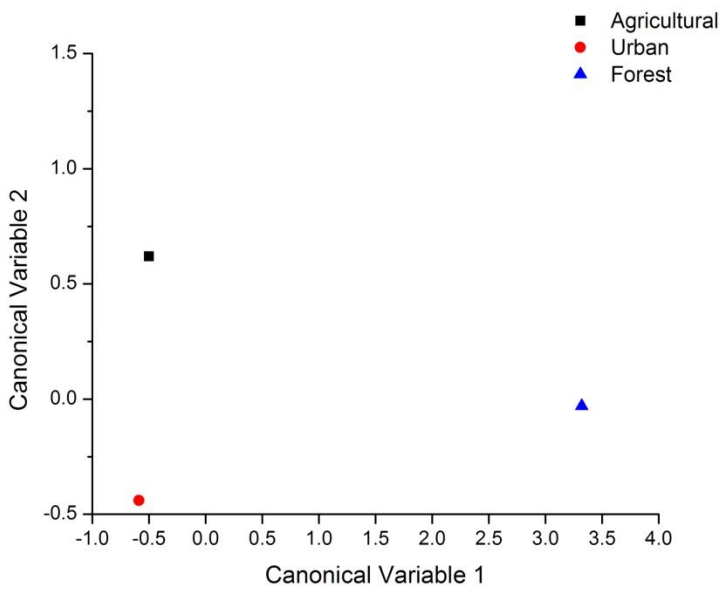

Figure 5.12. Canonical means of physiochemical soil parameters by land use

Table 5.6. Description of canonical structure for physiochemical soil properties

Canonical Variable	Variables Describing the Canonical Structure
Canonical Variable 1	pH (-0.89)
	Total Organic Carbon (0.63)
Canonical Variable 2	% Silt Fraction (0.59)
	Cation Exchange Capacity (-0.49)
	% Sand Fraction (-0.59)
	% Clay Fraction (-0.64)
	Water Holding Capacity (-0.77)

Influences of Physiochemical Soil Parameters on Water Quality

Canonical correlation analysis of physiochemical soil parameters and pathogen concentrations did not reveal any significant correlations, which was expected based on the failure to detect *E. coli* O157:H7 or *Shigella sp.* and the infrequent detection of *Giardia, Cryptosporidium* or bacteriophage. Although no significant correlations were observed between soil parameters and pathogen presence, physiochemical properties including matric potential, cation exchange capacity, and sorption processes can aid in the prevention of pathogen introduction into surface water. The low CEC values observed in this study combined with the ability of *Giardia* and *Cryptosporidium* to adhere to soil particle and enter the water as free individuals (Dai et al. 2003) may account for the observed protozoan concentrations in Sinking Creek.

Canonical discriminant analysis was also conducted to determine the influence of physiochemical soil parameters on fecal pollution in Sinking Creek and assess the usefulness of soil properties in predicting water quality. Physiochemical soil properties demonstrate predictive ability of surface water quality based on land use (Figure 5.13, Table 5.7). The strong separation of all land use groups suggests the influence of soil erosion and soil particle size on fecal pollution loading in Sinking Creek. The first canonical variable is influenced greatly by alkalinity and hardness. The ions that contribute to alkalinity and hardness concentrations in water may be introduced by the erosion of soil and geologic formations such as shale, sandstone, siltstone, and limestone, all of which are commonly found in Northeast Tennessee. Agricultural sites are most impacted by soil erosion, followed by urban sites. Forest land use sites are influenced by surface runoff to a lesser extent than are agricultural and urban land use

sites. The presence and erosion of sandy soils at these sites likely contribute to fecal coliform and *E. coli* loading into Sinking Creek, as microorganisms will adsorb more to finer textured soils than to coarse textured soils (Abu-Ashour et al. 1998; Hijnen et al. 2005). Compared to silt and clay particles, sand particles have a smaller surface area and thus less potential for microbial adsorption (Aislabie et al. 2001; Chu et al. 2003). This becomes particularly important as microbial movement through soil is primarily affected by adsorption and filtration processes (McLeod et al. 2001). Without these processes, microorganisms can move quickly through soils into receiving waters.

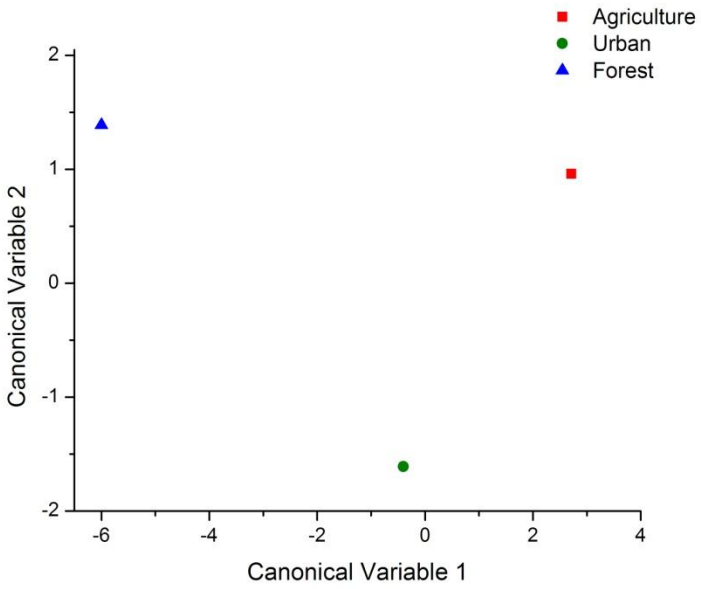

Figure 5.13. Canonical means of physiochemical soil parameters and water quality parameters by land use

Table 5.7. Description of canonical structure for physiochemical soil properties and water quality variables

Canonical Variable	Variables Describing the Canonical Structure
Canonical Variable 1	Hardness (0.96) Alkalinity (0.92) Soil pH (0.66) Total Coliforms in Water (0.48) Colilert (0.46) Fecal Coliforms in Water (0.44) Nitrates (0.41) Fecal Coliforms in Sediment (0.34) Soil Total Organic Carbon (-0.56)
Canonical Variable 2	% Silt Fraction (0.46) Colilert (0.39) Soil Total Organic Carbon (0.35) Fecal Coliforms in Water (0.34) Soil pH (-0.39) % Sand Fraction (-0.45) % Clay Fraction (-0.50)

The second canonical variable is defined by soil particle size measures. Urban sites are characterized by higher clay fractions compared to agricultural and forest land use sites, and likely result in greater microbial retention as a result of adsorption and filtration processes. The influence of soil particle size and pH along the second canonical variable may account for differences in fecal coliform and *E. coli*

concentrations between urban and agriculture land use patterns. Total organic carbon concentrations also influence the second canonical variable, and it would appear that organic matter has a strong influence on microbial fate and transport in soil in the Sinking Creek watershed at forest and agricultural land use sites compared to urban land use sites. Organic matter has been shown to be a significant factor influencing microbial transport through soil, even more so than CEC (Stevik et al. 1999). The inclusion of total organic carbon in the canonical discriminant structure suggests the influence of organic matter on microbial fate and transport in soil at these land use sites. Leaf litter incorporated at the soil surface may be significantly contributing to microbial surface runoff compared to subsurface transport and deposition because of the reliance of surface transport through the soil matrix by microorganisms (Agnelli, 2004; Atalay et al. 2007). These results suggest that physiochemical soil properties influence the observed water quality and that soil characteristics have some predictive value in determining fecal coliform and *E. coli* loading in Sinking Creek, as soil erosion, particle size, and total organic carbon concentrations influence the transport of fecal pollution from source to receiving waters.

Microbial Soil Analysis/Carbon Use

Transformed AWCD values were analyzed at the land use level using principal component analysis to determine carbon use patterns based on land use type. Distinct patterns of carbon use were observed based on land use (Figure 5.14). Similar PC scores were observed for the first principal component, which explains 66.2% of the total variance in microbial activity. The use of N-acetyl-D-galactosamine, adonitol, D-

arabitol, L-fucose, m-inositol, lactulose, cis-aconitic acid, citric acid, β-hydroxybutyric acid, α-ketogluaric acid, D-saccharic acid, 2,3-butanediol, L-aspartic acid, L-theronine, inosine, bromocuccinic acid, and 2-aminoethanol did not differ based on land use patterns. The poor correlation of a these carbon sources to land use does not necessarily indicate that the carbon source was poorly used at any particular land use type but that their use was not significantly different between land use patterns. It should be noted that the carbon sources used are a measure of functional potential and are not reflective of *in situ* microbial activity (Garland and Mills, 1991).

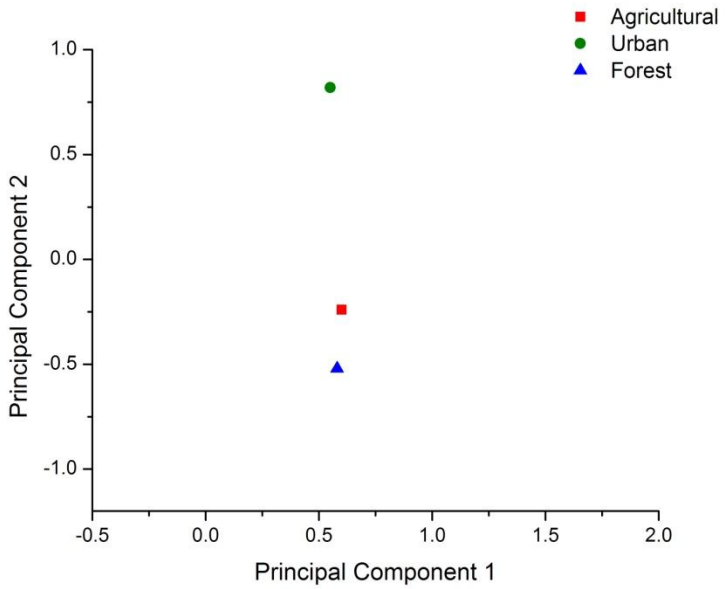

Figure 5.14. Ordination produced from principal component analysis of soil samples by land use pattern

The higher PC values for the urban land use classification along the second principal component indicate a greater response to particular carbon sources by these microbial communities and account for 19.3% of the variance in the data (Table 5.8).

Microbial communities at urban land use sites were able to use all of the 9 carbon source categories except for polymers. The carbohydrates more commonly and frequently used by microbial communities at urban land sites are ubiquitous in the environment or exist as a metabolite of carbohydrate use. Fructose, an isomer of glucose, can be produced by the hydrolysis of raffinose. Its use can result in the production of other metabolites that are frequently metabolized at urban land use sites. Lactose and melibiose can both be hydrolyzed to produce glucose and galactose that can then be used by the microbial community. Metabolism of melibiose can also result in the production of lactose, maltose, sucrose, and trehalose. Mannitol may be present as a metabolite of fructose or fermentation products and its oxidation results in the production of mannose, which is also used at urban land use sites. Sorbitol and L-arabinose are obtained by the reduction of glucose Maltose is produced by the breakdown of starch, a product of carbohydrate fermentation, and metabolites may include lactose, melibiose, sucrose, and trehalose.

Table 5.8. Carbon sources more commonly used at urban land use sites

Carbohydrates	**Amides**
L-Arabinose	Succinamic Acid
D-Fructose	Glucuronamide
D-Galactose	
Gentiobiose	**Phosphorylated Chemicals**
α-D-Glucose	D,L-α-Glycerol Phosphate
α-Lactose	
Maltose	**Amino Acids**
D-Mannitol	D-Alanine
D-Mannose	L-Alanyl-Glycine
D-Melibiose	L-Glutamic Acid
D-Psicose	Glycyl-L-Glutamic Acid
D-Raffinose	Hydroxy-L-Proline
L-Rhamnose	L-Leucine
D-Sorbitol	L-Phenylalanine
Sucrose	L-Proline
	L-Pyroglutamic Acid
Carboxylic Acids	
Formic Acid	**Aromatic Chemicals**
D-Galactonic Acid Lactone	Thymidine
D-Galacturonic Acid	Uridine
D-Gluconic Acid	
D-Glucoronic Acid	**Amines**
γ-Hydroxybutyric Acid	Phenylethylamine
D,L-Lactic Acid	
Propionic Acid	**Esters**
Succinic Acid	Methylpyruvate
Alcohols	
Glycerol	

Carboxylic acids are weak organic acids that are metabolized using the Krebs Cycle. The carboxylic acids used are typically the weak acids of carbohydrates that were more commonly used at urban land use sites. The amides used at these sites are the hydrolyzed forms of their carboxylic acids and the ester used is also a product of condensation of an alcohol with a carboxylic acid. Amino acids are used to build proteins, provide energy, and produce aromatic chemicals. Some of the amino acids more easily used at these sites suggest anthropogenic influences on functional potential. Glutamic acids are commonly used as food additives (MSG) and as pesticides, L-proline is used in pharmaceutical and biotechnical applications, and L-

pyroglutamic acid is used as dietary supplements. The ability of the microbial community to use aromatic chemicals including thymidine and uridine produced from amino acids is evident at urban sites. The increased use of thymidine and leucine has been associated with an increase in heavy metals (Díaz-Raviña and Bååth, 1996). In addition to potential anthropogenic influences from amino acids, amines such as phenylethylamine are also associated with therapeutic drugs (Paetsch and Greenshaw 1993).

Similarly, the lower PC values for the agricultural and forest sites compared to those at urban sites along the second principal component indicate lower responses to particular carbon sources by the microbial communities (Table 5.9). Carbon source use at these sites was different from that at urban sites, as microbial were able to use all 9 of the carbon source categories. Those carbohydrates more frequently used at these sites tend to be more complex sugars. Cellobiose is the product of the microbial metabolism of cellulose, a component of plant cell walls that is introduced as detritus. The ability of the microbial communities to use cellobiose suggests that microbial communities are frequently processing organic matter and detritus at these sites. Sugar alcohols were also frequently used as carbon sources, including *i*-erythritol and xylitol. The phosphorylated chemicals that are used are intermediates of glycolysis and the pentose phosphate pathway. The ability of the microbial communities to use polymers in addition to phosphorylated chemicals may suggest the microbial communities are capable of degrading complex sugars or that these carbon sources are metabolized when additional carbon sources are not available. Carbon sources may be limited at agricultural land use sites depending on seasonality and the removal of vegetation

resulting in a limited availability of organic matter. Similarly, seasonal effects of detritus may account for the use of these compounds at forest sites. The carboxylic acids and amino acids used at these sites also reflect metabolites of the carbohydrates that are more commonly used. The ability of the microbial communities to use urocanic acid from histadine catabolism is often associated with mammalian skin and sweat and suggests the influence of livestock and wildlife populations at these land use sites. The influence of wildlife is also supported by the use of putrescine at these land use sites, indicating the ability of the microbial communities to use decomposing organisms (Paczowski and Schütz, 2011).

Table 5.9. Carbon sources more commonly used at agriculture and forest land use sites

Carbohydrates	**Amino Acids**
N-Acetyl-D-Glucosamine	L-Alanine
Cellobiose	L-Asparagine
i-Erythritol	Glycyl-L-Aspartic Acid
α-D-Glucose	L-Histadine
β-Methylglucoside	L-Ornithine
D-Trehalose	D-Serine
Turanose	L-Serine
Xylitol	D,L-Carnitine
	γ-Aminobutyric Acid
Carboxylic Acids	
Acetic Acid	**Aromatic Chemicals**
D-Gluconic Acid	Urocanic Acid
D-Glucosaminic Acid	
α-Hydroxybuteric Acid	**Amines**
p-Hydroxyphenylacetic Acid	Putrescine
Itaconic Acid	
α-Ketovaleric Acid	**Esters**
Malonic Acid	Mono-Methylsuccinate
Quinic Acid	
Sebacic Acid	**Polymers**
	Glycogen
Amides	α-Cyclodextrin
L-Alaninamide	Dextrin
	Tween 80
Phosphorylated Chemicals	Tween 40
Glucose-1-Phosphate	
Glucose-6-Phosphate	

The difference in carbon source use by microbial communities by land use patterns indicates the ability of these microbial communities to use an array of carbon sources. Although all of the carbon sources were able to be used by the microbial communities, some microbial communities were more successful in the use of particular carbon sources than others. Overall, the more complex carbon sources were used by the microbial communities at agricultural and forest land use sites, suggesting more specialized microbial communities compared to those at urban land use sites that used simpler carbon sources more readily. Although the ability of the microbial communities to use some carbon sources associated with anthropogenic activity, these results should be interpreted with caution as the carbon utilization patterns are a measure of functional potential rather than of *in situ* activities.

Conclusion

Because fecal pollution in the Sinking Creek watershed has been associated with surface runoff, it is necessary to understand the role of soil in the fate and transport of pathogens from sources to receiving waters. The objective of this group of experiments was to examine the physical and chemical soil properties at the 14 established water sampling sites on Sinking Creek to better understand the interactions between the soil structure and pathogens. Based on the coarse soil texture and presence of organic matter on the soil surface, it can be suggested that soil contributes to the introduction of fecal pollution into Sinking Creek. Understanding these interactions can lead to better design and implementation of BMPs to remediate and prevent fecal contamination in the Sinking Creek. Analysis of soil microbial activities indicates the ability of the

microbial communities along Sinking Creek to use an array of sole carbon sources. Preferential use of these carbon sources is evident, as the microbial communities at urban land use sites tend to use simpler carbon sources and their metabolites while microbial communities at agricultural and forest land use sites appear to be more specialized in their ability to use complex carbon sources. The functional ability of these microbial communities to use carbon sources may help prevent the introduction of unwanted organic matter and fecal pollution in Sinking Creek. Future research should focus on the comparison and correlation of carbon sources used by microbial communities in stream sediments to those used by microbial communities in soil to further suggest sources of fecal pollution.

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CHAPTER 6

DEVELOPMENT OF MULTIPLE REGRESSION MODELS TO PREDICT SOURCES OF FECAL POLLUTION IN THE WATAUGA RIVER WATERSHED

K.K. Hall and P.R. Scheuerman

Abstract

The increased listings of surface waters on 303d lists and the need to address these through the Total Maximum Daily Load (TMDL) process has resulted in increased research to identify methods that effectively and universally identify the types and sources of fecal pollution to avoid adverse human health outcomes associated with fecal contamination of surface waters. In addition to correctly identifying the nature of pollutants and their sources, these methods should also be efficient and cost effective to ensure the maximum use of available resources to improve surface water quality. The current method of TMDL development is based on a watershed approach to identify stressors and monitor remediation efforts. This decision-making tool uses a strategic approach to quantify point and nonpoint sources of pollution and focuses on improved management decisions to implement the most effective best management practices (BMPs) to improve water quality and remove impaired waters from 303d lists. The objective of this experiment was to assess the usefulness of the watershed scale approach to TMDL development by developing and applying multiple regression models based on the Sinking Creek data collected in this study and determine if the developed model correctly classified land use patterns using 7 additional creeks within the Watauga River watershed. Correct land use classification using a multiple regression model for an entire watershed can help in the selection and implementation of effective

BMPs based on water quality within the Watauga River watershed to remove waters from the 303d list.

Introduction

The watershed approach to TMDL development as described by the United States Environmental Protection Agency (USEPA) takes a comprehensive approach to water resource management by focusing on the identification of stressors using monitoring data and ongoing water quality assessments to assess remediation efforts at the watershed level (USEPA, 1995). Watershed assessments involve (1) targeting priority problems, (2) using the efforts of stakeholders, (3) developing integrative solutions, and (4) measuring the success of the program (USEPA 1995). The ultimate goal of this decision-making tool is to effectively identify and quantify point and nonpoint sources of pollution to develop effective TMDLs that will improve water quality resulting in delisting of the water body from the 303d list, resulting in the protection of public and environmental health. This approach relies heavily on the application of strategic programs involving state water quality, health agencies, and stakeholders to identify, prioritize, and remediate water quality issues. The foundation of the watershed approach involves programs and activities to control point sources, restore habitats, monitor water quality, develop TMDLs, and enforce regulations to ultimately protect human and environmental health (Figure 6.1). The Tennessee Department of Environment and Conservation (TDEC) is currently involved in the identification of priority problems through water quality assessments and subsequent development of TMDLs for impaired watersheds. The development of TMDLs at the watershed level, as

opposed to individual water bodies, has been recommended by the USEPA in an effort to assess water quality management decisions more efficiently and allow for the focused application of financial resources on priority areas.

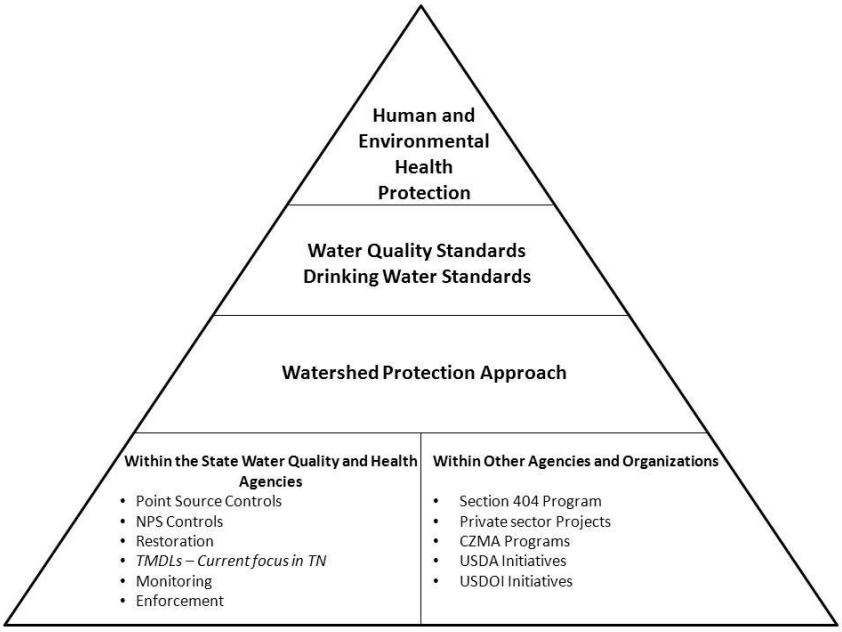

Figure 6.1. Framework for achieving the goals of the Clean Water Act (reproduced from USEPA 841-R-95-004, 1995)

The debate over what methods are able to effectively and efficiently address the quantity and sources of impairment in a watershed as it pertains to TMDL development has been ongoing. Several methods including ribotyping, pulsed-field gel electrophoresis, and antibiotic resistance analysis have been applied to correctly identify nonpoint sources of fecal pollution in surface waters. Ribotyping and pulsed-field gel electrophoresis allow for the discrimination between human and nonhuman sources of fecal pollution but rely on large geographically specific genetic databases to correctly classify sources (Tynkkynen et al. 1999; Carson et al. 2001). Similar to

ribotyping and pulsed-field gel electrophoresis, antibiotic resistance analysis also allows for the classification of fecal pollution sources based on antibiotic resistance of bacteria from human and animal sources. A major disadvantage of antibiotic resistance analysis is that it requires a large database that may be geographically specific (Wiggins et al. 1999). Although these methods may be regionally successful at identifying sources of fecal pollution, they cannot be universally applied to effectively identify and remediate fecal pollution to protect surface waters and public health.

The successful approach for the accurate identification of pollution sources to develop TMDLs that effectively reduce pollution is reliant on understanding the water quality variables and watershed characteristics that are most influencing water quality. Current pathogen TMDL development is based on the limited 30-day geometric mean that does not take into consideration seasonal effects, variability in land use patterns, or the influence of runoff events on water quality. TMDLs developed on a based on the 30-day geometric mean do not provide sufficient data to identify the presence of pathogens or sources of fecal pollution because they are based on a small sample size that may overlook sources of variability within the watershed.

The shortcomings of conventional methods of source identification suggest that alternative methods of water quality monitoring program design and data analysis are needed to better protect surface water resources. This research has suggested the use of canonical correlation and canonical discriminant analyses based on land use patterns to understand the influences of spatial and temporal variability on fecal pollution in Sinking Creek located in the Watauga River watershed. This approach for identifying the water quality variables that are most associated with fecal pollution may be more

successful at predicting water quality than more common data analysis methods, including multiple regression analysis.

An extension of simple linear regression, multiple regression analysis is a multivariate statistical tool that allows for the determination of a single dependent response variable based on several explanatory variables as described by:

$$y = a + b_1x_1 + b_2x_2 + \ldots + b_px_p \quad \text{(Eq. 6.1)}$$

where *y* is the predictor value, $a, b_1, b_2 \ldots b_p$ are constants and $x_1, x_2 \ldots x_p$ are the variables from which the prediction is made. The model is developed based on the variables that significantly contribute to the correct identification of the land use patterns (agriculture, urban, and forest). A successful model should be able to correctly classify the predictor variable based on the input of water quality data. Multiple regression models are commonly applied to water quality data to identify those water quality variables that are associated with fecal pollution (Ellis and Rodrigues, 1995; Mehaffey et al. 2005; Schoonover and Lockaby, 2005; Ham et al. 2009; Desai et al. 2010).

The successful development and application of a single multiple regression model from one water body to predict land use patterns, and the types and sources of pollution associated with those land use patterns, to others within a watershed can help meet the goals of the watershed approach to water resource management (Mehaffey et al. 2005). The simplicity of applying one model that correctly predicts land use patterns across an entire watershed can help reduce of the number of resources necessary to identify sources of impairment within individual bodies of water. This can further lead to the development and implementation of watershed TMDLs that have successfully

quantified point source and nonpoint source pollutants and identified their sources using time and cost effective methods. TMDLs that accurately reflect the extent and sources of pollution, and the variables contributing to water quality within the watershed are more likely to be successful at reducing pollution through the identification of priority areas and the implementation of successful BMPs to remove waters from 303d lists.

The objective of this experiment was to determine if a multiple regression model developed from one creek within the watershed was successful in predicting land use patterns and fecal pollution sources in additional creeks in the Watauga River watershed. Three multiple regression models were developed using the chemical and microbial water quality data collected during this study to assess the usefulness of multiple regression analysis compared to canonical discriminant analysis to classify land uses. The first regression model included all of the monitored chemical and microbial water quality parameters. The second model included only those chemical and microbial water quality parameters that were significant based on stepwise regression ($p < 0.05$), and the third model used those chemical and microbial water quality parameters identified by canonical discriminant analysis as most influencing water quality by land use. These multiple regression models were then applied to water quality data previously collected from 8 creeks within the Watauga River watershed (including Sinking Creek) to assess their ability to correctly classify land use classifications.

Materials and Methods

Sample Collection

The Watauga River watershed (HUC 06010103) is located in Carter, Johnson, Sullivan, Unicoi, and Washington Counties in Eastern Tennessee. Since 2003, creeks within the watershed were monitored to assess overall physical, chemical, and microbial water quality and to identify sources of impairment (Table 6.1). Sampling sites for each creek were selected using a targeted sampling approach and land use patterns were identified at each site (Tables 6.2 – 6.9). Ten sites on Boones Creek were monitored monthly from April 2005 to March 2006 and quarterly until December 2008. Twelve sites on Buffalo Creek were monitored monthly from June 2004 to June 2005 and quarterly until December 2008. Four sites on Carroll Creek and five sites on Reedy Creek were monitored monthly from June 2006 to May 2007 and quarterly until February 2008. Nine sites on Cash Hollow were monitored monthly from June 2003 to May 2004 and quarterly until October 2008. Eight sites on Knob Creeks were monitored monthly from June 2007 to April 2008. Fourteen sites on Sinking Creek were monitored monthly from June 2003 to May 2004 and quarterly until August 2011.

Table 6.1. Creeks monitored in this study within the Watauga River watershed

Creek	Waterbody ID	Location	Land Use
Boones Creek	TN 06010103006–1000	Washington	Combination of agricultural and urban
Buffalo Creek	TN 06010103011–1000	Carter	Combination of agricultural and urban
Carroll Creek	TN 06010103006–0100	Washington	Combination of agricultural and urban
Cash Hollow Creek	TN 06010103635–0100	Washington	Transition from urban to agricultural
Knob Creek	TN 06010103635–1000	Washington	Transition from agricultural to urban land use
Reedy Creek	TN 06010103061–1000	Washington	Transition from agricultural to urban
Sinking Creek	TN 06010103046–1000	Washington/Carter	Transition from forest to urban to agricultural

.

Table 6.2. Sampling locations on Boones Creek

Site Number	Site Description and Location	Land Use	Creek Characteristics
1	Upstream of bridge on Tavern Hill Road N 36°18.947', W 82°28.940'	Agricultural	Fine sediment
2	Downstream of first bridge on Hales Road N 36°19.216', W 82°28.702'	Agriculture	Fine sediment
3	Downstream of bridge at tributary on Hales Road N 36°19.209', W 82°28.221'	Agriculture	Fine sediment
4	Downstream of bridge on Bugaboo Springs Road N 36°19.956', W 82°28.065'	Agricultural	Fine sediment with cobbles
5	Upstream of bridge on Ridges Club Drive N 36°20.463', W 82°27.425'	Urban	Fine sediment with cobble
6	Downstream of bridge on Highland Church Road N 36°21.166', W 82°26.766'	Agricultural	Fine sediment
7	Downstream of I26 overpass on Memory Gardens Road N 36°22.774', W 82°25.491'	Urban	Fine sediment with gravel and cobble
8	Downstream of bridge off Quality Circle N 36°22.912', W 82°24.930'	Urban	Gravel, cobble and boulders
9	Downstream of bridge on Flourville Road N 36°23.511', W 82°24.086'	Agricultural	Fine sediment with cobble and boulders
10	Mouth of Boones Creek at Boone Lake N 36°23.460', W 82°23.752'	Urban	Gravel, cobble and boulders

Table 6.3. Sampling locations on Buffalo Creek

Site Number	Site Description and Location	Land Use	Creek Characteristics
1	US23 at Howard Gouge Road N 36°12.596', W 82°20.815'	Urban	Gravel
2	Downstream of pump station on US23 N 36°12.864', W 82°20.630'	Urban	Fine sediment with cobble
3	Downstream of output pipe on Sugar Hollow Road N 36°13.283', W 82°20.384'	Urban	Cobble and boulders
4	Upstream of bridge on Golf Course Drive at Buffalo Mountain Resort N 36°13.287', W 82°19.916'	Urban	Cobble
5	Downstream from golf course outflow at Country Club Drive N 36°14.114, W 82°19.690	Urban	Cobble
6	Upstream of bridge on Marbleton Road N 36°15.085', W 82°19.257'	Agricultural	Cobble
7	Wiseman Feed and Seed next to Fagan Road N 36°15.461', W 82°19.254'	Agricultural	Cobble
8	Downstream of Dave Renfro Bridge N 36°15.922', W 82°18.977'	Agricultural	Fine sediment with cobble and boulders
9	Upstream of bridge at Okalona Road and Bishop Road intersection N 36°17.111', W 82°18.505'	Urban	Gravel and cobbles
10	Walking bridge at Milligan College N 36°18.042', W 82°17.835'	Urban	Gravel and cobbles
11	Downstream of bridge on Reeser Road N 36°18.443', W 82°17.503'	Urban	Gravel and cobbles
12	Elizabethton Little League Park N 36°19.548', W 82°16.335'	Urban	Gravel and cobbles

Table 6.4. Sampling locations on Carroll Creek

Site Number	Site Description and Location	Land Use	Creek Characteristics
1	Upstream of bridge on Carroll Creek Road at Tara Court N 36°21.627', W 82°24.929'	Agricultural	Gravel and cobble
2	Upstream of overpass on Carroll Creek Road behind Food City N 36°22.638', W 82°24.548'	Agricultural	Gravel and cobble
3	Upstream from tree at Carroll Creek Road at Ranch Road N 36°22.940', W 82°24.068'	Agricultural	Gravel and cobble with boulders
4	Cedar Point Road at Cedar Point Place N 36°23.184', W 82°23.585'	Urban	Gravel and cobble with boulders

Table 6.5. Sampling locations on Cash Hollow Creek

Site Number	Site Description and Location	Land Use	Creek Characteristics
1	Upstream of crossing under Woodland Avenue N 36°20.881', W 82°20.795'	Urban	Fine sediment with cobble
2	Upstream of crossing under Crystal Springs Road N 36°20.877', W 82°20.804'	Urban	Fine sediment with cobble
3	Downstream of crossing under Crystal Springs Road N 36°20.883', W 82°20.806'	Urban	Fine sediment with cobble
4	Upstream of crossing under Lakeview Avenue N 36°21.135', W 82°20.686'	Urban	Fine sediment
5	Upstream of inflow from Convenience Center for Household Waste N 36°21.712', W 82°20.280'	Urban	Cobble
6	Downstream of inflow from Convenience Center for Household Waste N 36°21.715', W 82°20.280'	Urban	Cobble
7	Upstream of Morning Star Church on Cash Hollow Road N 36°22.022', W 82°20.527'	Urban	Cobble
8	Downstream of small bridge on Cash Hollow Road N 36°22.683', W 82°21.043'	Agricultural	Fine sediment with cobble
9	Upstream of boundary fence on Cash Hollow Road and Austin Springs Road N 36°22.829', W 82°21.286'	Agricultural	Fine sediment with gravel

Table 6.6. Sampling locations for Cobb Creek

Site Number	Site Description and Location	Land Use	Creek Characteristics
1	Downstream of bridge near Mountcastle Shopping Center N 36°20.328, W 82°22.106’	Urban	Cobbles and boulders
2	Upstream of overpass on Silverdale Drive N 36°21.072’, W 82°22.421’	Urban	Fine sediment with cobbles
3	Downstream of trees on West Brook Lane and Oakland Avenue N 36°21.214’, W 82°21.503	Urban	Fine sediment
4	Upstream of bridge on Austin Springs Road at Mary’s Salads N 36°22.081’, W 82°21.275’	Urban	Fine sediment
5	Downstream of Brush Creek Wastewater Treatment Plant N 36°22.376’, W 82°21.296’	Urban	Fine sediment and cobbles

Table 6.7. Sampling locations on Knob Creek

Site Number	Site Description and Location	Land Use	Creek Characteristics
1	Downstream of bridge on John France Road N 36°19.12.7', W 82°28.13.2'	Agricultural	Fine sediment
2	Downstream of bridge at intersection of Claude Simmons Road and Moss Circle N 36°19'.447', W 82°25.392'	Agricultural	Fine sediment
3	Downstream of bridge at gauging station next to Headtown Road N 36°19.127', W 82°28.132'	Agricultural	Fine sediment with cobble
4	Downstream from stream intersection at Knob Creek Road and Fairridge Road N 36°20.275', W 82°24.387'	Agricultural	Cobble
5	Downstream from gauging station next to tributary on Knob Creek Road N 36°20.283', W 82°24.330'	Urban	Fine sediment with cobble and boulders
6	Parking area at Café Pacifica on Oakland Avenue N 36°20.556', W 82°24.162'	Urban	Cobble and boulders
7	Northeast intersection of Oakland Avenue and N. Roan Street N 36°21.379', W 82°23.148'	Urban	Fine sediment with cobble
8	Big Valley Road N 36°2.211', W 82°22.304'	Urban	Cobble and boulders

Table 6.8. Sampling locations on Reedy Creek

Site Number	Site Description and Location	Land Use	Creek Characteristics
1	Stream crossing at Old Stage Road N 36°22.410', W 82°27.030'	Agricultural	Fine sediment
2	Boone Road off Old Stage Road N 36°23.043', W 82°26.319'	Agricultural	Cobble
3	Old Gray Station Road at The Ruritan Turkey Shoot Club N 36°23.753, W 82°26.449	Agricultural	Cobble
4	Downstream of bridge on White Street N 36°24.328', W 82°24.605'	Agricultural	Cobble
5	Cove entrance to Boone Lake on Crouch Road N 36°23.297, W 82°24.345	Urban	Cobble and boulders

Table 6.9. Sampling locations on Sinking Creek

Site Number	Site Description and Location	Land Use	Creek Characteristics
1	Downstream of Sinking Creek pump station on Sinking Creek Road N 36°20.118', W 82°18.035'	Agricultural	Cobble and boulders
2	Upstream of Bob Peoples bridge on Sinking Creek Road N 36°9.837', W 82°18.254'	Agricultural	Gravel and cobble
3	Upstream of Sinking Creek Church and North Road N 36°9.662', W 82°18.447'	Agricultural	Gravel and cobble
4	Upstream of crossing on Joe Carr Road N 36°9.594', W 82°18.579'	Agricultural	Fine sediment with cobble and boulders
5	Upstream of bridge on Dave Buck Road N 36°9.113', W 82°19.290'	Agricultural	
6	Downstream of bridge on Daytona Drive, old Sinking Creek pump station N 36°8.788', W 82°19.625'	Urban	Cobble and boulders
7	Upstream of bridge on Miami Drive, King Springs Baptist Church N 36°8.772', W 82°19.685'	Urban	Cobble
8	Upstream of Bosch NPDES discharge point N 36°8.472', W 82°19.948'	Urban	Cobble
9	Upstream of Twin Oaks golf Course storage area on Lafe Cox Drive N 36°7.887', W 82°20.741'	Urban	Cobble
10	Upstream of bridge crossing Sinking Creek at Hickory Springs Road N 36°17.431', W 82°21.397'	Urban	Gravel with cobble and boulders
11	Upstream of crossing at Miller Lane N 36°17.105', W 82°21.800'	Urban	Cobble and boulders
12	Upstream of tributary on David Miller Road N 36°16.967', W 82°21.970'	Urban	Cobble
13	Upstream of road crossing on Jim McNeese Road N 36°16.035', W 82°22.163'	Forest	Cobble and boulders
14	Downstream of path crossing at Dry Springs Road N 36°14.800', W 82°22.033'	Forest	Gravel with cobble and boulders

Sample Collection

Water samples for total and fecal coliform bacteria (TC/FC), standard plate counts (SPC), analyses were collected and analyzed in triplicate (SPC samples analyzed in duplicate) in sterile, 1-L Nalgene™ bottles. Water samples for Colilert® analyses were collected in sterile 100ml plastic bottles (IDEXX Laboratories, Westbrook, Maine). Water samples for nitrates (NO_3^-), phosphates (PO_4^-), ammonia (NH_3^+), 5-day biochemical oxygen demand (BOD_5), alkalinity, and hardness were collected and analyzed in triplicate in sterile 2-L Nalgene™ bottles. Sediment samples for TC/FC in water, microbial enzyme activity (MEA), and acridine orange direct counts (AODC) were collected in 2oz sterile Whirl-Pak™ bags. All samples were transported to the laboratory on ice and analyzed within the holding times described in Table 6.10. Field measurements for pH, air and water temperature, dissolved oxygen, and conductivity were also collected at each site.

Table 6.10. Physical, chemical, and microbial water quality parameters measured

Parameter	Abbreviation	Units	Holding Time
pH	pH	pH	Field measurement
Water temperature	WT	^{o}C	Field measurement
Air temperature	AT	^{o}C	Field measurement
Dissolved oxygen	DO	mg/l as O_2	Field measurement
Conductivity	Cond	µmohs	Field measurement
Fecal coliform in water	FCW	CFU/100ml	6h
Total coliform in water	TCW	CFU/100ml	6h
Fecal coliform in sediment	FCS	CFU/100ml	6h
Total coliform in sediment	TCS	CFU/100ml	6h
Colilert	Colilert	CFU/100ml	6h
Standard plate count	SPC	CFU/ml	6h
Acridine orange direct counts	AODC	cells/g sediment	6h
Acid phosphatase	AcidP	µg/g sediment	24h
Alkaline phosphatase	AlkP	µg/g sediment	24h
Dehydrogenase	DHA	µg/g sediment	24h
Galactosidase	Gal	µg/g sediment	24h
Glucosidase	Glu	µg/g sediment	24h
Nitrates	NO_3	mg/l	24h
Phosphates	PO_4^{2-}	mg/l	24h
Ammonia	NH_3^{+}	mg/l	24h
Biochemical oxygen demand	BOD_5	mg/l	24h
Hardness	Hard	mg/l	48h
Alkalinity	Alk	mg/l	24h

Quality assurance and quality control (QA/QC) practices included the analysis of chemical parameters consisted of one trip blank, one field blank, a negative control, one replicate, one spiked sample, and one quality control standard. QA/QC practices included in the analysis of microbial parameters included the analysis of one trip blank, one field blank, a negative control, and a positive control. A secondary wastewater effluent sample was used as the positive control for TC/FC, Colilert®, SPC, and bacteriophage analyses. Laboratory strains of *E. coli* O157:H7 and *Shigella flexneri*

(ATCC® Number 43895™ and ATCC® 12022™, respectively) were used to seed water samples that served as a positive control for PCR analysis.

Microbial Analyses

TC/FC analyses for water samples were conducted according to Standard Methods for Examination of Water and Wastewater (APHA, 1992). Briefly, 0.5ml of water were filtered through a 0.45µm membrane filter (EMD Millipore, Billerica, MA) and the filter placed in a petri dish containing an absorbent pad (EMD Millipore, Billerica, MA) with 2ml of m-Endo media for total coliform analysis or m-FC media for fecal coliform analysis. All plates were inverted and enumerated following 24h incubation at 37^{o}C and 44.5^{o}C for total coliform and fecal coliforms, respectively. For TC/FC sediment analyses, 0.5g of sediment was added to 25ml of sterile water + 1% Tween 80. The samples were vortexed and allowed to settle for 30 minutes, and 0.5ml of the buffer suspension was filtered according to Standard Methods for Examination of Water and Wastewater as described above (APHA, 1992).

SPC were conducted according to Standard Methods for Examination of Water and Wastewater (APHA, 1992) using R2A agar. One milliliter of water was placed in the center of a sterile petri dish (Fisher Scientific, Pittsburgh, PA) and 10ml of R2A agar was added to the dish. The plate was swirled in a figure eight motion to allow the sample to disperse in the media and cover the plate. Plates were allowed to solidify and were enumerated following incubation at 25^{o}C for 48h. *Escherichia coli* concentrations were determined using the Colilert® Quanti-Tray method (APHA, 1995). To each 100ml water sample, a packet of Defined Substrate Technology® (DST®)

reagent (IDEXX Laboratories, Westbrook, Maine) was added and mixed. The sample was then poured into a Quanti-Tray®, sealed using the Quanti-Tray® sealer, and incubated for 24h at 37^{o}C. *E. coli* were then enumerated using the Standard Method most probable number (MPN) procedure. Samples for water TC/FC were processed in triplicate and samples for sediment TC/FC were processed in duplicate. SPC were processed in duplicate and one Colilert® sample was processed for each site.

MEA analyses were conducted and included acid and alkaline phosphatases, glucosidase, galactosidase, and dehydrogenase activities. For each enzyme analyzed, 1g of sediment was added to a test tube containing a specific buffer and enzyme. Sediment samples for acid phosphatase were mixed with 4ml of 1M TRIS buffer (pH 4.8) and 4ml of 1M TRIS buffer (pH) 8.4 for alkaline phosphatase. For both acid and alkaline phosphatase, 1ml of 1M TRIS buffer with 0.1% phosphatase substrate (pH 7.6) was added to each tube (Sayler et al. 1979). Sediment samples for galactosidase and glucosidase activities were mixed with 4ml of 0.1M phosphate buffer (pH 9.0). Galactosidase activity was measured by adding 1ml of 0.01M phosphate buffer with 0.15% p-nitrophenyl-β-D-galactopyranoside as an indicator of galactosidase activity. One milliliter of 0.01M phosphate buffer with 0.15% 4-nitrophenyl-β-D-glucopyranoside was used as an indicator to assess glucosidase activities (Morrison et al. 1977). Following addition of buffers and indicators, all tubes were vortexted and incubated at 25^{o}C for 24h. Acid and alkaline phosphatase, galactosidase, and glucosidase activities were determined using a spectrophotometer at an absorbance of 418nm.

For dehydrogenase (DHA) activity, 1g of sediment was added to a test tube containing 2ml of 0.1M phosphate buffer (pH 7.6) and 1ml of 0.5% iodonitrotetrazolium

chloride (INT) salt solution. The samples were vortexed and incubated in the dark at 25^{o}C for 45 minutes. One milliliter of the sample was filtered through a 0.22µm porosity cellulose membrane (GE Water and Process Technologies, Trevose, PA) and allowed to dry at room temperature. The membrane, was then added to a test tube containing 5ml of dimethyl sulfoxide, vortexted to dissolve the membrane, and incubated in the dark at 25^{o}C for 24h. Dehydrogenase activity was then determined using a spectrophotometer at an absorbance of 460nm.

AODC analysis was performed as described by Ghiorse and Balkwill (1983). Three hundred milligrams of sediment was added to 30ml of sterile PBS+Tween 80, vortexed for 60s, and allowed to settle for 3h. Two hundred fifty microliters of the suspension was mixed with 5ml sterile water + 500µl acridine orange stain, and samples were vortexed for 30s. Samples were filtered using 25mm, 0.2µm pore polycarbonate nucleopore filters (Osmonics, Inc., Minnetonka, MN), and the filters were mounted and fixed on slides for enumeration at 1000X using the Olympus BH2 epifluorescent microscope (Olympus, New Hyde Park, NY). One sediment sample was processed per site and 3 microscopic fields were enumerated on each slide.

Chemical Analyses

NO_3^-, PO_4^-, NH_3^+, alkalinity, and hardness analyses were performed in triplicate using colorimetric HACH™ methods and HACH™ reagents as described by the manufacturer (HACH Company, Loveland, CO). Briefly, NO_3^-, PO_4^-, NH_3^+ analyses were conducted by adding 10ml of water to a vial containing the appropriate reagent packet; NitraVer5, PhosVer3 and salicylate/ammonia cyanurate reagents, respectively.

The vials were shaken to dissolve the reagent and samples were analyzed using pocket colorimeters specific to the nutrient of interest. Alkalinity and hardness analyses were conducted using 100ml sample volumes and a digital titrator. For alkalinity determination, 1 packet of phenolthalein indicator and bromcresol green-methyl red indicator were added to the sample and mixed. The sample was then titrated with 1.6N sulfuric acid to a grey-green endpoint. For hardness determination, 1 packet of ManVer2 reagent and 2ml of hardness buffer (pH 10) were added to the 100ml sample and mixed. The sample was then titrated with 0.8N Ethylenediaminetetraacetic acid (EDTA) to a blue endpoint. BOD_5 analyses were conducted according to Standard Methods for Examination of Water and Wastewater (APHA, 1992). Wheaton BOD bottles (Wheaton Science Products, Millville, NJ) were completely filled with sample water and capped with glass stoppers to ensure no air bubbles were present. Initial (Day 0) and final (Day 5) dissolved oxygen concentrations were measured using the YSI Model 5000 dissolved oxygen meter (YSI Inc., Yellow Springs, OH).

Statistical Analyses

Three multiple regression models were developed using the Sinking Creek data collected in this study in SAS/STAT software v.9.2 (SAS Institute, Cary, NC). The first model (model 1) contained all of the measured chemical and microbial water quality parameters (Table 6.11). The second model (model 2) contained only significant variables identified by stepwise regression (Table 6.12), and the third model (model 3) contained significant variables identified by canonical discriminant analysis (Table 6.13). All water quality data collected from Sinking Creek during this study and from the

additional creeks in the Watauga River watershed were log transformed to achieve a normal distribution and land use patterns were coded as follows: (1) = agriculture, (2) = urban, and (3) = forest. Only those parameters that were significant at the $p < 0.05$ level were considered significant and included in the stepwise regression model and canonical discriminant model. The multiple regression equations were then applied to water quality data collected from Boones, Buffalo, Carroll, Cash Hollow, Cobb, Knob, Reedy, and Sinking Creeks to assess the ability of the models to correctly classify land use patterns within the Watauga River watershed. Data from these creeks were also pooled and the ability of the Sinking Creek model to predict land use patterns was assessed at the watershed level.

Table 6.11. Chemical and microbial water quality parameters included in the full regression model

Variable	Abbreviation
Fecal coliform in water	FCW
Total coliform in water	TCW
Fecal coliform in sediment	FCS
Total coliform in sediment	TCS
Colilert	Colilert
Standard plate count	SPC
Acridine orange direct counts	AODC
Acid phosphatase	AcidP
Alkaline phosphatase	AlkP
Dehydrogenase	DHA
Galactosidase	Gal
Glucosidase	Glu
Nitrates	NO_3
Phosphates	PO_4^{2-}
Ammonia	NH_3^+
Biochemical oxygen demand	BOD_5
Alkalinity	Alk
Hardness	Hard

Table 6.12. Significant chemical and microbial water quality parameters included in the stepwise regression model 2

Variable	Abbreviation
Fecal coliform water	FCW
Fecal coliform sediment	FCS
Colilert	Colilert
Nitrates	NO_3^-
Biochemical Oxygen Demand	BOD
Alkalinity	Alk
Hardness	Hard

Table 6.13. Chemical and microbial water quality parameters identified by canonical discriminant analysis included in model 3

Variable	Abbreviation
Fecal coliform water	FCW
Colilert	Colilert
Alkalinity	Alk
Hardness	Hard

Results and Discussion

Regression equations for the 3 models are in Table 6.14. The first model contained all of the measured chemical and microbial water quality parameters. The second model contained only significant variables identified by stepwise regression ($p < 0.05$), and the third model contained significant variables identified by canonical discriminant analysis. All of the regression models were statistically significant ($p < 0.0001$), suggesting the ability of the models to successfully predict land use patterns in Sinking Creek based on the measured water quality parameters.

Table 6.14. Regression equations for the 3 developed models to predict land use in the Watauga River watershed

Model	Regression Equation	Adjusted r^2	p - value
1	LU = (log(FCW)*-0.20 - log(TCW)*0.02 + log(FCS)*0.18 – log(TCS)*0.06 – log(Colilert)*0.24 – log(NO_3)*0.35 – log(PO_4)*0.06 + log(NH_3)*0.03 – log(BOD)*0.35 – log(Alk)*0.55 – log(Hard)*0.70 – log(SPC)*0.17 – log(AODC)*0.04 – log(AcidP)*0.04 + log(AlkP)*0.02 + log(DHA)*0.12 + log(Galact)*0.02 – log(Gluc)*0.03) + 5.62	r^2 = 0.87	< 0.0001
2	LU = (log(FCW)*-0.20 + log(FCS)*0.18 – log(Colilert)*0.24 – log(NO_3)*0.35 – log(BOD)*0.35 – log(Alkalinity)*0.55 – log(Hardness)*0.70) + 5.62	r^2 = 0.88	< 0.0001
3	LU = (log(Colilert)*-0.28 – log(NO_3)*0.34 - log(Hardness)*0.94 – log(FCW)*0.12 – log(Alkalinity)*0.34) + 5.15	r^2 = 0.85	< 0.0001

Abbreviations: FCW = fecal coliforms in water, TCW = total coliforms in water, FCS = fecal coliforms in sediment, TCS = total coliforms in sediment, Colilert = *E. coli*, NO_3^- = nitrates, PO_4^{2-} = phosphates, NH_3= ammonia, BOD = biochemical oxygen demand, Alk = alkalinity, Hard = hardness, SPC = standard plate count, AODC = acridine orange direct counts, AcidP = acid phosphatase, AlkP = alkaline phosphatase, DHA = dehydrogenase, Galact = galactosidase, Gluc = glucosidase

The models were then applied at the watershed level to water quality data collected from 8 creeks within the Watauga River watershed (Table 6.15). All 3 models remained statistically significant ($p < 0.0001$) when applied to the Watauga River watershed data. Despite their significance, models 1 and 2 were only able to describe a relatively small amount of the variability within the data set based on their r^2 values. These low r^2 values reflect the influence of variability between water bodies within the same watershed. The water quality variables that are most influential in determining sources of impairment based on land use patterns in Sinking Creek are not the same throughout the watershed. For example, the variables influential in Boones, Cash Hollow, Cobb, Knob, and Reedy Creeks were similar and included total and fecal coliforms in water and sediment, nitrates, phosphates, alkalinity, hardness, and galactosidase. The variables influential in Buffalo and Carroll Creeks included fecal coliforms in water and sediment, hardness, and biochemical oxygen demand. The lower r^2 in model 2 compared to the model 1 reflects the influence of those chemical and microbial parameters throughout the entire watershed that were found to be insignificant during stepwise regression analysis of the collected Sinking Creek data. Those parameters identified as insignificant in model 2 include: total coliform bacteria in water and sediment, standard plate counts, acridine orange direct counts, acid and alkaline phosphatase, dehydrogenase, galactosidase, glucosidase, phosphates, and ammonia.

Table 6.15. Multiple regression statistics for the 3 multiple regression models applied to data from the Watauga River watershed

Model	p - value	Adjusted r^2
1	$p < 0.0001$	0.02
2	$p < 0.0001$	0.01
3	$p < 0.0001$	0.35

Model 3 was also significant and accounted for more variability at the watershed level compared to models 1 and 2. This model was developed using those variables found to be significantly contributing to the discrimination between land use patterns in Sinking Creek based on canonical discriminant analysis. This result suggests that prior determination of the chemical and microbial water quality variables that are most associated with degraded water quality as they pertain to land use patterns in one stream are similar to those variables contributing to degraded water quality throughout the entire watershed. This result highlights the combined usefulness of multivariate statistical analyses such as canonical discriminant and multiple regression analyses.

The multiple regression models were also applied at the creek level to determine if the model could successfully predict land use patterns and subsequent sources of impairment (Table 6.16). Models 1 and 2 were unable to predict land use patterns in all of the creeks except for Sinking Creek. The inability of a these models to accurately identify and classify sources of water quality impairment based on land use patterns suggests that the variables that are associated with water quality impairments within and between the surface waters of the watershed are different and that a simple multiple regression model may not be sufficient to identify sources of impairment as

they relate to land use. The ability of these regression models to predict land use patterns in previously collected data from Sinking Creek from 2003 – 2011 demonstrates that those variables most influencing water quality in Sinking Creek are influenced to some extent by temporal variability. Seasonality and succession of the stream system over time likely contribute to the inability of the models to account for all of the variability in Sinking Creek.

Table 6.16. Regression statistics for the 3 developed models as applied to each creek to predict fecal pollution source

Model	Creek	Adjusted r^2	p – value
1	Boones Creek	0.0003	p = 0.27
	Buffalo Creek	0.002	p = 0.11
	Carroll Creek	0.0003	p = 0.81
	Cash Hollow Creek	0.001	p = 0.51
	Knob Creek	0.001	p = 0.28
	Reedy Creek	0.001	p = 0.59
	Sinking Creek	0.08	p < 0.0001
2	Boones Creek	0.0004	p = 0.26
	Buffalo Creek	0.0007	p = 0.21
	Carroll Creek	0.0003	p = 0.81
	Cash Hollow Creek	0.0001	p = 0.83
	Knob Creek	0.0008	p = 0.37
	Reedy Creek	0.0002	p = 0.83
	Sinking Creek	0.34	p < 0.0001
3	Boones Creek	0.02	p = 0.04
	Buffalo Creek	0.04	p = 0.0012
	Carroll Creek	0.05	p = 0.10
	Cash Hollow Creek	0.004	p = 0.78
	Knob Creek	0.08	p = 0.008
	Reedy Creek	0.25	p < 0.0001
	Sinking Creek	0.74	p < 0.0001

Model 3 was more successful at predicting land use patterns at the creek level compared to models 1 and 2. This model included the variables that were identified through canonical discriminant analysis as those that allow for the most discrimination

between land use classifications based on water quality in Sinking Creek during 2011. Model 3 was able to predict land use patterns in all creeks with the exception of Carroll and Cash Hollow Creeks, with the greatest amount of variability accounted for within the previously collected Sinking Creek data. The failure of model 3 to predict land use and sources of impairment in Carroll and Cash Hollow Creeks is most likely due to the influence of unidentified nonpoint sources of fecal pollution in these creeks. Although all 3 models were statistically significant, model 3 developed using the chemical and microbial water quality variables that discriminate based on land use in Sinking Creek accounted for the most variability at the watershed and creek level. This finding suggests that canonical discriminant and multiple regression analyses can be used together to analyze water quality data and determine sources of impairment based on land use patterns.

The inability of models 1 and 2 and limited predictability of model 3 to successfully predict the land use classifications of these creeks agrees with results of previous studies conducted in the Watauga River watershed. These studies indicate that there is variability in the extent and sources of pollution within the watershed, and that the application of multivariate statistical analyses to water quality data can help identify those variables that contribute to degraded surface water quality differ based on land use patterns (Hall et al. 2007; 2008; 2011). The inability of these regression models to predict land use classifications throughout the watershed further supports these previous findings and suggests that those variables related to fecal pollution may vary spatially and temporally within a watershed.

The watershed TMDL approach does not account for sources of variability within the entire watershed and are currently based on a limited 30-day geometric mean. Canonical discriminant analysis can be used to address these sources of variability by identifying those variables that are most influencing water quality. It has been reported that multiple regression models developed from data collected from creeks should be used with caution as they may not be representative of all streams within the watershed or reflect true watershed dynamics (Schoonover and Lockaby, 2006; Toor et al. 2008; Kang et al. 2010). The results of this study support these findings and further suggest that TMDL development may require long term monitoring to correctly identify and quantify pollution sources using multivariate statistics methods such as canonical discriminant analysis. It can be argued that the use of long-term water quality monitoring at multiple sites and multivariate data analyses for each creek within a watershed are neither time nor cost effective for successful TMDL development. However, the use of resources to ensure the effective identification and quantification of sources of impairment and accounting for variability within the watershed may demonstrate long-term cost effectiveness. Correctly identifying and classifying sources of fecal pollution using multivariate statistical tools and understanding sources of variability can help in the development of effective TMDLs. If an ineffective TMDL is developed based on limited data that does not reflect true watershed dynamics, successful BMPs cannot be implemented to prevent and remediate surface water impairment for an entire watershed.

The objectives of the watershed approach as described by the USEPA for effective and efficient water resource management involves the identification of priority

areas, the development and implementation of integrative solutions, and the measurement of the success of the program. The additional key component in this process involves the inclusion of stakeholders throughout the process, as they are the individuals who stand to benefit most from the water resource. One of the main benefits of this approach to water resource management is the efficient use of limited time and financial resources in assessing water quality, determining sources of impairment, preventing future pollution events, and remediating current degraded surface waters to remove them from impaired waters lists.

However, the foundation of this watershed approach involves the accurate identification of point and nonpoint sources of pollutants and addressing these through the development of TMDLs to protect human and environmental health. The success of this watershed approach is contingent on the development of TMDLs that accurately quantify point and nonpoint sources of pollution and that reflect true watershed dynamics by accounting for those sources of variability within and between the surface waters composing the watershed. This study has demonstrated that the failure to consider sources of variability including land use patterns and differences in the water quality parameters that most influence overall water quality can set the stage for the failure of the watershed approach to manage water resources.

Conclusions

Current water quality assessment and protection is involved the development of TMDLs at the watershed level to address these degraded resources. However, the effectiveness of watershed TMDLs to address water quality impairments through the

development and implementation of BMPs involving stakeholders has yet to be determined. This study suggests that the development of TMDLs at the watershed level may not accurately reflect true watershed dynamics and that the failure to consider sources of variability within and between water bodies in the same watershed may impede the development and implementation of successful BMPs to remove water bodies from the State of Tennessee's 303d list. The failure to consider sources of variability within and between water bodies in the same watershed can lead to incorrectly identification and quantification of surface water pollutants. This ultimately has the potential to hinder the effectiveness of TMDLs by requiring additional time and money to be spent re-assessing priority areas, identifying sources of impairment and implementing applicable BMPs to restore and protect water quality. As a result, the use of the watershed approach to address surface water quality issues may require more time and money to correctly identify and reduce water pollutants following their failure to remove impaired surface waters from 303d lists. It is therefore imperative that TMDL development focus on sources of variability within and between surface waters. Giving consideration to these sources of variability using targeted, long-term monitoring programs, and canonical discriminant analysis combined with multiple regression analysis can improve our identification and quantification of nonpoint sources of pollution, thus allowing us to assess the effectiveness of TMDLs and implement the appropriate BMPs that result in the greatest reduction of water pollutants in an effort to protect human and environmental health.

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CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

Using a combination of a targeted water quality monitoring program and multivariate statistical analyses to identify sources of anthropogenic stress, the following conclusions can be made:

1. Linear regression analyses of fecal indicator organisms and pathogens were statistically significant but low ($r^2 \leq 0.12$ for *Cryptosporidium* and $\underline{\leq}$ 0.05 for *Giardia*) for protozoan pathogens but not statistically significant for bacterial or viral pathogens. This suggests that the use of fecal indicators may not accurately estimate the risk of pathogen exposure in Sinking Creek.
2. Spatial and temporal variability in the amounts and types of pollution, including fecal indicator bacteria, indicate that TMDL development may require multi-year data at multiple sampling points rather than the limited 30-day geometric mean to more accurately reflect pollution loadings and patterns in Sinking Creek.
3. A better understanding of loading patterns and temporal and spatial distribution using canonical correlation and canonical discriminant analyses may lead to the correct identification of nonpoint sources of fecal pollution in relation to land use patterns. This data analysis approach can be applied to other watersheds to identify common patterns associating pollution types to various sources, and to effectively develop and implement BMPs to prevent and remediate the effects of rapid urbanization.

4. Understanding the influence of physical, chemical, and microbial soil properties in soil adjacent to each stream on water quality can lead to better design and implementation of BMPs to remediate and prevent fecal contamination in the Sinking Creek. It is likely that physiochemical soil properties including coarse soil texture and presence of organic matter on the soil surface contribute to the introduction of fecal pollution into Sinking Creek. The functional ability of soil microbial communities to use a variety of carbon sources may help prevent the introduction of unwanted organic matter and fecal pollution into surface waters.
5. Failure to consider sources of variability within and between water bodies in the same watershed may impede the development and implementation of successful BMPs to protect and remediate impaired surface waters. TMDLs developed at the watershed level that do not consider sources of variability may not accurately reflect true watershed dynamics.
6. Considering sources of physical, chemical, and microbial variability in surface waters using targeted long-term monitoring programs, and canonical discriminant analysis combined with multiple regression analysis can improve our identification and quantification of nonpoint sources of pollution. This understanding can allow for the assessment of the effective TMDLs and implementation of the appropriate BMPs that result in the greatest reduction of water pollutants to protect human and environmental health.

Recommendations for future research include the application of this alternative method of water quality monitoring to additional watersheds to further assess its usefulness in identifying nonpoint sources of fecal pollution. In addition to using this

approach in relation to land use patterns, it is also suggested that this data analysis approach could be used to identify nonpoint sources of fecal pollution as they relate to habitat assessment. The use of habitat assessment scores instead of land use patterns take into consideration site specific characteristics such as riparian buffers, substrate composition, bank stability, and vegetation. Future research should focus on the comparison and correlation of carbon sources used by microbial communities in stream sediments to those used by microbial communities in soil to further suggest sources of fecal pollution.

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APPENDICES

Appendix A: Media and Reagents

Acridine Orange Stain, 0.1%

0.1g of AO
100mL of dH_2O.
Filter sterilize through a 0.2 µm filter into a sterile glass bottle
Store at 4°C

ATCC 271 Agar, 0.7%

Prepare ATCC 271 broth as described above with the addition of 1.4g agar/L

ATCC 271 Agar, 1.5%

Prepare ATCC 271 broth as described with the addition of 18g agar/L

ATCC 271 Broth

10g tryptone
1g yeast extract
8g NaCl
1L dH_2O
Autoclave at 121°C for 15 minutes and add the following reagents after autoclaving
10ml of 10% glucose solution
2ml of 1M $CaCl_2$
1ml of 10mg/ml thiamine

Diethylpyrocarbonate Treated Water, 0.05%

50µl diethylpyrocarbonate
100ml sterile dH_2O
Filter sterilize through a 0.2 µm filter into a sterile glass bottle
Store at 4°C

Elution Buffer for Envirocheck™ Filter Capsules

10ml of 10% Laureth-12 solution
10ml of 1M Tris (pH 7.4)
2ml of 0.5M EDTA (pH 8.0)
150µl Antifoam A solution

Iodonitrotetrazolium Chloride Solution, 0.5%

0.5g of INT (iodonitrotetrazolium chloride)
90mL of dH_2O
Mix INT in the dark for 30 minutes and bring volume to 100ml
Filter sterilize through a 0.2µm filter into a sterile glass bottle
Store in the dark at 4°C

m-Endo Medium

4.8g of the m-Endo broth base
2ml 95% ethanol
98ml dH_2O
Heat to boiling then promptly remove from hot plate

m-FC Medium

3.7g of m-FC broth base
1ml 1% rosolic acid
99ml dH_2O
Heat to boiling then promptly remove from hot plate

Phosphate Buffer, 0.1M, pH 7.6

1.56g NaH_2PO_4 (or 1.79 g of $NaH_2PO_4 \cdot H_2O$)
12.35g Na_2HPO_4 (or 23.30 g of $Na_2HPO_4 \cdot 7H_2O$)
1L dH_2O
Autoclave at 121°C for 15 minutes
Store at 4°C

Phosphate Buffer, 0.1M, pH 9.0

1.84 g of Na_2HPO_4
1L dH_2O
Autoclave at 121°C for 15 minutes
Store at 4°C

Phosphate Buffer with 0.15% Galactosidase Indicator, pH 7.6

0.156g of NaH_2PO_4 (or 0.179 g of $NaH_2PO_4 \cdot H_2O$)
1.235g of Na_2HPO_4 (or 2.330 g of $Na_2HPO_4 \cdot 7H_2O$)
0.151 g of p-nitrophenyl-B-D-galactopyranoside
100ml dH_2O
Filter sterilize through a 0.2 µm filter into a sterile glass bottle
Store at 4°C

Phosphate Buffer with 0.15% Glucosidase Indicator, pH 7.6

0.156g of NaH_2PO_4 (or 0.179 g of $NaH_2PO_4 \cdot H_2O$)
1.235g of Na_2HPO_4 (or 2.330 g of $Na_2HPO_4 \cdot 7H_2O$)
0.151 g of 4-nitrophenyl-B-D-glucopyranoside
100ml dH_2O
Filter sterilize through a 0.2 µm filter into a sterile glass bottle
Store at 4°C

Phosphate Buffered Saline, pH 7.4

8g NaCl
0.2g KCl
1.44g Na_2HPO_4
0.24g KH_2PO_4
1L dH_2O
Autoclave at 121°C for 15 minutes
Store at 4°C

Phosphate Buffered Saline + Tween 80, pH 7.2

140 mL of 0.2 M NaH_2PO_4
360 mL of 0.2 M Na_2HPO_4
10ml Tween 80
1L dH_2O
Autoclave at 121°C for 15 minutes
Store at 4°C

Phosphate Buffered Water

10g PBW powder
1L dH_2O
Autoclave at 121°C for 15 minutes
Store at 4°C

R2A Agar for Standard Plate Counts

18.2g R2A agar
1L dH_2O
Autoclave at 121°C for 15 minutes

Tris Buffer, 1M, pH 4.8

0.60g of TRIZMA Base
15.76g of TRIZMA HCl
500ml of dH_2O
Autoclave at 121^{o}C for 15 minutes
Store at 4^{o}C

Tris Buffer, 1M, pH 8.6

6.06g of TRIZMA Base
1.92g of TRIZMA HCl
500ml dH_2O
Autoclave at 121^{o}C for 15 minutes
Store at 4^{o}C

Tris Buffer with 0.1% Phosphatase Substrate, 1M, pH 7.6

0.21g TRIZMA Base
1.21g of TRIZMA HCl
0.1 g of phosphatase substrate
100ml dH_2O
Filter sterilize through a 0.2 µm filter into a sterile glass bottle
Store at 4^{o}C

Tween 80, 1%

5ml Tween 80
1L dH_2O
Autoclave at 121^{o}C for 15 minutes
Store at 4^{o}C

Appendix B: Water Quality Summary Statistics

Table 1. Summary statistics for January 2011, site 2

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	6.8	0	1
Water Temperature (^{o}C)	8.1	0	1
pH	7.2	0	1
Conductivity (µmohs)	322	0	1
Dissolved Oxygen (mg/L as O_2)	10.8	0	1
Discharge (m^3/sec)	0.17	0	1
Fecal Coliform – Water (CFU/100ml)	3433.3	665.8	3
Total Coliform – Water (CFU/100ml)	4466.7	0.08	3
Fecal Coliform – Sediment (CFU/100ml)	25.0	3931.1	2
Total Coliform – Sediment (CFU/100ml)	387.5	0	2
Colilert (MPN/100ml)	1299.7	512.7	1
Nitrates (mg/L)	1.3	0	1
Phosphates (mg/L)	.44	0.37	3
Ammonia (mg/L)	.09	0.03	3
Biochemical Oxygen Demand (mg/L as O_2)	1.5	0.11	3
Alkalinity (mg/L as $CaCO_3$)	117.3	2.1	3
Hardness (mg/L as $CaCO_3$)	176.3	24.6	3
Standard Plate Count (CFU/ml)	500.0	8.5	2
Acridine Orange Direct Counts (cells/g)	2.7×10^8	6.5×10^7	1
Acid Phosphatase (µg/g)	50.1	10.8	3
Alkaline Phosphatase (µg/g)	71.5	66.0	3
Dehydrogenase (µg/g)	48.5	16.7	3
Galactosidase (µg/g)	36.5	16.9	3
Glucosidase (µg/g)	92.3	018.5	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	1.0×10^4	1.7×10^4	3
Giardia sp.(cysts/L)	9.5	0	1
Cryptosporidium sp. (cysts/L)	4.8	0	1

Table 2. Summary statistics for January 2011, site 4

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	5.3	0	1
Water Temperature (^{o}C)	8.2	0	1
pH	7.0	0	1
Conductivity (µmohs)	295.0	0	1
Dissolved Oxygen (mg/L as O_2)	10.5	0	1
Discharge (m^3/sec)	0.49	0	1
Fecal Coliform – Water (CFU/100ml)	2933.3	1078.6	3
Total Coliform – Water (CFU/100ml)	8033.3	568.6	3
Fecal Coliform – Sediment (CFU/100ml)	250.0	70.7	2
Total Coliform – Sediment (CFU/100ml)	1375.0	1449.6	2
Colilert (MPN/100ml)	57.8	0	1
Nitrates (mg/L)	0.93	0.32	1
Phosphates (mg/L)	0.97	0.48	3
Ammonia (mg/L)	0.10	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	1.7	0.06	3
Alkalinity (mg/L as $CaCO_3$)	103.3	3.1	3
Hardness (mg/L as $CaCO_3$)	126.7	4.5	3
Standard Plate Count (CFU/ml)	488.0	36.8	2
Acridine Orange Direct Counts (cells/g)	1.0×10^8	1.1×10^8	1
Acid Phosphatase (µg/g)	31.5	5.6	3
Alkaline Phosphatase (µg/g)	156.4	56.1	3
Dehydrogenase (µg/g)	47.1	10.4	3
Galactosidase (µg/g)	42.6	27.6	3
Glucosidase (µg/g)	166.6	56.1	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	6.36	3.1	3
Giardia sp.(cysts/L)	28.0	0	1
Cryptosporidium sp. (cysts/L)	16.0	0	1

Table 3. Summary statistics for January 2011, site 7

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	8.1	0	1
Water Temperature (^{o}C)	8.6	0	1
pH	6.7	0	1
Conductivity (µmohs)	214.0	0	1
Dissolved Oxygen (mg/L as O_2)	10.5	0	1
Discharge (m^3/sec)	0.41	0	1
Fecal Coliform – Water (CFU/100ml)	50.0	0	3
Total Coliform – Water (CFU/100ml)	50.0	0	3
Fecal Coliform – Sediment (CFU/100ml)	75.0	35.6	2
Total Coliform – Sediment (CFU/100ml)	1337.5	1856.1	2
Colilert (MPN/100ml)	1.0	0	1
Nitrates (mg/L)	1.43	0.32	1
Phosphates (mg/L)	0.67	0.67	3
Ammonia (mg/L)	0.09	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	1.8	0.17	3
Alkalinity (mg/L as $CaCO_3$)	72.7	2.1	3
Hardness (mg/L as $CaCO_3$)	96.7	1.5	3
Standard Plate Count (CFU/ml)	88.0	39.6	2
Acridine Orange Direct Counts (cells/g)	1.2×10^8	5.8×10^7	1
Acid Phosphatase (µg/g)	18.7	10.7	3
Alkaline Phosphatase (µg/g)	57.4	34.1	3
Dehydrogenase (µg/g)	10.6	6.7	3
Galactosidase (µg/g)	16.1	11.8	3
Glucosidase (µg/g)	15.4	10.8	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	2.1	2.9	3
Giardia sp.(cysts/L)	116.0	0	1
Cryptosporidium sp. (cysts/L)	68.0	0	1

Table 4. Summary statistics for January 2011, site 10

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	8.9	0	1
Water Temperature (^{o}C)	7.7	0	1
pH	7.0	0	1
Conductivity (µmohs)	123.1	0	1
Dissolved Oxygen (mg/L as O_2)	11.5	0	1
Discharge (m^3/sec)	0.71	0	1
Fecal Coliform – Water (CFU/100ml)	100.0	68.6	3
Total Coliform – Water (CFU/100ml)	283.3	225.5	3
Fecal Coliform – Sediment (CFU/100ml)	25.0	0	2
Total Coliform – Sediment (CFU/100ml)	50.0	0	2
Colilert (MPN/100ml)	6.3	0	1
Nitrates (mg/L)	1.4	0.21	1
Phosphates (mg/L)	0.20	0.06	3
Ammonia (mg/L)	0.08	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	2.0	0.16	3
Alkalinity (mg/L as $CaCO_3$)	46.0	1.7	3
Hardness (mg/L as $CaCO_3$)	56.3	3.2	3
Standard Plate Count (CFU/ml)	275.0	41.0	2
Acridine Orange Direct Counts (cells/g)	7.5×10^7	2.2×10^7	1
Acid Phosphatase (µg/g)	53.8	1	3
Alkaline Phosphatase (µg/g)	288.5	27.6	3
Dehydrogenase (µg/g)	36.4	165.2	3
Galactosidase (µg/g)	32.3	18.9	3
Glucosidase (µg/g)	140.4	23.0	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	34.1	57.1	3
Giardia sp.(cysts/L)	28.0	0	1
Cryptosporidium sp. (cysts/L)	16.0	0	1

Table 5. Summary statistics for January 2011, site 13

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	8.3	0	1
Water Temperature (^{o}C)	6.0	0	1
pH	6.5	0	1
Conductivity (µmohs)	33.1	0	1
Dissolved Oxygen (mg/L as O_2)	11.7	0	1
Discharge (m^3/sec)	0.27	0	1
Fecal Coliform – Water (CFU/100ml)	83.3	28.9	3
Total Coliform – Water (CFU/100ml)	133.3	57.7	3
Fecal Coliform – Sediment (CFU/100ml)	37.5	17.7	2
Total Coliform – Sediment (CFU/100ml)	37.5	17.7	2
Colilert (MPN/100ml)	18.9	0	1
Nitrates (mg/L)	0.40	0.20	1
Phosphates (mg/L)	0.33	0.13	3
Ammonia (mg/L)	0.07	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	1.8	0.23	3
Alkalinity (mg/L as $CaCO_3$)	13.3	2.9	3
Hardness (mg/L as $CaCO_3$)	16.3	2.5	3
Standard Plate Count (CFU/ml)	168.0	39.6	2
Acridine Orange Direct Counts (cells/g)	6.4×10^7	1.7×10^7	1
Acid Phosphatase (µg/g)	56.2	37.5	3
Alkaline Phosphatase (µg/g)	301.8	162.6	3
Dehydrogenase (µg/g)	16.2	2.4	3
Galactosidase (µg/g)	11.6	6.3	3
Glucosidase (µg/g)	77.9	33.9	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	8.75	0	1
Cryptosporidium sp. (cysts/L)	12.3	0	1

Table 6. Summary statistics for January 2011, site 14

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	8.3	0	1
Water Temperature (^{o}C)	5.7	0	1
pH	6.2	0	1
Conductivity (µmohs)	24.1	0	1
Dissolved Oxygen (mg/L as O_2)	11.5	0	1
Discharge (m^3/sec)	0.14	0	1
Fecal Coliform – Water (CFU/100ml)	50	0	3
Total Coliform – Water (CFU/100ml)	216.6	144.3	3
Fecal Coliform – Sediment (CFU/100ml)	37.5	17.7	2
Total Coliform – Sediment (CFU/100ml)	37.5	17.7	2
Colilert (MPN/100ml)	17.3	0	1
Nitrates (mg/L)	0.80	0.30	1
Phosphates (mg/L)	1.1	0.78	3
Ammonia (mg/L)	0.06	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	2.3	0.35	3
Alkalinity (mg/L as $CaCO_3$)	8.3	0.58	3
Hardness (mg/L as $CaCO_3$)	15.7	3.1	3
Standard Plate Count (CFU/ml)	166.0	2.8	2
Acridine Orange Direct Counts (cells/g)	1.3×10^8	6.7×10^7	1
Acid Phosphatase (µg/g)	64.2	8.6	3
Alkaline Phosphatase (µg/g)	173.3	152.2	3
Dehydrogenase (µg/g)	34.0	21.3	3
Galactosidase (µg/g)	15.2	11.7	3
Glucosidase (µg/g)	122.0	15.4	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	2.0	0	1
Cryptosporidium sp. (cysts/L)	1.0	0	1

Table 7. Summary statistics for February 2011, site 2

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	14.6	0	1
Water Temperature (^{o}C)	10.7	0	1
pH	8.2	0	1
Conductivity (µmohs)	307.0	0	1
Dissolved Oxygen (mg/L as O_2)	9.3	0	1
Discharge (m^3/sec)	0.03	0	1
Fecal Coliform – Water (CFU/100ml)	629.6	0	3
Total Coliform – Water (CFU/100ml)	148.1	357.2	3
Fecal Coliform – Sediment (CFU/100ml)	25.0	64.2	2
Total Coliform – Sediment (CFU/100ml)	5950.0	0	2
Colilert (MPN/100ml)	84.5	8343.86	1
Nitrates (mg/L)	0.40	0	1
Phosphates (mg/L)	0.40	0.17	3
Ammonia (mg/L)	.012	0.18	3
Biochemical Oxygen Demand (mg/L as O_2)	1.9	0.06	3
Alkalinity (mg/L as $CaCO_3$)	182.7	0.17	3
Hardness (mg/L as $CaCO_3$)	183.3	3.5	3
Standard Plate Count (CFU/ml)	530.0	14.1	2
Acridine Orange Direct Counts (cells/g)	1.5×10^8	3.8×10^7	1
Acid Phosphatase (µg/g)	68.7	11.3	3
Alkaline Phosphatase (µg/g)	207.4	12.4	3
Dehydrogenase (µg/g)	21.6	11.6	3
Galactosidase (µg/g)	9.7	7.4	3
Glucosidase (µg/g)	288.3	47.3	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	2.0	0	1
Cryptosporidium sp. (cysts/L)	8.0	0	1

Table 8. Summary statistics for February 2011, site 4

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	14.8	0	1
Water Temperature (^{o}C)	10.6	0	1
pH	8.2	0	1
Conductivity (µmohs)	288.0	0	1
Dissolved Oxygen (mg/L as O_2)	9.7	0	1
Discharge (m^3/sec)	0.17	0	1
Fecal Coliform – Water (CFU/100ml)	1296.3	357.2	3
Total Coliform – Water (CFU/100ml)	1407.4	1218.9	3
Fecal Coliform – Sediment (CFU/100ml)	25.0	0	2
Total Coliform – Sediment (CFU/100ml)	1600.0	2121.3	2
Colilert (MPN/100ml)	110.6	0	1
Nitrates (mg/L)	1.43	0.78	1
Phosphates (mg/L)	0.26	0.04	3
Ammonia (mg/L)	0.11	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	2.2	0.11	3
Alkalinity (mg/L as $CaCO_3$)	169.0	1.7	3
Hardness (mg/L as $CaCO_3$)	189.3	15.4	3
Standard Plate Count (CFU/ml)	534.0	65.1	2
Acridine Orange Direct Counts (cells/g)	1.6×10^8	8.7×10^7	1
Acid Phosphatase (µg/g)	69.6	20.7	3
Alkaline Phosphatase (µg/g)	167.2	92.5	3
Dehydrogenase (µg/g)	17.0	3.5	3
Galactosidase (µg/g)	7.9	4.2	3
Glucosidase (µg/g)	70.3	56.1	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	334.2	576.6	3
Giardia sp.(cysts/L)	7.7	0	1
Cryptosporidium sp. (cysts/L)	2.6	0	1

Table 9. Summary statistics for February 2011, site 7

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	15.0	0	1
Water Temperature (^{o}C)	12.3	0	1
pH	7.7	0	1
Conductivity (µmohs)	238.0	0	1
Dissolved Oxygen (mg/L as O_2)	8.7	0	1
Discharge (m^3/sec)	0.22	0	1
Fecal Coliform – Water (CFU/100ml)	55.6	0	3
Total Coliform – Water (CFU/100ml)	55.6	0	3
Fecal Coliform – Sediment (CFU/100ml)	25.0	0	2
Total Coliform – Sediment (CFU/100ml)	125.0	35.4	2
Colilert (MPN/100ml)	1.0	0	1
Nitrates (mg/L)	1.4	0.42	3
Phosphates (mg/L)	0.34	0.16	3
Ammonia (mg/L)	0.08	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	1.8	0.10	3
Alkalinity (mg/L as $CaCO_3$)	143.3	2.1	3
Hardness (mg/L as $CaCO_3$)	152.3	7.4	3
Standard Plate Count (CFU/ml)	172.0	17.0	2
Acridine Orange Direct Counts (cells/g)	1.4×10^8	1.4×10^7	1
Acid Phosphatase (µg/g)	51.8	25.6	3
Alkaline Phosphatase (µg/g)	236.7	83.7	3
Dehydrogenase (µg/g)	18.5	12.4	3
Galactosidase (µg/g)	3.5	0.27	3
Glucosidase (µg/g)	32.0	27.2	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	667.0	1154.4	3
Giardia sp.(cysts/L)	4.0	0	1
Cryptosporidium sp. (cysts/L)	2.0	0	1

Table 10. Summary statistics for February 2011, site 10

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	14.6	0	1
Water Temperature (^{o}C)	11.0	0	1
pH	8.0	0	1
Conductivity (µmohs)	150.9	0	1
Dissolved Oxygen (mg/L as O_2)	10.2	0	1
Discharge (m^3/sec)	0.1	0	1
Fecal Coliform – Water (CFU/100ml)	129.6	84.9	3
Total Coliform – Water (CFU/100ml)	111.13	0	3
Fecal Coliform – Sediment (CFU/100ml)	337.5	17.7	2
Total Coliform – Sediment (CFU/100ml)	3650.0	565.7	2
Colilert (MPN/100ml)	330.9	0	1
Nitrates (mg/L)	19	0.36	3
Phosphates (mg/L)	0.36	0.13	3
Ammonia (mg/L)	0.06	0.05	3
Biochemical Oxygen Demand (mg/L as O_2)	2.01	0.10	3
Alkalinity (mg/L as $CaCO_3$)	95.7	0.58	3
Hardness (mg/L as $CaCO_3$)	114.0	2.0	3
Standard Plate Count (CFU/ml)	400.0	62.2	2
Acridine Orange Direct Counts (cells/g)	1.8×10^8	5.5×10^7	1
Acid Phosphatase (µg/g)	36.4	22.9	3
Alkaline Phosphatase (µg/g)	71.9	23.4	3
Dehydrogenase (µg/g)	18.4	2.3	3
Galactosidase (µg/g)	10.6	4.1	3
Glucosidase (µg/g)	83.0	46.7	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	7.0	11.3	3
Giardia sp.(cysts/L)	14.0	0	1
Cryptosporidium sp. (cysts/L)	4.0	0	1

Table 11. Summary statistics of February 2011, site 13

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	15.7	0	1
Water Temperature (^{o}C)	8.3	0	1
pH	7.8	0	1
Conductivity (µmohs)	41.1	0	1
Dissolved Oxygen (mg/L as O_2)	10.9	0	1
Discharge (m^3/sec)	0.09	0	1
Fecal Coliform – Water (CFU/100ml)	55.5	0	3
Total Coliform – Water (CFU/100ml)	129.6	84.7	3
Fecal Coliform – Sediment (CFU/100ml)	25.0	0	2
Total Coliform – Sediment (CFU/100ml)	50.0	0	2
Colilert (MPN/100ml)	13.5	0	1
Nitrates (mg/L)	0.40	0.26	3
Phosphates (mg/L)	0.51	0.56	3
Ammonia (mg/L)	0.10	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	2.3	0.02	3
Alkalinity (mg/L as $CaCO_3$)	25.7	1.5	3
Hardness (mg/L as $CaCO_3$)	51.0	1.0	3
Standard Plate Count (CFU/ml)	380.0	33.9	2
Acridine Orange Direct Counts (cells/g)	1.7×10^8	1.5×10^7	1
Acid Phosphatase (µg/g)	134.1	51.3	3
Alkaline Phosphatase (µg/g)	37.9	5.6	3
Dehydrogenase (µg/g)	20.3	6.5	3
Galactosidase (µg/g)	37.5	4.2	3
Glucosidase (µg/g)	21.0	3.7	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	11.8	0	1
Cryptosporidium sp. (cysts/L)	7.1	0	1

Table 12. Summary statistics for February 2011, site 14

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	14.8	0	1
Water Temperature (^{o}C)	8.4	0	1
pH	7.9	0	1
Conductivity (µmohs)	18.5	0	1
Dissolved Oxygen (mg/L as O_2)	9.9	0	1
Discharge (m^3/sec)	0.02	0	1
Fecal Coliform – Water (CFU/100ml)	55.5	0	3
Total Coliform – Water (CFU/100ml)	166.7	147.0	3
Fecal Coliform – Sediment (CFU/100ml)	37.5	17.7	2
Total Coliform – Sediment (CFU/100ml)	25.0	0	2
Colilert (MPN/100ml)	1.0	0	1
Nitrates (mg/L)	0.77	0.29	3
Phosphates (mg/L)	0.19	0.05	3
Ammonia (mg/L)	0.08	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	2.5	0.12	3
Alkalinity (mg/L as $CaCO_3$)	12.0	1.0	3
Hardness (mg/L as $CaCO_3$)	34.3	8.1	3
Standard Plate Count (CFU/ml)	134.0	42.4	2
Acridine Orange Direct Counts (cells/g)	1.3×10^8	1.0×10^8	1
Acid Phosphatase (µg/g)	79.2	17.6	3
Alkaline Phosphatase (µg/g)	201.3	36.7	3
Dehydrogenase (µg/g)	24.2	9.1	3
Galactosidase (µg/g)	33.8	3.1	3
Glucosidase (µg/g)	20.2	17.2	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	0	0	0
Cryptosporidium sp. (cysts/L)	0	0	0

Table 13. Summary statistics for March 2011, site 2

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	17.0	0	1
Water Temperature (^{o}C)	12.1	0	1
pH	8.4	0	1
Conductivity (µmohs)	140.0	0	1
Dissolved Oxygen (mg/L as O_2)	10.0	0	1
Discharge (m^3/sec)	0.84	0	1
Fecal Coliform – Water (CFU/100ml)	450.0	377.5	3
Total Coliform – Water (CFU/100ml)	466.7	208.2	3
Fecal Coliform – Sediment (CFU/100ml)	37.5	17.7	2
Total Coliform – Sediment (CFU/100ml)	1075.0	1308.2	2
Colilert (MPN/100ml)	214.3	0	1
Nitrates (mg/L)	1.3	0.12	3
Phosphates (mg/L)	0.26	0.06	3
Ammonia (mg/L)	0.08	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	0.89	0.11	3
Alkalinity (mg/L as $CaCO_3$)	154.7	11.0	3
Hardness (mg/L as $CaCO_3$)	186.7	5.9	3
Standard Plate Count (CFU/ml)	458.0	8.5	2
Acridine Orange Direct Counts (cells/g)	1.3×10^8	6.8×10^7	1
Acid Phosphatase (µg/g)	22.0	3.4	3
Alkaline Phosphatase (µg/g)	113.5	82.2	3
Dehydrogenase (µg/g)	84.4	6.7	3
Galactosidase (µg/g)	67.2	10.4	3
Glucosidase (µg/g)	274.6	206.0	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	22.0	0	1
Cryptosporidium sp. (cysts/L)	10.0	0	1

Table 14. Summary statistics for March 2011, site 4

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	14.3	0	1
Water Temperature (^{o}C)	12.2	0	1
pH	8.3	0	1
Conductivity (µmohs)	128.0	0	1
Dissolved Oxygen (mg/L as O_2)	9.8	0	1
Discharge (m^3/sec)	0.68	0	1
Fecal Coliform – Water (CFU/100ml)	766.7	152.8	3
Total Coliform – Water (CFU/100ml)	900.0	1300.0	3
Fecal Coliform – Sediment (CFU/100ml)	25.0	0	2
Total Coliform – Sediment (CFU/100ml)	2775.0	1449.6	2
Colilert (MPN/100ml)	461.1	0	1
Nitrates (mg/L)	1.4	0.21	3
Phosphates (mg/L)	0.39	0.05	3
Ammonia (mg/L)	0.08	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	1.0	0.06	3
Alkalinity (mg/L as $CaCO_3$)	136.0	1.7	3
Hardness (mg/L as $CaCO_3$)	168.4	4.5	3
Standard Plate Count (CFU/ml)	412.0	39.6	2
Acridine Orange Direct Counts (cells/g)	1.0×10^8	8.5×10^7	1
Acid Phosphatase (µg/g)	54.1	39.3	3
Alkaline Phosphatase (µg/g)	30.0	17.0	3
Dehydrogenase (µg/g)	16.2	22.0	3
Galactosidase (µg/g)	68.9	10.4	3
Glucosidase (µg/g)	504.2	326.1	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	12.0	0	1
Cryptosporidium sp. (cysts/L)	4.0	0	1

Table 15. Summary statistics for March 2011, site 7

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	15.6	0	1
Water Temperature (^{o}C)	12.5	0	1
pH	7.9	0	1
Conductivity (µmohs)	102.0	0	1
Dissolved Oxygen (mg/L as O_2)	9.1	0	1
Discharge (m^3/sec)	0.46	0	1
Fecal Coliform – Water (CFU/100ml)	50.0	0	3
Total Coliform – Water (CFU/100ml)	300.0	264.6	3
Fecal Coliform – Sediment (CFU/100ml)	25.0	0	2
Total Coliform – Sediment (CFU/100ml)	150.0	70.7	2
Colilert (MPN/100ml)	1.0	0	1
Nitrates (mg/L)	1.3	0.38	3
Phosphates (mg/L)	0.43	0.09	3
Ammonia (mg/L)	0.11	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	0.95	0.12	3
Alkalinity (mg/L as $CaCO_3$)	131.3	1.5	3
Hardness (mg/L as $CaCO_3$)	138.0	3.5	3
Standard Plate Count (CFU/ml)	246.0	127.3	2
Acridine Orange Direct Counts (cells/g)	1.0×10^8	3.3×10^7	1
Acid Phosphatase (µg/g)	85.4	68.9	3
Alkaline Phosphatase (µg/g)	50.5	31.5	3
Dehydrogenase (µg/g)	28.5	7.23	3
Galactosidase (µg/g)	58.6	316.7	3
Glucosidase (µg/g)	272.2	3189.6	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	10.0	0	1
Cryptosporidium sp. (cysts/L)	2.0	0	1

Table 16. Summary statistics for March 2011, site 10

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	17.1	0	1
Water Temperature (^{o}C)	12.1	0	1
pH	8.0	0	1
Conductivity (µmohs)	68.0	0	1
Dissolved Oxygen (mg/L as O_2)	9.9	0	1
Discharge (m^3/sec)	0.30	0	1
Fecal Coliform – Water (CFU/100ml)	166.7	115.5	3
Total Coliform – Water (CFU/100ml)	133.3	144.3	3
Fecal Coliform – Sediment (CFU/100ml)	25.0	0	2
Total Coliform – Sediment (CFU/100ml)	50.0	0	2
Colilert (MPN/100ml)	21.6	0	1
Nitrates (mg/L)	1.3	0.17	3
Phosphates (mg/L)	0.12	0.03	3
Ammonia (mg/L)	0.06	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	0.82	0.03	3
Alkalinity (mg/L as $CaCO_3$)	92.0	5.2	3
Hardness (mg/L as $CaCO_3$)	100.0	7.2	3
Standard Plate Count (CFU/ml)	260.0	56.6	2
Acridine Orange Direct Counts (cells/g)	6.7×10^7	2.2×10^7	1
Acid Phosphatase (µg/g)	56.5	12.8	3
Alkaline Phosphatase (µg/g)	85.5	19.0	3
Dehydrogenase (µg/g)	49.5	44.5	3
Galactosidase (µg/g)	52.6	7.2	3
Glucosidase (µg/g)	420.0	36.3	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	2.0	0	1
Cryptosporidium sp. (cysts/L)	2.0	0	1

Table 17. Summary statistics for March 2011, site 13

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	18.1	0	1
Water Temperature (^{o}C)	12.5	0	1
pH	8.0	0	1
Conductivity (µmohs)	17.0	0	1
Dissolved Oxygen (mg/L as O_2)	9.2	0	1
Discharge (m^3/sec)	0.1	0	1
Fecal Coliform – Water (CFU/100ml)	50.0	0	3
Total Coliform – Water (CFU/100ml)	83.3	28.7	3
Fecal Coliform – Sediment (CFU/100ml)	25.0	0	2
Total Coliform – Sediment (CFU/100ml)	37.5	17.7	2
Colilert (MPN/100ml)	16.1	0	1
Nitrates (mg/L)	0.33	0.15	3
Phosphates (mg/L)	0.26	0.05	3
Ammonia (mg/L)	0.07	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	0.84	0.11	3
Alkalinity (mg/L as $CaCO_3$)	63.3	7.2	3
Hardness (mg/L as $CaCO_3$)	32.0	3.6	3
Standard Plate Count (CFU/ml)	224.0	0	2
Acridine Orange Direct Counts (cells/g)	7.8×10^7	3.3×10^7	1
Acid Phosphatase (µg/g)	34.6	23.2	3
Alkaline Phosphatase (µg/g)	37.7	33.7	3
Dehydrogenase (µg/g)	71.8	35.5	3
Galactosidase (µg/g)	45.7	10.0	3
Glucosidase (µg/g)	407.7	319.5	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	16.0	0	1
Cryptosporidium sp. (cysts/L)	12.0	0	1

Table 18. Summary statistics for March 2011, site 14

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	18.7	0	1
Water Temperature (^{o}C)	10.8	0	1
pH	8.0	0	1
Conductivity (µmohs)	9.0	0	1
Dissolved Oxygen (mg/L as O_2)	9.8	0	1
Discharge (m^3/sec)	0.02	0	1
Fecal Coliform – Water (CFU/100ml)	50.0	0	3
Total Coliform – Water (CFU/100ml)	166.7	115.5	3
Fecal Coliform – Sediment (CFU/100ml)	25.0	0	2
Total Coliform – Sediment (CFU/100ml)	37.5	17.7	2
Colilert (MPN/100ml)	4.1	0	1
Nitrates (mg/L)	0.80	0.30	3
Phosphates (mg/L)	0.20	0.13	3
Ammonia (mg/L)	0.07	0	3
Biochemical Oxygen Demand (mg/L as O_2)	1.3	0.32	3
Alkalinity (mg/L as $CaCO_3$)	49.0	3.6	3
Hardness (mg/L as $CaCO_3$)	25.3	1.5	3
Standard Plate Count (CFU/ml)	174.0	8.5	2
Acridine Orange Direct Counts (cells/g)	8.3×10^7	6.5×10^7	1
Acid Phosphatase (µg/g)	77.9	14.1	3
Alkaline Phosphatase (µg/g)	119.8	95.4	3
Dehydrogenase (µg/g)	72.0	33.8	3
Galactosidase (µg/g)	124.5	32.3	3
Glucosidase (µg/g)	267.9	69.7	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	10.0	0	1
Cryptosporidium sp. (cysts/L)	4.0	0	1

Table 19. Summary statistics for April 2011, site 2

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	14.2	0	1
Water Temperature (^{o}C)	11.5	0	1
pH	7.4	0	1
Conductivity (µmohs)	244.0	0	1
Dissolved Oxygen (mg/L as O_2)	9.8	0	1
Discharge (m^3/sec)	1.0	0	1
Fecal Coliform – Water (CFU/100ml)	2100.0	500.0	3
Total Coliform – Water (CFU/100ml)	4666.7	4446.7	3
Fecal Coliform – Sediment (CFU/100ml)	50.0	0	2
Total Coliform – Sediment (CFU/100ml)	137.5	159.1	2
Colilert (MPN/100ml)	187.2	0	1
Nitrates (mg/L)	1.5	0.91	3
Phosphates (mg/L)	0.45	0.08	3
Ammonia (mg/L)	0.07	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	1.6	0.12	3
Alkalinity (mg/L as $CaCO_3$)	140.3	1.5	3
Hardness (mg/L as $CaCO_3$)	1177.7	10.3	3
Standard Plate Count (CFU/ml)	680.0	84.9	2
Acridine Orange Direct Counts (cells/g)	2.1×10^8	1.2×10^8	1
Acid Phosphatase (µg/g)	8.2	2.9	3
Alkaline Phosphatase (µg/g)	37.7	15.7	3
Dehydrogenase (µg/g)	26.3	6.7	3
Galactosidase (µg/g)	3.2	2.1	3
Glucosidase (µg/g)	11.1	4.8	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	24.0	0	1
Cryptosporidium sp. (cysts/L)	18.0	0	1

Table 20. Summary statistics for April 2011, site 4

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	13.8	0	1
Water Temperature (^{o}C)	11.6	0	1
pH	6.9	0	1
Conductivity (µmohs)	209.0	0	1
Dissolved Oxygen (mg/L as O_2)	10.0	0	1
Discharge (m^3/sec)	1.0	0	1
Fecal Coliform – Water (CFU/100ml)	533.3	152.8	3
Total Coliform – Water (CFU/100ml)	1633.3	2227.9	3
Fecal Coliform – Sediment (CFU/100ml)	175.0	176.8	2
Total Coliform – Sediment (CFU/100ml)	2975.0	3924.4	2
Colilert (MPN/100ml)	116.2	0	1
Nitrates (mg/L)	1.2	0.58	3
Phosphates (mg/L)	0.39	0.13	3
Ammonia (mg/L)	0.09	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	1.5	0.10	3
Alkalinity (mg/L as $CaCO_3$)	130.3	2.1	3
Hardness (mg/L as $CaCO_3$)	147.3	3.2	3
Standard Plate Count (CFU/ml)	775.0	77.8	2
Acridine Orange Direct Counts (cells/g)	1.3×10^8	7.9×10^7	1
Acid Phosphatase (µg/g)	8.6	8.1	3
Alkaline Phosphatase (µg/g)	39.9	15.2	3
Dehydrogenase (µg/g)	38.7	15.3	3
Galactosidase (µg/g)	4.5	1.5	3
Glucosidase (µg/g)	49.6	24.5	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	8.0	0	1
Cryptosporidium sp. (cysts/L)	1.0	0	1

Table 21. Summary statistics for April 2011, site 7

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	15.3	0	1
Water Temperature (^{o}C)	12.2	0	1
pH	7.2	0	1
Conductivity (µmohs)	171.7	0	1
Dissolved Oxygen (mg/L as O_2)	9.6	0	1
Discharge (m^3/sec)	0.01	0	1
Fecal Coliform – Water (CFU/100ml)	66.7	28.9	3
Total Coliform – Water (CFU/100ml)	1000.0	1645.5	3
Fecal Coliform – Sediment (CFU/100ml)	25.0	0	2
Total Coliform – Sediment (CFU/100ml)	1500.0	282.8	2
Colilert (MPN/100ml)	5.2	0	1
Nitrates (mg/L)	1.4	0.44	3
Phosphates (mg/L)	0.27	0.04	3
Ammonia (mg/L)	0.08	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	1.8	0.12	3
Alkalinity (mg/L as $CaCO_3$)	97.0	1.0	3
Hardness (mg/L as $CaCO_3$)	121.3	3.1	3
Standard Plate Count (CFU/ml)	260.0	169.7	2
Acridine Orange Direct Counts (cells/g)	1.6×10^8	1.6×10^8	1
Acid Phosphatase (µg/g)	30.9	11.7	3
Alkaline Phosphatase (µg/g)	3.3	0.75	3
Dehydrogenase (µg/g)	67.0	13.6	3
Galactosidase (µg/g)	13.2	3.3	3
Glucosidase (µg/g)	7.7	6.3	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	6.7	2.9	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	4.0	0	1
Cryptosporidium sp. (cysts/L)	4.0	0	1

Table 22. Summary statistics for April 2011, site 10

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	16.6	0	1
Water Temperature (^{o}C)	11.9	0	1
pH	7.6	0	1
Conductivity (µmohs)	112.1	0	1
Dissolved Oxygen (mg/L as O_2)	10.1	0	1
Discharge (m^3/sec)	0.40	0	1
Fecal Coliform – Water (CFU/100ml)	100.0	86.6	3
Total Coliform – Water (CFU/100ml)	1700.0	2771.3	3
Fecal Coliform – Sediment (CFU/100ml)	37.5	17.7	2
Total Coliform – Sediment (CFU/100ml)	1612.5	2245.1	2
Colilert (MPN/100ml)	40.2	0	1
Nitrates (mg/L)	1.2	0.45	3
Phosphates (mg/L)	0.41	0.02	3
Ammonia (mg/L)	0.07	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	1.4	0.1	3
Alkalinity (mg/L as $CaCO_3$)	67.3	2.5	3
Hardness (mg/L as $CaCO_3$)	93.3	6.7	3
Standard Plate Count (CFU/ml)	555.0	63.6	2
Acridine Orange Direct Counts (cells/g)	2.2×10^8	1.3×10^8	1
Acid Phosphatase (µg/g)	39.8	8.1	3
Alkaline Phosphatase (µg/g)	37.4	9.2	3
Dehydrogenase (µg/g)	51.4	13.6	3
Galactosidase (µg/g)	1.5	1.0	3
Glucosidase (µg/g)	9.7	6.8	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	6.7	2.9	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	4.0	0	1
Cryptosporidium sp. (cysts/L)	1.0	0	1

Table 23. Summary statistics for April 2011, site 13

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	18.7	0	1
Water Temperature (^{o}C)	11.2	0	1
pH	7.2	0	1
Conductivity (µmohs)	29.4	0	1
Dissolved Oxygen (mg/L as O_2)	9.8	0	1
Discharge (m^3/sec)	0.43	0	1
Fecal Coliform – Water (CFU/100ml)	50.0	0	3
Total Coliform – Water (CFU/100ml)	583.3	880.8	3
Fecal Coliform – Sediment (CFU/100ml)	62.5	53.0	2
Total Coliform – Sediment (CFU/100ml)	37.5	17.7	2
Colilert (MPN/100ml)	7.5	0	1
Nitrates (mg/L)	0.90	0.30	3
Phosphates (mg/L)	0.22	0.06	3
Ammonia (mg/L)	0.07	0	3
Biochemical Oxygen Demand (mg/L as O_2)	1.5	0.10	3
Alkalinity (mg/L as $CaCO_3$)	17.3	2.1	3
Hardness (mg/L as $CaCO_3$)	41.3	3.5	3
Standard Plate Count (CFU/ml)	205.0	77.8	2
Acridine Orange Direct Counts (cells/g)	1.5×10^8	3.7×10^7	1
Acid Phosphatase (µg/g)	53.3	15.9	3
Alkaline Phosphatase (µg/g)	110.5	38.4	3
Dehydrogenase (µg/g)	34.0	22.3	3
Galactosidase (µg/g)	4.8	1.6	3
Glucosidase (µg/g)	19.1	9.8	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	6.7	2.9	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	2.0	0	1
Cryptosporidium sp. (cysts/L)	4.0	0	1

Table 24. Summary statistics for April 2011, site 14

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	18.0	0	1
Water Temperature (^{o}C)	11.1	0	1
pH	6.5	0	1
Conductivity (µmohs)	17.3	0	1
Dissolved Oxygen (mg/L as O_2)	9.9	0	1
Discharge (m^3/sec)	0.04	0	1
Fecal Coliform – Water (CFU/100ml)	66.7	28.9	3
Total Coliform – Water (CFU/100ml)	150.0	132.3	3
Fecal Coliform – Sediment (CFU/100ml)	25.0	0	2
Total Coliform – Sediment (CFU/100ml)	187.5	229.8	2
Colilert (MPN/100ml)	27.9	0	1
Nitrates (mg/L)	0.77	0.40	3
Phosphates (mg/L)	0.37	0.30	3
Ammonia (mg/L)	0.07	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	1.5	0.10	3
Alkalinity (mg/L as $CaCO_3$)	10.4	0.58	3
Hardness (mg/L as $CaCO_3$)	31.4	7.2	3
Standard Plate Count (CFU/ml)	125.0	7.1	2
Acridine Orange Direct Counts (cells/g)	2.0×10^8	1.1×10^8	1
Acid Phosphatase (µg/g)	33.8	14.0	3
Alkaline Phosphatase (µg/g)	4.3	0.83	3
Dehydrogenase (µg/g)	66.9	43.0	3
Galactosidase (µg/g)	8.7	5.5	3
Glucosidase (µg/g)	19.0	8.9	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	8.3	2.9	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	2.0	0	1
Cryptosporidium sp. (cysts/L)	2.0	0	1

Table 25. Summary statistics for May 2011, site 2

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	18.5	0	1
Water Temperature (^{o}C)	13.6	0	1
pH	8.0	0	1
Conductivity (µmohs)	274.0	0	1
Dissolved Oxygen (mg/L as O_2)	9.3	0	1
Discharge (m^3/sec)	1.2	0	1
Fecal Coliform – Water (CFU/100ml)	2366.7	378.6	3
Total Coliform – Water (CFU/100ml)	6066.7	8548.9	3
Fecal Coliform – Sediment (CFU/100ml)	562.5	194.5	2
Total Coliform – Sediment (CFU/100ml)	1100.0	1520.3	2
Colilert (MPN/100ml)	435.2	0	1
Nitrates (mg/L)	1.2	0.31	3
Phosphates (mg/L)	0.14	0.05	3
Ammonia (mg/L)	0.09	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	0.76	0.04	3
Alkalinity (mg/L as $CaCO_3$)	164.3	2.5	3
Hardness (mg/L as $CaCO_3$)	177.3	4.5	3
Standard Plate Count (CFU/ml)	1175.0	190.9	2
Acridine Orange Direct Counts (cells/g)	4.8×10^8	2.7×10^8	1
Acid Phosphatase (µg/g)	21.9	8.1	3
Alkaline Phosphatase (µg/g)	22.3	3.0	3
Dehydrogenase (µg/g)	10.2	2.2	3
Galactosidase (µg/g)	2.7	1.7	3
Glucosidase (µg/g)	39.7	2.6	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	12.0	0	1
Cryptosporidium sp. (cysts/L)	2.0	0	1

Table 26. Summary statistics for May 2011, site 4

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	16.2	0	1
Water Temperature (^{o}C)	13.6	0	1
pH	8.2	0	1
Conductivity (µmohs)	224.0	0	1
Dissolved Oxygen (mg/L as O_2)	9.4	0	1
Discharge (m^3/sec)	0.46	0	1
Fecal Coliform – Water (CFU/100ml)	4200.0	1113.6	3
Total Coliform – Water (CFU/100ml)	5300.0	5915.2	3
Fecal Coliform – Sediment (CFU/100ml)	100.0	35.4	2
Total Coliform – Sediment (CFU/100ml)	2037.5	2846.1	2
Colilert (MPN/100ml)	101.2	0	1
Nitrates (mg/L)	1.1	0.52	3
Phosphates (mg/L)	0.07	0.06	3
Ammonia (mg/L)	0.10	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	0.87	0.17	3
Alkalinity (mg/L as $CaCO_3$)	149.0	4.0	3
Hardness (mg/L as $CaCO_3$)	162.3	2.1	3
Standard Plate Count (CFU/ml)	975.0	7.1	2
Acridine Orange Direct Counts (cells/g)	3.5×10^8	2.2×10^8	1
Acid Phosphatase (µg/g)	20.2	8.2	3
Alkaline Phosphatase (µg/g)	17.3	6.2	3
Dehydrogenase (µg/g)	23.5	3.9	3
Galactosidase (µg/g)	1.4	0.9	3
Glucosidase (µg/g)	71.0	33.7	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	8.0	0	1
Cryptosporidium sp. (cysts/L)	2.0	0	1

Table 27. Summary statistics for May 2011, site 7

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	18.2	0	1
Water Temperature (^{o}C)	14.1	0	1
pH	9.7	0	1
Conductivity (µmohs)	203.0	0	1
Dissolved Oxygen (mg/L as O_2)	9.2	0	1
Discharge (m^3/sec)	0.42	0	1
Fecal Coliform – Water (CFU/100ml)	433.3	251.7	3
Total Coliform – Water (CFU/100ml)	1383.3	1376.9	3
Fecal Coliform – Sediment (CFU/100ml)	287.5	53.0	2
Total Coliform – Sediment (CFU/100ml)	4950.0	318.2	2
Colilert (MPN/100ml)	8.6	0	1
Nitrates (mg/L)	0.90	0.36	3
Phosphates (mg/L)	0.16	0.04	3
Ammonia (mg/L)	0.26	0.30	3
Biochemical Oxygen Demand (mg/L as O_2)	0.95	0.24	3
Alkalinity (mg/L as $CaCO_3$)	122.3	4.2	3
Hardness (mg/L as $CaCO_3$)	130.7	1.2	3
Standard Plate Count (CFU/ml)	450.0	56.6	2
Acridine Orange Direct Counts (cells/g)	6.7×10^8	1.1×10^8	1
Acid Phosphatase (µg/g)	17.9	4.6	3
Alkaline Phosphatase (µg/g)	73.5	75.6	3
Dehydrogenase (µg/g)	26.3	6.5	3
Galactosidase (µg/g)	2.0	0.42	3
Glucosidase (µg/g)	77.0	94.1	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	3.7	5.5	3
Giardia sp.(cysts/L)	6.0	0	1
Cryptosporidium sp. (cysts/L)	10.0	0	1

Table 28. Summary statistics for May 2011, site 10

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	20.8	0	1
Water Temperature (^{o}C)	13.6	0	1
pH	7.6	0	1
Conductivity (µmohs)	123.8	0	1
Dissolved Oxygen (mg/L as O_2)	9.6	0	1
Discharge (m^3/sec)	0.32	0	1
Fecal Coliform – Water (CFU/100ml)	1200.0	173.2	3
Total Coliform – Water (CFU/100ml)	3233.3	3010.5	3
Fecal Coliform – Sediment (CFU/100ml)	275.0	70.7	2
Total Coliform – Sediment (CFU/100ml)	3762.5	5285.6	2
Colilert (MPN/100ml)	29.5	0	1
Nitrates (mg/L)	1.3	0.30	3
Phosphates (mg/L)	0.29	0.07	3
Ammonia (mg/L)	0.08	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	0.72	0.04	3
Alkalinity (mg/L as $CaCO_3$)	76.3	2.1	3
Hardness (mg/L as $CaCO_3$)	82.0	5.3	3
Standard Plate Count (CFU/ml)	815.0	21.2	2
Acridine Orange Direct Counts (cells/g)	7.0×10^7	6.3×10^7	1
Acid Phosphatase (µg/g)	54.6	29.8	3
Alkaline Phosphatase (µg/g)	109.2	30.6	3
Dehydrogenase (µg/g)	28.4	3.7	3
Galactosidase (µg/g)	2.7	1.8	3
Glucosidase (µg/g)	174.3	23.3	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	8.0	0	1
Cryptosporidium sp. (cysts/L)	2.0	0	1

Table 29. Summary statistics for May 2011, site 13

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	18.9	0	1
Water Temperature (^{o}C)	13.8	0	1
pH	7.9	0	1
Conductivity (µmohs)	35.2	0	1
Dissolved Oxygen (mg/L as O_2)	9.3	0	1
Discharge (m^3/sec)	0.10	0	1
Fecal Coliform – Water (CFU/100ml)	1066.7	763.8	3
Total Coliform – Water (CFU/100ml)	1566.7	1150.4	3
Fecal Coliform – Sediment (CFU/100ml)	175.0	106.1	2
Total Coliform – Sediment (CFU/100ml)	1131.3	1582.2	2
Colilert (MPN/100ml)	127.4	0	1
Nitrates (mg/L)	0.57	0.31	3
Phosphates (mg/L)	0.35	0.45	3
Ammonia (mg/L)	0.08	0	3
Biochemical Oxygen Demand (mg/L as O_2)	0.82	0.22	3
Alkalinity (mg/L as $CaCO_3$)	18.0	1.0	3
Hardness (mg/L as $CaCO_3$)	21.3	1.5	3
Standard Plate Count (CFU/ml)	535.0	63.6	2
Acridine Orange Direct Counts (cells/g)	2.9×10^8	7.3×10^7	1
Acid Phosphatase (µg/g)	91.0	10.4	3
Alkaline Phosphatase (µg/g)	221.9	13.0	3
Dehydrogenase (µg/g)	14.6	9.7	3
Galactosidase (µg/g)	5.4	1.5	3
Glucosidase (µg/g)	56.5	11.1	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	6.0	0	1
Cryptosporidium sp. (cysts/L)	10.0	0	1

Table 30. Summary statistics for May 2011, site 14

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	19.3	0	1
Water Temperature (^{o}C)	12.1	0	1
pH	7.7	0	1
Conductivity (µmohs)	19.8	0	1
Dissolved Oxygen (mg/L as O_2)	9.6	0	1
Discharge (m^3/sec)	0.01	0	1
Fecal Coliform – Water (CFU/100ml)	233.3	317.5	3
Total Coliform – Water (CFU/100ml)	566.7	503.3	3
Fecal Coliform – Sediment (CFU/100ml)	62.5	53.0	2
Total Coliform – Sediment (CFU/100ml)	2050.0	1520.3	2
Colilert (MPN/100ml)	8.6	0	1
Nitrates (mg/L)	0.73	0.42	3
Phosphates (mg/L)	0.27	0.08	3
Ammonia (mg/L)	0.11	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	0.70	0.04	3
Alkalinity (mg/L as $CaCO_3$)	8.0	2.0	3
Hardness (mg/L as $CaCO_3$)	13.0	7.8	3
Standard Plate Count (CFU/ml)	280.0	113.1	2
Acridine Orange Direct Counts (cells/g)	4.1×10^8	5.8×10^7	1
Acid Phosphatase (µg/g)	190.9	246.4	3
Alkaline Phosphatase (µg/g)	96.9	2.9	3
Dehydrogenase (µg/g)	30.3	3.9	3
Galactosidase (µg/g)	4.6	3.5	3
Glucosidase (µg/g)	41.4	19.3	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	6.0	0	1
Cryptosporidium sp. (cysts/L)	4.0	0	1

Table 31. Summary statistics from June 2011, site 2

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	18.6	0	1
Water Temperature (^{o}C)	17.2	0	1
pH	7.3	0	1
Conductivity (µmohs)	217.0	0	1
Dissolved Oxygen (mg/L as O_2)	8.7	0	1
Discharge (m^3/sec)	0.80	0	1
Fecal Coliform – Water (CFU/100ml)	2516.7	2141.5	3
Total Coliform – Water (CFU/100ml)	10216.7	11063.9	3
Fecal Coliform – Sediment (CFU/100ml)	125.0	106.1	2
Total Coliform – Sediment (CFU/100ml)	175.0	174.8	2
Colilert (MPN/100ml)	615.2	0	1
Nitrates (mg/L)	0.87	0.12	3
Phosphates (mg/L)	0.10	0.06	3
Ammonia (mg/L)	0.20	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	0.47	0.24	3
Alkalinity (mg/L as $CaCO_3$)	174.7	0.58	3
Hardness (mg/L as $CaCO_3$)	189.7	7.8	3
Standard Plate Count (CFU/ml)	1200.0	212.1	2
Acridine Orange Direct Counts (cells/g)	7.5×10^7	8.4×10^6	1
Acid Phosphatase (µg/g)	107.4	71.2	3
Alkaline Phosphatase (µg/g)	203.6	28.6	3
Dehydrogenase (µg/g)	24.5	4.9	3
Galactosidase (µg/g)	10.6	3.7	3
Glucosidase (µg/g)	16.0	0.24	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	18.8	0	1
Cryptosporidium sp. (cysts/L)	18.8	0	1

Table 32. Summary statistics for June 2011, site 4

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	19.1	0	1
Water Temperature (^{o}C)	17.1	0	1
pH	7.3	0	1
Conductivity (µmohs)	287.0	0	1
Dissolved Oxygen (mg/L as O_2)	9.0	0	1
Discharge (m^3/sec)	0.29	0	1
Fecal Coliform – Water (CFU/100ml)	14900.0	1670.3	3
Total Coliform – Water (CFU/100ml)	16300.0	10431.2	3
Fecal Coliform – Sediment (CFU/100ml)	825.0	388.9	2
Total Coliform – Sediment (CFU/100ml)	6050.0	7566.0	2
Colilert (MPN/100ml)	522.6	0	1
Nitrates (mg/L)	1.9	0.15	3
Phosphates (mg/L)	0.08	0.03	3
Ammonia (mg/L)	0.20	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	0.48	0.06	3
Alkalinity (mg/L as $CaCO_3$)	167.0	2.0	3
Hardness (mg/L as $CaCO_3$)	179.0	5.3	3
Standard Plate Count (CFU/ml)	765.0	91.9	2
Acridine Orange Direct Counts (cells/g)	4.7×10^7	5.3×10^7	1
Acid Phosphatase (µg/g)	75.3	27.0	3
Alkaline Phosphatase (µg/g)	449.5	329.8	3
Dehydrogenase (µg/g)	15.2	9.6	3
Galactosidase (µg/g)	3.9	1.7	3
Glucosidase (µg/g)	10.6	4.5	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	3.7	5.5	3
Giardia sp.(cysts/L)	15.8	0	1
Cryptosporidium sp. (cysts/L)	10.5	0	1

Table 33. Summary statistics for June 2011, site 7

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	19.7	0	1
Water Temperature (^{o}C)	16.3	0	1
pH	8.4	0	1
Conductivity (µmohs)	234.0	0	1
Dissolved Oxygen (mg/L as O_2)	8.5	0	1
Discharge (m^3/sec)	0.25	0	1
Fecal Coliform – Water (CFU/100ml)	333.3	321.5	3
Total Coliform – Water (CFU/100ml)	2900.0	1708.8	3
Fecal Coliform – Sediment (CFU/100ml)	625.0	530.3	2
Total Coliform – Sediment (CFU/100ml)	11575.0	2934.5	2
Colilert (MPN/100ml)	24.4	0	1
Nitrates (mg/L)	1.5	0.06	3
Phosphates (mg/L)	0.03	0.03	3
Ammonia (mg/L)	0.19	0.04	3
Biochemical Oxygen Demand (mg/L as O_2)	0.40	0.05	3
Alkalinity (mg/L as $CaCO_3$)	140.3	3.1	3
Hardness (mg/L as $CaCO_3$)	149.0	1.0	3
Standard Plate Count (CFU/ml)	370.0	127.3	2
Acridine Orange Direct Counts (cells/g)	1.0×10^8	6.3×10^7	1
Acid Phosphatase (µg/g)	70.5	9.0	3
Alkaline Phosphatase (µg/g)	208.5	55.5	3
Dehydrogenase (µg/g)	21.3	19.4	3
Galactosidase (µg/g)	0.48	0.15	3
Glucosidase (µg/g)	12.9	2.0	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	22.0	0	1
Cryptosporidium sp. (cysts/L)	11.0	0	1

Table 34. Summary statistics for June 2011, site 10

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	19.5	0	1
Water Temperature (^{o}C)	16.0	0	1
pH	8.2	0	1
Conductivity (µmohs)	234.0	0	1
Dissolved Oxygen (mg/L as O_2)	9.6	0	1
Discharge (m^3/sec)	0.18	0	1
Fecal Coliform – Water (CFU/100ml)	500.0	100.0	3
Total Coliform – Water (CFU/100ml)	4366.7	4554.5	3
Fecal Coliform – Sediment (CFU/100ml)	225.0	247.5	2
Total Coliform – Sediment (CFU/100ml)	4700.0	6364.0	2
Colilert (MPN/100ml)	42.2	0	1
Nitrates (mg/L)	0.60	0.17	3
Phosphates (mg/L)	0.07	0.06	3
Ammonia (mg/L)	0.22	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	0.36	0.07	3
Alkalinity (mg/L as $CaCO_3$)	103.3	0.60	3
Hardness (mg/L as $CaCO_3$)	109.7	1.5	3
Standard Plate Count (CFU/ml)	330.0	28.3	2
Acridine Orange Direct Counts (cells/g)	1.1×10^8	3.5×10^7	1
Acid Phosphatase (µg/g)	73.1	16.4	3
Alkaline Phosphatase (µg/g)	30.3	34.7	3
Dehydrogenase (µg/g)	27.7	2.4	3
Galactosidase (µg/g)	6.4	4.5	3
Glucosidase (µg/g)	9.7	6.8	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	14.0	0	1
Cryptosporidium sp. (cysts/L)	28.0	0	1

Table 35. Summary statistics for June 2011, site 13

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	19.1	0	1
Water Temperature (^{o}C)	17.5	0	1
pH	8.5	0	1
Conductivity (µmohs)	71.3	0	1
Dissolved Oxygen (mg/L as O_2)	8.6	0	1
Discharge (m^3/sec)	0.10	0	1
Fecal Coliform – Water (CFU/100ml)	366.7	115.5	3
Total Coliform – Water (CFU/100ml)	1933.3	1616.6	3
Fecal Coliform – Sediment (CFU/100ml)	62.5	53.0	2
Total Coliform – Sediment (CFU/100ml)	1300.0	1767.8	2
Colilert (MPN/100ml)	14.8	0	1
Nitrates (mg/L)	1.2	0.20	3
Phosphates (mg/L)	0.13	0.06	3
Ammonia (mg/L)	0.09	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	0.95	0.10	3
Alkalinity (mg/L as $CaCO_3$)	28.7	1.2	3
Hardness (mg/L as $CaCO_3$)	41.3	4.0	3
Standard Plate Count (CFU/ml)	230.1	99.0	2
Acridine Orange Direct Counts (cells/g)	6.1×10^7	1.3×10^7	1
Acid Phosphatase (µg/g)	96.2	24.8	3
Alkaline Phosphatase (µg/g)	133.0	68.0	3
Dehydrogenase (µg/g)	30.6	10.3	3
Galactosidase (µg/g)	6.0	1.7	3
Glucosidase (µg/g)	3.4	1.7	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	26.0	0	1
Cryptosporidium sp. (cysts/L)	19.0	0	1

Table 36. Summary statistics for June 2011, site 14

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	19.8	0	1
Water Temperature (^{o}C)	17.8	0	1
pH	8.4	0	1
Conductivity (µmohs)	23.4	0	1
Dissolved Oxygen (mg/L as O_2)	7.2	0	1
Discharge (m^3/sec)	0.10	0	1
Fecal Coliform – Water (CFU/100ml)	50.0	0	3
Total Coliform – Water (CFU/100ml)	1833.3	2050.2	3
Fecal Coliform – Sediment (CFU/100ml)	525.0	459.6	2
Total Coliform – Sediment (CFU/100ml)	4700.0	4949.5	2
Colilert (MPN/100ml)	32.2	0	1
Nitrates (mg/L)	0.83	0.06	3
Phosphates (mg/L)	0.90	0	3
Ammonia (mg/L)	0.14	0.03	3
Biochemical Oxygen Demand (mg/L as O_2)	0.81	0.04	3
Alkalinity (mg/L as $CaCO_3$)	10.3	1.2	3
Hardness (mg/L as $CaCO_3$)	11.0	1.0	3
Standard Plate Count (CFU/ml)	85.0	21.2	2
Acridine Orange Direct Counts (cells/g)	6.4×10^7	4.6×10^7	1
Acid Phosphatase (µg/g)	266.2	362.1	3
Alkaline Phosphatase (µg/g)	67.2	47.3	3
Dehydrogenase (µg/g)	28.9	6.2	3
Galactosidase (µg/g)	2.9	2.8	3
Glucosidase (µg/g)	5.7	4.5	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	7.3	0	1
Cryptosporidium sp. (cysts/L)	15.0	0	1

Table 37. Summary statistics for July 2011, site 2

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	20.5	0	1
Water Temperature (^{o}C)	17.0	0	1
pH	6.7	0	1
Conductivity (µmohs)	325.0	0	1
Dissolved Oxygen (mg/L as O_2)	8.3	0	1
Discharge (m^3/sec)	0.52	0	1
Fecal Coliform – Water (CFU/100ml)	7066.7	261.6	3
Total Coliform – Water (CFU/100ml)	14933.3	14204.7	3
Fecal Coliform – Sediment (CFU/100ml)	350.0	70.7	2
Total Coliform – Sediment (CFU/100ml)	8275.0	9693.4	2
Colilert (MPN/100ml)	730.8	0	1
Nitrates (mg/L)	0.57	0.31	3
Phosphates (mg/L)	0.16	0.04	3
Ammonia (mg/L)	0.06	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	0.67	0.09	3
Alkalinity (mg/L as $CaCO_3$)	196.3	1.2	3
Hardness (mg/L as $CaCO_3$)	219.0	52.2	3
Standard Plate Count (CFU/ml)	1625.0	261.6	2
Acridine Orange Direct Counts (cells/g)	2.5×10^8	7.9×10^7	1
Acid Phosphatase (µg/g)	6.9	11.8	3
Alkaline Phosphatase (µg/g)	29.8	22.5	3
Dehydrogenase (µg/g)	21.2	8.2	3
Galactosidase (µg/g)	2.2	1.7	3
Glucosidase (µg/g)	27.9	8.3	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	22.5	0	1
Cryptosporidium sp. (cysts/L)	52.5	0	1

Table 38. Summary statistics for July 2011, site 4

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	20.8	0	1
Water Temperature (^{o}C)	17.3	0	1
pH	7.3	0	1
Conductivity (µmohs)	293.0	0	1
Dissolved Oxygen (mg/L as O_2)	8.5	0	1
Discharge (m^3/sec)	0.23	0	1
Fecal Coliform – Water (CFU/100ml)	1933.3	702.4	3
Total Coliform – Water (CFU/100ml)	9553.3	8333.9	3
Fecal Coliform – Sediment (CFU/100ml)	175.0	53.4	2
Total Coliform – Sediment (CFU/100ml)	3150.0	1626.4	2
Colilert (MPN/100ml)	164.0	0	1
Nitrates (mg/L)	1.5	0.91	3
Phosphates (mg/L)	0.10	0.01	3
Ammonia (mg/L)	0.32	0.41	3
Biochemical Oxygen Demand (mg/L as O_2)	0.77	0.06	3
Alkalinity (mg/L as $CaCO_3$)	179.7	2.5	3
Hardness (mg/L as $CaCO_3$)	214.7	18.2	3
Standard Plate Count (CFU/ml)	855.0	162.6	2
Acridine Orange Direct Counts (cells/g)	2.1×10^8	1.1×10^8	1
Acid Phosphatase (µg/g)	0.10	0	3
Alkaline Phosphatase (µg/g)	22.4	4.5	3
Dehydrogenase (µg/g)	15.7	6.3	3
Galactosidase (µg/g)	1.7	1.6	3
Glucosidase (µg/g)	20.2	3.7	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	38.0	0	1
Cryptosporidium sp. (cysts/L)	14.3	0	1

Table 39. Summary statistics for July 2011, site 7

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	21.2	0	1
Water Temperature (^{o}C)	16.1	0	1
pH	7.4	0	1
Conductivity (µmohs)	223.0	0	1
Dissolved Oxygen (mg/L as O_2)	8.2	0	1
Discharge (m^3/sec)	0.15	0	1
Fecal Coliform – Water (CFU/100ml)	333.3	115.5	3
Total Coliform – Water (CFU/100ml)	4000.0	3704.1	3
Fecal Coliform – Sediment (CFU/100ml)	62.5	53.0	2
Total Coliform – Sediment (CFU/100ml)	3350.0	2474.9	2
Colilert (MPN/100ml)	10.4	0	1
Nitrates (mg/L)	0.90	0.30	3
Phosphates (mg/L)	0.10	0.03	3
Ammonia (mg/L)	0.31	0.42	3
Biochemical Oxygen Demand (mg/L as O_2)	0.32	0.40	3
Alkalinity (mg/L as $CaCO_3$)	152.7	1.5	3
Hardness (mg/L as $CaCO_3$)	166.7	8.6	3
Standard Plate Count (CFU/ml)	345	134.4	2
Acridine Orange Direct Counts (cells/g)	2.2×10^8	3.8×10^7	1
Acid Phosphatase (µg/g)	0.15	0.10	3
Alkaline Phosphatase (µg/g)	40.4	9.2	3
Dehydrogenase (µg/g)	16.8	7.0	3
Galactosidase (µg/g)	1.62	0.53	3
Glucosidase (µg/g)	24.2	3.1	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	24.0	0	1
Cryptosporidium sp. (cysts/L)	12.0	0	1

Table 40. Summary statistics for July 2011, site 10

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	25.7	0	1
Water Temperature (^{o}C)	16.4	0	1
pH	7.2	0	1
Conductivity (µmohs)	124.3	0	1
Dissolved Oxygen (mg/L as O_2)	9.4	0	1
Discharge (m^3/sec)	0.07	0	1
Fecal Coliform – Water (CFU/100ml)	700.0	519.6	3
Total Coliform – Water (CFU/100ml)	3333.3	4738.5	3
Fecal Coliform – Sediment (CFU/100ml)	450.0	212.1	2
Total Coliform – Sediment (CFU/100ml)	5675.0	1803.1	2
Colilert (MPN/100ml)	58.4	0	1
Nitrates (mg/L)	1.3	0.21	3
Phosphates (mg/L)	0.17	0.01	3
Ammonia (mg/L)	0.11	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	0.75	0.06	3
Alkalinity (mg/L as $CaCO_3$)	123.3	2.5	3
Hardness (mg/L as $CaCO_3$)	123.0	5.2	3
Standard Plate Count (CFU/ml)	320.0	42.4	2
Acridine Orange Direct Counts (cells/g)	2.7×10^8	1.4×10^8	1
Acid Phosphatase (µg/g)	4.6	4.8	3
Alkaline Phosphatase (µg/g)	56.3	14.5	3
Dehydrogenase (µg/g)	13.8	7.7	3
Galactosidase (µg/g)	2.8	0.34	3
Glucosidase (µg/g)	32.4	3.7	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	3.8	0	1
Cryptosporidium sp. (cysts/L)	15.0	0	1

Table 41. Summary statistics for July 2011, site 13

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	24.9	0	1
Water Temperature (^{o}C)	19.1	0	1
pH	7.9	0	1
Conductivity (µmohs)	73.4	0	1
Dissolved Oxygen (mg/L as O_2)	8.8	0	1
Discharge (m^3/sec)	0.06	0	1
Fecal Coliform – Water (CFU/100ml)	366.7	378.6	3
Total Coliform – Water (CFU/100ml)	9..3	808.3	3
Fecal Coliform – Sediment (CFU/100ml)	362.5	477.3	2
Total Coliform – Sediment (CFU/100ml)	7150.0	1484.9	2
Colilert (MPN/100ml)	8.2	0	1
Nitrates (mg/L)	0.50	0.50	3
Phosphates (mg/L)	0.19	0.03	3
Ammonia (mg/L)	0.06	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	0.080	0.12	3
Alkalinity (mg/L as $CaCO_3$)	38.7	1.2	3
Hardness (mg/L as $CaCO_3$)	42.0	5.6	3
Standard Plate Count (CFU/ml)	230.0	56.6	2
Acridine Orange Direct Counts (cells/g)	2.5×10^8	1.4×10^8	1
Acid Phosphatase (µg/g)	20.7	6.9	3
Alkaline Phosphatase (µg/g)	75.9	9.1	3
Dehydrogenase (µg/g)	15.3	1.3	3
Galactosidase (µg/g)	3.9	1.2	3
Glucosidase (µg/g)	28.9	3.4	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	1.4	0	1
Cryptosporidium sp. (cysts/L)	5.5	0	1

Table 42. Summary statistics for July 2011, site 14

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	24.5	0	1
Water Temperature (^{o}C)	19.3	0	1
pH	8.3	0	1
Conductivity (µmohs)	27.6	0	1
Dissolved Oxygen (mg/L as O_2)	7.1	0	1
Discharge (m^3/sec)	0.004	0	1
Fecal Coliform – Water (CFU/100ml)	100	0	3
Total Coliform – Water (CFU/100ml)	933.3	757.2	3
Fecal Coliform – Sediment (CFU/100ml)	37.5	17.7	2
Total Coliform – Sediment (CFU/100ml)	1350.0	212.1	2
Colilert (MPN/100ml)	19.4	0	1
Nitrates (mg/L)	0.67	0.40	3
Phosphates (mg/L)	0.19	0.06	3
Ammonia (mg/L)	0.04	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	0.83	0.05	3
Alkalinity (mg/L as $CaCO_3$)	10.0	2.0	3
Hardness (mg/L as $CaCO_3$)	11.3	0.58	3
Standard Plate Count (CFU/ml)	320.0	70.7	2
Acridine Orange Direct Counts (cells/g)	2.4×10^8	1.2×10^8	1
Acid Phosphatase (µg/g)	31.5	5.9	3
Alkaline Phosphatase (µg/g)	41.1	19.7	3
Dehydrogenase (µg/g)	13.5	2.0	3
Galactosidase (µg/g)	3.2	1.1	3
Glucosidase (µg/g)	35.6	4.5	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	30.0	0	1
Cryptosporidium sp. (cysts/L)	7.5	0	1

Table 43. Summary statistics for August 2011, site 2

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	18.1	0	1
Water Temperature (^{o}C)	17.7	0	1
pH	7.4	0	1
Conductivity (µmohs)	321.0	0	1
Dissolved Oxygen (mg/L as O_2)	8.9	0	1
Discharge (m^3/sec)	2.4	0	1
Fecal Coliform – Water (CFU/100ml)	3400.0	800.0	3
Total Coliform – Water (CFU/100ml)	16133.3	3028.8	3
Fecal Coliform – Sediment (CFU/100ml)	950.0	70.7	2
Total Coliform – Sediment (CFU/100ml)	1440.0	1979.9	2
Colilert (MPN/100ml)	275.0	0	1
Nitrates (mg/L)	1.2	0.10	3
Phosphates (mg/L)	0.17	0.05	3
Ammonia (mg/L)	0.08	0.03	3
Biochemical Oxygen Demand (mg/L as O_2)	0.26	0.06	3
Alkalinity (mg/L as $CaCO_3$)	186.3	3.1	3
Hardness (mg/L as $CaCO_3$)	205.7	2.3	3
Standard Plate Count (CFU/ml)	770.0	183.8	2
Acridine Orange Direct Counts (cells/g)	4.2×10^7	1.9×10^7	1
Acid Phosphatase (µg/g)	12.9	5.2	3
Alkaline Phosphatase (µg/g)	50.0	17.4	3
Dehydrogenase (µg/g)	6.1	4.5	3
Galactosidase (µg/g)	1.0	0.33	3
Glucosidase (µg/g)	32.2	5.3	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	17.5	0	1
Cryptosporidium sp. (cysts/L)	17.5	0	1

Table 44. Summary statistics for August 2011, site 4

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	17.0	0	1
Water Temperature (^{o}C)	17.4	0	1
pH	7.4	0	1
Conductivity (µmohs)	3000.0	0	1
Dissolved Oxygen (mg/L as O_2)	9.0	0	1
Discharge (m^3/sec)	0.35	0	1
Fecal Coliform – Water (CFU/100ml)	3133.3	1137.3	3
Total Coliform – Water (CFU/100ml)	20000.0	6428.1	3
Fecal Coliform – Sediment (CFU/100ml)	675.0	106.1	2
Total Coliform – Sediment (CFU/100ml)	12800.0	282.4	2
Colilert (MPN/100ml)	301.0	0	1
Nitrates (mg/L)	1.3	0.12	3
Phosphates (mg/L)	0.08	0.05	3
Ammonia (mg/L)	0.10	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	0.34	0.02	3
Alkalinity (mg/L as $CaCO_3$)	173.3	2.1	3
Hardness (mg/L as $CaCO_3$)	193.3	1.2	3
Standard Plate Count (CFU/ml)	500.0	56.6	2
Acridine Orange Direct Counts (cells/g)	9.6×10^7	4.4×10^7	1
Acid Phosphatase (µg/g)	10.9	6.8	3
Alkaline Phosphatase (µg/g)	42.4	17.7	3
Dehydrogenase (µg/g)	5.0	4.1	3
Galactosidase (µg/g)	4.2	2.4	3
Glucosidase (µg/g)	38.4	7.9	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	15.0	0	1
Cryptosporidium sp. (cysts/L)	7.0	0	1

Table 45. Summary statistics for August 2011, site 7

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	18.0	0	1
Water Temperature (^{o}C)	15.8	0	1
pH	7.0	0	1
Conductivity (µmohs)	258.0	0	1
Dissolved Oxygen (mg/L as O_2)	8.8	0	1
Discharge (m^3/sec)	0.50	0	1
Fecal Coliform – Water (CFU/100ml)	1266.7	416.3	3
Total Coliform – Water (CFU/100ml)	6333.3	2858.9	3
Fecal Coliform – Sediment (CFU/100ml)	475.0	35.4	2
Total Coliform – Sediment (CFU/100ml)	3400.0	3252.7	2
Colilert (MPN/100ml)	41.0	0	1
Nitrates (mg/L)	1.8	0.17	3
Phosphates (mg/L)	0.16	0.04	3
Ammonia (mg/L)	0.09	0.05	3
Biochemical Oxygen Demand (mg/L as O_2)	0.31	0.04	3
Alkalinity (mg/L as $CaCO_3$)	152.7	3.5	3
Hardness (mg/L as $CaCO_3$)	169.7	1.5	3
Standard Plate Count (CFU/ml)	310.0	28.3	2
Acridine Orange Direct Counts (cells/g)	6.0×10^7	1.9×10^7	1
Acid Phosphatase (µg/g)	10.2	8.6	3
Alkaline Phosphatase (µg/g)	55.7	9.0	3
Dehydrogenase (µg/g)	34.4	7.0	3
Galactosidase (µg/g)	1.8	0.68	3
Glucosidase (µg/g)	34.7	8.7	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	6.8	0	1
Cryptosporidium sp. (cysts/L)	27.0	0	1

Table 46. Summary statistics from August 2011, site 10

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	20.9	0	1
Water Temperature (^{o}C)	16.4	0	1
pH	7.6	0	1
Conductivity (µmohs)	192.8	0	1
Dissolved Oxygen (mg/L as O_2)	9.1	0	1
Discharge (m^3/sec)	0.08	0	1
Fecal Coliform – Water (CFU/100ml)	1200.0	529.1	3
Total Coliform – Water (CFU/100ml)	9133.3	3177.0	3
Fecal Coliform – Sediment (CFU/100ml)	375.0	247.5	2
Total Coliform – Sediment (CFU/100ml)	15050.0	6576.1	2
Colilert (MPN/100ml)	171.0	0	1
Nitrates (mg/L)	1.1	0.25	3
Phosphates (mg/L)	0.07	0.04	3
Ammonia (mg/L)	0.11	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	0.30	0.03	3
Alkalinity (mg/L as $CaCO_3$)	121.3	0.58	3
Hardness (mg/L as $CaCO_3$)	129.3	3.2	3
Standard Plate Count (CFU/ml)	360.0	42.4	2
Acridine Orange Direct Counts (cells/g)	8.5×10^7	4.0×10^7	1
Acid Phosphatase (µg/g)	6.4	4.5	3
Alkaline Phosphatase (µg/g)	50.6	11.8	3
Dehydrogenase (µg/g)	36.5	16.6	3
Galactosidase (µg/g)	2.5	1.8	3
Glucosidase (µg/g)	52.0	15.6	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	15.0	0	1
Cryptosporidium sp. (cysts/L)	7.0	0	1

Table 47. Summary statistics for August 2011, site 13

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	18.9	0	1
Water Temperature (^{o}C)	18.4	0	1
pH	7.0	0	1
Conductivity (µmohs)	79.5	0	1
Dissolved Oxygen (mg/L as O_2)	8.5	0	1
Discharge (m^3/sec)	0.07	0	1
Fecal Coliform – Water (CFU/100ml)	466.7	305.5	3
Total Coliform – Water (CFU/100ml)	7400.0	1249.0	3
Fecal Coliform – Sediment (CFU/100ml)	1175.0	176.8	2
Total Coliform – Sediment (CFU/100ml)	10450.0	70.7	2
Colilert (MPN/100ml)	41.0	0	1
Nitrates (mg/L)	0.27	0.06	3
Phosphates (mg/L)	0.13	0.05	3
Ammonia (mg/L)	0.06	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	0.50	0.33	3
Alkalinity (mg/L as $CaCO_3$)	42.0	1.7	3
Hardness (mg/L as $CaCO_3$)	48.3	2.5	3
Standard Plate Count (CFU/ml)	330.0	127.3	2
Acridine Orange Direct Counts (cells/g)	5.3×10^7	1.9×10^7	1
Acid Phosphatase (µg/g)	33.4	2.6	3
Alkaline Phosphatase (µg/g)	61.3	30.5	3
Dehydrogenase (µg/g)	33.5	14.1	3
Galactosidase (µg/g)	3.6	0.73	3
Glucosidase (µg/g)	25.9	6.7	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	3.8	0	1
Cryptosporidium sp. (cysts/L)	7.5	0	1

Table 48. Summary statistics for August 2011, site 14

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	18.8	0	1
Water Temperature (^{o}C)	18.1	0	1
pH	7.0	0	1
Conductivity (µmohs)	28.2	0	1
Dissolved Oxygen (mg/L as O_2)	8.2	0	1
Discharge (m^3/sec)	0.01	0	1
Fecal Coliform – Water (CFU/100ml)	333.3	115.5	3
Total Coliform – Water (CFU/100ml)	3400.0	1907.9	3
Fecal Coliform – Sediment (CFU/100ml)	275.0	35.4	2
Total Coliform – Sediment (CFU/100ml)	150.0	70.7	2
Colilert (MPN/100ml)	171.0	0	1
Nitrates (mg/L)	0.60	0.53	3
Phosphates (mg/L)	0.18	0.08	3
Ammonia (mg/L)	0.08	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	0.36	0.12	3
Alkalinity (mg/L as $CaCO_3$)	10.3	0.58	3
Hardness (mg/L as $CaCO_3$)	14.3	1.2	3
Standard Plate Count (CFU/ml)	375.0	35.4	2
Acridine Orange Direct Counts (cells/g)	1.4×10^8	4.5×10^7	1
Acid Phosphatase (µg/g)	33.7	10.6	3
Alkaline Phosphatase (µg/g)	66.6	10.3	3
Dehydrogenase (µg/g)	14.1	10.1	3
Galactosidase (µg/g)	1.1	0.42	3
Glucosidase (µg/g)	31.6	7.7	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.67	0.29	3
Giardia sp.(cysts/L)	10.8	0	1
Cryptosporidium sp. (cysts/L)	21.7	0	1

Table 49. Summary statistics for September 2011, site 2

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	17.0	0	1
Water Temperature (^{o}C)	16.3	0	1
pH	6.8	0	1
Conductivity (µmohs)	457.0	0	1
Dissolved Oxygen (mg/L as O_2)	14.2	0	1
Discharge (m^3/sec)	1.1	0	1
Fecal Coliform – Water (CFU/100ml)	2266.7	1154.7	3
Total Coliform – Water (CFU/100ml)	9066.7	10515.4	3
Fecal Coliform – Sediment (CFU/100ml)	625.0	247.5	2
Total Coliform – Sediment (CFU/100ml)	16800.0	3111.3	2
Colilert (MPN/100ml)	90.0	0	1
Nitrates (mg/L)	2.0	1.0	3
Phosphates (mg/L)	0.15	0.04	3
Ammonia (mg/L)	0.08	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	0.82	0.12	3
Alkalinity (mg/L as $CaCO_3$)	192.7	0.58	3
Hardness (mg/L as $CaCO_3$)	198.0	7.8	3
Standard Plate Count (CFU/ml)	605.0	162.6	2
Acridine Orange Direct Counts (cells/g)	2.3×10^8	4.1×10^7	1
Acid Phosphatase (µg/g)	89.8	40.5	3
Alkaline Phosphatase (µg/g)	348.7	49.0	3
Dehydrogenase (µg/g)	27.7	3.6	3
Galactosidase (µg/g)	20.5	11.8	3
Glucosidase (µg/g)	266.2	162.1	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	19.5	0	1
Cryptosporidium sp. (cysts/L)	19.5	0	1

Table 50. Summary statistics for September 2011, site 4

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	17.8	0	1
Water Temperature (^{o}C)	16.3	0	1
pH	7.2	0	1
Conductivity (µmohs)	414.0	0	1
Dissolved Oxygen (mg/L as O_2)	8.3	0	1
Discharge (m^3/sec)	0.17	0	1
Fecal Coliform – Water (CFU/100ml)	2400.0	1000.0	3
Total Coliform – Water (CFU/100ml)	12133.3	5636.8	3
Fecal Coliform – Sediment (CFU/100ml)	400.0	70.7	2
Total Coliform – Sediment (CFU/100ml)	7600.0	2828.4	2
Colilert (MPN/100ml)	65.4	0	1
Nitrates (mg/L)	1.1	0.42	3
Phosphates (mg/L)	0.14	0.06	3
Ammonia (mg/L)	0.09	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	0.95	0.13	3
Alkalinity (mg/L as $CaCO_3$)	184.7	3.8	3
Hardness (mg/L as $CaCO_3$)	194.3	1.5	3
Standard Plate Count (CFU/ml)	595.0	332.4	2
Acridine Orange Direct Counts (cells/g)	1.9×10^8	9.4×10^7	1
Acid Phosphatase (µg/g)	60.3	38.8	3
Alkaline Phosphatase (µg/g)	246.6	123.9	3
Dehydrogenase (µg/g)	28.2	3.5	3
Galactosidase (µg/g)	15.9	2.8	3
Glucosidase (µg/g)	338.8	12.8	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	18.8	0	1
Cryptosporidium sp. (cysts/L)	3.8	0	1

Table 51. Summary statistics for September 2011, site 7

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	19.3	0	1
Water Temperature (^{o}C)	15.2	0	1
pH	6.7	0	1
Conductivity (µmohs)	358.0	0	1
Dissolved Oxygen (mg/L as O_2)	7.3	0	1
Discharge (m^3/sec)	0.08	0	1
Fecal Coliform – Water (CFU/100ml)	200.0	173.2	3
Total Coliform – Water (CFU/100ml)	2466.7	1553.5	3
Fecal Coliform – Sediment (CFU/100ml)	250.0	282.8	2
Total Coliform – Sediment (CFU/100ml)	5650.0	212.1	2
Colilert (MPN/100ml)	19.0	0	1
Nitrates (mg/L)	1.0	0.46	3
Phosphates (mg/L)	0.26	0.24	3
Ammonia (mg/L)	0.12	0.03	3
Biochemical Oxygen Demand (mg/L as O_2)	0.87	0.12	3
Alkalinity (mg/L as $CaCO_3$)	154.3	2.5	3
Hardness (mg/L as $CaCO_3$)	168.7	4.0	3
Standard Plate Count (CFU/ml)	135.0	49.5	2
Acridine Orange Direct Counts (cells/g)	2.4×10^8	6.0×10^7	1
Acid Phosphatase (µg/g)	27.4	25.0	3
Alkaline Phosphatase (µg/g)	367.9	31.4	3
Dehydrogenase (µg/g)	29.9	6.7	3
Galactosidase (µg/g)	16.6	4.5	3
Glucosidase (µg/g)	136.2	67.0	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	18.8	0	1
Cryptosporidium sp. (cysts/L)	18.8	0	1

Table 52. Summary statistics for September 2011, site 10

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	20.3	0	1
Water Temperature (^{o}C)	16.4	0	1
pH	6.3	0	1
Conductivity (µmohs)	290.0	0	1
Dissolved Oxygen (mg/L as O_2)	8.0	0	1
Discharge (m^3/sec)	0.06	0	1
Fecal Coliform – Water (CFU/100ml)	466.7	305.5	3
Total Coliform – Water (CFU/100ml)	6200.0	2986.6	3
Fecal Coliform – Sediment (CFU/100ml)	150.0	70.7	2
Total Coliform – Sediment (CFU/100ml)	2075.0	742.5	2
Colilert (MPN/100ml)	49.2	0	1
Nitrates (mg/L)	0.87	0.40	3
Phosphates (mg/L)	0.14	0.07	3
Ammonia (mg/L)	0.14	0.07	3
Biochemical Oxygen Demand (mg/L as O_2)	0.82	0.04	3
Alkalinity (mg/L as $CaCO_3$)	130.0	2.6	3
Hardness (mg/L as $CaCO_3$)	134.0	3.0	3
Standard Plate Count (CFU/ml)	445.0	49.5	2
Acridine Orange Direct Counts (cells/g)	2.6×10^8	4.1×10^7	1
Acid Phosphatase (µg/g)	40.8	31.5	3
Alkaline Phosphatase (µg/g)	364.0	30.4	3
Dehydrogenase (µg/g)	19.6	3.3	3
Galactosidase (µg/g)	14.9	12.4	3
Glucosidase (µg/g)	483.2	14.6	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	16.5	0	1
Cryptosporidium sp. (cysts/L)	11.0	0	1

Table 53. Summary statistics for September 2011, site 13

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	20.4	0	1
Water Temperature (^{o}C)	17.5	0	1
pH	7.0	0	1
Conductivity (µmohs)	84.5	0	1
Dissolved Oxygen (mg/L as O_2)	7.2	0	1
Discharge (m^3/sec)	0.06	0	1
Fecal Coliform – Water (CFU/100ml)	400.0	200.0	3
Total Coliform – Water (CFU/100ml)	5933.3	1942.5	3
Fecal Coliform – Sediment (CFU/100ml)	625.0	106.1	2
Total Coliform – Sediment (CFU/100ml)	4625.0	388.9	2
Colilert (MPN/100ml)	52.4	0	1
Nitrates (mg/L)	1.1	0.20	3
Phosphates (mg/L)	0.19	0.17	3
Ammonia (mg/L)	0.07	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	0.89	0.22	3
Alkalinity (mg/L as $CaCO_3$)	51.3	2.1	3
Hardness (mg/L as $CaCO_3$)	52.3	1.5	3
Standard Plate Count (CFU/ml)	205.0	7.1	2
Acridine Orange Direct Counts (cells/g)	1.9×10^8	1.3×10^8	1
Acid Phosphatase (µg/g)	81.9	15.4	3
Alkaline Phosphatase (µg/g)	248.7	75.8	3
Dehydrogenase (µg/g)	26.7	0.17	3
Galactosidase (µg/g)	106.4	8.1	3
Glucosidase (µg/g)	195.9	112.6	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	6.0	0	1
Cryptosporidium sp. (cysts/L)	3.0	0	1

Table 54. Summary statistics from September 2011, site 14

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	20.5	0	1
Water Temperature (^{o}C)	17.3	0	1
pH	6.7	0	1
Conductivity (µmohs)	30.1	0	1
Dissolved Oxygen (mg/L as O_2)	7.1	0	1
Discharge (m^3/sec)	0.02	0	1
Fecal Coliform – Water (CFU/100ml)	133.3	57.7	3
Total Coliform – Water (CFU/100ml)	3466.7	1404.8	3
Fecal Coliform – Sediment (CFU/100ml)	62.5	53.0	2
Total Coliform – Sediment (CFU/100ml)	300.0	282.8	2
Colilert (MPN/100ml)	24.2	0	1
Nitrates (mg/L)	1.0	0.20	3
Phosphates (mg/L)	0.27	0.19	3
Ammonia (mg/L)	0.10	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	0.62	0.10	3
Alkalinity (mg/L as $CaCO_3$)	13.0	0	3
Hardness (mg/L as $CaCO_3$)	13.0	0	3
Standard Plate Count (CFU/ml)	245.0	162.6	2
Acridine Orange Direct Counts (cells/g)	1.4×10^8	4.7×10^7	1
Acid Phosphatase (µg/g)	84.9	9.6	3
Alkaline Phosphatase (µg/g)	268.2	44.3	3
Dehydrogenase (µg/g)	24.8	2.1	3
Galactosidase (µg/g)	21.4	7.0	3
Glucosidase (µg/g)	223.0	21.6	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	4.3	0	1
Cryptosporidium sp. (cysts/L)	8.5	0	1

Table 55. Summary statistics from October 2011, site 2

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	8.6	0	1
Water Temperature (^{o}C)	12.6	0	1
pH	6.7	0	1
Conductivity (µmohs)	399.0	0	1
Dissolved Oxygen (mg/L as O_2)	9.4	0	1
Discharge (m^3/sec)	0.37	0	1
Fecal Coliform – Water (CFU/100ml)	600.0	200.0	3
Total Coliform – Water (CFU/100ml)	3466.7	1026.3	3
Fecal Coliform – Sediment (CFU/100ml)	150.0	141.4	2
Total Coliform – Sediment (CFU/100ml)	3575.0	247.5	2
Colilert (MPN/100ml)	145.0	0	1
Nitrates (mg/L)	1.27	0.32	3
Phosphates (mg/L)	0.10	0.10	3
Ammonia (mg/L)	0.06	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	0.65	0.13	3
Alkalinity (mg/L as $CaCO_3$)	184.0	2.6	3
Hardness (mg/L as $CaCO_3$)	191.0	5.6	3
Standard Plate Count (CFU/ml)	1160.0	127.3	2
Acridine Orange Direct Counts (cells/g)	2.1×10^8	8.4×10^7	1
Acid Phosphatase (µg/g)	96.5	32.8	3
Alkaline Phosphatase (µg/g)	683.5	370.8	3
Dehydrogenase (µg/g)	28.0	6.1	3
Galactosidase (µg/g)	45.0	25.4	3
Glucosidase (µg/g)	297.0	67.1	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	11.3	0	1
Cryptosporidium sp. (cysts/L)	11.3	0	1

Table 56. Summary statistics from October 2011, site 4

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	8.5	0	1
Water Temperature (^{o}C)	12.3	0	1
pH	7.0	0	1
Conductivity (µmohs)	351.0	0	1
Dissolved Oxygen (mg/L as O_2)	9.0	0	1
Discharge (m^3/sec)	0.15	0	1
Fecal Coliform – Water (CFU/100ml)	366.7	251.7	3
Total Coliform – Water (CFU/100ml)	7133.3	3711.2	3
Fecal Coliform – Sediment (CFU/100ml)	75.0	35.4	2
Total Coliform – Sediment (CFU/100ml)	1925.0	883.9	2
Colilert (MPN/100ml)	73.3	0	1
Nitrates (mg/L)	1.7	0.62	3
Phosphates (mg/L)	0.24	0.13	3
Ammonia (mg/L)	0.05	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	0.56	0.06	3
Alkalinity (mg/L as $CaCO_3$)	177.0	2.0	3
Hardness (mg/L as $CaCO_3$)	184.3	3.2	3
Standard Plate Count (CFU/ml)	785.0	162.6	2
Acridine Orange Direct Counts (cells/g)	4.6×10^8	6.3×10^7	1
Acid Phosphatase (µg/g)	138.8	36.5	3
Alkaline Phosphatase (µg/g)	233.0	104.7	3
Dehydrogenase (µg/g)	13.9	11.8	3
Galactosidase (µg/g)	33.7	5.0	3
Glucosidase (µg/g)	149.0	0.88	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	2.4	0	1
Cryptosporidium sp. (cysts/L)	14.3	0	1

Table 57. Summary statistics for October 2011, site 7

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	8.0	0	1
Water Temperature (^{o}C)	13.7	0	1
pH	6.8	0	1
Conductivity (µmohs)	350.	0	1
Dissolved Oxygen (mg/L as O_2)	801	0	1
Discharge (m^3/sec)	0.18	0	1
Fecal Coliform – Water (CFU/100ml)	100.0	0	3
Total Coliform – Water (CFU/100ml)	1200.0	0	3
Fecal Coliform – Sediment (CFU/100ml)	87.5	88.4	2
Total Coliform – Sediment (CFU/100ml)	4925.0	4348.7	2
Colilert (MPN/100ml)	13.5	0	1
Nitrates (mg/L)	1.6	0.61	3
Phosphates (mg/L)	0.11	0.07	3
Ammonia (mg/L)	0.67	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	0.51	0.23	3
Alkalinity (mg/L as $CaCO_3$)	151.3	1.2	3
Hardness (mg/L as $CaCO_3$)	161.3	5.7	3
Standard Plate Count (CFU/ml)	85.0	21.2	2
Acridine Orange Direct Counts (cells/g)	1.7×10^8	8.6×10^7	1
Acid Phosphatase (µg/g)	84.9	33.0	3
Alkaline Phosphatase (µg/g)	410.0	58.0	3
Dehydrogenase (µg/g)	31.3	7.4	3
Galactosidase (µg/g)	22.0	8.9	3
Glucosidase (µg/g)	237.8	70.4	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	7.5	0	1
Cryptosporidium sp. (cysts/L)	1.9	0	1

Table 58. Summary statistics for October 2011, site 10

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	7.7	0	1
Water Temperature (^{o}C)	12.8	0	1
pH	7.3	0	1
Conductivity (µmohs)	178.9	0	1
Dissolved Oxygen (mg/L as O_2)	8.6	0	1
Discharge (m^3/sec)	0.07	0	1
Fecal Coliform – Water (CFU/100ml)	100.0	0	3
Total Coliform – Water (CFU/100ml)	3200.0	721.1	3
Fecal Coliform – Sediment (CFU/100ml)	25.0	0	2
Total Coliform – Sediment (CFU/100ml)	475.0	388.9	2
Colilert (MPN/100ml)	56.3	0	1
Nitrates (mg/L)	0.55	0.44	3
Phosphates (mg/L)	0.21	0.07	3
Ammonia (mg/L)	0.08	0.01	3
Biochemical Oxygen Demand (mg/L as O_2)	0.52	0.03	3
Alkalinity (mg/L as $CaCO_3$)	125.0	4.4	3
Hardness (mg/L as $CaCO_3$)	135.7	9.1	3
Standard Plate Count (CFU/ml)	360.0	183.8	2
Acridine Orange Direct Counts (cells/g)	1.3×10^8	4.9×10^7	1
Acid Phosphatase (µg/g)	58.7	10.1	3
Alkaline Phosphatase (µg/g)	204.5	50.5	3
Dehydrogenase (µg/g)	23.0	5.9	3
Galactosidase (µg/g)	12.6	11.1	3
Glucosidase (µg/g)	170.9	75.0	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.67	0.28	3
Giardia sp.(cysts/L)	4.8	0	1
Cryptosporidium sp. (cysts/L)	4.8	0	1

Table 59. Summary statistics for October 2011, site 13

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	7.6	0	1
Water Temperature (^{o}C)	10.2	0	1
pH	6.9	0	1
Conductivity (µmohs)	64.4	0	1
Dissolved Oxygen (mg/L as O_2)	8.1	0	1
Discharge (m^3/sec)	0.20	0	1
Fecal Coliform – Water (CFU/100ml)	100.0	0	3
Total Coliform – Water (CFU/100ml)	1200.0	1216.6	3
Fecal Coliform – Sediment (CFU/100ml)	25.0	0	2
Total Coliform – Sediment (CFU/100ml)	800.0	636.4	2
Colilert (MPN/100ml)	6.3	0	1
Nitrates (mg/L)	1.13	0.94	3
Phosphates (mg/L)	0.24	0.06	3
Ammonia (mg/L)	0.07	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	0.67	0.02	3
Alkalinity (mg/L as $CaCO_3$)	45.0	4.4	3
Hardness (mg/L as $CaCO_3$)	45.3	1.5	3
Standard Plate Count (CFU/ml)	160	99.0	2
Acridine Orange Direct Counts (cells/g)	1.4×10^8	9.8×10^7	1
Acid Phosphatase (µg/g)	84.2	43.8	3
Alkaline Phosphatase (µg/g)	815.2	168.0	3
Dehydrogenase (µg/g)	23.2	15.0	3
Galactosidase (µg/g)	24.1	6.9	3
Glucosidase (µg/g)	173.5	10.4	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	5.5	0	1
Cryptosporidium sp. (cysts/L)	11.0	0	1

Table 60. Summary statistics for October 2011, site 14

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	7.0	0	1
Water Temperature (^{o}C)	9.8	0	1
pH	6.8	0	1
Conductivity (µmohs)	23.3	0	1
Dissolved Oxygen (mg/L as O_2)	8.2	0	1
Discharge (m^3/sec)	0.02	0	1
Fecal Coliform – Water (CFU/100ml)	200.0	0	3
Total Coliform – Water (CFU/100ml)	2466.7	2893.7	3
Fecal Coliform – Sediment (CFU/100ml)	25.0	0	2
Total Coliform – Sediment (CFU/100ml)	112.5	123.7	2
Colilert (MPN/100ml)	14.6	0	1
Nitrates (mg/L)	0.83	0.31	3
Phosphates (mg/L)	0.18	0.09	3
Ammonia (mg/L)	0.07	0.03	3
Biochemical Oxygen Demand (mg/L as O_2)	0.97	0.06	3
Alkalinity (mg/L as $CaCO_3$)	12.7	3.5	3
Hardness (mg/L as $CaCO_3$)	12.3	0.58	3
Standard Plate Count (CFU/ml)	1980.0	495.0	2
Acridine Orange Direct Counts (cells/g)	1.9×10^8	2.8×10^7	1
Acid Phosphatase (µg/g)	144.6	29.6	3
Alkaline Phosphatase (µg/g)	297.8	158.2	3
Dehydrogenase (µg/g)	23.2	13.4	3
Galactosidase (µg/g)	15.6	7.6	3
Glucosidase (µg/g)	170.5	18.1	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.66	0.28	3
Giardia sp.(cysts/L)	21.3	0	1
Cryptosporidium sp. (cysts/L)	8.5	0	1

Table 61. Summary statistics from November 2011, site 2

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	-1.5	0	1
Water Temperature (^{o}C)	7.9	0	1
pH	7.3	0	1
Conductivity (µmohs)	329.0	0	1
Dissolved Oxygen (mg/L as O_2)	11.6	0	1
Discharge (m^3/sec)	0.51	0	1
Fecal Coliform – Water (CFU/100ml)	1300.0	1044.0	3
Total Coliform – Water (CFU/100ml)	7666.7	2759.2	3
Fecal Coliform – Sediment (CFU/100ml)	425.0	247.5	2
Total Coliform – Sediment (CFU/100ml)	4325.0	883.9	2
Colilert (MPN/100ml)	141.4	0	1
Nitrates (mg/L)	1.6	0.46	3
Phosphates (mg/L)	0.20	0.05	3
Ammonia (mg/L)	0.07	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	1.1	0.43	3
Alkalinity (mg/L as $CaCO_3$)	128.7	18.9	3
Hardness (mg/L as $CaCO_3$)	174.7	2.1	3
Standard Plate Count (CFU/ml)	460.0	0	2
Acridine Orange Direct Counts (cells/g)	1.5×10^8	3.3×10^7	1
Acid Phosphatase (µg/g)	62.9	8.8	3
Alkaline Phosphatase (µg/g)	234.7	101.7	3
Dehydrogenase (µg/g)	30.7	4.6	3
Galactosidase (µg/g)	12.7	11.6	3
Glucosidase (µg/g)	94.9	26.2	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	4.9	7.6	3
Giardia sp.(cysts/L)	5.3	0	1
Cryptosporidium sp. (cysts/L)	10.5	0	1

Table 62. Summary statistics for November 2011, site 4

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	-1.3	0	1
Water Temperature (^{o}C)	7.9	0	1
pH	7.3	0	1
Conductivity (µmohs)	299.0	0	1
Dissolved Oxygen (mg/L as O_2)	10.2	0	1
Discharge (m^3/sec)	0.24	0	1
Fecal Coliform – Water (CFU/100ml)	800.0	400.0	3
Total Coliform – Water (CFU/100ml)	10933.3	2830.8	3
Fecal Coliform – Sediment (CFU/100ml)	150.0	141.4	2
Total Coliform – Sediment (CFU/100ml)	2100.0	919.2	2
Colilert (MPN/100ml)	151.0	0	1
Nitrates (mg/L)	1.5	0.85	3
Phosphates (mg/L)	0.44	0.06	3
Ammonia (mg/L)	0.07	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	1.6	0.02	3
Alkalinity (mg/L as $CaCO_3$)	128.0	5.6	3
Hardness (mg/L as $CaCO_3$)	161.0	1.7	3
Standard Plate Count (CFU/ml)	575.0	91.9	2
Acridine Orange Direct Counts (cells/g)	1.8×10^8	2.9×10^7	1
Acid Phosphatase (µg/g)	62.1	8.8	3
Alkaline Phosphatase (µg/g)	313.0	88.8	3
Dehydrogenase (µg/g)	22.2	5.6	3
Galactosidase (µg/g)	29.0	5.2	3
Glucosidase (µg/g)	56.0	7.2	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	4.0	6.0	3
Giardia sp.(cysts/L)	11.0	0	1
Cryptosporidium sp. (cysts/L)	5.0	0	1

Table 63. Summary statistics for November 2011, site 7

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	1.5	0	1
Water Temperature (^{o}C)	9.9	0	1
pH	7.7	0	1
Conductivity (µmohs)	276.0	0	1
Dissolved Oxygen (mg/L as O_2)	9.1	0	1
Discharge (m^3/sec)	0.41	0	1
Fecal Coliform – Water (CFU/100ml)	133.3	57.7	3
Total Coliform – Water (CFU/100ml)	1333.3	305.5	3
Fecal Coliform – Sediment (CFU/100ml)	175.0	35.4	2
Total Coliform – Sediment (CFU/100ml)	3350.0	70.7	2
Colilert (MPN/100ml)	8.5	0	1
Nitrates (mg/L)	1.9	0.20	3
Phosphates (mg/L)	0.18	0.02	3
Ammonia (mg/L)	0.09	0.04	3
Biochemical Oxygen Demand (mg/L as O_2)	1.4	0.10	3
Alkalinity (mg/L as $CaCO_3$)	104.0	5.2	3
Hardness (mg/L as $CaCO_3$)	129.0	3.6	3
Standard Plate Count (CFU/ml)	125.0	35.4	2
Acridine Orange Direct Counts (cells/g)	1.7×10^8	4.0×10^7	1
Acid Phosphatase (µg/g)	75.5	22.1	3
Alkaline Phosphatase (µg/g)	474.4	214.4	3
Dehydrogenase (µg/g)	23.7	4.5	3
Galactosidase (µg/g)	43.9	1.9	3
Glucosidase (µg/g)	89.7	43.4	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.94	0.76	3
Giardia sp.(cysts/L)	4.5	0	1
Cryptosporidium sp. (cysts/L)	4.5	0	1

Table 64. Summary statistics for November 2011, site 10

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	1.3	0	1
Water Temperature (^{o}C)	8.7	0	1
pH	7.6	0	1
Conductivity (µmohs)	136.1	0	1
Dissolved Oxygen (mg/L as O_2)	8.0	0	1
Discharge (m^3/sec)	0.26	0	1
Fecal Coliform – Water (CFU/100ml)	400.0	200.0	3
Total Coliform – Water (CFU/100ml)	3933.3	832.7	3
Fecal Coliform – Sediment (CFU/100ml)	75.0	35.4	2
Total Coliform – Sediment (CFU/100ml)	1125.0	106.1	2
Colilert (MPN/100ml)	193.5	0	1
Nitrates (mg/L)	1.2	0.12	3
Phosphates (mg/L)	0.17	0.07	3
Ammonia (mg/L)	0.09	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	1.3	0.15	3
Alkalinity (mg/L as $CaCO_3$)	65.3	1.2	3
Hardness (mg/L as $CaCO_3$)	84.3	2.1	3
Standard Plate Count (CFU/ml)	530.0	141.2	2
Acridine Orange Direct Counts (cells/g)	6.8×10^7	5.3×10^7	1
Acid Phosphatase (µg/g)	37.7	24.1	3
Alkaline Phosphatase (µg/g)	283.8	122.7	3
Dehydrogenase (µg/g)	16.6	3.7	3
Galactosidase (µg/g)	29.5	13.8	3
Glucosidase (µg/g)	134.0	64.1	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.64	0.24	3
Giardia sp.(cysts/L)	45.0	0	1
Cryptosporidium sp. (cysts/L)	30.0	0	1

Table 65. Summary statistics for November 2011, site 13

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	3.1	0	1
Water Temperature (^{o}C)	6.5	0	1
pH	7.3	0	1
Conductivity (µmohs)	82.3	0	1
Dissolved Oxygen (mg/L as O_2)	7.6	0	1
Discharge (m^3/sec)	0.23	0	1
Fecal Coliform – Water (CFU/100ml)	100.0	0	3
Total Coliform – Water (CFU/100ml)	933.3	503.3	3
Fecal Coliform – Sediment (CFU/100ml)	75.0	35.4	2
Total Coliform – Sediment (CFU/100ml)	1025.0	176.8	2
Colilert (MPN/100ml)	9.7	0	1
Nitrates (mg/L)	1.4	0.40	3
Phosphates (mg/L)	0.15	.06	3
Ammonia (mg/L)	0.06	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	1.2	0.08	3
Alkalinity (mg/L as $CaCO_3$)	16.7	4.9	3
Hardness (mg/L as $CaCO_3$)	24.0	2.0	3
Standard Plate Count (CFU/ml)	125.0	35.4	2
Acridine Orange Direct Counts (cells/g)	1.0×10^8	5.5×10^7	1
Acid Phosphatase (µg/g)	111.1	25.4	3
Alkaline Phosphatase (µg/g)	858.6	367.7	3
Dehydrogenase (µg/g)	13.6	3.9	3
Galactosidase (µg/g)	48.3	19.3	3
Glucosidase (µg/g)	196.5	26.0	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	1.0	0.87	3
Giardia sp.(cysts/L)	4.5	0	1
Cryptosporidium sp. (cysts/L)	4.5	0	1

Table 66. Summary statistics for November 2011, site 14

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	5.5	0	1
Water Temperature (^{o}C)	6.5	0	1
pH	7.4	0	1
Conductivity (µmohs)	18.8	0	1
Dissolved Oxygen (mg/L as O_2)	7.5	0	1
Discharge (m^3/sec)	0.04	0	1
Fecal Coliform – Water (CFU/100ml)	100.0	0	3
Total Coliform – Water (CFU/100ml)	500.0	360.6	3
Fecal Coliform – Sediment (CFU/100ml)	25.0	0	2
Total Coliform – Sediment (CFU/100ml)	25.0	0	2
Colilert (MPN/100ml)	2.0	0	1
Nitrates (mg/L)	1.4	0	3
Phosphates (mg/L)	0.19	0.09	3
Ammonia (mg/L)	0.05	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	0.31	0.14	3
Alkalinity (mg/L as $CaCO_3$)	12.0	1.0	3
Hardness (mg/L as $CaCO_3$)	10.0	1.0	3
Standard Plate Count (CFU/ml)	225.0	120.2	2
Acridine Orange Direct Counts (cells/g)	1.4×10^8	7.3×10^7	1
Acid Phosphatase (µg/g)	57.2	45.2	3
Alkaline Phosphatase (µg/g)	348.7	17.6	3
Dehydrogenase (µg/g)	9.5	3.6	3
Galactosidase (µg/g)	128.0	27.4	3
Glucosidase (µg/g)	250.6	85.7	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.63	0.23	3
Giardia sp.(cysts/L)	9.0	0	1
Cryptosporidium sp. (cysts/L)	18.0	0	1

Table 67. Summary statistics for December 2011, site 2

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	-1.5	0	1
Water Temperature (^{o}C)	7.4	0	1
pH	6.6	0	1
Conductivity (µmohs)	354.0	0	1
Dissolved Oxygen (mg/L as O_2)	12.5	0	1
Discharge (m^3/sec)	0.33	0	1
Fecal Coliform – Water (CFU/100ml)	266.7	115.5	3
Total Coliform – Water (CFU/100ml)	3400.0	2800.0	3
Fecal Coliform – Sediment (CFU/100ml)	100.0	0	2
Total Coliform – Sediment (CFU/100ml)	2500.0	1060.7	2
Colilert (MPN/100ml)	113.7	0	1
Nitrates (mg/L)	2.7	0.98	3
Phosphates (mg/L)	0.21	0.06	3
Ammonia (mg/L)	0.09	0.04	3
Biochemical Oxygen Demand (mg/L as O_2)	1.6	0.36	3
Alkalinity (mg/L as $CaCO_3$)	163.7	14.2	3
Hardness (mg/L as $CaCO_3$)	190.7	5.0	3
Standard Plate Count (CFU/ml)	170.0	42.4	2
Acridine Orange Direct Counts (cells/g)	1.6×10^8	1.7×10^7	1
Acid Phosphatase (µg/g)	130.1	83.9	3
Alkaline Phosphatase (µg/g)	507.2	113.2	3
Dehydrogenase (µg/g)	27.0	17.5	3
Galactosidase (µg/g)	45.3	25.0	3
Glucosidase (µg/g)	154.7	28.7	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	24.0	0	1
Cryptosporidium sp. (cysts/L)	36.0	0	1

Table 68. Summary statistics for December 2011, site 4

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	-1.6	0	1
Water Temperature (^{o}C)	7.3	0	1
pH	7.5	0	1
Conductivity (µmohs)	331.0	0	1
Dissolved Oxygen (mg/L as O_2)	11.7	0	1
Discharge (m^3/sec)	0.37	0	1
Fecal Coliform – Water (CFU/100ml)	223.3	152.8	3
Total Coliform – Water (CFU/100ml)	3666.7	2275.5	3
Fecal Coliform – Sediment (CFU/100ml)	37.5	17.7	2
Total Coliform – Sediment (CFU/100ml)	575.0	176.8	2
Colilert (MPN/100ml)	104.3	0	1
Nitrates (mg/L)	1.8	0.56	3
Phosphates (mg/L)	0.42	0.22	3
Ammonia (mg/L)	0.06	0.05	3
Biochemical Oxygen Demand (mg/L as O_2)	1.3	0.11	3
Alkalinity (mg/L as $CaCO_3$)	145.3	4.0	3
Hardness (mg/L as $CaCO_3$)	186.0	2.6	3
Standard Plate Count (CFU/ml)	200.0	42.4	2
Acridine Orange Direct Counts (cells/g)	6.1×10^7	1.3×10^7	1
Acid Phosphatase (µg/g)	40.4	24.1	3
Alkaline Phosphatase (µg/g)	335.7	184.4	3
Dehydrogenase (µg/g)	29.5	5.1	3
Galactosidase (µg/g)	24.7	16.8	3
Glucosidase (µg/g)	67.5	78.1	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	9.0	0	1
Cryptosporidium sp. (cysts/L)	9.0	0	1

Table 69. Summary statistics for December 2011, site 7

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	0.80	0	1
Water Temperature (^{o}C)	11.0	0	1
pH	7.2	0	1
Conductivity (µmohs)	309.0	0	1
Dissolved Oxygen (mg/L as O_2)	10.2	0	1
Discharge (m^3/sec)	0.26	0	1
Fecal Coliform – Water (CFU/100ml)	100.0	0	3
Total Coliform – Water (CFU/100ml)	333.3	230.7	3
Fecal Coliform – Sediment (CFU/100ml)	100.0	70.7	2
Total Coliform – Sediment (CFU/100ml)	16400.0	3394.1	2
Colilert (MPN/100ml)	5.2	0	1
Nitrates (mg/L)	1.6	0.17	3
Phosphates (mg/L)	0.19	0.01	3
Ammonia (mg/L)	0.12	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	1.2	0.09	3
Alkalinity (mg/L as $CaCO_3$)	117.7	1.5	3
Hardness (mg/L as $CaCO_3$)	149.3	6.7	3
Standard Plate Count (CFU/ml)	55.0	21.2	2
Acridine Orange Direct Counts (cells/g)	1.1×10^8	1.7×10^7	1
Acid Phosphatase (µg/g)	86.3	20.4	3
Alkaline Phosphatase (µg/g)	522.5	32.6	3
Dehydrogenase (µg/g)	16.4	11.8	3
Galactosidase (µg/g)	20.1	9.4	3
Glucosidase (µg/g)	79.8	52.4	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	7.5	0	1
Cryptosporidium sp. (cysts/L)	15.0	0	1

Table 70. Summary statistics for December 2011, site 10

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	2.4	0	1
Water Temperature (^{o}C)	9.2	0	1
pH	6.8	0	1
Conductivity (µmohs)	134.3	0	1
Dissolved Oxygen (mg/L as O_2)	10.8	0	1
Discharge (m^3/sec)	0.22	0	1
Fecal Coliform – Water (CFU/100ml)	100.0	0	3
Total Coliform – Water (CFU/100ml)	2066.7	2386.1	3
Fecal Coliform – Sediment (CFU/100ml)	37.5	17.7	2
Total Coliform – Sediment (CFU/100ml)	437.5	583.4	2
Colilert (MPN/100ml)	18.3	0	1
Nitrates (mg/L)	1.4	0.40	3
Phosphates (mg/L)	0.24	0.07	3
Ammonia (mg/L)	0.09	0.04	3
Biochemical Oxygen Demand (mg/L as O_2)	1.1	0.06	3
Alkalinity (mg/L as $CaCO_3$)	84.7	0.58	3
Hardness (mg/L as $CaCO_3$)	103.3	1.2	3
Standard Plate Count (CFU/ml)	75.0	35.4	2
Acridine Orange Direct Counts (cells/g)	9.8×10^7	4.2×10^7	1
Acid Phosphatase (µg/g)	72.2	40.4	3
Alkaline Phosphatase (µg/g)	721.9	381.0	3
Dehydrogenase (µg/g)	33.8	24.6	3
Galactosidase (µg/g)	8.6	6.8	3
Glucosidase (µg/g)	106.7	49.7	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	6.3	0	1
Cryptosporidium sp. (cysts/L)	6.3	0	1

Table 71. Summary statistics for December 2011, site 13

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	5.3	0	1
Water Temperature (^{o}C)	4.7	0	1
pH	7.4	0	1
Conductivity (µmohs)	34.9	0	1
Dissolved Oxygen (mg/L as O_2)	11.5	0	1
Discharge (m^3/sec)	0.08	0	1
Fecal Coliform – Water (CFU/100ml)	100.0	0	3
Total Coliform – Water (CFU/100ml)	333.3	115.5	3
Fecal Coliform – Sediment (CFU/100ml)	37.5	17.7	2
Total Coliform – Sediment (CFU/100ml)	350.0	424.3	2
Colilert (MPN/100ml)	4.1	0	1
Nitrates (mg/L)	1.2	0.40	3
Phosphates (mg/L)	0.21	0.03	3
Ammonia (mg/L)	0.10	0.02	3
Biochemical Oxygen Demand (mg/L as O_2)	1.5	0.19	3
Alkalinity (mg/L as $CaCO_3$)	20.0	1.0	3
Hardness (mg/L as $CaCO_3$)	27.7	1.2	3
Standard Plate Count (CFU/ml)	30.0	14.1	2
Acridine Orange Direct Counts (cells/g)	8.5×10^7	1.7×10^7	1
Acid Phosphatase (µg/g)	124.5	28.2	3
Alkaline Phosphatase (µg/g)	835.6	16.7	3
Dehydrogenase (µg/g)	20.8	11.8	3
Galactosidase (µg/g)	33.8	18.0	3
Glucosidase (µg/g)	74.8	15.9	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	17.5	0	1
Cryptosporidium sp. (cysts/L)	17.5	0	1

Table 72. Summary statistics for December 2011, site 14

Variable	Mean	Std Dev	N
Air Temperature (^{o}C)	4.8	0	1
Water Temperature (^{o}C)	5.3	0	1
pH	6.9	0	1
Conductivity (µmohs)	18.2	0	1
Dissolved Oxygen (mg/L as O_2)	11.6	0	1
Discharge (m^3/sec)	0.02	0	1
Fecal Coliform – Water (CFU/100ml)	100.0	0	3
Total Coliform – Water (CFU/100ml)	666.7	808.3	3
Fecal Coliform – Sediment (CFU/100ml)	25.0	0	2
Total Coliform – Sediment (CFU/100ml)	175.0	106.1	2
Colilert (MPN/100ml)	1.0	0	1
Nitrates (mg/L)	1.8	0.51	3
Phosphates (mg/L)	0.20	0.05	3
Ammonia (mg/L)	0.06	0	3
Biochemical Oxygen Demand (mg/L as O_2)	1.4	0.10	3
Alkalinity (mg/L as $CaCO_3$)	13.3	2.1	3
Hardness (mg/L as $CaCO_3$)	13.0	0	3
Standard Plate Count (CFU/ml)	45.0	21.2	2
Acridine Orange Direct Counts (cells/g)	1.1×10^8	2.8×10^7	1
Acid Phosphatase (µg/g)	113.9	52.5	3
Alkaline Phosphatase (µg/g)	450.9	90.2	3
Dehydrogenase (µg/g)	18.1	9.7	3
Galactosidase (µg/g)	11.8	9.3	3
Glucosidase (µg/g)	119.8	9.1	3
E. coli O157:H7 (CFU/100ml)	12.5	0	3
Shigella sp. (CFU/100ml)	5.0	0	3
Bacteriophage (PUF/ml)	0.50	0	3
Giardia sp.(cysts/L)	22.5	0	1
Cryptosporidium sp. (cysts/L)	15.0	0	1

Table 73. Depth, width, velocity and discharge measurements by month and site

Date Samples Collected	Site	Mean Depth (m)	Width (m)	Velocity (m/s)	Discharge (m^3/s)
January 2011	2	0.27	7.1	0.2	0.117
	4	0.18	8.1	1.1	0.498
	7	0.22	3.3	1.9	0.414
	10	0.16	4.1	3.5	0.714
	13	0.08	4.4	2.5	0.268
	14	0.09	3.2	1.5	0.137
February 2011	2	0.17	5.8	0.03	0.030
	4	0.12	7.4	0.18	0.167
	7	0.15	3.1	0.46	0.217
	10	0.07	4.2	0.27	0.084
	13	0.07	2.4	0.52	0.091
	14	0.08	3.1	0.09	0.024
March 2011	2	0.13	10.6	0.61	0.840
	4	0.42	7.7	0.21	0.685
	7	0.19	3.3	0.73	0.459
	10	0.09	4.5	0.76	0.297
	13	0.07	5.1	0.27	0.098
	14	0.06	3.0	0.09	0.017
April 2011	2	0.15	11.3	0.58	1.003
	4	0.40	7.7	0.34	1.033
	7	0.28	3.2	0.91	0.810
	10	0.20	4.5	0.46	0.405
	13	0.14	5.4	0.58	0.427
	14	0.06	3.4	0.18	0.037
May 2011	2	0.23	11.9	0.46	1.233
	4	0.31	8.2	0.18	0.460
	7	0.21	3.4	0.58	0.420
	10	0.21	4.6	0.34	0.319
	13	0.10	5.6	0.18	0.099
	14	0.07	3.4	0.06	0.015
June 2011	2	0.18	11.1	0.40	0.792
	4	0.30	5.2	0.18	0.288
	7	0.15	3.5	0.46	0.245
	10	0.15	4.5	0.27	0.181
	13	0.07	5.8	0.24	0.104
	14	0.03	3.0	0.06	0.005
July 2011	2	0.16	11.5	0.27	0.515
	4	0.31	6.8	0.12	0.260
	7	0.19	3.2	0.24	0.148
	10	0.14	4.3	0.12	0.072
	13	0.05	5.7	0.24	0.065
	14	0.05	2.7	0.03	0.004

Table 73 (continued)

Date Samples Collected	Site	Mean Depth (m)	Width (m)	Velocity (m/s)	Discharge (m^3/s)
August 2011	2	0.19	11.6	0.34	2.382
	4	0.22	7.9	0.06	0.353
	7	0.18	3.1	0.27	0.502
	10	0.15	4.1	0.13	0.077
	13	0.07	5.6	0.20	0.075
	14	0.05	2.6	0.07	0.008
September 2011	2	0.14	11.3	0.67	1.055
	4	0.16	7.4	0.14	0.173
	7	0.19	3.2	0.13	0.076
	10	0.10	4.2	0.14	0.058
	13	0.06	6.0	0.17	0.063
	14	0.05	2.9	0.10	0.015
October 2011	2	0.19	11.4	0.17	0.367
	4	0.14	7.7	0.14	0.150
	7	0.23	3.2	0.25	0.184
	10	0.13	4.6	0.11	0.068
	13	0.10	7.9	0.25	0.198
	14	0.07	3.7	0.06	0.015
November 2011	2	0.15	11.3	0.30	0.505
	4	0.17	7.7	0.18	0.239
	7	0.25	3.2	0.52	0.415
	10	0.19	4.,4	0.30	0.259
	13	0.12	5.8	0.34	0.233
	14	0.06	4.1	0.18	0.042
December 2011	2	0.11	10.8	0.27	0.326
	4	0.31	7.9	0.15	0.377
	7	0.22	3.3	0.37	0.262
	10	0.22	4.1	0.24	0.223
	13	0.08	6.0	0.18	0.084
	14	0.05	3.4	0.09	0.016

Appendix C: Shigella spp. and E. coli O157:H7 Gel Electrophoresis Pictures

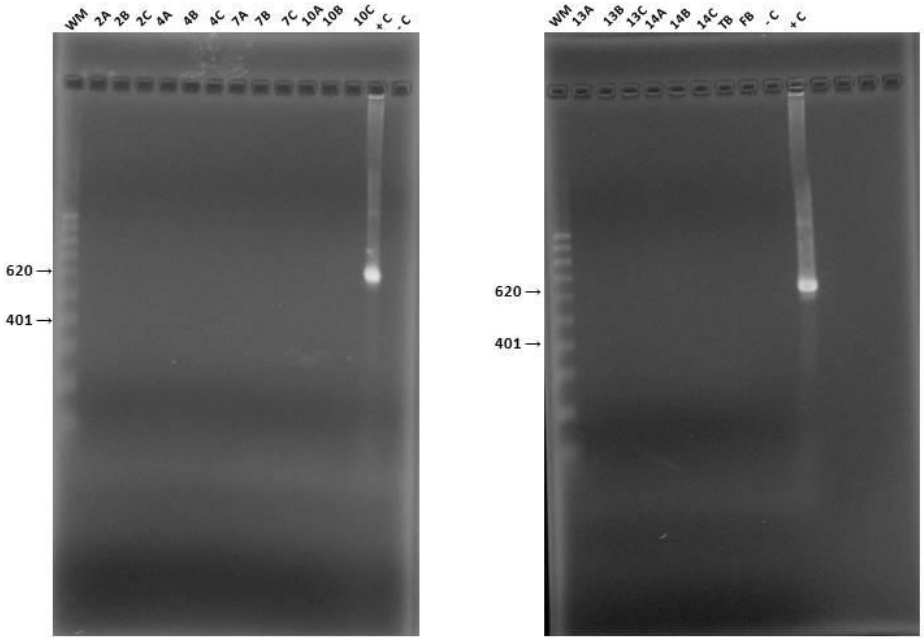

Figure 1. Gel electrophoresis of *Shigella sp.* PCR products, January 2011.

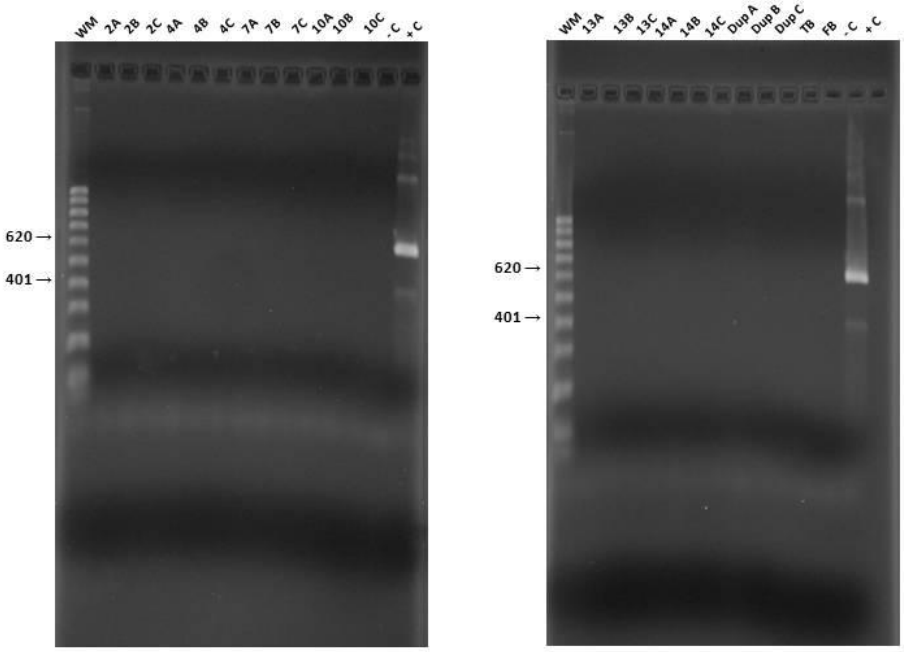

Figure 2. Gel electrophoresis of *Shigella sp.* PCR products, February 2011.

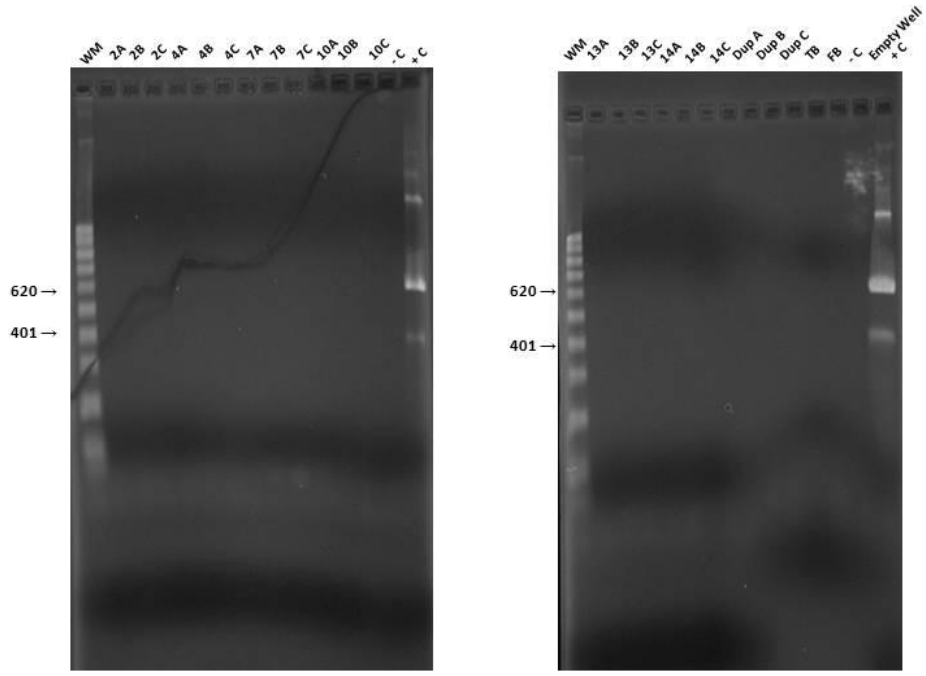

Figure 3. Gel electrophoresis of *Shigella sp.* PCR products, March 2011.

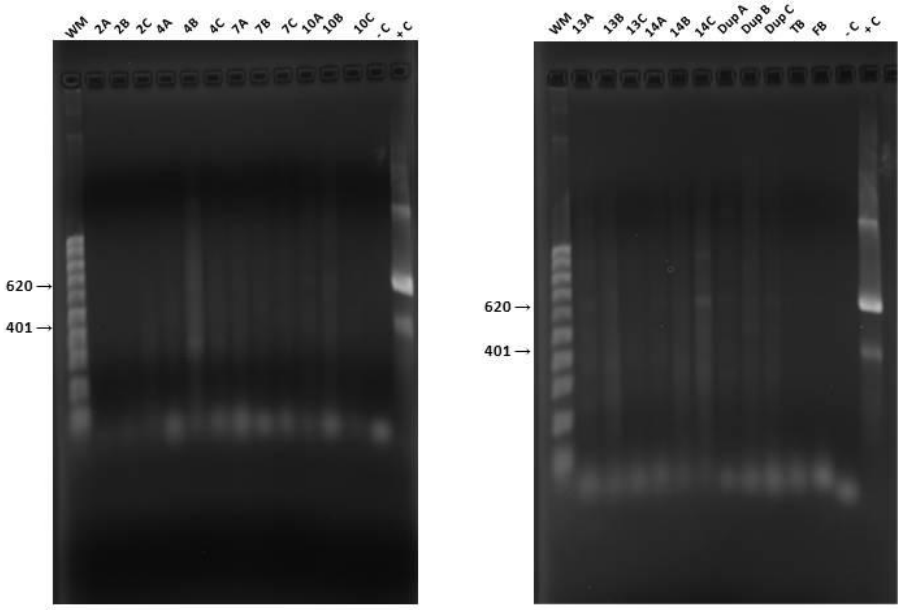

Figure 4. Gel electrophoresis of *Shigella sp.* PCR products, April 2011.

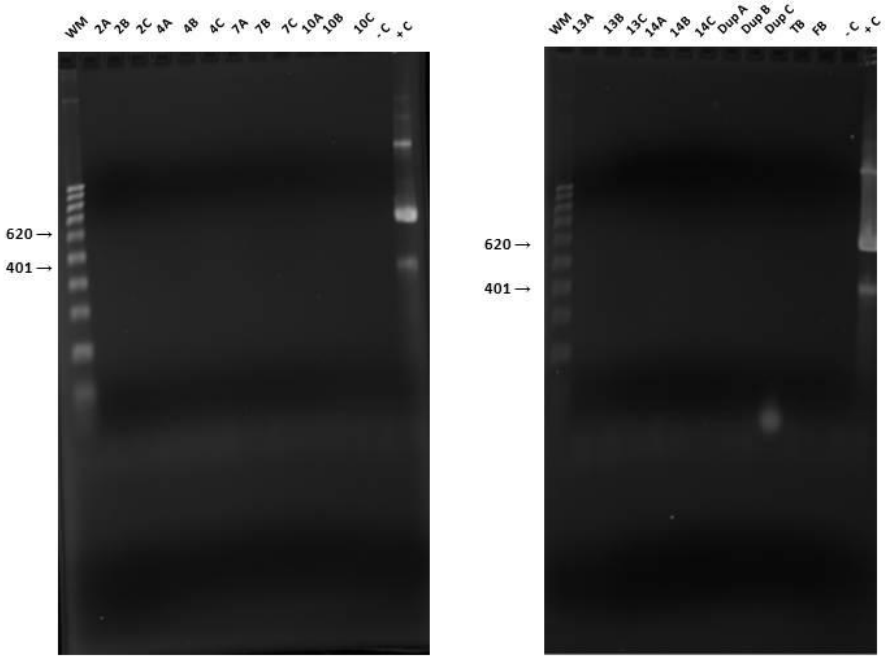

Figure 5. Gel electrophoresis of *Shigella sp.* PCR products, May 2011.

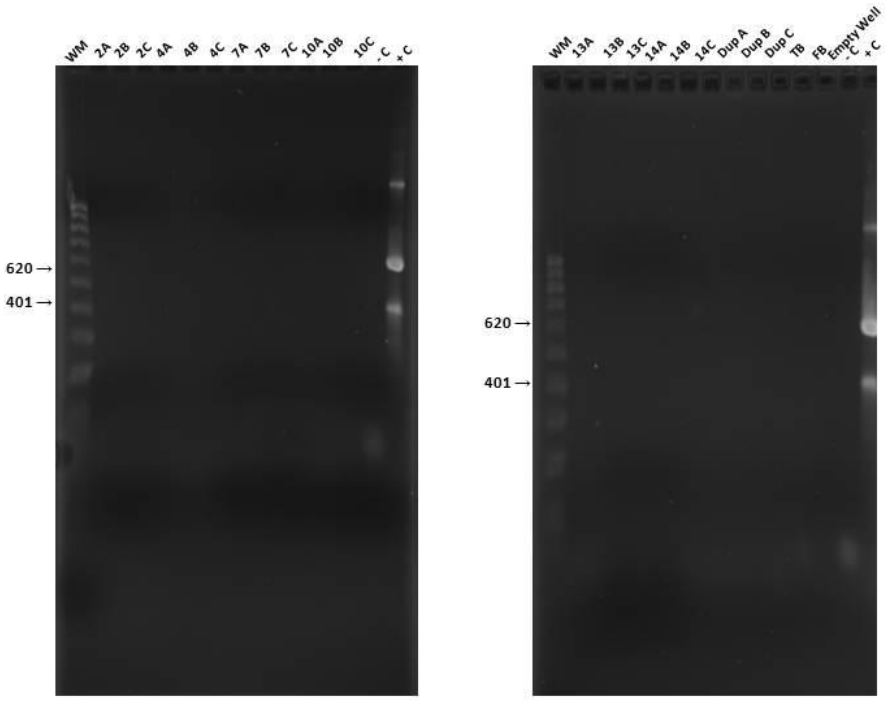

Figure 6. Gel electrophoresis of *Shigella sp.* PCR products, June 2011.

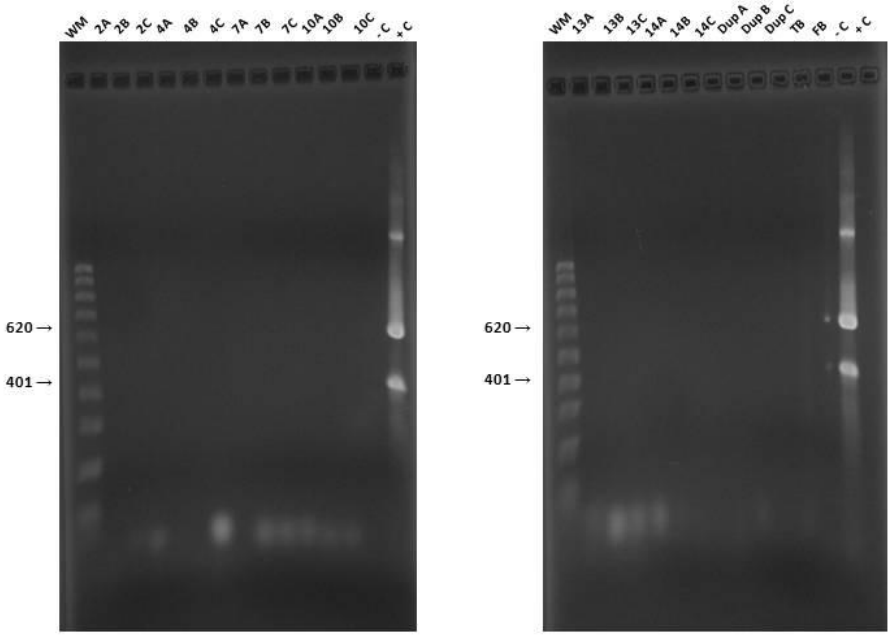

Figure 7. Gel electrophoresis of *Shigella sp.* PCR products, July 2011.

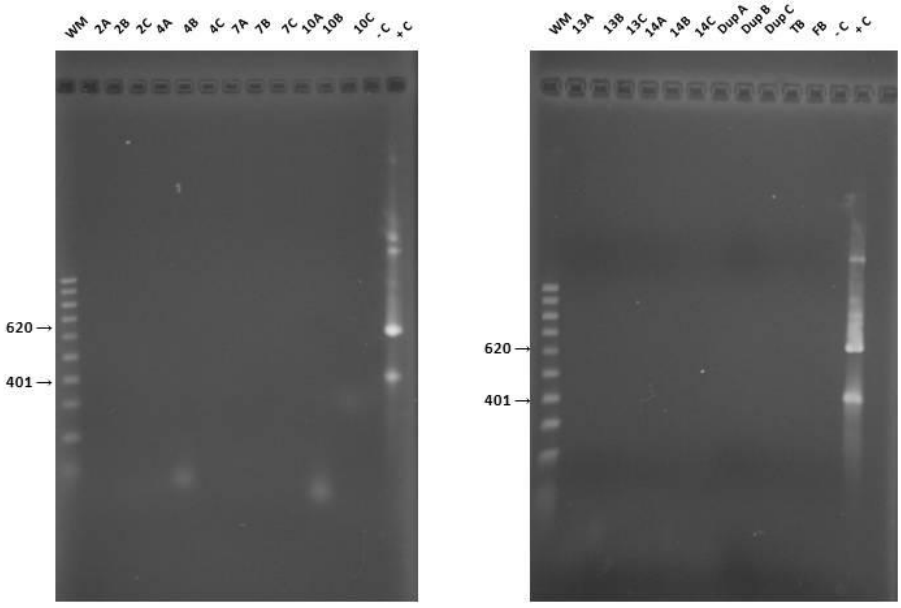

Figure 8. Gel electrophoresis of *Shigella sp.* PCR products, August 2011.

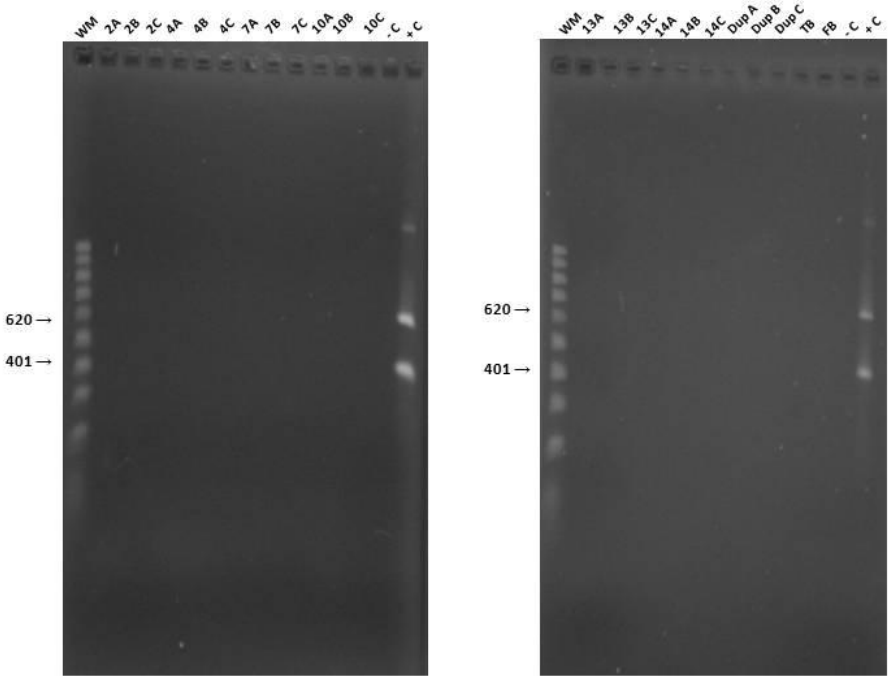

Figure 9. Gel electrophoresis of *Shigella sp.* PCR products, September 2011.

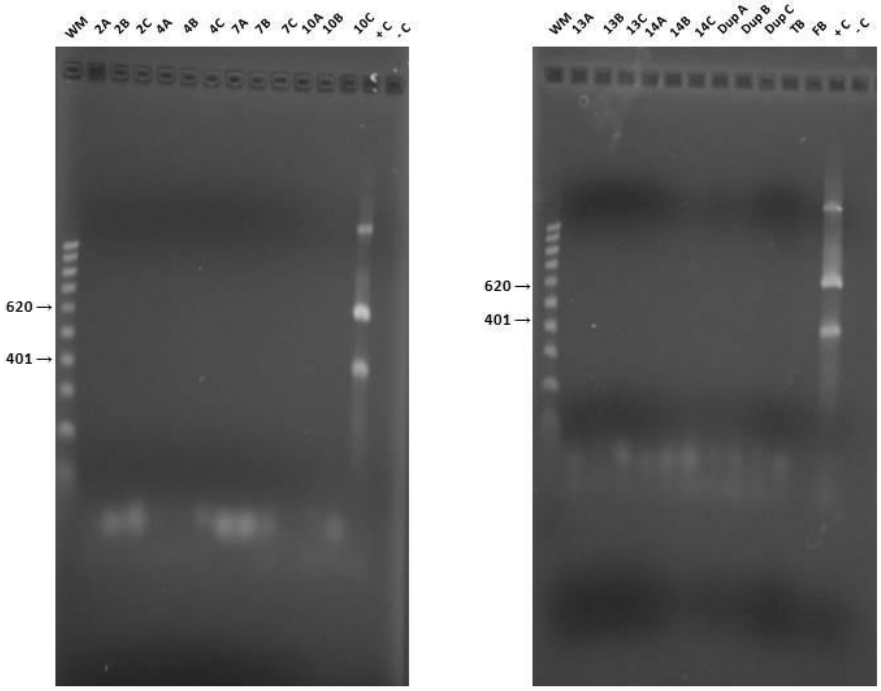

Figure 10. Gel electrophoresis of *Shigella sp.* PCR products, October 2011.

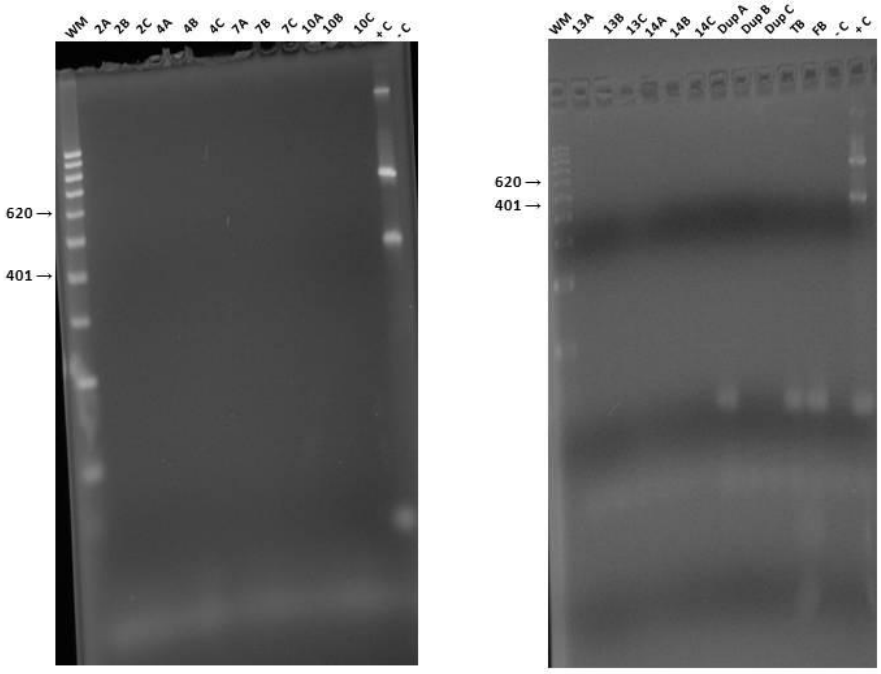

Figure 11. Gel electrophoresis of *Shigella sp.* PCR products, November 2011.

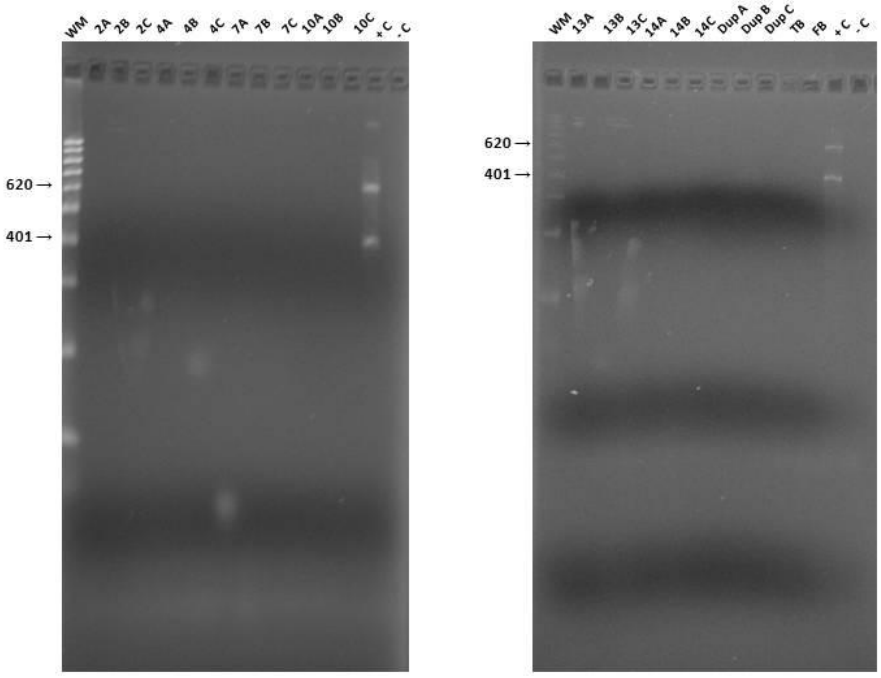

Figure 12. Gel electrophoresis of *Shigella sp.* PCR products, December 2011.

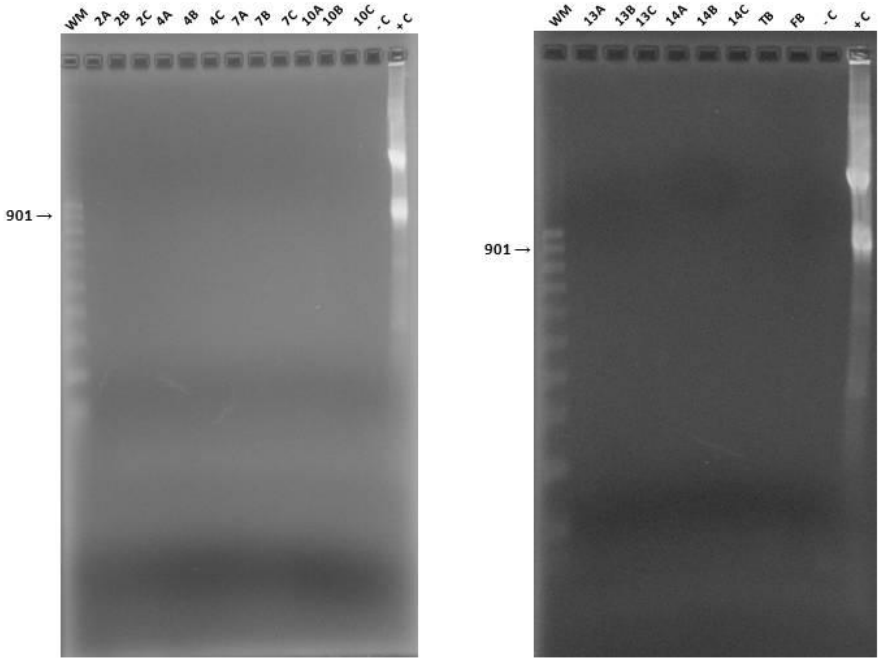

Figure 13. Gel electrophoresis of *E. coli* O157:H7 PCR products, January 2011.

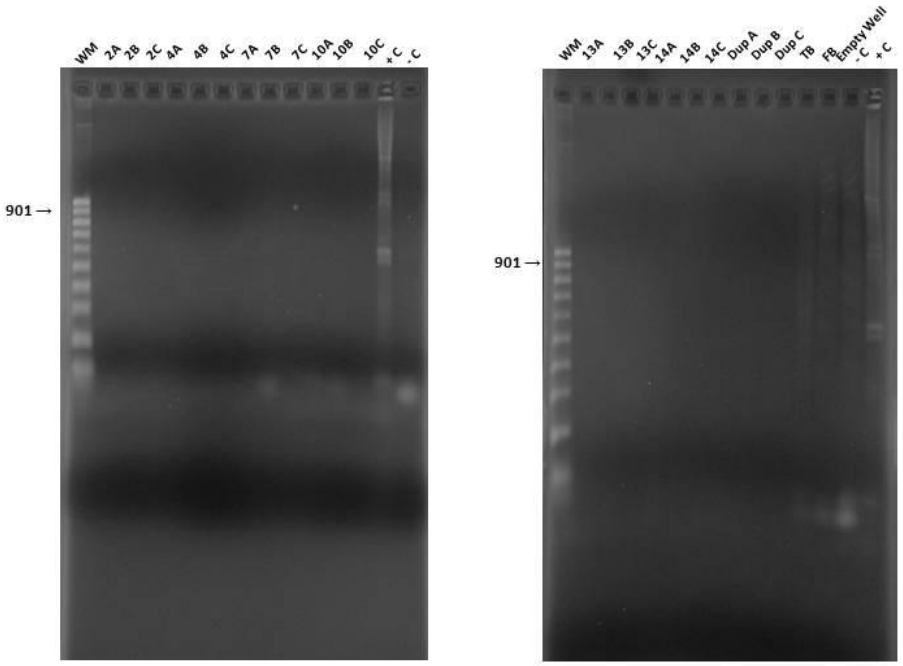

Figure 14. Gel electrophoresis of *E. coli* O157:H7 PCR products, February 2011.

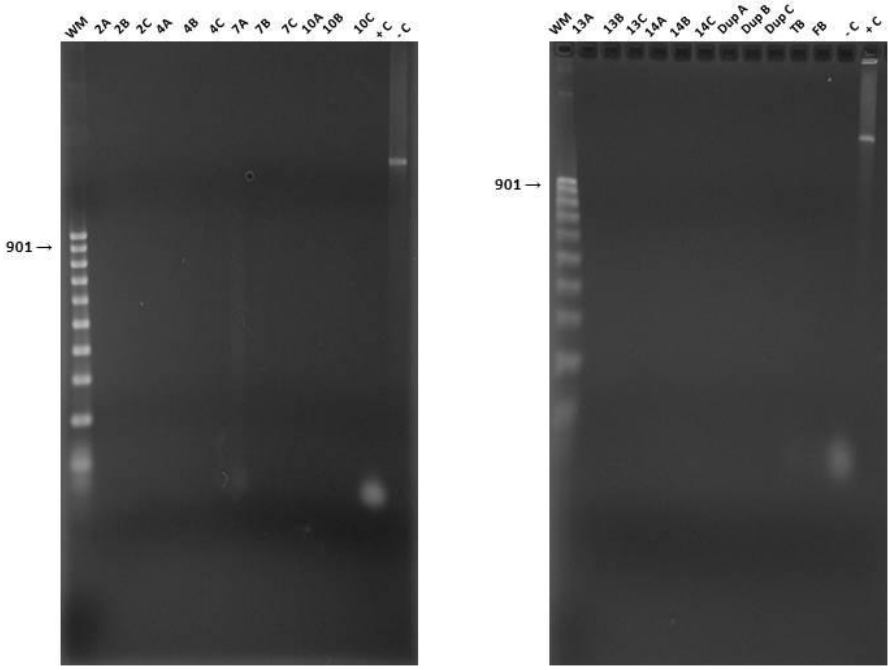

Figure 15. Gel electrophoresis of *E. coli* O157:H7 PCR products, March 2011.

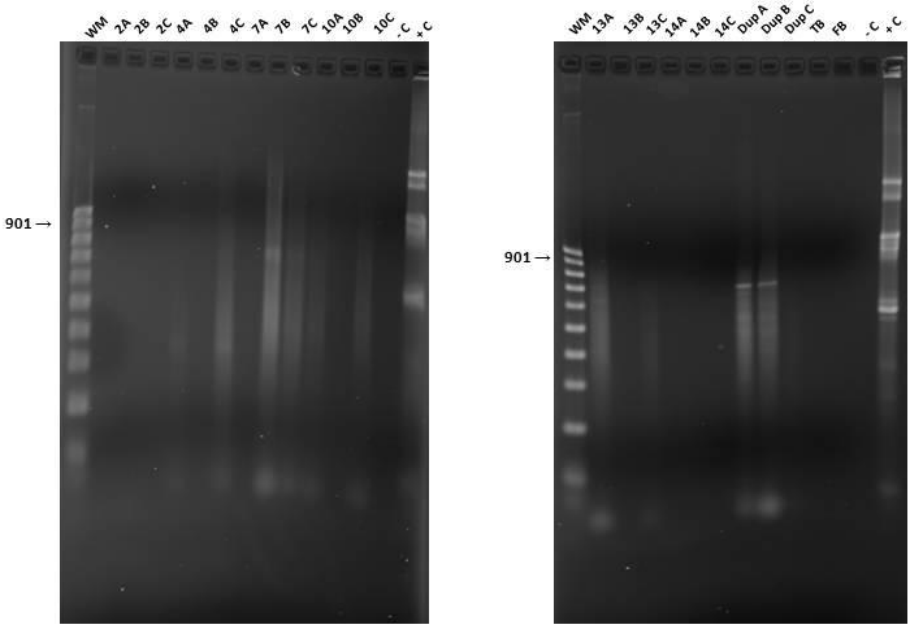

Figure 16. Gel electrophoresis of *E. coli* O157:H7 PCR products, April 2011.

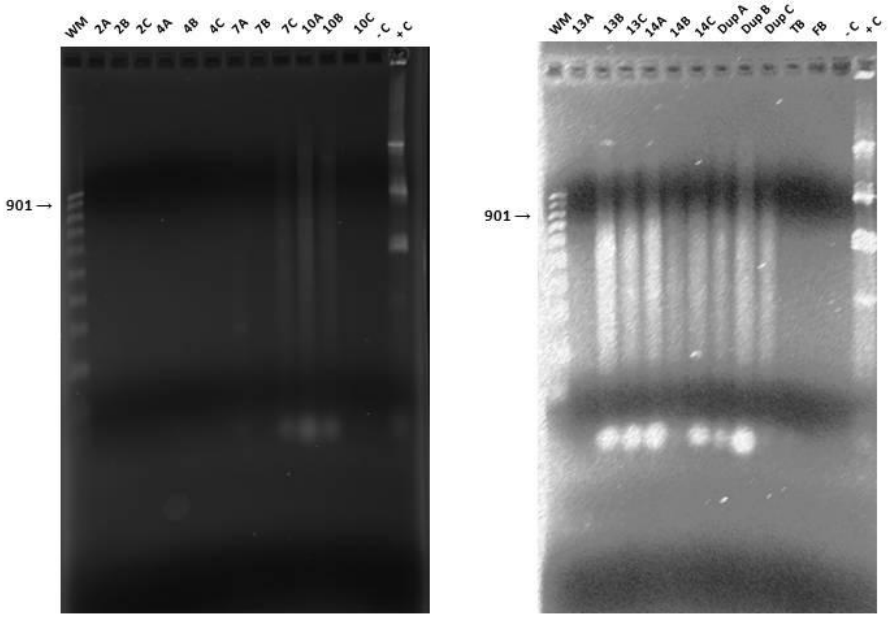

Figure 17. Gel electrophoresis of *E. coli* O157:H7 PCR products, May 2011.

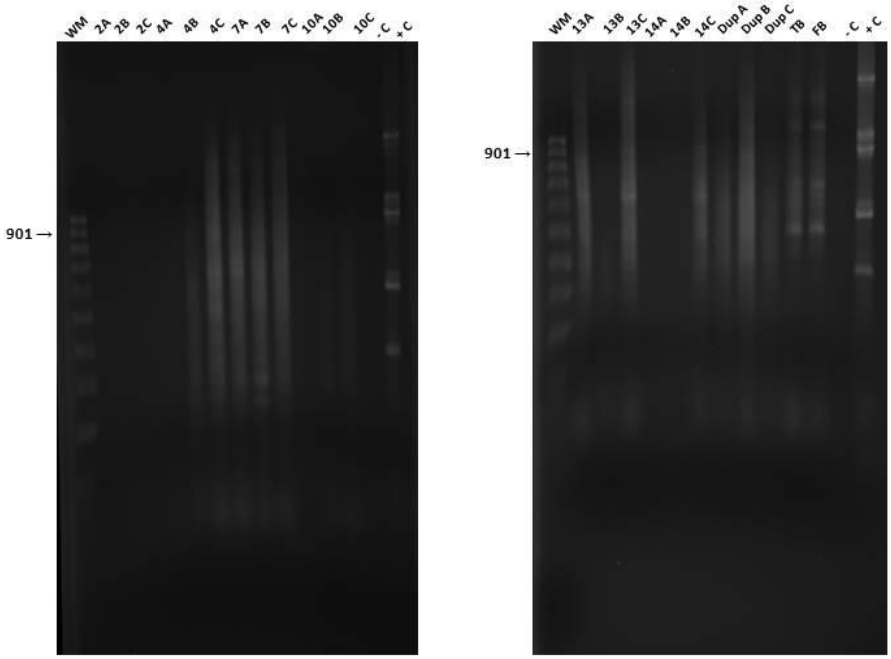

Figure 18. Gel electrophoresis of *E. coli* O157:H7 PCR products, June 2011.

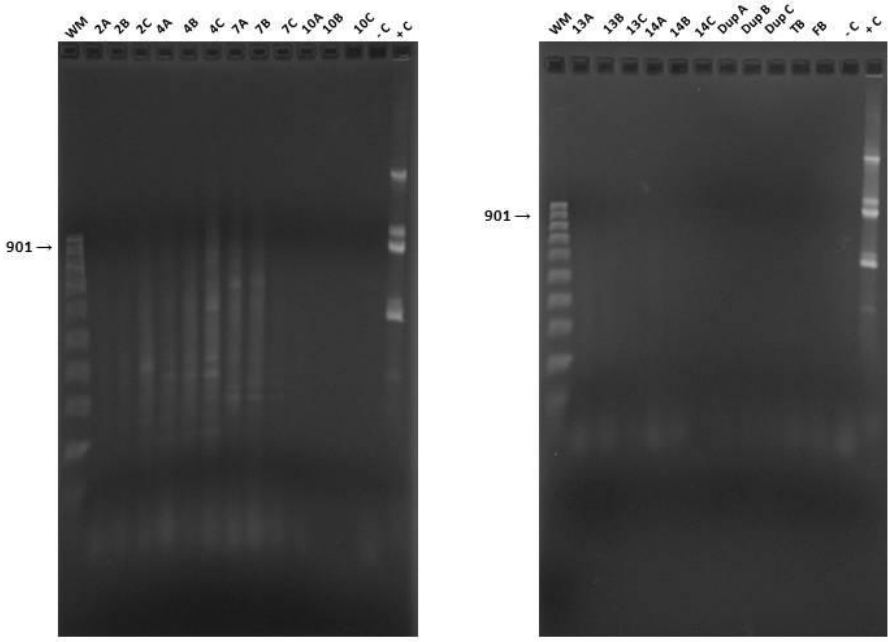

Figure 19. Gel electrophoresis of *E. coli* O157:H7 PCR products, July 2011.

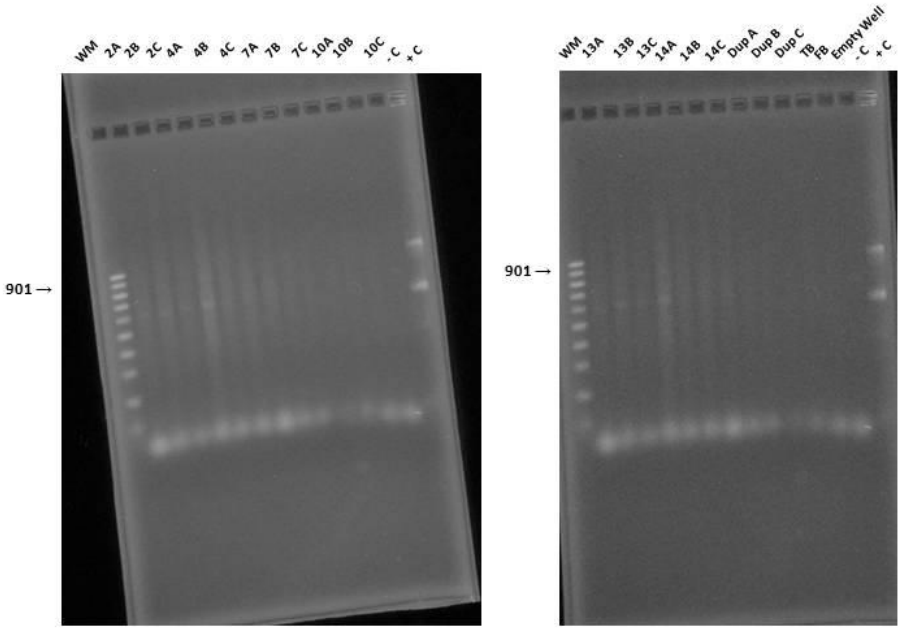

Figure 20. Gel electrophoresis of *E. coli* O157:H7 PCR products, August 2011.

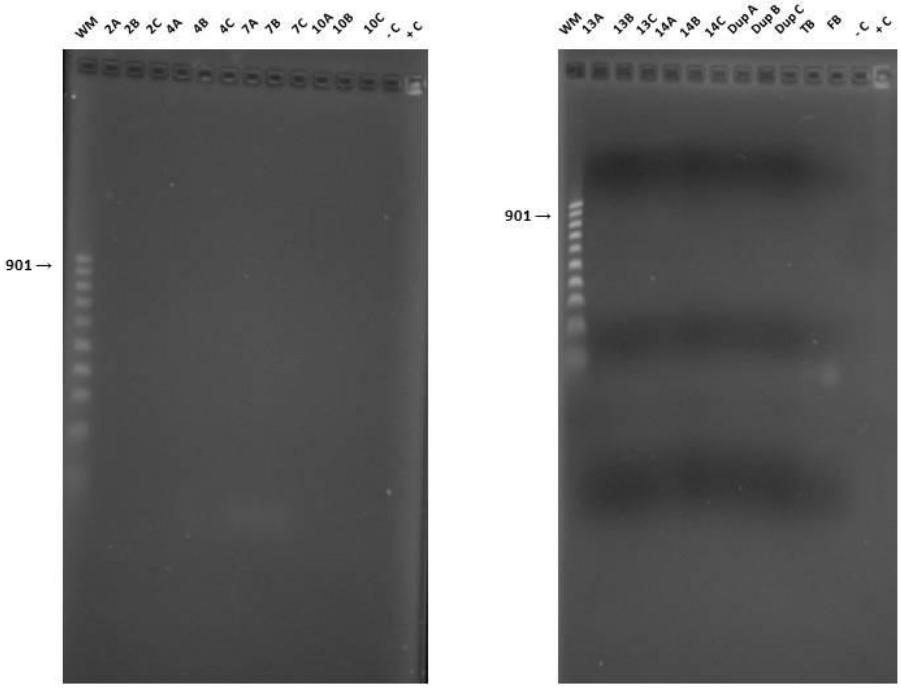

Figure 21. Gel electrophoresis of *E. coli* O157:H7 PCR products, September 2011.

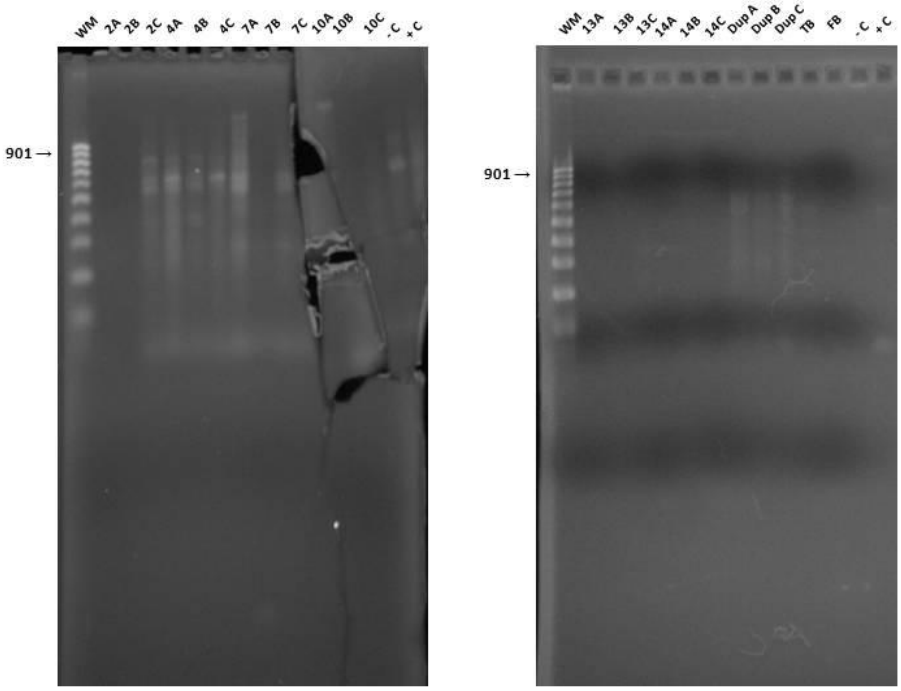

Figure 22. Gel electrophoresis of *E. coli* O157:H7 PCR products, October 2011.

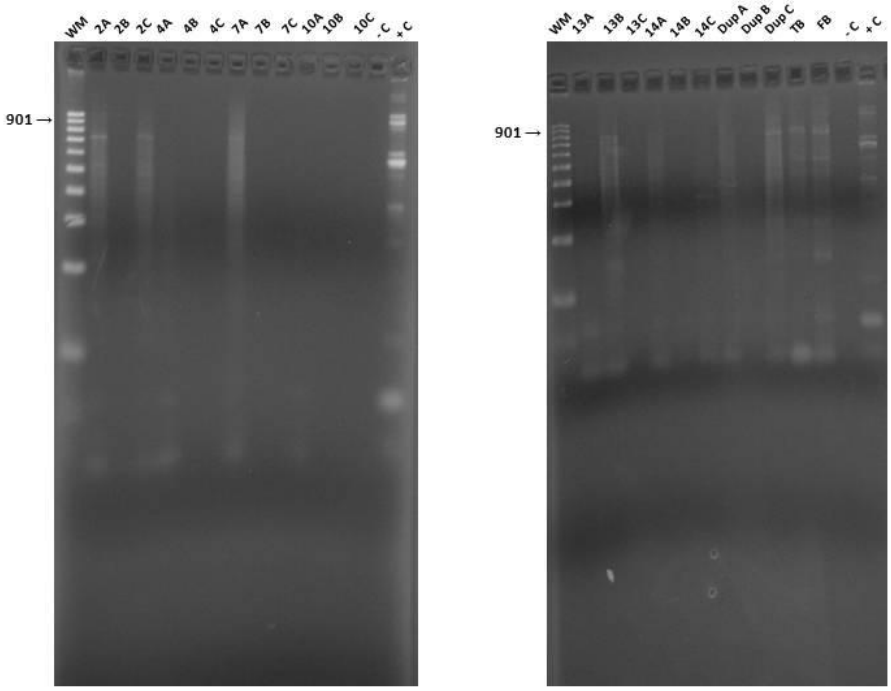

Figure 23. Gel electrophoresis of *E. coli* O157:H7 PCR products, November 2011.

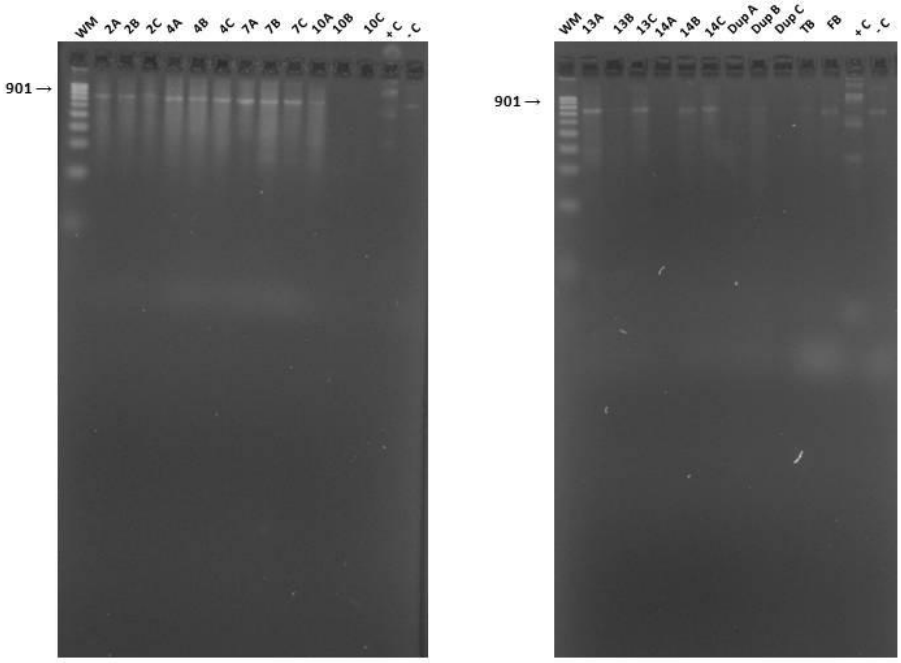

Figure 24. Gel electrophoresis of *E. coli* O157:H7 PCR products, December 2011.

Appendix D: Sinking Creek Habitat Assessments

Table 1. Habitat assessment of site 2

Land Use	Agriculture
Dominant Vegetation	Grasses
Erosion	Moderate
Inorganic Substrate Components (%)	50% Cobble 25% Gravel 10% Sand 10% Silt 5% Clay
Detritus (%)	10%
Mud/Muck (%)	0%
Marl (%)	5%
Epifaunal Substrate	7
Embeddedness	13
Velocity and Depth Regime	8
Sediment Deposition	10
Channel Flow Status	14
Channel Alteration	13
Frequency of Riffles	8
Bank Stability	
Right Bank	7
Left Bank	7
Vegetative Protection	
Right Bank	4
Left Bank	5
Riparian Vegetative Zone Width	
Right Bank	3
Left Bank	5
Total Score (%)	**52%**

Table 2. Habitat assessment for site 4

Land Use	Agriculture
Dominant Vegetation	Grasses
Erosion	Moderate
Inorganic Substrate Components (%)	25% Boulder 25% Cobble 10% Gravel 20% Sand 15% Silt 5% Clay
Detritus (%)	10%
Mud/Muck (%)	0%
Marl (%)	0%
Epifaunal Substrate	11
Embeddedness	7
Velocity and Depth Regime	13
Sediment Deposition	8
Channel Flow Status	13
Channel Alteration	11
Frequency of Riffles	14
Bank Stability	
Right Bank	1
Left Bank	1
Vegetative Protection	
Right Bank	2
Left Bank	3
Riparian Vegetative Zone Width	
Right Bank	1
Left Bank	1
Total Score (%)	**43%**

Table 3. Habitat assessment for site 7

Land Use	Urban
Dominant Vegetation	Grasses
Erosion	Heavy
Inorganic Substrate Components (%)	10% Boulder 60% Cobble 10% Gravel 10% Sand 5% Silt 5% Clay
Detritus (%)	10%
Mud/Muck (%)	0%
Marl (%)	5%
Epifaunal Substrate	18
Embeddedness	11
Velocity and Depth Regime	4
Sediment Deposition	13
Channel Flow Status	19
Channel Alteration	2
Frequency of Riffles	18
Bank Stability Right Bank Left Bank	 5 10
Vegetative Protection Right Bank Left Bank	 2 1
Riparian Vegetative Zone Width Right Bank Left Bank	 0 2
Total Score (%)	**53%**

Table 4. Habitat assessment for site 10

Land Use	Urban
Dominant Vegetation	Grasses
Erosion	Heavy
Inorganic Substrate Components (%)	40% Boulder 40% Cobble 10% Gravel 3% Sand 3% Silt 4% Clay
Detritus (%)	5%
Mud/Muck (%)	0%
Marl (%)	5%
Epifaunal Substrate	18
Embeddedness	9
Velocity and Depth Regime	11
Sediment Deposition	13
Channel Flow Status	13
Channel Alteration	6
Frequency of Riffles	18
Bank Stability	
Right Bank	7
Left Bank	7
Vegetative Protection	
Right Bank	4
Left Bank	4
Riparian Vegetative Zone Width	
Right Bank	2
Left Bank	2
Total Score (%)	**57%**

Table 5. Habitat assessment for site 13

Land Use	Forest
Dominant Vegetation	Trees
Erosion	None
Inorganic Substrate Components (%)	25% Boulder 50% Cobble 10% Gravel 10% Sand 3% Silt 2% Clay
Detritus (%)	40%
Mud/Muck (%)	0%
Marl (%)	5%
Epifaunal Substrate	19
Embeddedness	19
Velocity and Depth Regime	3
Sediment Deposition	18
Channel Flow Status	15
Channel Alteration	16
Frequency of Riffles	18
Bank Stability	
Right Bank	7
Left Bank	7
Vegetative Protection	
Right Bank	3
Left Bank	3
Riparian Vegetative Zone Width	
Right Bank	7
Left Bank	7
Total Score (%)	**71%**

Table 6. Habitat assessment for site 14

Land Use	Forest
Dominant Vegetation	Trees
Erosion	None
Inorganic Substrate Components (%)	25% Boulder 25% Cobble 25% Gravel 15% Sand 5% Silt 5% Clay
Detritus (%)	10%
Mud/Muck (%)	0%
Marl (%)	0%
Epifaunal Substrate	18
Embeddedness	18
Velocity and Depth Regime	4
Sediment Deposition	18
Channel Flow Status	18
Channel Alteration	19
Frequency of Riffles	19
Bank Stability	
Right Bank	9
Left Bank	9
Vegetative Protection	
Right Bank	7
Left Bank	7
Riparian Vegetative Zone Width	
Right Bank	10
Left Bank	10
Total Score (%)	**83%**

Figure 1. Site 2 – Bob Peoples Bridge on Sinking Creek Road

Figure 2. Site 4 – Joe Carr Road

Figure 3. Site 7 – Miami Drive, King Springs Baptist Church

Figure 4. Site 10 – Hickory Springs Road

Figure 5. Site 13 – Jim McNeese Road

Figure 6. Site 14 – Dry Springs Road

VITA

KIMBERLEE K HALL

Personal Data:	Date of Birth: May 14, 1982
	Place of Birth: Shelby, Michigan
Eductation:	B.S. Biology/Ecology and Environmental Biology, Applalachian State University, May 2004
	Ph.D. Environmental Health Sciences East Tennessee State University, August 2012
Professional Experience:	Graduate Research Assistant, East Tennessee State University, Department of Environmental Health, 2004 - 2006
	Graduate Research Assistant and Teaching Associate, East Tennessee State University, Department of Environmental Health, 2006 – 2012
Poster Presentations:	Hall KK, Evanshen BG, Maier KJ, Scheureman PR. 2008. Application of multivariate statistical analyses to microbial water quality parameters in four geographically similar creeks in Northeast Tennessee to identify patterns associating land use to fecal pollution sources. Abstract, 107th Annual Meeting for the American Society for Microbiology, Toronto, Ontario, Canada
	Hall KK, Evanshen BG, Maier KJ, Scheureman PR. 2011. Analysis of water quality data using multivariate statistics to patterns associating land use to fecal pollution sources. 111th Annual Meeting for the American Society for Microbiology, New Orleans, LA USA
Awards:	ETSU Graduate Council Teaching Excellence Award, East Tennessee State University, April, 2009