Bacterial Source Tracking and Survival of *Escherichia coli*

by

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Abstract

Surface water is used for drinking by many people around the world. *E. coli* is the most frequently used bacterial indicator used for assessing water quality. The survival, sources, and concentrations of *E. coli* were examined through a series of experiments that investigated the survival of beef cattle *E. coli* on land and in water, and used bacterial source tracking (BST) to determine the sources of fecal contamination diurnally and annually in multiple watersheds in British Columbia.

A fecal pat experiment was conducted to examine the survival of *E. coli* under 4 levels of solar exposure. *E. coli* survived longer with increasing shade. Age of fecal pats, as well as exposure to solar radiation negatively influenced the survival of *E. coli*. The survival of *E. coli* in stream water was examined in filtered and unfiltered stream water at 3 different temperatures (6, 20 and 26 ºC). There was no significant difference in the survival of *E. coli* in filtered versus non-filtered stream water. Lower water temperatures (6 ºC) increased the survival of *E. coli*. The addition of manure to the water substantially increased the nutrient concentrations and organics.

BST is a rapidly growing area of research and technology development and many methods are being developed and tested. The choice of method used for BST depends on: question(s) to be answered, scale of identification needed, available expertise, cost of analysis, turnaround time, and access to facilities. The spatial, diurnal, and annual sources and concentrations of *E. coli* were investigated in several watersheds in British Columbia. Fecal coliforms and *E. coli* concentrations varied throughout the day, as well as by site, month and year. Ribotyping identified many different sources of *E. coli* within

the watersheds. The majority of *E. coli* isolates classified were from wildlife sources in each watershed even though they had different land-use.

Supervisors: Dr. Asit Mazumder and Dr. Rick Nordin, (Department of Biology)

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Dedication

This thesis is dedicated to the loving memory of my grandparents, Harry and Jessie Adamson.

Chapter 1: Introduction

Introduction

Microbial contamination is a major environmental and health issue with drinking water in British Columbia (BC), Canada, and worldwide (BC Gov. 2001, WHO 2003). A significant proportion of surface water in the United States exceeds fecal bacterial water quality standards (USEPA 2005). Awareness and concern for water quality issues has increased due to pathogenic outbreaks involving *Cryptosporidium*, *Giardia*, and *Escherichia coli* (*E. coli*) O157:H7 (Rosen 2000, Davies and Mazumder 2003, Hashsham et al. 2004). Water quality has become such a large concern that millions of dollars are being spent across British Columbia and Canada to upgrade and develop better domestic water disinfection, filtration and distribution systems to improve the quality of drinking water. One of the most common health concerns from drinking water is the high numbers of fecal bacteria in source and tap water. Health concerns relating to water quality, and increased pressures from multiple-use in water supply watersheds have created conflicts between user groups and their demands on water resources. The resolution of these conflicts and the adoption of better management practices requires solid scientific data on the sources of *E. coli* and knowledge of its life history and survival in a watershed.

Bacterial indicators, including total coliforms, fecal coliforms, *E. coli*, and enterococci are currently used for assessing water quality (USEPA 1986, Rosen 2000, BC Gov. 2001, USEPA 2002, WHO 2004). These indicator organisms are prevalent in the intestines and feces of warm-blooded mammals, including humans, but are not usually pathogenic (USEPA 2005). Indicator bacteria are used for monitoring because they are less expensive and easier to culture than the pathogens. There is considerable debate

regarding the appropriateness of various indicator organisms, since no strong associations or relationships between indicator species and pathogens have been found (Bernard and Field 2000, Griffin et al. 2001). The presence of fecal bacteria does however demonstrate fecal contamination, although not the source. Scott et al. (2002) suggest that an ideal indicator would be non-pathogenic, rapidly detected, easily enumerated, and have survival characteristics similar to those pathogens of concern. An ideal indicator should also have a predictive relationship with the pathogens of concern.

Livestock grazing on watersheds in BC is a common practice that is often met with opposition from groups concerned with the impacts on water quality. Despite many studies on livestock management and water quality, results are inconsistent and contradictory. Information on the movement, survival, and sources of microorganisms in the environment is essential to understanding livestock impacts. There have been many studies on the impact of cattle grazing and land application of manure on bacterial runoff, but often, the source of contamination in streams (wildlife, livestock, or human) has not been determined specifically (Edwards et al. 1997, Bicudo and Goyal 2003). BST can help determine more specifically the impacts of the potential sources of fecal pollution at a site. BST can help determine if the pollutants at a given site in a stream are from the cattle in the area or from other upstream sources. Once the sources of fecal pollution are identified, management systems can be developed and implemented to reduce fecal loading in a stream.

Research Objectives

The research presented in this thesis focuses on 5 main areas: review of bacterial source tracking (Chapter 2); survival of beef cattle *E. coli* on land (Chapter 3); survival of beef cattle *E. coli* in water (Chapter 4); diurnal variability of *E. coli* concentrations and sources (Chapter 5); and spatial and annual concentrations and sources of *E. coli* on multiple watersheds in British Columbia (Chapter 6). Understanding the sources and survival of fecal bacteria in the environment is critical for the development of meaningful monitoring programs and best management strategies to reduce *E. coli* input to streams, thereby improving water quality and reducing the risk to human health.

Source Tracking Methods (Chapter 2)

Watersheds that provide surface water used for drinking water supplies are vulnerable to fecal pollution from many sources within the watershed including wildlife, livestock, and humans. Currently, there is no standard method for tracking the sources of fecal bacteria. Source tracking of bacterial contamination in drinking water is a rapidly growing area of research and technology development and many methods are being developed and evaluated (Scott et al. 2002, Simpson et al. 2002, Meays et al. 2004, USEPA 2005). Chapter 2 reviews several source tracking methods that are in current use for determining the source of fecal bacteria in the environment and discusses the advantages and disadvantages of each method. Applying BST for monitoring, assessment, and hypothesis testing represents a promising means of determining pollution sources.

Survival of E. coli (Chapter 3 and Chapter 4)

Understanding bacterial dynamics including transport and viability within the environment is important for protecting and managing surface waters (Rosen 2000, Ferguson et al. 2003, Meays et al. 2004, Meays et al. 2005). Information on the survival and growth of bacteria and waterborne pathogens is limiting and often contradictory (Alexander 1986, Park et al. 1991, Ferguson et al. 2003). *E. coli* is a well studied organisms, but the majority of research has been conducted on pure cultures in the laboratory, or inoculated into livestock waste (Avery et al. 2004). Park et al. (1991) suggested that the survival and optimum conditions for an organism in a laboratory experiment may be very different to what happens under various environmental conditions. Both laboratory and field experiments are needed in order to understand the survival of these organisms.

In a series of field experiments, Buckhouse and Gifford (1976) and Bohn and Buckhouse (1985) suggested that coliforms could survive for at least a year in cattle feces. Other studies have suggested that fecal bacteria can survive and grow in the environment (Gerba and McLeod 1976, Tassoula 1997, Byappanahalli and Fujioka 1998, Topp and Scott 2003, Topp et al. 2003, Unc and Goss 2003). Many factors, or combinations of factors influence the survival of fecal bacteria. Sunlight (Chamberlin and Mitchell 1978, Fujioka et al. 1981, Davies and Evison 1991, Meays et al. 2005), temperature (McFeters and Stuart 1972, Alexander et al. 1986, Ferguson et al. 2003), and nutrient or organic limitation (McFeters and Stuart 1972, Gerba and McLeod 1976, Tassoula 1997, Byappanahalli and Fujioka 1998, Holben et al. 1992) have been cited as being the most influential factors to the survival of fecal coliforms and *E. coli* in water

and feces. Other factors that have been shown or suggested to influence the survival of bacteria include pH (McFeters and Stuart 1972, Alexander et al. 1986), salinity (Davies and Evison 1991, Tassoula 1997), sedimentation (Gerba and McLeod 1976, Davies and Evison 1991, Sherer et al. 1992), predators especially protozoan (Scheuerman et al. 1988, Gonzalez et al. 1990, Gurijala and Alexander 1990), and competition with other organisms (Holben et al. 1992).

Cattle are often cited as having a negative impact on water quality (Kauffman and Krueger 1984, Belsky et al. 1999). However, most studies investigating fecal pollution and survival of *E. coli* have concentrated on intensive agriculture or manure slurries for study purposes (Entry et al. 2000a, Entry et al. 2000b, Rosen 2000). Chapter 3 examines the survival of *E. coli* in beef cattle fecal pats under different levels of solar exposure in order to characterize what would be closer to reality for a rangeland situation where feces were surface deposited, in smaller pats and not incorporated into the soil. The survival of natural populations of *E. coli* from beef cattle manure in stream water at different temperatures is examined in Chapter 4.

E. coli concentrations and sources in watersheds (Chapter 5 and Chapter 6)

Wildlife, livestock, and humans all contribute fecal bacteria to surface waters within a watershed. Currently, most monitoring programs collect bacterial water samples to determine the levels of fecal pollution in a water body, however, these samples do not provide any information on the source of pollution. New molecular and biochemical methods for detecting the sources of fecal contamination have and are being developed (Scott et al. 2002, Simpson et al. 2002, Meays et al. 2004) but little information is available on the natural variability of concentrations and sources of fecal pollutants in

natural waters. Monitoring programs vary widely with variable numbers of samples being collected for analysis and evaluation for decisions (Whitman and Nevers 2004). Unfortunately, sampling designs are rarely based on empirical or anticipated variation, accuracy or precision even though there is a high amount of variation between samples (Whitman and Nevers 2004). Managing and developing meaningful monitoring programs for optimal water quality requires sound scientific data on the variability of fecal contaminants, their concentrations, and their sources. Chapter 5 investigates the diurnal variability in concentrations and sources of *E. coli* in 3 streams. Chapter 6 addresses the variability in concentrations and sources of *E. coli* in 4 watersheds with different land-use over a 2-year period.

Chapter 2: Source Tracking Fecal Bacteria in Water: A Critical Review of Current Methods

Abstract

Many molecular and biochemical methods and techniques are being developed to track sources of bacteria in water and food. Currently, there is no standard method proposed for source tracking. This manuscript is a critical evaluation of the various methods used in watersheds, and highlights some of the advantages and disadvantages of each method. Making a decision on a single or combination of methods to use under a particular situation will depend on a number of factors including: question(s) to be answered, scale of identification (broad scale vs. specific species identification), available expertise, cost of analysis, turnaround time, and access to facilities. This manuscript reviews several source tracking methodologies which are in current use for source tracking fecal bacteria in the environment including: ribotyping, pulse-field gel electrophoresis (PFGE), denaturing-gradient gel electrophoresis (DGGE), repetitive DNA sequences (Rep-PCR), host-specific 16S rDNA genetic markers, and antibiotic resistance analysis.

Introduction

Water is central to all life, and safe drinking water is essential. Concerns with water quality have increased in recent years, in part due to the more frequent contamination of drinking water by *Cryptosporidium*, *Giardia*, *E. coli* O157:H7 and other pathogens (Rosen 2000, Davies and Mazumder 2003, Hashsham et al. 2004). Health concerns relating to water quality, and multiple-use in watersheds have created conflicts between user groups and their demands on land and water resources. Non-point source pollution is difficult to quantify and determine the source, and therefore opposing interest groups often identify others for causing the problem without any technical basis. Resolution of conflicts and the adoption of better management practices and policies require sound scientific data on the sources of bacterial pathogens and knowledge of their life history and survival in watersheds.

Currently total coliforms, fecal coliforms, *E. coli*, and enterococci are bacterial indicators used in water quality and health risk assessments (USEPA 1986, Rosen 2000, BC Gov. 2001, USEPA 2002, WHO 2004). Each group of bacteria is normally prevalent in the intestines and feces of warm-blooded mammals, including wildlife, livestock, and humans (USEPA 2005). The indicator bacteria themselves are usually not pathogenic. Indicator bacteria such as fecal coliforms, fecal streptococci and *E. coli* are used because they are much easier and less costly to detect and enumerate than the pathogens themselves. Fecal bacteria are enumerated using either the membrane filter technique or the multiple-tube fermentation test (APHA 1998). There is ongoing debate on which organism should be used as an indicator, as no strong association exists between indicators and the pathogens they are supposed to indicate (Bernard and Field

2000, Griffin et al. 2001). The presence of *E. coli* or fecal coliform bacteria in water does however indicate that fecal contamination has occurred. Scott et al. (2002) suggest that an ideal indicator would be non-pathogenic, rapidly detected, easily enumerated, and have survival characteristics that are similar to those pathogens of concern. An ideal microbial source tracking (MST) microorganism would have all of the above mentioned qualities as well as discriminatory power between hosts (Farber 1996).

Although, it does not fall directly within the context of this paper, another challenge is to understand the dynamics of fecal coliforms, including the factors determining their transport and viability in the environment. Even with the best and most robust methods for identifying and tracking sources of contamination, it will remain difficult to protect water sources and manage fecal contamination unless there is an understanding of the bacterial dynamics in the natural environment. The potential sources, as well as the survival of pathogens will vary substantially by source water ecosystems and regional climatic conditions.

Bacterial source tracking (BST), or MST, includes several methodologies used to determine sources of fecal bacteria (the major groups being wildlife, humans, and domestic livestock) from environmental samples. The term "bacterial source tracking" was first coined by Hagedorn and Wiggins in their website (http://www.bsi.bt.edu/biol_4684/BST/BST.html) which describes various sub-typing methods (Harwood 2002). Methods for BST fall into three groups: molecular, biochemical, and chemical. For this review paper, the focus will be on describing the methods used for bacterial/microbial source tracking that can be applied on a watershed scale. Currently, there is no standard method that has been adopted for source tracking.

Source tracking of bacterial contamination in drinking water is a rapidly growing area of research and technology development. As a result, it is critical to understand the pros and cons of various tools currently applied to tracking bacterial sources, and this review makes an attempt to enhance the understanding for general readers and understanding and applying bacterial source tracking for monitoring, assessment and hypothesis testing.

Common source tracking methods

In the past, fecal coliform/fecal streptococci (FC/FS) ratios have been used to assess the general source of non-point fecal pollution, with FC/FS > 4 indicating humans, FC/FS between 0.1 and 0.6 indicating domestic animals, and FC/FS < 0.1 indicating wild animals as the source (Geldreich 1976). However, studies have found that the FC/FS ratio was difficult to use in agricultural settings (Howell et al. 1996), and the American Public Health Association (APHA) no longer recommends the use of the FC/FS ratio as a means of differentiating human and animal sources of pollution (APHA 1998).

Today, there are a number of different molecular and biochemical methods proposed for BST including: ribotyping (Samadpour and Chechowitz 1995, Farber 1996, Tynkkynen et al. 1999, Parveen et al. 1999, Carson et al. 2001, Farag et al. 2001, Hager 2001a, Samadpour 2002, Hartel et al. 2002, Simpson et al. 2002, Scott et al. 2002); pulse-field gel electrophoresis (PFGE) (Tynkkynen et al. 1999, Simmons et al. 2000, Hager 2001b, Simpson et al. 2002, Scott et al. 2002); randomly amplified polymorphic DNA (RAPD) (Tynkkynen et al. 1999); denaturing-gradient gel electrophoresis (DGGE) (Farnleitner et al. 2000, Buchan et al. 2001, Chee-Sanford et al. 2001, Simpson et al. 2002); repetitive DNA sequences (Rep-PCR) (Dombek et al. 2000, Holloway 2001); length heterogeneity PCR (LH-PCR) (Suzuki et al. 1998, Bernhard and Field

2000a, Bernhard and Field 2000b); terminal restriction fragment length polymorphism analysis (T-RFLP) (Bernhard and Field 2000a, Bernhard and Field 2000b); host specific 16S rDNA (Suzuki et al. 1998, Bernhard and Field 2000a, Bernhard and Field 2000b,); toxin biomarkers (Hager 2001a, Olson et al. 2002); reverse transcriptase PCR (Hager 2001b); phage analysis (Hager 2001b, Sobsey 2002); and antibiotic resistance analysis (ARA) (Wiggins 1996, Parveen et al. 1997, Wiggins et al. 1999, Hagedorn et al. 1999, Harwood et al. 2000, Hager 2001a).

Several of the molecular and biochemical techniques have been applied or suggested for use in watershed studies (Simpson et al. 2002). Table 1 summarizes proposed molecular methods including the advantages and disadvantages of each method. Table 2 summarizes the non-molecular techniques. Below are more detailed descriptions of only those methods that have been proposed for use in watershed studies.

Ribotyping

Ribotyping, also referred to as "molecular fingerprinting", is a way of identifying microorganisms from the analysis of DNA fragments generated from restriction enzyme digestion of genes encoding their 16S rRNA (Farber 1996, Aarnisalo et al. 1999, Samadpour 2002). The ribotyping procedure provides a DNA fingerprint of bacterial genes coding for ribosomal ribonucleic acids (rRNA), which are highly conserved in microorganisms (Farber 1996, Samadpour 2002). Unique strains of *E. coli* are adapted to their own specific environment (intestines of host species), and as a result differ from other strains found in other host species. To use the BST/MST method, collections of potential source material (fecal samples of all potential sources in the watershed) must be collected and sub-typed. The genetic fingerprints of the bacterial isolates from the water

samples can then be compared to those of the bacteria from the suspected animal sources (Samadpour 2002). Ribotyping does not involve sequencing, instead, it measures the unique pattern generated when DNA from a specific organism is subject to restriction enzyme digestion and the fragments are separated and probed with a ribosomal RNA probe (Farber 1996, Samadpour 2002). Ribotyping is a specific method of bacterial identification and the procedure is illustrated in Figure 1.

The BST methodology for ribotyping has been under development for the past 12 years by Samadpour and colleagues, and it has been applied to over 80 studies in the U.S. and Canada (Samadpour 2002). Samadpour (2002) has shown that the choice in restriction enzymes used for ribotyping is critical, and that double enzyme analysis should be used to identify clones to a higher degree of accuracy, as single enzyme digestion is insufficient (Table 3).

Carson et al. (2001) and Parveen et al. (1999) have tested the average rate of correct classification (ARCC) achieved by ribotyping when differentiating between human and nonhuman sources of fecal pollution. Carson et al. (2001) found that when using discriminant analysis the rate of correct classification from each of eight known sources (human, cattle, pig, horse, dog, chicken, turkey, and goose) ranged between 49% and 96%. They found a higher classification accuracy when the analysis was limited to three host sources (i.e. cattle, pigs and humans), and were able to achieve a 97% ARCC by grouping the nonhuman riboprints and comparing them to human riboprints. Carson et al. (2001) only used one restriction enzyme (*Hin*dIII) in their study. Parveen et al. (1999) used discriminant analysis of ribotype profiles to correctly classify 97% and 67% of the nonhuman and human source isolates using the ribotype method. Their ARCC was

82%. Parveen et al. (1999) used a number of restriction enzymes including *Hin*dIII, *Eco*RI, *Sal*I, and *Bgl*I.

Pulse-field gel electrophoresis (PFGE)

PFGE is a DNA "fingerprinting" technique that uses rare-cutting restriction enzymes on the entire DNA genome (Tynkkynen et al. 1999, Simmons et al. 2000, Hager 2001b). The large genomic fragments are then separated by subjecting them to alternately pulsed, perpendicularly oriented electrical fields (King and Stansfield 2002). Figure 2 illustrates the PFGE procedure.

PFGE is similar to ribotyping, but instead of analyzing rRNA, it uses the whole DNA genome. Bacterial DNA analyzed through PFGE are embedded in agarose plugs, which are then placed in hollow combs of the electrophoresis gel where they become part of the gel as the gel moves over the combs. Following electrophoresis and staining of the gels, banding patterns emerge (Hager 2001b).

Tynkkynen et al. (1999) compared ribotyping, PFGE, and randomly amplified polymorphic DNA (RAPD) for typing two strains of *Lactobacillus* (*L. rhamnosus* and *L. casei*). They found that PFGE was the most discriminatory method followed by ribotyping and RAPD revealing 17 (71%), 15 (63%), and 12 (50%) genotypes respectively for the 24 strains studied. However, they only used one restriction enzyme-*Eco*RI, for the ribotyping procedure. As mentioned earlier, the use of two restriction enzymes is highly recommended to improve accuracy (Samadpour 2002).

Denaturing-gradient gel electrophoresis (DGGE)

DGGE is an electrophoretic technique that separates genes of the same size that differ in base sequence (Madigan et al. 2003). A gradient of DNA denaturant is used to

"melt" a double-stranded DNA fragment moving across the gel, which stops migration. The differences in melting properties are controlled largely by differences in base sequences. Therefore, each band observed on the gel, represents a specific sequence of a gene that may vary by as little as one nucleotide in their sequences (Madigan et al. 2003). This method gives a detailed picture of the number of phylotypes (distinct 16S rRNA genes) present in a sample. These bands can then be sequenced and compared with sequences of known species available in an appropriate database, thereby revealing the actual species present in a community. This method coupled with PCR amplification of rDNA genes has been used primarily to determine the genetic fingerprints of microbial communities (Chee-Sanford et al. 2001). This method is still in development for application to fecal source tracking experiments (Chee-Sanford et al. 2001, Farnleitner et al. 2000). Farnleitner et al. (2000) adapted PCR-DGGE technology for the specific detection and profiling of *E. coli* populations differing in a fragment of the functional *uidA* gene. Their results indicate that PCR-DGGE could simultaneously detect and differentiate *E. coli* populations from environmental freshwater samples and generate a species-specific community fingerprint. Further studies need to be conducted on PCR-DGGE to test its potential to discriminate *uidA* profiles of mixed *E. coli* populations from different sources.

Buchan et al. (2001) applied DGGE to the 16S-23S rRNA intergenic spacer region, which is under minimal selection pressure and often varies among strains (Figure 3). They found a high diversity of *E. coli* among environmental isolates, and therefore could not pinpoint the source of contamination in the watershed studied. They concluded that applying DGGE to the intergenic spacer region was an effective method

for strain level differentiation of bacteria, but further studies are needed to improve the application to the field.

Repetitive DNA sequences (Rep-PCR)

Rep-PCR is a DNA fingerprint technique that uses repetitive intergenic DNA sequences to differentiate between sources of fecal pollution (Dombek et al. 2000). With this technique, DNA between adjacent repetitive extragenic elements is amplified using PCR to produce various size DNA fragments (Farber 1996, Dombek et al. 2000). The PCR products are then size-fractionated by agarose-gel electrophoresis to produce specific DNA fingerprint patterns. These fingerprint patterns can then be analyzed using pattern recognition computer software (Dombek et al. 2000). Dombek et al. (2000) used rep-PCR with BOX A1R primer to differentiate between human and six species of animal (cows, pigs, sheep, chickens, geese and ducks) fecal pollution. They analyzed 154 isolates using Jaccard similarity coefficients and Jackknife analysis, and were able to correctly classify 100% of the chicken and cow isolates and between 78 and 90% of the other isolates (Table 4). Overall, they suggested that rep-PCR is a useful method for differentiating and grouping *E. coli* isolates from animals and humans. Carson et al. (2003) conducted a comparison study of ribotyping and rep-PCR on eight host classes (human, cattle, pig, horse, dog, chicken, turkey, and goose) and found that the ARCC for ribotyping was 73% versus 88% for rep-PCR. They concluded that rep-PCR was more accurate, reproducible, and efficient than ribotyping. However, in their comparison study, they only used one restriction enzyme (*Hin*dIII) for ribotyping versus two as recommended by Samadpour (2002). Holloway (2001), using the protocol of Dombek et al. (2000), used rep-PCR to determine animal host type for 91 *E. coli* and 68

Enterococcus fecalis strains from human, cattle, swine and poultry feces. In contrast to the results from Dombek et al. (2000) and Carson *et al.* (2003), Holloway (2001) did not observe any significant clustering of *E. coli* or *Enterococcus fecalis* strains by animal type. Holloway (2001) suggested that too few strains may have been tested in his study. In conclusion Holloway (2001) stated that this technique is not ready and reliable for the identification of the source of fecal contamination in water and that a large sample size may be necessary for this approach.

Host-specific 16S rDNA genetic markers

The host-specific 16S ribosomal DNA (rDNA) genetic markers technique distinguishes members of mixtures of bacterial gene sequences by detecting differences in the number of base pairs in a particular gene fragment (Bernhard and Field 2000a, Bernard and Field 2000b). Length heterogeneity PCR (LH-PCR) separates PCR products for host specific genetic markers based on length of amplicons (Bernhard and Field 2000a). LH-PCR can quickly provide a profile of amplicon diversity in complex mixtures of PCR products (Suzuki et al. 1998). Terminal restriction fragment length polymorphism analysis (T-RFLP) uses restriction enzymes on PCR amplicons to determine unique size fragments among fluorescently labeled terminal end fragments (Bernhard and Field 2000a). LH-PCR and T-RFLP analyze differences in the lengths of gene fragments due to insertions and deletions to estimate the relative abundance of each fragment (Bernhard and Field 2000a). This method helps to decrease some of the problems associated with the under sampling of diversity in a microbial community and the uncertainty of bias due to the reannealing kinetics in the cloning process by PCR (Suzuki et al. 1998). Bernhard and Field (2000a) developed 16S rDNA markers that

were based on fecal anaerobes (*Bacteroides* and *Bifidobacterium*) to distinguish human and cow fecal pollution. Strict anaerobes were chosen because they are restricted to warm-blooded animals, make up a large portion of the fecal bacteria, and do not survive long once deposited in waters. *Bacteroides* and *Bifidobacterium* have had limited use as indicators of fecal pollution because they are difficult to grow in culture media. The use of molecular methods versus culture-based methods improved the ability for their use in water quality monitoring (Bernhard and Field 2000a). Bernhard and Field (2000a) found that the *Bacteroides-Prevotella* group was a better indicator than the *Bifidobacterium* species due to the ease of detection and longer survival in water. Bernhard and Field (2000b) also tested their approach on feces from human, sewage and cattle sources and found their method was successful in being able to distinguish sources (Table 5). Since only human and cattle markers were studied, further research needs to be conducted on other sources of fecal contamination such as wildlife and domestic animals other than cattle.

Antibiotic resistance analysis (ARA)

ARA is a method that is based on patterns of antibiotic resistance of bacteria from human and animal sources. The premise behind this method is that human fecal bacteria will have greater resistance to specific antibiotics followed by livestock and wildlife, and that livestock will have greater resistance to other antibiotics (Hager 2001a). These differences occur because humans are exposed to different antibiotics than cattle versus pigs versus poultry versus wildlife etc. Isolates of fecal streptococci and/or *E. coli* are taken from various sources (human, livestock, and wildlife), and these isolates are grown

on a variety of antibiotics. Following incubation, isolates are scored as "growth/no growth" for each concentration of an antibiotic (Hager 2001a). The resistance pattern of an organism is used to identify its source. A database of antibiotic resistant patterns from known sources within a watershed is needed to compare sample isolate patterns to. It is still not known how many isolates are needed to be representative of a watershed. Either sample-level analysis or isolate-level analysis can be used with this method. If it was assumed that a sample came from a single major source, then sample-level analysis could be used, however it is very unlikely that a water sample taken from a watershed would contain only one source. If a sample was assumed to be contaminated by more than one source, isolate level analysis should be used (Wiggins et al. 1999). ARA is a low cost method that only requires basic microbiology training to perform (Hager 2001a). Several studies with average rates of correct classification ranging from 62 to 84% cite the ARA method as a useful tool in assessing sources of fecal contamination (Wiggins 1996, Parveen et al. 1997, Wiggins et al. 1999, Hagedorn et al. 1999, Harwood et al. 2000). Pooling sources (i.e. turkey and chicken pooled as poultry, or all animal sources pooled versus human) has been found to improve ARCC (Wiggins 1996, Hagedorn et al. 1999). Wiggins (1996) found that separation between human and wild isolates had an ARCC of 98%, and therefore in recreation waters not impacted by agriculture this would be a useful method to distinguish between human and wild sources of fecal pollution. In a study by Harwood et al. (2000), the percent of correct classification of various sources of known fecal streptococcus isolates ranged from 34 to 89% (Table 6).

This method has been criticized because the grouping of isolates may be influenced by a strain's prior exposure to antibiotics (Dombek et al. 2000). Also, the

assumption that all livestock will have been exposed to some level of antibiotics is not true. Another problem could be that wildlife often live in close proximity to livestock and consume their feed and therefore may be exposed to antibiotics used for livestock. *E. coli* from wildlife exposed to livestock feed could be incorrectly classified. This method is also not useful for differentiating wildlife sources. Finally, further research is needed to determine if this method can accurately identify sources of fecal pollution from mixed (more than one source) samples (Wiggins et al. 1999).

Conclusion

As discussed above, many methods and techniques are being developed and refined for use of BST. Each method appears to have distinct advantages and disadvantages. No one method has been proposed as a standard method for source tracking. Determining which method or combination of methods to use for any given situation will depend on a number of factors including: specific question to be answered, detail required to answer the question (i.e. broad scale results – human/non-human versus detailed results – human, livestock species, wildlife species), availability of resources (cost of analysis varies depending on technique used, and size of the watershed), time constraints and turnaround time, and ability to access a lab or facilities with expertise to analyze the samples. A critical criterion in selecting a method would depend on the complexity of the watershed and associated multiple potential sources of bacterial contamination of source water. More comparison studies on source tracking methods are needed in order to determine which method works best for watershed studies. More research is also needed to determine within any particular method which primers and organism provide the greatest specificity (Myoda et al. 2003). PFGE and ribotyping have

had differing success depending on the restriction enzyme(s) used (Myoda et al. 2003). Comparison studies on restriction enzymes and standardization of protocol within a method would be beneficial for the interpretation of comparison studies between methods. Field protocol including number of water samples collected, number of isolates identified, and location and number of sites needed in a watershed also requires further studies. Finally, Myoda et al. (2003) in their comparison study of PFGE, rep-PCR, and ribotyping found that none of the methods stood out as being superior, and that the main differences were investigator dependent, and there is a need to optimize analytical and statistical methods and minimize sources of error.

Table 1. Comparison of molecular microbial source tracking methods used for watershed experiments (adapted from Simpson et al. 2002, and Scott et al. 2002).

Method	Description	Advantages	Disadvantages	References
Ribotyping	Southern blot of genomic DNA cut with restriction enzymes, probed with ribosomal sequences; discriminates species	Highly reproducible; classify isolates from multiple sources	Complex; expensive; labour intensive; geographically specific; database required; variations in methodology	Samadpour and Chechowitz 1995, Farber 1996, Tynkkynen et al. 1999, Parveen et al. 1999, Farag et al. 2001, Hager 2001a, Carson et al. 2001, Hartel et al. 2002, Samadpour 2002, Scott et al. 2003
Pulse Field Gel Electrophoresis (PFGE)	DNA fingerprinting with rare cutting restriction enzymes coupled with electrophoretic analysis; discriminates species	Extremely sensitive to minute genetic differences; highly reproducible	May be too sensitive to broadly discriminate source; long assay time; limited simultaneous processing; database required	Tynkkynen et al. 1999, Simmons et al. 2000, Hager 2001b, King and Stansfield 2002
Denaturing-Gradient Gel Electrophoresis (DGGE)	Electrophoresis analysis of PCR products based on melting properties of the amplified DNA sequences; discriminates species	Works on isolates	Still early development; technically demanding; time consuming; limited simultaneous processing; not good on environmental isolates; database required	Farnleitner et al. 2000, Buchan et al. 2001, Chee-Sanford et al. 2001
Repetitive DNA Sequences (Rep-PCR)	PCR used to amplify palindromic DNA sequences coupled with electrophoretic analysis; discriminates species	Simple and rapid	Reproducibility a concern; cell culture required; large database required; variability increases as database increases	Dombek et al. 2000, Holloway 2001
Length heterogeneity PCR (LH-PCR)	Separates PCR products for host specific genetic markers based on length	Does not require culturing or a database	Expensive equipment; technically demanding	Suzuki et al. 1998, Bernhard and Field 2000a, Bernhard and Field 2000b
Terminal restriction fragment length polymorphism analysis (T-RFLP)	Uses restriction enzymes coupled with PCR in which only fragments containing a fluorescent tag are detected	Does not require culturing or a database	Expensive equipment; technically demanding	Bernhard and Field 2000a, Bernhard and Field 2000b
Host-Specific 16S rDNA	Combine LH-PCR and T-RFLP methods on fecal anaerobes (*Bacteroides* and *Bifidobacterium*); discriminates human and cattle, other markers being developed	Does not require culturing or a database; indicator of recent pollution	Only tested on human and cattle markers; limited simultaneous processing; expensive equipment; technically demanding; little known about survival of *Bacteriodes spp*. in environment	Bernhard and Field 2000a, Bernhard and Field 2000b

Table 2. Comparison of non-molecular microbial source tracking methods (adapted from Simpson et al. 2002, and Scott et al. 2002).

Method	Description	Advantages	Disadvantages	Reference
Antibiotic Resistance Analysis (ARA) or Multiple Antibiotic Resistance (MAR)[a]	Biochemical technique. Differentiates bacteria (*E. coli* or fecal streptococci) from sources using antibiotics associated with human and animal therapy and animal feed. Discriminates between human, livestock, and wildlife. Does not discriminate wildlife species.	Rapid; discriminates isolates from multiple animal sources	Requires reference database; geographically specific; isolates that show no antibiotic resistance cannot be typed; can be highly prone to false positives; AR genes typically encoded on plasmids often lost with environmental conditions; grouping of isolates may be influenced by strain's prior exposure to antibiotics; difficulty with mixed samples.	Wiggins 1996, Parveen et al. 1997, Wiggins et al. 1999, Hagedorn et al. 1999, Harwood et al. 2000, Hager 2001a, Buchan et al. 2001
Optical brighteners	Found in laundry detergents; indicates human pollution	Simple, fast, low cost	Provide limited information. May not reflect recent pollution	Hagedorn 2001
Caffeine	Water samples tested for presence of caffeine; indicates human pollution	Indicates impact from human pollution	Expensive; easily degraded by soil microbes; sensitivity issues	Hagedorn 2001

[a]Methods that have been used on watershed experiments.

Table 3. Summary of Ribotype Totals for Single and Double Enzyme Analysis (Samadpour 2002).

Enzyme	Total Ribotypes	Source Specific Ribotypes	Source Related Ribotypes	Transient Ribotypes
*Pvu*II	514	221 (43%)	31 (6%)	262 (51%)
*Eco*RI	723	368 (51%)	38 (5%)	317 (44%)
*Pvu*II and *Eco*RI	873	823 (94%)	18 (2%)	32 (4%)

Table 4. Classification of isolates to animal source groups by using BOX PCR DNA fingerprints and Jackknife analysis (Dombek et al. 2000).

	% of *E.coli* isolates in assigned group[a]						
Assigned Group	Human	Goose	Duck	Sheep	Pig	Chicken	Cow
Human	**82.8**	0.0	0.0	0.0	0.0	0.0	0.0
Goose	6.9	**81.0**	4.3	5.3	0.0	0.0	0.0
Duck	3.4	0.0	**78.3**	0.0	0.0	0.0	0.0
Sheep	0.0	4.8	8.7	**89.5**	4.8	0.0	0.0
Pig	6.9	0.0	4.3	5.3	**81.0**	0.0	0.0
Chicken	0.0	9.5	0.0	0.0	4.8	**100**	0.0
Cow	0.0	4.8	4.3	0.0	9.5	0.0	**100**

[a]Values in boldface indicate percentages of isolates correctly assigned to source groups.

Table 5. Distribution of host-specific genetic markers in feces from targeted sources (Bernard and Field 2000b).

		No. of positive PCR results[a]			
		Human markers		Cow markers	
Target	No. samples tested	HF8 cluster	HF10 cluster	CF123 cluster	CF151 cluster
Human	13	11	6	0	0
Sewage	3	3	1	0	0
Cow	19	0	1	19	19

[a]PCR results are from two rounds of 25 cycles each.

Table 6. Classification of known fecal streptococcus isolates by source based on antibiotic resistance patterns (adapted from Harwood et al. 2000).

	No. (%) of database isolates assigned to each source category[a]					
Fecal source (n)	Humans	Chicken	Cow	Dog	Pig	Wild
Human (1 653)	**1 000 (60.5)**	159 (9.6)	134 (8.1)	157 (9.5)	81 (4.9)	122 (7.4)
Chicken (844)	171 (20.3)	**290 (34.4)**	61 (7.2)	24 (2.8)	35 (4.2)	263 (31.1)
Cow (1 112)	234 (21.0)	79 (7.1)	**495 (44.5)**	132 (11.9)	40 (3.6)	132 (11.9)
Dog (153)	4 (2.6)	6 (3.9)	9 (5.9)	**116 (75.8)**	0 (0)	18 (11.8)
Pig (520)	22 (4.2)	7 (1.4)	14 (2.7)	0 (0)	**462 (88.9)**	15 (2.8)
Wild (337)	35 (10.3)	17 (5.1)	17 (5.1)	12 (3.6)	22 (6.5)	**234 (69.4)**

[a]Values in boldface indicate isolates (%) correctly assigned to source groups.

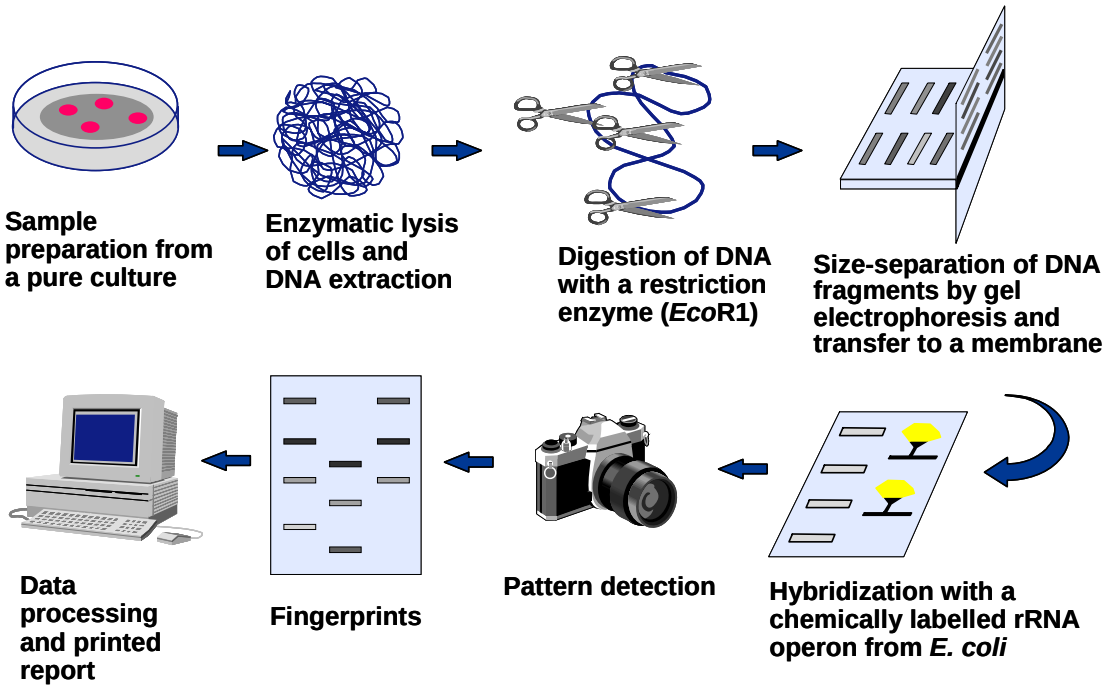

Figure 1. Illustration of the ribotyping procedure (with permission Aarnisalo et al. 1999).

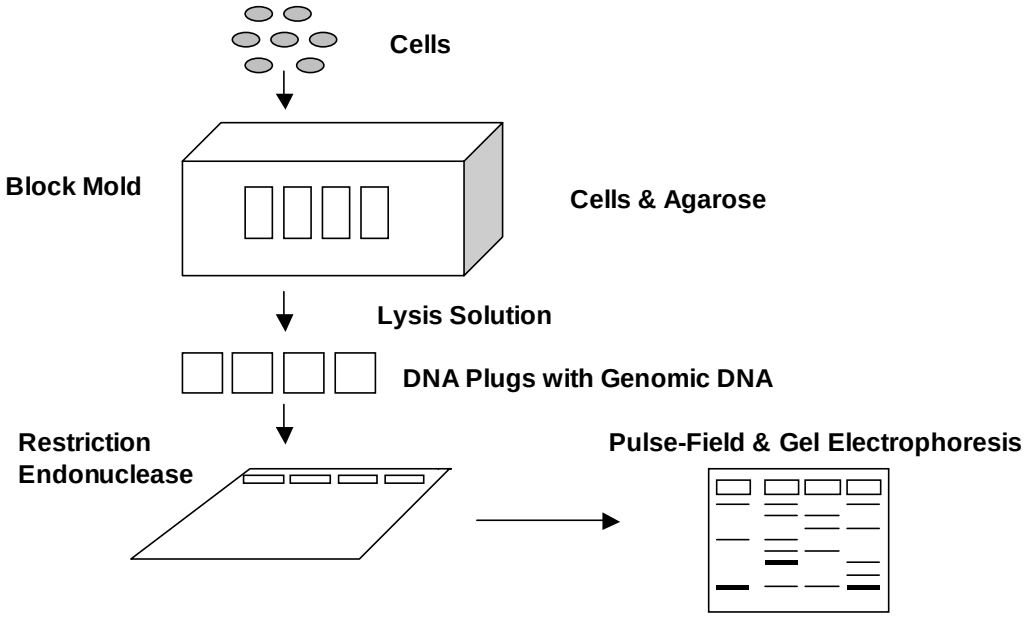

Figure 2. Pulse-field gel electrophoresis procedure (redrawn from Farber 1996).

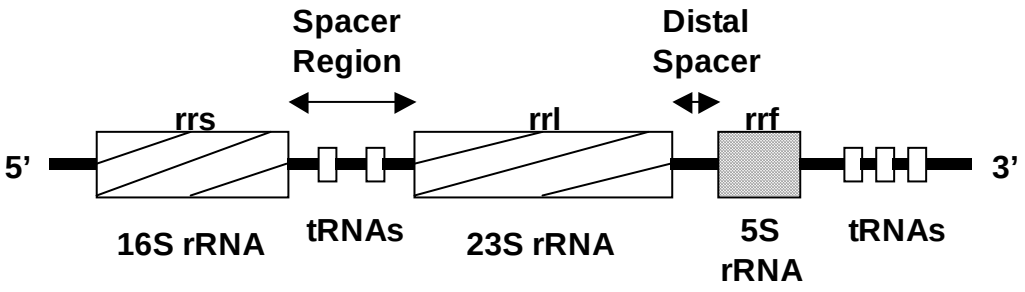

Figure 3. Schematic diagram of a typical ribosomal rRNA operon (redrawn from Farber 1996).

Chapter 3: Survival of *Escherichia coli* in Beef Cattle Fecal Pats Under Different Levels of Solar Exposure.

Abstract

Understanding the survival and transport of *E. coli* in feces on land and in water is important when trying to assess contamination of water by grazing animals. A fecal pat experiment was conducted in July and August of 2003, to investigate the survival of *E. coli* under four levels of solar exposure controlled by using shade cloth. Fresh beef cattle manure was uniformly blended to produce 2.5 and 1.6 kg fecal pats in trays or in contact with the soil and placed under 0, 40, 80, and 100 % shade cloth treatments and replicated five times. Samples from each fecal pat were collected at time zero to establish *E. coli* levels, day one, day three, and approximately weekly thereafter for 45 days to determine die-off. *E. coli* concentrations and percent moisture were measured for each fecal sampling. At the end of the experiment, fecal pats under the 0 % shade cloth had the lowest *E. coli* concentrations followed by the 40, 80, and 100 % treatments (0.018, 0.040, 0.11, and 0.44 x 10^6 colony forming units (CFU) g^{-1} respectively). Fecal pat size was significant only on day 17, with large fecal pats having higher concentrations of *E. coli* ($P < .0001$). There was no significant difference ($P = 0.43$) in *E. coli* concentration between the fecal pats in contact with the soil versus plastic trays. Percent moisture of fecal pats was not a good covariate. Age of fecal pats, as well as exposure to solar radiation negatively influences the survival of *E. coli*. From a management perspective, *E. coli* in fecal pats under forested situations would survive longer than in open grasslands due to shading, and any possible contamination by *E. coli* would be greatest within 7 days of removing cattle from a riparian area or pasture.

Introduction

Fecal coliforms and *Escherichia coli* (*E. coli*) are used in water quality as indicators of fecal contamination and potential pathogens (Rosen 2000). Although these indicators are not usually pathogenic and may not correlate well with the pathogens that they are meant to indicate, they are easier and less costly to detect and enumerate. Fecal contamination of water can come from many sources (wildlife, livestock, and humans). New molecular and biochemical methods for detecting the sources of fecal contamination are being developed (Scott et al. 2002, Simpson et al. 2002, Meays et al. 2004) but information on the survival and growth of bacteria and waterborne pathogens is limiting and often contradictory (Alexander 1986, Park et al. 1991, Ferguson et al. 2003). Microbial contamination of source water is a major environmental and health issue with drinking water in British Columbia (BC), Canada, and worldwide (BC Gov. 2001, WHO 2003). Many people rely on surface water from watersheds with multiple uses (forestry, mining, agriculture, wildlife, urban development and recreation) as the source of their drinking water. Maintaining sustainable clean water supplies requires sound scientific data on the pollutants that affect water quality. *E. coli* is probably one of the most studied organisms but the majority of research has been conducted on pure cultures in the laboratory, or inoculated into livestock waste (Avery et al. 2004). Park et al. (1991) argued that the survival and optimum conditions for an organism in a laboratory experiment may be very different to what happens under various environmental conditions. Both laboratory and field experiments are needed in order to understand the survival of these organisms.

In a series of field experiments looking at fecal coliforms, Buckhouse and Gifford (1976) and Bohn and Buckhouse (1985) suggested that cattle feces could provide a protective medium for coliforms to survive for at least a year. Buckhouse and Gifford (1976) also concluded that bacteria did not travel farther than 1.0 m on a sandy loam range site located in Southeastern Utah. Doyle et al. (1975) studied forested buffer strips in controlling bacterial transport on a gravelly silt loam soil and observed no significant movement of bacteria beyond 3.8 m. In a laboratory experiment simulating overland flow and bacterial movement across plots, Larsen et al. (1994) found that bacterial loads were reduced by 95 % if 2.13 m distance between the feces and collection point for overland flow were maintained. They found that even with a small buffer of 0.61 m the coliform count was reduced by 83 %. More studies are needed to look at *E. coli* and other fecal pathogens and their movement on and through soils under different environmental conditions.

Although it is generally thought that there are no significant environmental sources of *E. coli* and other bacteria unrelated to direct fecal contamination (Byappanahalli and Fujioka 1998), there have been studies supporting the idea that fecal bacteria can survive and grow in the environment (Gerba and McLeod 1976, Tassoula 1997, Byappanahalli and Fujioka 1998, Topp and Scott 2003, Topp et al. 2003, Unc and Goss 2003). Understanding the potential of fecal bacteria to survive and grow under certain circumstances is critical for managing watersheds or areas that have chronic high fecal counts.

Sunlight is reported to be one of the most detrimental factors to the survival of *E. coli* in water (Chamberlin and Mitchell 1978, Fujioka et al. 1981), whereas available

water has been suggested most critical in soils (Unc and Goss 2003). Other factors that have been shown or suggested to influence the survival of bacteria as mentioned above includes: temperature, pH, nutrients, predators, soil type, season and competition with other organisms (Chamberlin and Mitchell 1978, Fujioka et al. 1981, Alexander 1986, Sherr et al. 1987, Ferguson et al. 2003, Unc and Goss 2003). There is very little information available on whether the factors influencing microbial survival are the same for aquatic systems, manure, and soil matrices (Ferguson et al. 2003).

A more holistic approach to understanding fecal pollution is needed, which identifies the sources of fecal pollution, and determines the survival and transport of the pathogens on land and in water. Survival and transport of bacteria in the environment is very complex. The objectives of this study were to: 1) Determine the impact of shade on survival of *E. coli*; 2) Determine if size of fecal pat affects survival of *E. coli*; 3) Determine any differences in survival of *E. coli* in contact with soil vs. on plastic trays; and 4) Determine if there is a relationship with *E. coli* survival and the percent moisture of feces at time of sampling.

Materials and Methods

A fecal pat experiment was conducted in July and August of 2003 near the town of Armstrong in the south central interior of BC, to investigate the survival of *E. coli* under four levels of solar exposure controlled using shade cloth (Appendix 1). July and August broke records for being extremely hot and dry months in the southern interior of BC. The mean average maximum temperature for July and August was 30.0 and 31.1 ^{o}C, respectively. The temperatures for July and August were 3.4 and 4.9 ^{o}C above the long-

term average. The amount of precipitation for July and August was 3.9 % and 4.7 % of the long-term average for this region.

Field plots

Clear plastic tarps (0% direct solar block), ginseng tarps (40 and 80 % direct solar block) and reflective impenetrable solid silver coloured tarps (100% direct solar block) all 1.8 by 3 m^2, were suspended and anchored using metal posts, ropes and tent pegs to create tent like structures. Five tarps were draped and centered over each of four 12.5 m ropes (total of 20 tarps) and anchored approximately 0.3 m above the ground using tent pegs and rope to allow for air circulation. Spacing between the tarps was approximately 0.6 m apart. The five replicates of 0, 40, 80, and 100% shade were completely randomized in the field.

Source of E. coli

Natural populations of *E. coli* in fresh beef cattle manure were used for this experiment. Approximately 200 kg of fresh beef cattle manure was collected from two ranches using shovels and pails. Cattle manure was transported to the field plot site using four large plastic garbage cans. Manure was emptied from the garbage cans into a clean Rubbermaid® 450 L water trough and blended thoroughly using a drywall mud paddle attached to an electric drill. Fecal pats (2.5 kg and 1.6 kg wet weight) were made from the uniformly blended manure. Each size fecal pat was placed both directly on the ground as well as in pie-shaped plastic trays for a total of 4 fecal pats under each of the 20 tarps. The location of both the shade tarps as well as the location of the fecal pats under the tarps was completely randomized.

Sampling

Five replicate samples of blended fresh manure were collected and cultured to establish the initial *E. coli* concentration of the fresh manure at time zero. Samples approximately 1 to 2 g were taken from each fecal pat on day one, three, seven, and approximately weekly thereafter for 45 days to determine viable *E. coli* concentrations. Samples were taken from the middle of the fecal pat and transported to the laboratory in sterile individually labelled vials in a cooler with ice. Additional measurements taken included: percent moisture of feces at each sampling time, and hourly temperature measurements of fecal pats, air temperature and ground temperature using Onset HOBOS® and Tidbits®.

Statistical design

The design at each sampling time for this experiment was a split-plot with shade cloth as the main plot arranged as a completely randomized design, and fecal pat size and contact (ground or plate) the split-plot factors. Analysis was conducted at each sampling time using PROC MIXED in SAS® (1996). Fixed effects included: shade, pat size, contact, and time. Random effects were plot, and plot*pat size*contact. A variable named TIME was created which represented the day on which the samples were taken. Samples that were taken within one day of each other (small fecal pats one day and large fecal pats the next) were grouped. All factors (shade, pat size, contact, and time) were included in the model and comparisons were made with this arrangement of sampling time. The overall analysis with TIME indicated that a four-way interaction was significant. Therefore, an analysis was performed for each TIME and produced the LSMEANS for all the effects. The data was also analyzed using a repeated measures model with a UN(1) variance-covariance structure. The analysis indicated that the

variances among times were not the same, but there was no correlation among times. The results were similar to those obtained with the split-plot analysis, therefore, the data from the split-plot analysis for each TIME is presented.

Log_{10} values were used to perform the statistical analysis because of the large range in data for *E. coli* concentrations. Percent moisture was added as a covariate to the model, however the results did not converge. A linear relationship between log_{10} counts and moisture did not exist or was dependent on treatments only for certain times. It was concluded that moisture was not a very useful covariate.

Plating and enumerating

Samples were collected from fecal pats and taken on ice directly to the laboratory for culturing. Measuring spoons and spatulas were flame sterilized before and in between each fecal sample. A 1 ml volume of fecal material (the same amount as was put in the incubator to calculate percent moisture) was added to 90 ml of sterilized de-ionized water. The determined dry weight of the fecal material was used in the calculating of *E. coli* concentrations per dry weight gram of sample. The bottles that contained the water and fecal samples were vigorously shaken to suspend *E. coli*. The water samples were then placed in a walk-in cooler (approximately 4ºC) until they were ready to be filtered (approx. 2 hours). Bottles were inverted 10 times prior to being filtered using the membrane filtration technique. Volumes of 10, 100, and 1000 µl were filtered through a 0.45 µm pore membrane filter, and the filter placed in petri-dishes containing Millipore m-ColiBlue24® broth for coliform and *E. coli* detection. Petri-dishes were placed in an incubator at 35ºC for 24 hours and were enumerated by counting blue colonies (*E. coli*) on the filter paper. Colony counts per volume sampled were then converted to counts per

dry weight gram of feces for comparison purposes. Volumes filtered altered with sampling cycle. Early in the experiment when counts were extremely high, only volumes of 10 and 100 µl were filtered and later in the experiment when numbers decreased, larger volumes of water were filtered, up to 2 ml. Triplicate filtrations were performed on approximately 10-15% of the samples each sampling run to determine accuracy.

Results and Discussion

The primary objective of this study was to examine the impact of shade (direct solar radiation) on the survival of natural populations of *E. coli* in beef cattle fecal pats. Beef cattle pats were chosen because ranching and non-point source pollution by range cattle is often cited as having a negative impact on water quality (Kauffman and Krueger 1984, Belsky et al. 1999). However, most studies investigating fecal pollution and survival of *E. coli* have concentrated on intensive agriculture or manure slurries for study purposes (Entry et al. 2000a, Entry et al.2000b, Rosen 2000). This study attempted to characterize what would be closer to reality for a rangeland situation where feces were surface deposited, in smaller pats and not incorporated into the soil. I recognize that blending the manure and making fecal pats is not the same as direct deposit of fecal pats on the land surface. Blending the manure would increase the aeration, which could impact the survival of *E. coli*. However, the authors felt that with their experimental design, they could conduct a controlled experiment with replication and minimize the impacts of variables other than those under investigation.

Results show that shade was the only significant factor affecting the survival of *E. coli* from day 17 to day 45 (Table 7). Fecal pat size and contact (ground or plate) were only significantly different on day 17. This was unexpected as different moisture levels

and temperatures between the small and large fecal pats were observed. There was no significant difference in *E. coli* concentrations under the shade treatments for the large fecal pats for the first week of sampling (Figure 4). *E. coli* was able to survive for more than 45 days in the hot dry summer weather, and shade enhanced the survival of *E. coli* (Figure 5). At the end of the experiment, fecal pats under the 0 and 40 % shade cloth had significantly lower colony forming units (CFU) g^{-1} compared to the 80 and 100 % treatments, respectively.

Percent moisture of feces declined faster under the 0% shade tarp than the 100% shade tarp, but it was not a covariant that could be utilized to show a strong relationship with *E. coli* CFU g^{-1} as illustrated in Figure 6. By day 31 the percent moisture was still declining, whereas *E. coli* concentration under the 100 % shade treatment was increasing. It was suspected that this is likely due to survival, reproduction and persistence of possible different *E. coli* strains in the manure. Unc and Goss (2003) suggested that available moisture was the most important factor affecting bacterial survival and should be measured. For our experiment percent moisture of the feces was measured, which did not show a relationship.

E. coli levels were observed to increase on days 1 and 7 under the 40 and 0 % shade treatments respectively, suggesting that it may be possible for *E. coli* to replicate in the environment. Previous studies have also suggested that *E. coli* is capable of replicating in the environment (Gerba and McLeod 1976, Tassoula 1997, Byappanahalli and Fujioka 1998, Topp and Scott 2003, Topp et al. 2003, Unc and Goss 2003). Overall, time and exposure to sunlight had a significant negative impact on the survival of *E. coli* in beef cattle fecal pats. Further research is needed on the survival and behavior in the

environment of *E. coli* and other fecal bacteria and pathogens from different animal feces. Identifying similarities in survival characteristics in the environment between pathogenic organisms and indicators would also be very useful.

Management Implications

From a management perspective, *E. coli* in fecal pats under forested situations would survive longer than in open grasslands due to the shade potential. However, if there was not adequate forage in the denser forested areas, the cattle would spend more time in open areas or areas of available forage. Tate et al. (2003) found that cattle feces distribution patterns were significantly associated with the location of livestock attractants, slope percent and aspect, hydrologic position, and season. Understanding these patterns on different landscapes and using cattle distribution tools, ranchers or land managers can move livestock from areas of high risk to lower risk to minimize or eliminate fecal contamination of water supplies.

E. coli concentrations remained high during the first 7 days of exposure to the environment. However, it is not known what the transport potential of bacteria from the fecal pats would be on the different days throughout the study. Fecal pats crust over quickly and begin to dry out and it is not known how much precipitation or what intensity would be needed to move the bacteria from the pats into soils or water sources. One would expect that the drier or thicker the fecal pat crust, the more difficult it would be for movement of *E. coli*. Therefore, season of grazing may impact the ability of *E. coli* to move from fecal pats, since in the spring or fall the days are not as hot or dry, as compared to mid- to late-summer.

As indicated earlier, more studies are needed in order to understand both the survival and transport potential of fecal pathogens. Scientific knowledge from experiments directed at the survival and transport can be applied to improve management plans and reduce both the impact of fecal pollution on water quality, and risk associated with human health.

Table 7. Observed significance levels (P-values) for fixed effects for each day[2] (i.e. TIME) sampled.

Effect	Day 1	Day 17	Day 24	Day 31	Day 38	Day 45
Shade	NS[1]	0.07	0.008	0.057	0.0008	0.0006
Pat size	NS	<.0001	NS	NS	NS	NS
Shade*Pat size	NS	0.08	NS	NS	NS	NS
Contact	NS	0.005	NS	NS	NS	NS
Shade*contact	NS	0.02	0.0003	NS	NS	NS
Pat size*contact	NS	NS	0.02	NS	NS	NS
Shade*Pat*contact	NS	0.004	NS	NS	NS	NS

[1]NS = not significant, P > 0.1.

[2]Note: On days 3 and 7, only the large fecal pats were sampled due to a delay in shipping of laboratory supplies. Data for these days was analyzed separately since we only had one fecal pat size (large). There were no significant differences for the shade, and shade*contact effects (P>>0.05). On day 7, the large fecal pats in contact with the plate had a significantly higher colony forming units (CFU) g^{-1} than large fecal pats on the ground (P = 0.03).

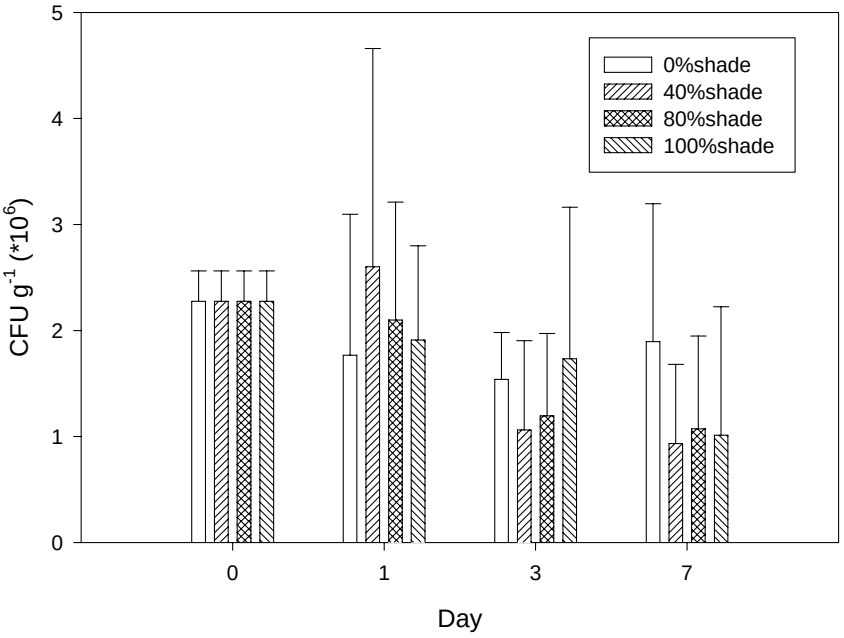

Figure 4. Mean *E. coli* concentrations (CFU g^{-1}dry weight manure) and SD for large fecal pats for days 0, 1, 3, and 7. There was no significant difference between shade treatment for each day sampled.

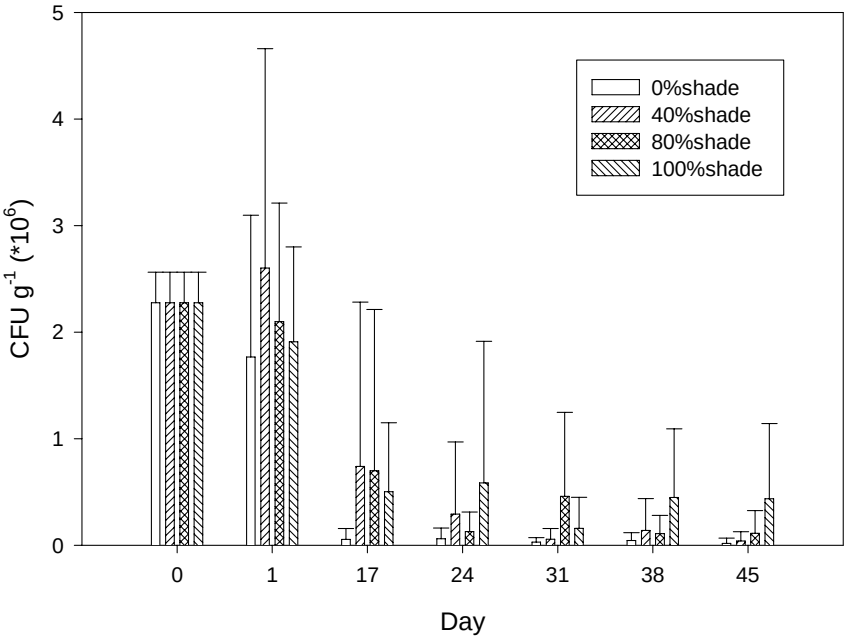

Figure 5. Mean *E. coli* concentrations (CFU g^{-1} dry weight manure) and SD of all fecal pats for each day (i.e. TIME) and shade treatment. From day 17 to day 45, percent shade was significant. Note that results for day 3 and 7 are not on this figure since only the large fecal pats were sampled (see Figure 4). Three outliers (day 1, 40 % shade treatment; day 24, 100% shade treatment; and day 31, 80 % shade treatment) were removed from the data set.

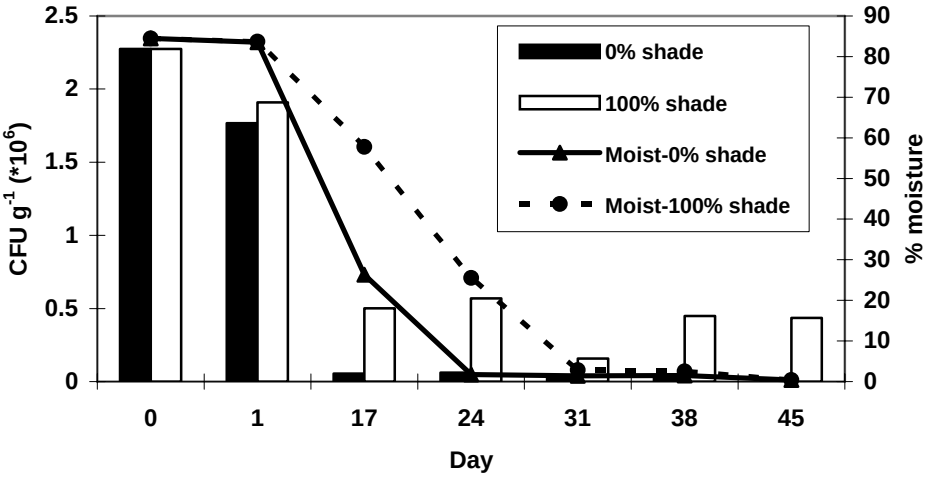

Figure 6. *E. coli* concentrations (CFU g^{-1} dry weight manure) and percent moisture of feces under the 0 and 100% shade tarps. Moist-0% shade is the percent moisture of feces under the 0% shade tarp, moist-100% shade is the percent moisture of feces under the 100% shade tarp.

Chapter 4: Survival of Beef Cattle *Escherichia coli* in Stream Water at Different Temperatures.

Abstract

Fecal contamination of drinking water is a major environmental and health issue worldwide. The understanding of the survival of fecal bacteria in the environment and the role of various environmental factors is not well understood. This experiment evaluated the survival of natural populations of *E. coli* from beef cattle manure in filtered and unfiltered stream water at 3 different temperatures (6, 20 and 26 ºC). There was no significant difference in the survival of *E. coli* in filtered vs. non-filtered stream water ($P = 0.29$). Day, and day by temperature interaction was significant on day 2 and 9 of the experiment ($P < 0.0001$). By day 9, there was approximately a 1.5-$\log_{10}$ decline in CFU g^{-1} for the 20 and 26 ºC treatments, whereas there was no decline in the 6 ºC treatment between days 2 and 9. By day 16, there was an approximate 2-$\log_{10}$ decrease in *E. coli* in all temperature treatments, which remained relatively constant to day 23. The addition of manure to the stream water in the experimental tanks substantially increased the organics and nutrient concentrations.

Introduction

Microbial contamination of source water is a major environmental and health issue with drinking water worldwide (WHO 2003). Surface water from watersheds with multiple uses such as: forestry, mining, agriculture, wildlife, urban development and recreation is used for drinking by many people around the world. Maintaining sustainable clean water supplies requires solid scientific data on the pollutants that affect water quality. A holistic approach to understanding fecal pollution is needed, which identifies the sources (Scott et al. 2002, Simpson et al. 2002, Meays et al. 2004), and determines the survival and transport of the pathogens on land and in water. Survival and transport of bacteria in the environment is very complex.

Understanding the survival of fecal bacteria in the environment and the role of various environmental factors regulating their survival are still in their infancy (Alexander 1986, Ferguson et al. 2003). Many factors, or combinations of factors can influence the survival of fecal bacteria. Sunlight (Chamberlin and Mitchell 1978, Fujioka et al. 1981, Davies and Evison 1991, Meays et al. 2005), temperature (McFeters and Stuart 1972, Alexander et al. 1986, Flint 1987, Ferguson et al. 2003), and nutrient or organic limitation (McFeters and Stuart 1972, Gerba and McLeod 1976, Flint 1987, Holben et al. 1992, Tassoula 1997, Byappanahalli and Fujioka 1998) have been cited as being the most influential factors to the survival of fecal coliforms and *E. coli* in water and feces. Other factors that have been shown or suggested to influence the survival of bacteria includes pH (McFeters and Stuart 1972, Alexander et al. 1986), salinity (Davies and Evison 1991, Tassoula 1997), sedimentation (Gerba and McLeod 1976, Davies and Evison 1991, Sherer et al. 1992), predators especially protozoan (Scheuerman et al. 1988,

Gonzalez et al. 1990, Gurijala and Alexander 1990), and competition with other organisms (Flint 1987, Holben et al. 1992).

Only a few studies examined more than one environmental stressor simultaneously, which would be more consistent with what happens in the natural environment (Ferguson et al. 2003). Many of the studies looking at survival of *E. coli* or fecal coliforms in water, use pure cultures (McFeters and Stuart 1972, Flint 1987, Bogosian et al. 1996, Darakas 2002), which may react differently than a range of natural populations adapted to the feces they were shed in. In the environment, *E. coli* would be deposited in the stream not in a pure culture, but in a fecal matrix exposed to complex and variable characteristics of stream water, for example temperature, nutrients, organics, and other organisms. Competition, temperature and nutrient availability have been suggested as the most important factors that influence the survival of *E. coli* in water (Flint 1987, Barnes and Gordon 2004). For this study experiments were set up under more natural conditions to evaluate the survival of natural populations of *E. coli* from beef cattle manure in filtered and unfiltered stream water at different temperatures. Only viable, culturable counts were reported for the purpose of this study. I recognize that while I attempted to run the experiment under more natural conditions using stream water and cattle feces, I did not mimic the turbulence of stream, dynamic ecosystem environments, or the natural fluctuations of temperatures and solar exposure.

Materials and Methods

A tank experiment was conducted for 23 days in July and August of 2003, to investigate the survival of *E. coli* in filtered and non-filtered stream water under 3 temperature treatments (6, 20, and 26±1ºC). Additional data collection and analysis of

the physical and chemical characteristics of the water and manure were conducted as per the Standard Methods for the Examination of Water and Wastewater (APHA 1998) and the British Columbia Environmental Laboratory Manual For the Analysis of Water, Wastewater, Sediment and Biological Materials (BC Gov. 1994).

Tanks

Styrofoam coolers (tanks) lined with plastic to avoid leaks were filled with 13.8 L of filtered or non-filtered stream water. Filtered stream water was passed through a 1 μm yarn-wound filter to remove any protozoan that range in size from 2-100 μm (Murray et al. 1998). Elite 799® air pumps (1000 cc/min) were used to oxygenize the tanks to represent a stream-like situation with ample oxygen. Ten tanks were used for each temperature treatment of 6, 20, and 26ºC. For the 6ºC temperature, tanks were placed in a walk-in cooler; for the 20ºC treatment tanks were left at room temperature; and for the 26ºC treatment, Hagen® Radiant Aquarium Heaters with Thermostat Controls were added to the tanks. Half of the tanks (5) from each temperature treatment contained filtered stream water and the other half contained non-filtered stream water. Lids were kept on the coolers except during sampling to minimize the effects of light and to help keep tanks at a constant temperature.

Source of E. coli

Natural populations of *E. coli* in fresh beef cattle manure were used for this experiment. Approximately 58 g wet mass (7.2 g dry mass) of fresh well-blended beef cattle manure was placed in each tank (roughly equivalent to a typical fecal pat of 2.5 kg wet weight deposited into a water body with a volume of 0.59 m^3). The manure was collected from a nearby ranch using shovels and a pail. The manure was blended

thoroughly using a drywall mud paddle attached to an electric drill. The blended manure was immediately transported to the laboratory and added to the tanks. Five sub-samples of the manure mixture were enumerated in order to establish the initial *E. coli* concentration for the fresh manure at the time of adding it to the tanks filled with stream water. The position of each of the 10 tanks under each temperature treatment was randomized.

Sampling

For each sampling, cooler lids were removed, and the stream water in the tank with manure added was stirred vigorously 50 times with a sterilized glass stir rod. Tanks were stirred in order to suspend any *E. coli* or organics that had settled to the bottom of the tank. Approximately 2 to 10 mL of tank water (depending on date sampled) was removed from each tank using a plastic pipette and placed into a sterilized glass vial. Water samples were processed for *E. coli* immediately after sampling.

Plating and enumerating

The glass vials containing the tank samples were inverted 5 times prior to being filtered using the membrane filtration technique. Volumes between 100 and 8000 µL were filtered through a 0.45 µm pore membrane filter, and the filter placed in petri-dishes containing Millipore m-ColiBlue24® broth for coliform and *E. coli* detection. Petri-dishes were placed in an incubator at 35ºC for 24 hours and enumerated by counting blue colonies (*E. coli*) on the filter paper. Colony counts per volume sampled were then converted to counts per dry weight gram of feces for comparison purposes. Volumes filtered altered with each sampling cycle. Early in the experiment when *E. coli* counts were higher, only volumes of 100 and 1000 µL were filtered and later in the experiment

when numbers decreased, larger volumes of water were filtered (up to 8 mL). Triplicate filtrations were performed on approximately 10-15% of the samples each sampling run to determine accuracy.

Statistical design

Log_{10}-transformed *E. coli* counts were statistically analyzed using the PROC MIXED procedure from SAS (SAS Institute Inc. 1999) with temperature, filter, day, and all interactions in the model as fixed effects. Since samples were repeatedly taken from the same tanks on different days in the experiment, day was treated as a repeated measure. Different variance-covariance structures were fitted in an attempt to best account for differences in variance estimates and correlations among the days according to the smallest AIC (Akaike's Information Criterion) value. Contrast statements were used to make comparisons among means of interest when the corresponding effect was significant. One outlier was removed from the results due to a zero value.

Results

There was a significant difference in *E. coli* concentrations between tanks at different temperatures only on days 2 and 9 (Tables 8 and 9). Similar to other studies (McFeters and Stuart 1972, Flint 1987, Darakas 2002), survival of *E. coli* was somewhat higher at the lower temperatures (6 versus 20 and 26 ºC). However, there was only a significant difference between temperature treatments on days 2 and 9, which is contrary to the other studies. By day 9, there was an approximate 1.5-log_{10} decline in CFU g^{-1} for the 20 and 26 ºC treatments whereas there was no decline in the 6 ºC treatment between days 2 and 9 (Figure 7). By day 16, we had an approximate 2-log_{10} decrease in *E. coli* for all temperature treatments, which remained relatively constant to day 23.

There was not a significant difference in *E. coli* concentrations between the filtered and unfiltered treatments. The addition of manure to the stream water substantially increased the nutrient concentrations (Tables 10 and 11). By the conclusion of the experiment, the 6 ºC temperature treatment had higher concentrations of colour, non-filterable residue, turbidity, dissolved organic carbon (DOC), total organic carbon (TOC), total Kjeldahl nitrogen, total nitrogen (TN), total organic nitrogen (TON), and ammonia nitrogen than the 20 or 26 ºC treatment. In contrast, the 6 ºC treatment had lower concentrations of nitrite nitrogen, nitrate nitrogen, ortho-phosphate (ortho-P), and total dissolved phosphorous (TDP) than the 20 or 26 ºC treatment.

Discussion

Flint (1987) found a rapid decline of *E. coli* in unfiltered and Whatman-filtered river water within 4 to 12 days at similar temperatures to this study. High counts of *E. coli* were observed in our study continuing up to day 23 (end of experiment). Flint (1987) found that in Millipore-filtered and autoclaved river water, survival was up to 120 d and greater than 260 d, respectively, suggesting that a biological component of the water was primarily responsible for the disappearance of *E. coli*, and temperature, although very important, was secondary. Other researchers have suggested that organic matter and nutrients support growth and survival of *E. coli* in water, sediment, and soil (McFeters and Stuart 1972, Gerba and McLeod 1976, Byappahalli and Fujioka 1988, Scheurerman et al. 1988, and Rosen 2000). Turbidity was high for all treatments in our experiment, especially the lower temperature treatments, when compared to the stream water before the addition of the manure. Nutrient concentrations were also much higher at the end of the experiment than what was found in the stream water prior to the addition

of the manure. A significant difference ($P < 0.0001$) in *E. coli* on days 2 and 9 between the temperature treatments was observed. Barnes and Gordon (2004) suggested that coliform cells are capable of cell division at a rate dependent on the concentration of some nutrient in a water body. Nutrients may have buffered the effect of temperature allowing *E. coli* to adjust, grow and survive longer under the 3 different temperature treatments in our study.

Predation has been shown to be an important factor in the survival of *E. coli* in natural waters (Scheuerman et al. 1988, Gonzalez et al. 1990, Gurijala and Alexander 1990). Gurijala and Alexander (1990) demonstrated that protozoa predation was a significant factor reducing the abundance of *E. coli* in lake water. Their data showed that protozoa increased during the phase of rapid decline of *E. coli,* and the addition of eucaryotic inhibitors delayed or eliminated bacterial decline. These findings were also supported by Scheuerman et al. (1988). Gurijala and Alexander (1990) found that protozoa developed more rapidly at 30 ºC than at 5, 10, or 15 ºC.

There was no significant difference in *E. coli* populations between filtered and unfiltered treatments in our experiment, which is similar to the results of Flint (1987). Flint (1987) found that there was little difference between survival of *E. coli* in untreated and Whatman-filtered water, which would remove protozoan and suspended material. Flint (1987) suggested that protozoa and attachment to particles appeared to have little influence on the disappearance of *E. coli* in river water samples. However, at temperatures of 25 ˚C and below, Flint (1987) found that survival was greatly enhanced with water that was Millipore-filtered (0.45 μm) leaving phage and ultramicrobacteria and removing most of the bacteria that would be competing for nutrients with *E. coli*.

Stream water used for this experiment was not tested for protozoa, and therefore it is possible that there were very few protozoa in the water, which would explain why there was no significant difference in *E. coli* between the filtered and unfiltered treatment. It is also possible that with the addition of the cattle feces to the water, there may not have been competition for nutrients between other bacteria and *E. coli*.

Several papers (Gerba and McLeod 1976, Tassoula 1997, Byappanahalli and Fujioka 1998) cite that *E. coli* is capable of growth in the environment if sufficient organics and/or nutrients are available. In this experiment, *E. coli* in the 6 ºC tanks showed a decline in the first day followed by a significant increase ($p = 0.0004$) on day 2, and were stable to day 9, followed by a more rapid decline by day 16. In contrast, *E. coli* populations in the 20 and 26 ºC treatments did not demonstrate growth, but showed a decline throughout the experiment.

McFeters and Stuart (1972) found that *E. coli* survival in the laboratory using water from the field had better survival for longer than in the field. However, it is difficult to conduct controlled experiments in the field where environmental conditions cannot be fixed. A controlled laboratory experiment with fluctuating water temperatures, solar exposure, and nutrient additions as would be found in the field would be useful for understanding impacts of a variable environment on fecal bacteria, although these experiments would be difficult to conduct.

Conclusions

Watershed managers and drinking water purveyors require information and understanding on the survival of fecal bacteria and pathogens in order to set up appropriate beneficial management practices to reduce the number of organisms for

treatment in water systems. Further research is needed on the survival and behaviour in the environment of *E. coli* and other fecal bacteria and pathogens found in contaminated drinking water sources under different environmental conditions. Scientific knowledge gained from experiments investigating survival and transport can be applied to improve watershed management plans and reduce both the impact of fecal pollution on water quality, and risk associated with human health.

Table 8. Tests of significance for temperature treatment, filter treatment, day and their interactions on $\log_{10}$ *E. coli* concentrations.

Effect	Num DF[2]	Den DF[3]	F-value	P-value
Temp	2	33	1.80	0.18
Filter[1]	1	33	1.14	0.29
Temp*Filter	2	33	0.72	0.50
Day	4	92	65.33	<0.0001
Temp*Day	8	94	11.44	<0.0001
Filter*Day	4	92	0.92	0.46
Temp*Filter*Day	8	94	0.54	0.82

[1]Filter compares filtered to non-filtered water.

[2]Numerator degrees of freedom.

[3]Denominator degrees of freedom

Table 9. *E. coli* concentration differences for the 6, 20 and 26 ºC temperature treatments for days 2 and 9.

Day	Temp. 1 (ºC)	Temp. 2 (ºC)	Difference[1]	Std Err	P-value
2	6	20	0.33	0.1863	0.08
	6	26	0.69	0.1863	0.0004
	20	26	0.36	0.1863	0.05
9	6	20	0.86	0.1863	<0.0001
	6	26	0.78	0.1863	<0.0001
	20	26	-0.08	0.1863	0.68

[1]Difference of $\log_{10}$ *E. coli* concentrations with different temperature tank stream water.

Table 10. Average (±SD[1]) water quality of triplicate unfiltered and filtered stream water samples before addition of manure.

Parameter	Unfiltered	SD	Filtered	SD
pH (pH units)	7.4	0	7.4	0
True Colour (Col. Unit)	60	0	60	0
Specific conductance (uS/cm)	38	0	37	0
Nonfilterable residue (mg/L)	<4	0	<4	0
Turbidity (NTU)	1.64	0.25	0.83	0.05
Dissolved Org. C (mg/L)	9.9	0.66	10.7	0.17
Total Org. C (mg/L)	8.93	0.06	9.63	0.21
Total Kjeldahl N (mg/L)	0.423	0.040	0.373	0.012
Total N (mg/L)	0.427	0.038	0.377	0.006
Total Org. N (mg/L)	0.410	0.044	0.363	0.012
Ammonia N (mg/L)	0.010	0.003	0.008	0.002
Dissolved Nitrate N (mg/L)	<0.02	0	<0.02	0
Nitrite N (mg/L)	<0.005	0	<0.005	0
Ortho-P (mg/L)	<0.005	0	0.006	0
Total Dissolved P (mg/L)	0.011	0.003	0.013	0.001
Total P (mg/L)	0.018	2.3E-10	0.016	0.00058

[1]Standard deviation.

Table 11. Water quality of filtered (F) and unfiltered (UF) stream water at 3 temperature treatments (6, 20, and 26 ºC) at conclusion of experiment[1].

Parameter	MDL[7]	UF-6[8]	F-6[9]	UF-20[10]	F-20[11]	UF-26[12]	F-26[13]
pH[2]	0.1	7.5	7.5	7.7	7.7	7.6	7.6
True Colour[3]	5	320	320	200	200	200	200
Specific conductance[4]	1	96	96	95	99	99	99
Nonfilterable residue[5]	4	37	28	8	8	18	17
Turbidity[6]	0.1	41.4	44	7.89	6.64	9.6	8.54
Dissolved Org. C[5]	0.5	22.4	24.3	18.4	18.6	16.9	17.9
Total Organic C[5]	0.5	18.7	21.7	16	15.9	14	17.4
Total Kjeldahl N[5]	0.02	3.36	3.33	1.9	2.12	1.63	1.75
Total N[5]	0.02	3.38	3.35	2.23	2.66	2.13	2.36
Total Organic N[5]	0.1	3.05	3.07	1.87	2.02	1.6	1.72
Ammonia N[5]	0.005	0.31	0.263	0.032	0.103	0.029	0.031
Dissolved Nitrate N[5]	0.02	<0.02	<0.02	0.05	0.02	0.05	0.04
Nitrite N[5]	0.005	0.011	0.01	0.277	0.519	0.462	0.573
Ortho-P[5]	0.005	0.03	0.02	0.33	0.54	0.51	0.61
Total Dissolved P[5]	0.005	0.421	0.437	0.445	0.505	0.606	0.597
Total P[5]	0.005	0.763	0.573	0.591	0.687	0.75	0.629

[1]120 mL of water was taken from each of the 5 tanks of the same temperature and filter treatment and blended for a total of 600 mL.

Units: [2]pH units, [3]Colour unit, [4]uS cm^{-1}, [5]mg L^{-1}, and [6]NTU

[7]Method detection limit.

[8]Unfiltered water, 6ºC.

[9]Filtered water, 6ºC.

[10]Unfiltered water, 20ºC.

[11]Filtered water, 20ºC.

[12]Unfiltered water, 26ºC.

[13]Filtered water, 26ºC.

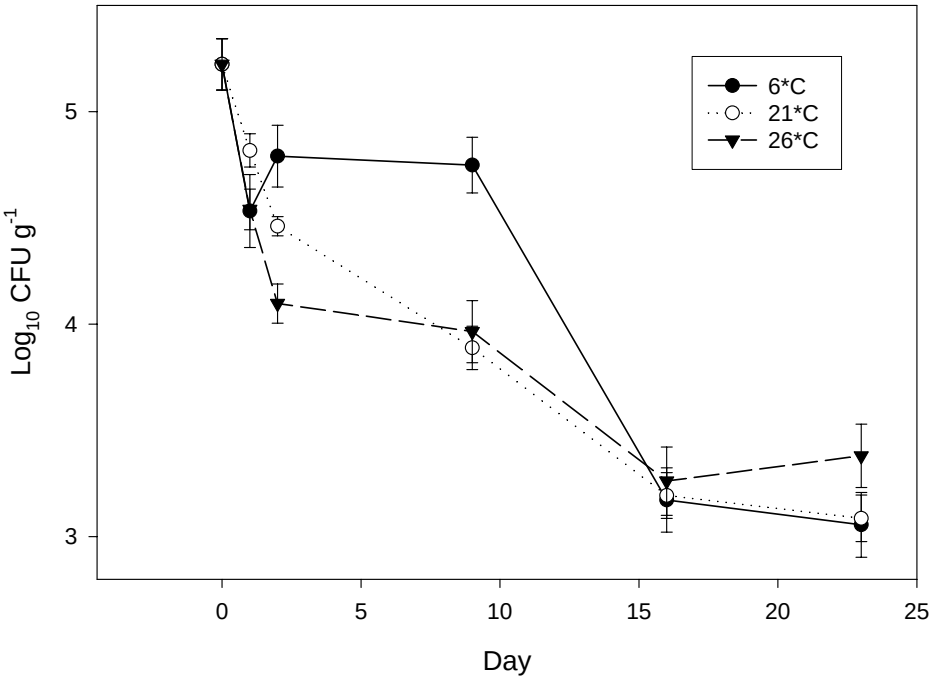

Figure 7. Mean $\log_{10}$ CFU g^{-1} *E. coli* of filtered and unfiltered samples for each day comparing temperature treatments of 6, 20 and 26±1 ºC.

Chapter 5: Diurnal Variability in Concentrations and Sources of *Escherichia coli* in 3 Streams.

Abstract

Microbial contamination is a major concern for drinking water worldwide. Many monitoring protocols using one or very few samples are inadequate and introduce a very large margin of error. An intensive sampling program needs to be conducted to characterize the *E. coli* concentrations of a source water stream prior to establishing a monitoring program so that the sample frequency can be determined statistically, based on an acceptable margin of error. Developing meaningful monitoring programs for managing water quality also requires scientific data on the sources of bacterial contaminants. In this study, 3 streams from drinking water watersheds were sampled every 15 minutes over a 24-hour period on 3 different days to determine concentrations of *E. coli* and to identify their sources using ribosomal RNA finger printing (ribotyping). Concentrations of *E. coli* varied throughout the day in each of the 3 streams. Ribotyping identified many different animal sources of *E. coli* in the samples. The sources of *E. coli* varied significantly with site ($P < 0.001$, df=16). The development of monitoring programs for watersheds need to consider individually the watershed, and care needs to be taken in determining appropriate sample sites, sampling regime and number of samples taken during each sampling period. It was demonstrated that there are significant variability in concentrations and sources of *E. coli* during a day, however, the mechanisms and processes associated with these variations need further research.

Introduction

Microbial contamination of water is a major environmental and health issue with drinking water worldwide (WHO 2003). Fecal contamination of water can come from many sources such as wildlife, livestock, and humans. New molecular and biochemical methods for detecting the sources of fecal contamination have been developed (Scott et al. 2002, Simpson et al. 2002, Meays et al. 2004) but little information is available on the natural variability of concentrations and sources of fecal pollutants in natural waters. Monitoring programs vary widely with variable numbers of samples being collected for analysis and evaluation for decisions (Whitman and Nevers 2004). Sampling designs are rarely based on empirical or anticipated variation, accuracy or precision even though there can be high variation between samples (Whitman and Nevers 2004). Whitman and Nevers (2004) suggested that because of these limitations with monitoring protocols, the effort, to provide accurate, reliable results should be maximized. Managing and developing meaningful monitoring programs for optimal water quality require sound scientific data on the variability of fecal contaminants, their concentrations, and their sources.

The objectives of this study were to evaluate the diurnal variability of *E. coli* concentrations (CFU 100 mL^{-1}) in 3 temperate streams, and to identify the sources of *E. coli* within a 24-hour period.

Materials and Methods

A field and laboratory experiment was conducted in July and August of 2004 to determine the natural variation in *E. coli* concentrations and sources in 3 streams over a

24-hour period. Three sampling sites were chosen on BX, Deer, and Duteau Creeks all near Vernon, British Columbia. The sampling sites were located in watersheds used for drinking water. Activities in the watersheds above the sampling sites on Deer and Duteau Creeks include cattle grazing, recreation (fishing and camping), forestry, and wildlife use. The average stocking rates for these 2 watersheds is approximately 1 AUM (animal unit month) per 2 ha (Wright 2005). The sampling site on BX Creek is located below a ski hill and provincial park that has summer and winter recreational activities, cattle grazing, wildlife and there is also private residential property located adjacent to the sampling site. Stocking rates on BX Creek is not known since there are several property owners with hobby farms along this stream.

Sampling

Ninety-six water samples were collected midstream at each sampling location by sampling every 15 minutes over a 24-hour period starting at midnight. Sterilized 250 mL plastic bottles were used. Samples were placed in a cooler with ice immediately after sampling and taken to the laboratory for analysis within 12 hours of sampling.

Culture conditions of E. coli

E. coli concentrations were enumerated by filtering volumes of 100 and 200 mL of stream water through a 0.45 μm membrane filter, and placing the filter in petri-dishes containing Millipore m-ColiBlue24® broth for coliform and *E. coli* detection. Petri-dishes were placed in an incubator at 35ºC for 24 hours and enumerated by counting blue colonies (*E. coli*) on the filters. Petri-dishes were then placed on ice and shipped to the Institute for Environmental Health Inc. in Seattle, WA for source tracking analysis.

Ribotyping and source determination

Colonies were picked from the shipped petri-dishes and plated to mFC plates. Individual blue colonies were picked at random from plates and streak plated to MacConkey agar to obtain isolated colonies. These were then streak plated to Tryptic Soy Agar. The pure culture was then tested for indole production, and citrate utilization and quadrant streaked to blood agar. After the isolate was verified as an *E. coli*, confluent growth from the blood agar plates was scraped with a sterile flat-headed toothpick and cell material was suspended in 800 µL 50mM Tris and 50mM EDTA (pH 8.0). Genomic DNA was extracted using the phenol/chloroform procedure (Sambrook et al. 1989). Restriction endonuclease digestion reactions were performed on between 1-2 ug of DNA using *Eco*RI and *Pvu*II (BoehringerMannheim GmbH, Germany) as per the manufacturer's protocol. DNA fragments were separated by electrophoresis at 33V for 12-14 h in a 0.8% agarose gel in 1X TBE (Tris-borate-EDTA). The DNA was transferred to a Nitran filter (Schleicher & Schuell, Keene, NH), baked at 80^{o}C for 1 h, and probed with ^{32} P-labeled copies of *E. coli* total rRNA produced using random hexanucleotide primers and Avian Myeloblastosis Virus reverse transcriptase (Strategene, La Jolla, CA) as per the manufacturer's protocol. After hybridization, the blots were washed in SSC buffer (44g L^{-1} sodium citrate, 97.7 g L^{-1} sodium chloride) and 0.1% sodium dodecylsulfate detergent at 65^{o}C. The hybridized bands were visualized by autoradiography using intensifying screens at -70^{o}C.

As previously described (Myoda et al. 2003, Samadpour et al. 2005), different ribotype patterns were assigned a number sequence. The number sequence was then checked against a database of over 40,000 known source ribotypes of human and animal origin. If the sequence was not located in the database (new) the number sequence was

entered and followed by the letter "a" and corresponding information attached. If the sequence was located, then the investigator would manually compare the gel to the other gels bearing that sequence. If a pattern was an identical match, it was given that letter designation as well. If the pattern was not a match, it was assigned the next letter of the alphabet and entered into the database along with corresponding information. Number sequence-letter combinations were then given a pattern number for the purpose of comparison within this study. A host source was assigned to an isolate that displayed a matching ribotype pattern to various known sources in the Samadpour laboratory ribotype database. Fecal samples of known local sources within the watersheds were also collected in order to add the ribotypes of known sources to the existing library. A Chi-square test using a 3 x 9 contingency table was used to test the association between stream and species that contributed *E. coli* into the stream (Townend 2004).

Results

E. coli concentrations.

In each stream, *E. coli* concentrations (CFU 100 mL^{-1}) varied between samples taken at 15-minute intervals (Figure 8). Duteau Creek had the lowest overall *E. coli* concentrations of the 3 watersheds evaluated. Concentrations ranged from 0 to 12 CFU 100 mL^{-1} with a mean of 4 and a standard deviation of 2.5 CFU 100 mL^{-1}. Deer Creek concentrations ranged from 6 to 79 CFU 100 mL^{-1} with a mean of 19 and a standard deviation of 11.9 CFU 100 mL^{-1}. Light precipitation fell in the early evening at the Deer Creek sampling site, however, there was no noticeable change in stream stage. BX Creek had the highest *E. coli* concentrations ranging from 22 to 696 CFU 100 mL^{-1} with a mean of 156 and a standard deviation of 181.4 CFU 100 mL^{-1}. A substantial increase in *E. coli*

occurred on BX Creek between 11:00 and 16:00. It started to rain at approximately 05:00 and slowed down to a drizzle by 11:00 at this sampling site. Rainfall records indicate that about 8 mm of rain fell on the day of sampling. Stream stage measurements increased during the rainfall event indicating higher stream flows in response to the precipitation.

Whitman and Nevers (2004) discussed some of the limitations of bacterial monitoring protocols. Data from this study supports the need to accurately determine the variation among samples when determining the number of samples that are needed in a monitoring program. For example, if a monitoring program had been set up to obtain a single sample on BX creek during the measured 24-hour period, the results for *E. coli* concentration could have been as low as 22 or as high as 696 CFU 100 mL^{-1}. The interpretation of the relative importance of fecal pollution in the stream would be very different based these two outermost results. There is variability between samples in a given day, as well as variability between days and sampling sites (Whitman and Nevers 2004) and therefore care needs to be taken in determining appropriate sample sites, sampling regime and number of samples taken during each sampling period. Tables 12 and 13 summarize the number of samples that would be needed based on standard deviations and margins of error for raw and $\log_{10}$ *E. coli* data for BX, Deer, and Duteau Creeks during a 24-hour period. If a monitoring program reports raw data, the range of sample size needed in a 24-hour period allowing for a margin of error between 50 % to 10 % would be: for BX Creek, 24 to 524 samples; for Deer Creek, 8 to 147 samples; and for Duteau Creek, 9 to 168 samples. Note that we collected 96 samples in a 24-hour period for each of the streams. If the monitoring program was to report $\log_{10}$ *E. coli* data

with the same margin of error, a sampler would have needed to collect 2 to 22 samples for BX Creek, 2 to 14 samples for Deer Creek, and 3 to 68 samples for Duteau Creek in a 24-hour period. These results demonstrate the importance of setting up an intensive sampling program to characterize the *E. coli* concentrations of a source water stream prior to establishing a monitoring program so that sample size needed based on acceptable margin of error can be calculated on an individual stream by stream basis. To obtain a reasonable margin of error a much larger number of samples would be needed than normally planned, and this would also impact the budget for sampling time, impact laboratory time and the funds allocated for analyses.

The current protocol of 1 or few samples that is often used in water quality monitoring introduces a margin of error greater than 50 %. Sampling plans need to be statistically sufficient for greater accuracy of reported data.

Temporal and spatial variability of E. coli sources

The sources of *E. coli* varied significantly with site ($P < 0.001$, df=16). For Duteau Creek, the main sources of *E. coli* for the 24 hour period were, in decreasing ranked order: avian, raccoon, rodent, unknown, canine, bovine, deer/elk, and bear (Table 14). For Deer Creek, the main source of *E. coli* was rodent, followed by avian, bovine, deer/elk, canine, unknown, raccoon, bear, and horse. For BX Creek, the main source of *E. coli* was canine, followed by raccoon, unknown, rodent, avian, bear, and bovine. Isolates classified as unknown were those that did not match 100 % to a sample in the source library. Of all the isolates classified, 11.1 % were classified as unknown. The majority of isolates ribotyped for each stream came from only 3 or 4 main sources, and most other source groups made only very small contribution to the total. Most sources

were present throughout most of the day. No trend was observed between time of day and *E. coli* concentrations or source.

Discussion

Non-point source pollution is often difficult to quantify and determine the sources, and therefore opposing interest groups often advocate that it is others that are creating the problem (Meays et al. 2004). Results from this study show that a wide range of *E. coli* sources were found in most samples taken. The majority of the ribotypes came from wildlife sources including canine (27.1 %), rodent (19.5 %) and avian (16.8 %) which are more difficult to manage within a watershed compared to domestic livestock. Overall, bovine contributed 7.1 % of the isolates classified.

There are many challenges in developing monitoring protocols. First, every stream is different and drains a unique watershed. Streamflow and potential runoff reaching a stream can change in response to current weather conditions as well as seasonally. As was demonstrated, there are many sources of *E. coli* entering a stream. There is also uncertainty about whether the sources being detected are from near the sampling location or from somewhere upstream. In a manure spike experiment, Biskie et al. (1988) found that 95% of fecal coliforms settle out of a stream within the first 50 m. More studies are needed to look at the impacts of stream characteristics including but not limited to velocity, size, sinuosity, exposure to sunlight, temperature, and substrate, as well as biological characteristics including grazing and filtration on the distance *E. coli* can travel in different watersheds. The survival and transport mechanisms of *E. coli* on land also require more research (Meays et al. 2005). Other challenges include the timing of sample collection; depending on when sampling is done, sampling might be coincident

with a fecal plume (i.e. an animal may have defecated above where you are sampling at the time of sampling so the result may be higher than if sampled earlier or later). If precipitation occurs at the time of or prior to sampling, the stream may respond with increased discharge, as was found in this study, which can stir up sediments and release fecal bacteria into the water column (Sherer et al. 1988). Also, increased precipitation can increase the amount of surface runoff or subsurface flows to the stream increasing the potential of fecal loading. As was found with this study, animals that are associated with water bodies such as rodents (including beaver) and avian, can provide a steady supply of *E. coli* to the water body. However, in this study we also found that other groups of animals that are not necessarily associated with water bodies such as canine and raccoon, also provided a source of *E. coli* to the stream throughout the whole day. Finally, more research needs to be conducted to determine what pathogens of concern are found in the animals detected in a given watershed in order to determine what the potential risk to human health would be.

A better understanding of all the potential sources of fecal pollution can be developed by using bacterial source tracking techniques (Scott et al. 2002, Simpson et al. 2002, Meays et al. 2004). Once sources are identified, management plans can be developed to attempt to reduce bacterial fecal loading in a water body. Watersheds need to be studied individually to determine bacterial sources and changes of these sources with season and location so that robust monitoring protocols and management prescriptions can be developed.

Table 12. Sample size (n) calculations based on standard deviation (SD) and margin of error (ME) for raw *E. coli* data collected over a 24-hour period using Lenth power and sample size program (Lenth 2005).

	Margin of Error (%)				
Stream	10	20	30	40	50
BX	524	133	61	36	24
Deer	147	39	19	12	8
Duteau	168	44	21	13	9

Table 13. Sample size (n) calculations based on standard deviation (SD) and margin of error (ME) for $\log_{10}$ *E. coli* data collected over a 24-hour period using Lenth power and sample size program (Lenth 2005).

	Margin of Error (%)				
Stream	10	20	30	40	50
BX	22	7	3	2	2
Deer	14	4	2	2	2
Duteau	68	19	10	6	3

Table 14. Total[1] and percentage (in parenthesis) of *E. coli* for each source by stream over a 24-hour period.

Source	BX	Deer	Duteau	Row
Avian	11 (5.6)	32 (17.1)	49 (29.9)	92 (16.8)
Bear	1 (<1)	6 (3.2)	5 (3.0)	12 (2.2)
Bovine	1 (<1)	25 (13.4)	13 (7.9)	39 (7.1)
Canine	114 (57.6)	15 (8.0)	20 (12.2)	149 (27.1)
Deer/Elk	0 (0)	19 (10.2)	11 (6.7)	30 (5.5)
Horse	0 (0)	1 (<1)	0 (0)	1 (<1)
Raccoon	24 (12.1)	12 (6.4)	22 (13.4)	58 (10.6)
Rodent[2]	23 (11.6)	62 (33.2)	22 (13.4)	107 (19.5)
Unknown	24 (12.1)	15 (8.0)	22 (13.4)	61 (11.1)
Sum	198	187	164	549

[1]Total is the total number of *E. coli* enumerated over the 24-hour period for each source for all 3 watersheds.

[2]Rodent includes beavers and all other rodents.

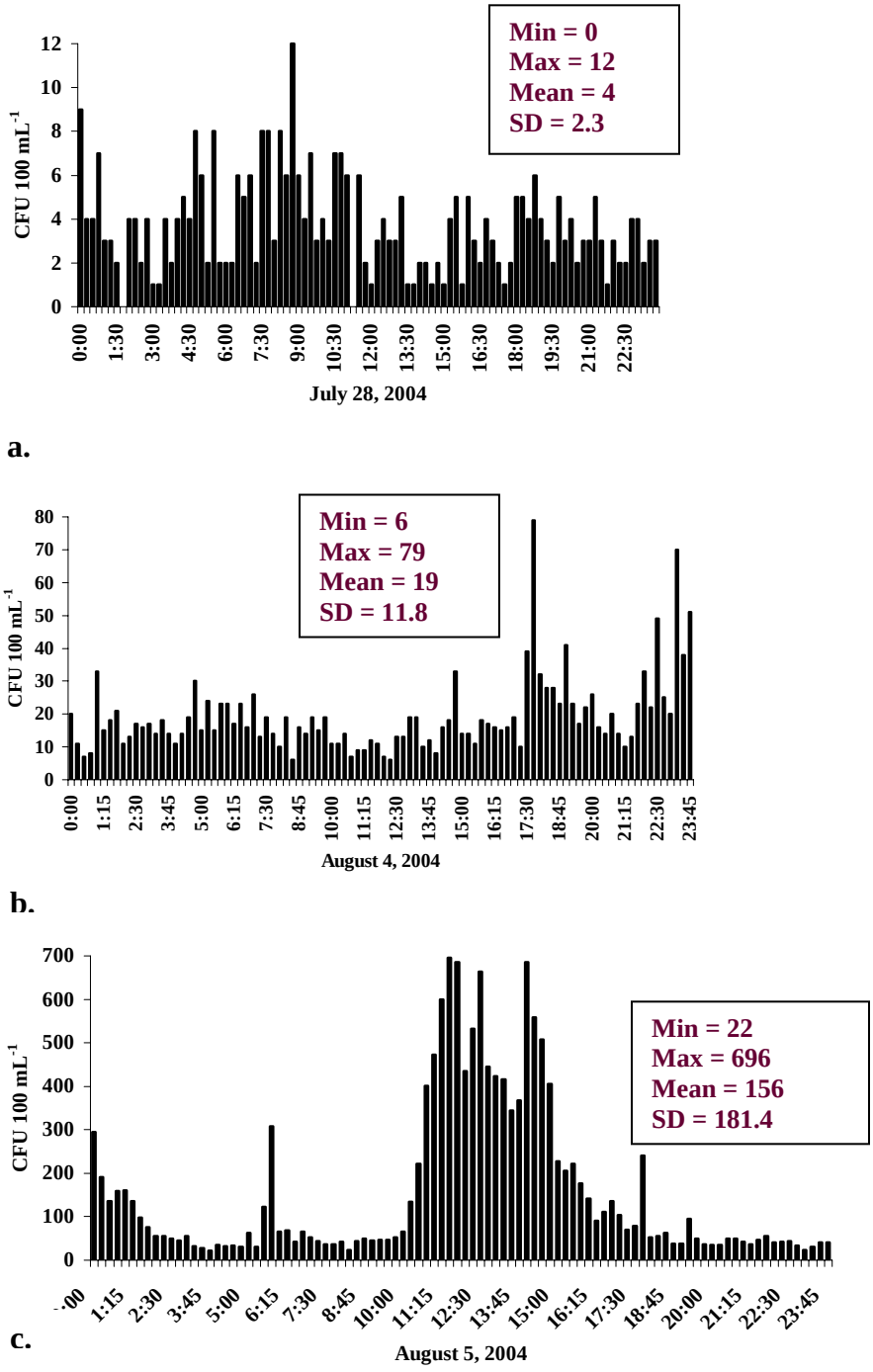

Figure 8. Duteau (a), Deer (b) and BX (c) Creeks *E. coli* concentrations over 24 hours (note different scale of y-axis for each site).

Chapter 6: Spatial and Annual Variability in Concentrations and Sources of *Escherichia coli* in Multiple Watersheds in British Columbia, Canada

Abstract

Non-point source fecal contamination is a concern for drinking water supplies worldwide. Often livestock grazing is cited as negatively impacting water quality even though most watersheds have many different user groups including forestry, mining, agriculture, wildlife, urban development and recreation. Identifying all the sources of fecal pollution is important so that management plans can be developed cooperatively by all user groups to reduce levels of fecal contaminants in surface waters. Bacterial source tracking (BST) is a useful tool that can be used for watershed management and risk assessment. In this study, 4 drinking water watersheds with different levels of land-use were sampled in 2003 and 2004 to determine concentrations of fecal bacteria and to identify their sources using ribosomal RNA finger printing (ribotyping). Concentrations of fecal coliforms varied by site, year, and month in each of the 4 watersheds. Ribotyping identified many different animal sources of *E. coli* in the samples. A wide range of *E. coli* sources were found in most samples taken over both years. The sources of *E. coli* varied significantly with stream for 2003 ($P < 0.001$, df=39) and 2004 ($P < 0.001$, df=39). The majority of *E. coli* isolates classified in 2003 came from wildlife sources including deer/elk, avian, and canine, whereas the majority of isolates classified in 2004 came from avian, bovine, and rodent sources. Fecal pollution arises from multiple sources and BST is a useful method for identifying the sources of non-point pollution in a watershed.

Introduction

Often the goals of watershed management are to enhance stream condition and control non-point source pollution. Livestock grazing is often cited as a land-use activity that if managed incorrectly, can have negative impacts on riparian areas and water quality (Kauffman and Krueger 1984, Skovlin 1984, Belsky et al. 1999). Water quality parameters of greatest concern associated with livestock grazing include sediment, nutrients, and pathogens. Unfortunately, there is little quantitative information available on watershed management and water quality. Despite many studies on livestock management and water quality, results are inconsistent and often contradictory (Larsen et al. 1998). Fecal contamination of water comes from many non-point sources such as wildlife, livestock, and humans. Managing and reducing fecal pollution levels in surface water is difficult because there are many sources and they are often not easy to identify. Evaluating studies on livestock management and water quality requires an understanding of the natural variability in the hydrologic cycle, the nutrient cycles, the pathogen cycles, and how upland and riparian management impacts these processes (Nader et al. 1998). Equally important is the ability to identify and quantify all the possible sources of fecal pollution in the watershed.

Bacteria from feces enter streams either by direct deposit, overland transport from a runoff event, or subsurface flows. Stephenson and Street (1978) found that highest concentrations of fecal coliforms are often related to runoff events. Sherer et al. (1988) found that high concentrations of coliforms may also come from re-suspending bacteria associated with the stream bottom sediments. Cattle defecate approximately 12 times per day with an average defecation of 2 to 3 kg, or 0.04 to 0.06% of their body weight

(Larsen et al. 1994). The amount of feces and urine produced by an animal and its nutrient content is dependent on the forage consumed (Nader et al. 1998). Reducing the time cattle spend near a stream would decrease the impact on water quality by cattle.

Atwill et al. (2002) developed a method for comparing environmental loading rates for different vertebrate hosts of *Cryptosporidium parvum.* They found that compared to cattle (where the vast majority of oocysts are shed in calves 1 to 30 days of age), the wildlife populations studied shed large amounts of oocysts for all age categories. Atwill et al. (2002) also found that both young and adult striped skunks, coyotes, California ground squirrels, and yellow-bellied marmots produced more oocysts per individual animal than beef or dairy cows. They concluded that regardless of age (or season), striped skunks, coyotes, California ground squirrels, and yellow-bellied marmots were substantial sources of *C. parvum* oocysts.

The protection of water quality from waterborne pathogens requires understanding and management of not only livestock in a watershed, but also the wildlife populations in the watershed. Barry et al. (1998) proposed developing a Hazard Analysis of Critical Control Points (HACCP) program for the Alameda Creek watershed in California to control *Cryptosporidium* and other waterborne pathogens. The HACCP program was originally developed approximately 30 years ago by the US Army and NASA to prevent identifiable food safety hazards by applying science-based controls throughout the production process (Barry et al. 1998). A multidisciplinary watershed team could be used to conduct a hazard analysis on a watershed and identify the critical control points in the collection, storage, and distribution of water (Barry et al. 1998). A watershed HACCP-based program for the protection of water quality could be greatly

enhanced with the use of tools such as bacterial source tracking (BST). BST identifies sources of fecal contamination within a watershed, and this information would be very useful when conducting a hazard analysis. Risk assessment can be determined more accurately by identifying the sources of fecal contamination and sampling the feces from the main contributors of fecal pollution for pathogens of concern. In order to protect water quality in a watershed, the role of not only livestock, but also wildlife and human fecal pathogen loading needs to be assessed. Once hazards and risks are identified within a watershed, a watershed team could use adaptive management, whereby management strategies are modified until water quality resource objectives are met (Elzinga et al. 1998), to develop strategies to reduce fecal contamination thereby reducing the risk to human health.

This study was conducted on 4 different watersheds with varying land-use (Appendix 11). Two of the watersheds (Duteau and Deer) were selected because they had managed cattle grazing activities from spring to fall as well as other land-use activities including recreation (fishing and camping), forestry, and wildlife. BX Creek watershed was chosen because it had more recreational land-use activities that included a ski hill (which is used for cross country and downhill skiing in the winter and hiking and mountain biking in the summer), as well as private residential properties, some grazing activity, and wildlife use. The fourth watershed (South Fortune) was chosen as a "control" watershed because it had very minimal land-use activity. The objectives of this study were to evaluate the variability of *E. coli* concentrations (CFU 100 mL^{-1}) in 4 temperate watersheds with different land-use from May to October over 2 years, and to determine the sources of *E. coli* and its variability in the watersheds.

Materials and Methods

A field and laboratory experiment was conducted from June to October of 2003 and May to September of 2004 to determine the natural variation in *E. coli* concentrations and sources in 4 streams used for drinking water. Three sampling sites were chosen on BX, Deer, and Duteau Creeks and 2 sampling sites were chosen on South Fortune (S. Fortune) Creek. All streams are located near Vernon, British Columbia (BC). The sampling sites were located in the headwaters (I), mid-elevation (II) and lower elevation (III) at the drinking water intake sites. S. Fortune Creek did not have a mid-elevation sampling site because of very difficult accessibility. Activities in the watersheds above the sampling sites on Deer and Duteau Creeks include cattle grazing, recreation (fishing and camping), forestry, and wildlife use. The average stocking rates for these 2 watersheds was approximately 1 animal unit month (AUM) per 2 ha (Wright 2005). BX Creek is located below a ski hill and provincial park that has summer and winter recreational activities, housing, cattle grazing, wildlife and there is also private residential property located adjacent to the stream between sites II and III. Stocking rates on BX Creek is not known since there are many property owners along this stream with hobby farms. S. Fortune Creek is a densely forested watershed with minimal use due to steep terrain and minimal access and is therefore the most pristine of the 4 watersheds. Most use in S. Fortune watershed would be from wildlife. Deer, Duteau, and S. Fortune Creeks originate from dammed reservoir sources at their headwaters. BX Creek originates from springs at its headwaters.

Stream length and distance between sample sites is summarized in Table 15, while Table 16 summarizes the elevation of the sample sites on each stream. Climatic

data (mean temperatures and total precipitation) is reported for 2003 and 2004 from the Environment Canada's Coldstream Ranch weather station (Figures 9 and 10). Daily average temperatures were higher in 2003 and 2004 than the long-term averages except for May 2003 and September 2004, where temperatures were the same as the long-term average. Precipitation in 2003 was substantially lower than the long-term average especially for May, July, August and September. Precipitation in May and June of 2004 was similar to the long-term average, however, July was much lower, and August through to October had higher precipitation. Stream flow data for the streams for 2003 and 2004 is shown in Appendix 1 and indicates that Deer Creek has the least amount of discharge with stream size increasing for BX, S. Fortune to Duteau.

Sampling

A total of 100 bacterial water samples were collected midstream at each sampling location each year using sterilized 250 mL plastic bottles. Sampling started in June and continued bi-weekly until October in 2003, and started in May 2004 and continued bi-weekly till the end of September 2004. Samples were collected 10 times each year. At each sampling time, 10 samples were collected 2 minutes apart to obtain a more representative sample and reduce the chances of sampling a fecal plume. Samples were immediately placed in a cooler with ice after sampling and shipped to the Institute for Environmental Health Inc. in Seattle, WA for *E. coli* analysis within 24 hours of sampling.

Water chemistry samples for pH, colour, turbidity, non-filterable residue (NFR), dissolved organic carbon (DOC), total N (TN), and total P (TP) were also collected at the time of sampling and were analyzed at an accredited laboratory using the Standard

Methods for the Examination of Water and Wastewater 20th Edition (APHA 1998). Appendix 2 shows additional water chemistry data collected.

Culture conditions of E. coli and source determination using ribotyping

Water samples were analyzed for fecal bacteria using the Membrane Filtration procedure according to the methodology outlined in the Standard Methods for the Examination of Water and Wastewater 20th Edition (APHA 1998). As previously described (Hyer and Moyer 2003, Stoeckel et al. 2004, Samadpour et al. 2005), individual blue colonies were selected at random from mFC plates and streak plated to MacConkey agar to obtain isolated colonies. These colonies were then streak plated to Tryptic Soy Agar. The pure culture was then tested for indole production, and citrate utilization and quadrant streaked to blood agar. After the isolate was verified as an *E. coli* colony, confluent growth from the blood agar plates was scraped with a sterile flat-headed toothpick and the cell material was suspended in 800 µL 50mM Tris and 50mM EDTA (pH 8.0). Genomic DNA was extracted using the phenol/chloroform procedure (Sambrook et al. 1989). Restriction endonuclease digestion reactions were performed on between 1-2 ug of DNA using *Eco*RI and *Pvu*II (BoehringerMannheim GmbH, Germany) as per the manufacturer's protocol. DNA fragments were separated by electrophoresis at 33V for 12-14 h in a 0.8% agarose gel in 1X TBE (Tris-borate-EDTA). The DNA was transferred to a Nitran filter (Schleicher and Schuell, Keene, NH), baked at 80°C for 1 h, and probed with ^{32} P-labeled copies of *E. coli* total rRNA produced using random hexanucleotide primers and Avian Myeloblastosis Virus reverse transcriptase (Strategene, La Jolla, CA) as per the manufacturer's protocol. After hybridization, the blots were washed in SSC buffer (44 g L^{-1} sodium citrate, 97.7 g L^{-1} sodium chloride) and

0.1% sodium dodecylsulfate detergent at 65^{o}C. The hybridized bands were visualized by autoradiography using intensifying screens at -70^{o}C.

As described elsewhere (Myoda et al. 2003, Samadpour et al. 2005), different ribotype patterns were assigned a number sequence. The number sequence was then checked against a database of over 40,000 known source ribotypes of human and animal origin. If the sequence was not located in the database (new) the number sequence was entered and followed by the letter "a" and corresponding information attached. If the sequence was located, then the investigator would manually compare the gel to the other gels bearing that sequence. If a pattern was an identical match, it was given that letter designation as well. If the pattern was not a match, it was assigned the next letter of the alphabet and entered into the database along with corresponding information. Number sequence-letter combinations were then given a pattern number for the purpose of comparison within this study. A host source was assigned to an isolate that displayed a matching ribotype pattern to various known sources in the Institute for Environmental Health Inc. ribotype database. Fecal samples of known local sources from within the different watersheds were also collected in order to add the ribotypes of known sources to the existing library from the study watersheds.

Statistics

Count data analysis was performed on the fecal coliform data. Since there were no replications with these data, the data were analyzed using log-linear analysis on the log-transformed counts. The SAS GENMOD procedure (SAS Institute Inc. 2005) was used for all analysis with a Poisson probability distribution. Data was summed over

SAMPLE and DAY in order to get sufficient counts to perform an analysis. Factors that were tested for differences include WATERSHED, SITE, YEAR, and MONTH.

For the source data, a Chi-square test using a 4 x 14 contingency table was used to test the association between stream and species that contributed *E. coli* into the stream (Townend 2004).

Water chemistry data were analyzed using PROC MIXED from SAS (SAS Institute Inc. 2005) with STREAM, YEAR, and their interaction treated as fixed effects and LOCATION, LOCATION*STREAM treated as random effects. This model was used because only comparisons among streams and years were of interest. Only locations I and III were used in these analyses since location II was missing from S. Fortune stream. Two sampling dates per month were included for June through September. The sampling dates were treated as unequally spaced repeated measures, and various variance-covariance structures were fitted and the one with the lowest AIC value was selected for the final analysis. The UNIVARIATE procedure was used to check the residuals for normality and for obvious outliers, which were removed before running the final analysis.

Results

Water chemistry data.

There was no significant difference between streams for pH (P = 0.11), specific conductance (P = 0.08), NFR (P = 0.86), and turbidity (P = 0.38). There was a significant difference between streams for colour (P= 0.001), DOC (P = 0.01), TN (P = 0.046), and TP (P = 0.005). There was no significant difference between years for specific conductance (P = 0.06), colour (P = 0.09), NFR (P = 0.25), turbidity (P = 0.09), and DOC

(P = 0.55). There was a significant difference between years for pH (P = 0.03), TN (P = 0.0002), and TP (P = 0.03). The streams had higher pH and TP in 2004 than 2003. BX, Deer and Duteau streams had higher TN in 2004 versus 2003, whereas there was no difference between years for S. Fortune Creek.

All the streams were within the acceptable limits for pH according to the BC Approved Water Quality Guidelines for drinking water (6.5 to 8.5 pH units) (Nagpal et al. 1998). Deer and Duteau Creeks had much higher colour than BX or S. Fortune Creeks and were above the approved guidelines for colour for drinking water use of 15 TCU (Table 17). All the streams had limits that were well below the drinking water use guideline of specific conductance of 700 $\mu S\ cm^{-1}$. Turbidity for all streams was below the aesthetic drinking water guideline of 5 NTU for both years. DOC, TN, and TP were much higher in Deer and Duteau Creeks than S. Fortune or BX Creeks.

Overall, all 4 streams were very similar in terms of general water chemistry (pH, specific conductance, non-filterable residue, and turbidity). The streams however, were different in terms of colour, DOC, TN and TP. Deer and Duteau Creeks were significantly higher in colour, DOC, TN and TP than S. Fortune or BX Creek. Nutrients or organic limitation have been cited as important factors in the survival and growth of *E. coli* in water (McFeters and Stuart 1972, Gerba and McLeod 1976, Flindt 1987, Tassoula 1997, Byappanahalli and Fujioka 1998). Therefore, if all other factors (stream temperature, exposure to solar radiation, competition with other bacteria, predators, etc.) were held constant, *E. coli* would likely survive for a longer period of time if deposited into Duteau or Deer Creek than if deposited in BX or S. Fortune Creeks. Water

chemistry is one of many factors that are needed in order to predict the survival and potential transport of fecal bacteria in water (McFeters and Stuart 1972).

Fecal bacteria concentrations.

In 2003 and 2004, 98 % (2,396 out of 2,453) and 97 % (2,406 out of 2,475) respectively, of fecal coliform isolates picked were confirmed as *E. coli*. For each site on each stream, there was a significant difference in fecal coliform counts for each year except for Duteau Creek in 2004, there was no significant difference ($P = 0.17$) in fecal coliform counts between sites II and III (Appendices 3 and 4). In 2003, the headwaters site on BX Creek (BX I) had significantly lower fecal coliform counts ($P < 0.0001$) than the mid-elevation (BX II) and lower elevation (BX III) sites. BX II had significantly higher fecal coliform counts ($P < 0.0001$) than BX III. In 2004, BX I had the lowest fecal coliform counts ($P < 0.0001$) and BX III had the highest fecal coliform counts ($P < 0.0001$). In 2003, both Deer and Duteau Creeks had the highest fecal coliform counts ($P < 0.0001$) at site II and the lowest fecal coliform counts ($P < 0.0001$) at site I. In 2004, Deer Creek had the highest fecal coliform counts ($P < 0.0001$) at site II and the lowest fecal coliform counts ($P < 0.0001$) at site I. In 2003, S. Fortune Creek had significantly higher counts of fecal coliforms ($P = 0.003$) at site I compared to site III. The reverse occurred in 2004, site III had significantly higher fecal coliform counts than site I ($P < 0.0001$). Overall, the mid- and lower elevation sites tended to have higher fecal coliform counts than the headwaters site, with the exception being S. Fortune Creek.

For each stream, there was a significant difference in monthly total fecal coliform counts between years (Appendices 5 to 7). Overall, there tended to be significantly higher fecal coliform counts in 2003 versus 2004 for BX and Deer Creeks, whereas there

tended to be significantly higher fecal coliform counts in 2004 versus 2003 for Duteau and S. Fortune Creeks. Finally, there was a significant difference in fecal coliform counts between streams each year (Appendices 8 and 9). For both years, Duteau had significantly higher fecal coliform counts ($P < 0.0001$) than any other stream (based on sites I and III only) followed by Deer, BX and S. Fortune.

As discussed in Chapter 5, there is a need to accurately determine the variation among samples when determining the number of samples that are needed in a monitoring program for a given stream. There is variability between samples in a given day (Chapter 5), as well as variability between days and sampling sites (Whitman and Nevers 2004) and therefore care needs to be taken in determining appropriate sample sites, sampling regime and number of samples taken during each sampling period.

Tables 18 and 19 summarize the number of samples that would be needed based on standard deviations and margins of error for raw and $\log_{10}$ fecal coliform data for BX, Deer, Duteau and S. Fortune Creeks for each year. Note that we collected 100 samples in each year for each site on each of the streams. Therefore, a total of 300 samples were collected each year for BX, Deer and Duteau Creeks, and 200 samples were collected for S. Fortune Creek. According to our sample size calculations, that would give us a margin of error of less than 20 % for this experiment in each watershed. As stated previously it is important to set up an intensive sampling program to characterize the bacterial (*E. coli* and/or fecal coliform) concentrations of a source water stream prior to establishing a monitoring program. Sample size needed can then be based on an acceptable margin of error that can be calculated for each individual stream (Meays et al. in review). It also indicates error based on samples taken if you cannot increase sample numbers.

Bacterial water quality monitoring programs that collect only a few bacterial samples will mostly be insufficient and introduce large margins of error exceeding 50 %. Sampling plans and number of samples need to be statistically sufficient for greater accuracy or reliability of reported data.

Temporal and spatial variability of E. coli sources

The sources of *E. coli* varied significantly with stream for both 2003 ($P < 0.001$, df=39) and 2004 ($P < 0.001$, df=39). For BX Creek, the main sources of *E. coli* in 2003 were, in ranked decreasing order, avian (26.5 %), canine (24.3 %), and deer/elk (19.1 %) (Table 20). The main source in 2004 was avian (24.3 %), followed by bovine (19.1 %), rodent (18.0 %) and unknown (11.5 %). For Deer Creek, the main source of *E. coli* for 2003 was deer/elk (21.1 %), followed by bear (17.0 %), canine (16.7 %), and unknown (16.1 %). The main source in 2004 was avian (28.1 %), followed by bovine (20.0 %), rodent (11.4 %), and canine (11.2 %). For Duteau Creek, the main source of *E. coli* for 2003 was deer/elk (28.3 %), followed by avian (21.4 %), and canine (20.3 %). The main source in 2004 was avian (24.7 %), followed by bovine (19.9 %), and rodent (18.1 %). For S. Fortune Creek, the main source of *E. coli* for 2003 was avian (40.7 %), followed by canine (14.1 %), rodent (13.3 %), and deer/elk (12.5 %). The main source in 2004 was avian (32.0 %), followed by rodent (22.6 %), deer/elk (11.3 %), and bovine (9.4 %). For each of the streams, a larger amount of bovine and rodent *E. coli* was detected in 2004 compared to 2003. The main source of *E. coli* detected from the classified isolates from all the watersheds was deer/elk in 2003 and avian in 2004. Isolates classified as unknown were those that did not match 100 % to a sample in the known source library.

Of all the isolates classified, 6.8 % were classified as unknown in 2003 and 7.0 % in 2004.

Similarly to a previous 24-hour study, the majority of isolates ribotyped for each stream came from only 3 or 4 main sources, whereas most other source groups made only a very small contribution (Meays et al. in review). Nearly all sources were present throughout most of the sampling dates. Bovine *E. coli* was detected at all of the headwater sites (BX I, Deer I, Duteau I, and S. Fortune I) in 2004 in mid-May before any cattle were released into the watersheds, indicating that bovine *E. coli* was able to survive over the winter.

Even though land-use differed between watersheds, and Deer and Duteau Creeks had more cattle grazing within the watersheds, wildlife was the main contributor of fecal pollution for each of the streams. In 2004, approximately 20% of the *E. coli* isolates on Deer and Duteau Creeks came from bovine sources. Even though livestock management plans are in place in these watersheds, additional livestock management tools and techniques could be adapted or improved in order to reduce fecal contamination by cattle. Livestock tools would include: off-stream watering, salt and mineral placement away from water sources, herding, fencing, culling animals that tend to spend more time in riparian areas, animal choice and grouping (age, type), and the timing, duration, and intensity of grazing.

Discussion

Non-point source pollution is often difficult to quantify and determine its sources, and therefore incorrect and inappropriate assumptions may be made on the main sources of fecal pollution (Meays et al. 2004, Meays et al. in review). Few experiments have

been conducted using BST in true watershed case studies as most results have been reported from feasibility studies (Scott et al. 2004). This watershed-based case study is the largest to date, and shows that a wide range of *E. coli* sources were found in most samples taken in each watershed over both years. The majority of isolates classified in 2003 came from wildlife sources including deer/elk (22.1 %), avian (21.5 %), and canine (19.9 %). In 2004, the majority of isolates classified came from avian (26.4 %), bovine (18.3 %), and rodent (16.9 %) sources. These findings are consistent with others who have found that no single source accounted for more than 30 % of the identified *E. coli* in a stream (Hyer and Moyer 2004). Similar to Hyer and Moyer (2004), there were many sources that contributed a minor (less than 10 %) portion of the total source, however, when these minor sources are added up, they can contribute a substantial amount of *E. coli* to the stream thereby impacting the water quality. Compared to other studies (Samadpour and Chechowitz 1995, Hyer and Moyer 2004), this study had a very low percentage of isolates that were classified as unknown (6.8 % in 2003, and 7.0 % in 2004). Scat samplings were undertaken in these watersheds throughout both years (as well as in previous years), which increased the ability to match isolates from water samples to the ribotyping database.

Results of this study demonstrate that the main contributors of fecal pollution shift from one year to another. Wildlife contributed the majority of *E. coli* in 2003 (> 84 %) and in 2004 (> 73 %) to the streams. These results are consistent with a previous 2001 study conducted on Duteau Creek which found that wildlife contributed approximately 69 % of *E. coli*, cattle contributed approximately 18 %, humans and domestic animals

contributed approximately 1 %, and unknown sources contributed approximately 12 % (Phippen 2002).

There were no distinctive differences between the main source contributors from one watershed to another, even though there was different land-use in each watershed. The highest percentage of isolates consistently found in each of the watersheds both years came from avian, canine, bear, deer/elk, rodent and bovine sources (Table 20). Others have noted similar sources in their studies (Farag et al. 2001, Hyer and Moyer 2004). In this study, sites were located from the headwaters to the lower elevation drinking water intake sites. There were no noticeable differences between the main sources identified from one site to another within a watershed.

Fecal coliform counts were lowest in S. Fortune Creek, which had the least amount of land-use and human activities followed by BX, Duteau and Deer Creeks, which tended to have more use (Appendix 3). For BX and Deer Creeks, the fecal coliform counts were higher in 2003 versus 2004, whereas fecal coliform counts were higher in 2004 for Duteau and S. Fortune Creeks. One would have expected to find higher counts in 2004 for all streams since it was a much wetter year than 2003, however precipitation and potential runoff appeared to play only a minor role. The amount of precipitation in a year may only have a minor effect on fecal coliform counts and other factors may be equally or more important such as stream flow and disturbance of sediment, subsurface flows to the stream, and direct deposit by wildlife and livestock in the riparian zone. Hyer and Moyer (2004) found that they had no differences in the contributing sources between low flow and high flow periods. In 2003, a 24-hour study was conducted at BX II to determine the sources of *E. coli* diurnally (Meays et al. in

review). During the 24 hours, it rained heavily (approximately 8 mm) increasing the stage measurements at the site. The main sources of *E. coli* during the 24-hour period were canine (57.6 %), raccoon (12.1 %), unknown (12.1 %), rodent (11.6 %) and avian (5.6 %). If we compare the sources found during the rainfall event to the overall sources identified for the site from June to October 2003, we see a decline in canine (57.6 to 23.2 %) and rodent (11.6 to 7.7 %) isolates identified, and an increase in avian (5.6 to 25.5 %), deer/elk (0 to 18.6 %) and bear (<1 to 11.5 %). However, these differences may be attributed to comparing a one-time sampling to an entire sampling season. The results suggest that the timing and duration of sampling can substantially change your observed results.

Fecal material can enter streams by direct deposit, overland flow, or by subsurface flows. While the transportation dynamics of fecal bacteria were not addressed in this study, more research is needed to better understand the environmental dynamics of *E. coli*. The survival of *E. coli* on land and in water also requires more research (Rosen 2000, Ferguson et al. 2003, Meays et al. 2004, Meays et al. 2005). As observed, *E. coli* appears to be able to survive the winter in the environment. Long-term survival of an organism used for monitoring can complicate the interpretation of data collected, since it would not be known if the fecal pollution was recent or not. Information gained on the survival and transport of fecal bacteria in the environment can be incorporated into management plans and used for developing appropriate buffer zones or areas of minimal use for cattle and wildlife in areas surrounding drinking water intake sites.

Atwill et al. (2002) demonstrated that many species of wildlife were substantial contributors of pathogens to the environment. This study demonstrates that fecal

pollution arises from multiple sources in watersheds. If buffer zones were developed to protect drinking water sources, as mentioned earlier, wildlife would also need to be restricted from these areas. Restricting wildlife use in a watershed area is much more difficult than restricting livestock use. BST is a useful tool to identify the main sources of fecal pollution entering these buffer zones.

In watersheds where cattle fecal pollution is a concern, off-stream water developments may be an effective strategy to reduce fecal loading in a riparian area. Studies have evaluated the effectiveness of having off-stream water developments as alternatives to livestock drinking directly from streams or fencing out riparian areas (Miner et al. 1992, Godwin and Miner 1996, Sheffield et al. 1997). Off-stream watering areas were shown to significantly reduce the time spent by livestock in riparian areas by more than 90%. Reducing the amount of time cattle spend in or near the stream would place the majority of cattle defecations away from the stream and thereby decrease the amount of fecal pollution directly entering the stream or being deposited within the riparian area. Off-stream watering is a viable alternative to total exclusion fencing along stream systems for improving water quality.

HACCP and watershed risk assessment can be aided with the use of BST information, to identify potential hazards in the watershed. Once the main sources of fecal contamination are identified in a watershed, feces can be collected directly from those sources and tested for pathogens of concern, thus providing more information about potential pathogenic risk.

Monitoring protocols and management strategies need to be developed specifically for individual watersheds taking into account the sources and concentrations

of fecal pollution. Challenges exist in the development of field monitoring protocols, as well as laboratory protocols including: cultivation methods for organisms (varies from lab to lab); survival of organisms detected (different in the lab versus the environment); and for ribotyping - database size, geographic distribution of isolates, choice and number of restriction enzymes, and number of isolates sampled (Meays et al. 2004, USEPA 2005).

Table 15. Stream length of study reach[1] and distance between sites[2].

Stream	Total length (km)	I to II	II to III
BX	13.2	5.2	8.0
Deer	7.1	1.6	5.5
Duteau	14.8	7.7	7.1
S. Fortune	7.4	-	-

[1]Length of the stream used for this study.

[2]I is the headwater site, II is mid-elevation and III is lower elevation drinking water site.

Table 16. Elevation (m) of sites for Creeks used for this study.

Stream	I (m)	II (m)	III (m)
BX	1627	948	497
Deer	1267	1108	607
Duteau	1351	1317	516
S. Fortune	1526	N/A[1]	358

[1]Not applicable.

Table 17. Water chemistry data for all streams for 2003 and 2004[1].

Parameter	BX	Deer	Duteau	S. Fortune[7]
2003				
pH[2]	8.0 ± 0.08	7.6 ± 0.25	7.3 ± 0.10	7.7 ± 0.33
True Colour[3]	9 ± 14	47 ± 18	45 ± 8	15 ± 11
Specific conductance[4]	271.0 ± 97.0	81.9 ± 64.1	36.4 ± 4.4	117.4 ± 70.7
Nonfilterable residue[5]	4.1 ± 0.40	4.4 ± 1.86	4.6 ± 2.18	4.0 ± 0
Turbidity[6]	0.53 ± 0.72	1.66 ± 1.05	1.77 ± 0.57	1.08 ± 1.45
Dissolved Org. C[5]	1.64 ± 0.77	8.97 ± 2.33	8.31 ± 1.02	3.37 ± 2.22
Total N[5]	0.09 ± 0.05	0.35 ± 0.09	0.30 ± 0.04	0.17 ± 0.10
Total P[5]	0.007 ± 0.004	0.018 ± 0.006	0.016 ± 0.005	0.007 ± 0.004
2004				
pH[2]	8.1 ± 0.09	7.6 ± 0.27	7.4 ± 0.12	7.8 ± 0.33
True Colour[3]	7 ± 7	53 ± 17.9	46 ± 10	10 ± 9
Specific conductance[4]	249.7 ± 80.3	78.8 ± 47.2	41.4 ± 5.2	119.2 ± 62.1
Nonfilterable residue[5]	5.0 ± 3.6	4.0 ± 0.39	4.7 ± 1.8	6.6 ± 7.5
Turbidity[6]	1.36 ± 1.83	1.96 ± 1.15	2.08 ± 1.03	3.93 ± 7.20
Dissolved Org. C[5]	2.33 ± 1.51	11.20 ± 2.87	9.66 ± 1.38	3.59 ± 1.86
Total N[5]	0.10 ± 0.06	0.43 ± 0.08	0.35 ± 0.04	0.16 ± 0.11
Total P[5]	0.007 ± 0.004	0.022 ± 0.006	0.021 ± 0.008	0.010 ± 0.009

[1]Sampling occurred in June to October 2003, and May to September 2004.

Units: [2]pH units, [3]Colour unit, [4]uS cm^{-1}, [5]mg L^{-1}, and [6]NTU

[7]Data was averaged over 2 sites on S. Fortune Creek versus 3 sites on each of the other streams.

Table 18. Sample size (n) calculations based on standard deviation (SD) and margin of error (ME) for raw fecal coliform data collected from June to October 2003 and May to September 2004 using Lenth power and sample size program (Lenth 2005).

	Margin of Error (%)				
Stream	10	20	30	40	50
2003					
BX	718	182	82	48	32
Deer	1422	358	161	92	60
Duteau	1334	336	151	86	56
S. Fortune	842	213	96	55	36
2004					
BX	1026	259	117	67	44
Deer	830	210	95	55	36
Duteau	1484	373	168	96	62
S. Fortune	1100	277	125	72	47

Table 19. Sample size (n) calculations based on standard deviation (SD) and margin of error (ME) for $\log_{10}$ fecal coliform data collected June to October 2003 and May to September 2004 using Lenth power and sample size program (Lenth 2005).

	Margin of Error (%)				
Stream	10	20	30	40	50
2003					
BX	184	48	23	14	10
Deer	254	66	31	19	13
Duteau	129	34	17	11	7
S. Fortune	476	121	56	33	22
2004					
BX	169	45	21	13	9
Deer	177	46	22	14	10
Duteau	136	36	18	11	8
S. Fortune	344	88	41	24	17

Table 20. Total[1] and percentage (in parenthesis) of *E. coli* for each source by stream for 2003 and 2004.

	BX	Deer	Duteau	S.Fortune	Total
2003					
Avian	220 (26.5)	81 (10.1)	201 (21.4)	107 (40.7)	609 (21.5)
Bear	65 (7.8)	136 (17.0)	56 (6.0)	15 (5.7)	272 (9.6)
Bovine	11 (1.3)	72 (9.0)	87 (9.2)	13 (4.9)	183 (6.5)
Canine	202 (24.3)	134 (16.7)	191 (20.3)	37 (14.1)	564 (19.9)
Deer/Elk	159 (19.1)	169 (21.1)	266 (28.3)	33 (12.5)	627 (22.1)
Feline	31 (3.7)	5 (<1)	16 (1.7)	3 (1.1)	55 (1.9)
Horse	26 (3.1)	2 (<1)	2 (<1)	0 (0)	30 (1.1)
Human	4 (<1)	14 (1.7)	8 (<1)	0 (0)	26 (<1)
Moose	0 (0)	0 (0)	0 (0)	1 (<1)	1 (<1)
Rabbit	2 (<1)	2 (<1)	0 (0)	2 (<1)	6 (<1)
Raccoon	21 (2.5)	11 (1.4)	29 (3.1)	6 (2.3)	67 (2.4)
Rodent	65 (7.8)	47 (5.9)	55 (5.8)	35 (13.3)	202 (7.1)
Squirrel	1 (<1)	0 (0)	0 (0)	1 (<1)	2 (<1)
Unknown	24 (2.9)	129 (16.1)	30 (3.2)	10 (3.8)	193 (6.8)
Sum	831 (100)	802 (100)	941 (100)	263 (100)	2837 (100)
2004					
Avian	131 (24.3)	145 (28.1)	161 (24.7)	85 (32.0)	522 (26.4)
Bear	28 (5.2)	22 (4.3)	57 (8.7)	21 (7.9)	128 (6.5)
Bovine	103 (19.1)	103 (20.0)	130 (19.9)	25 (9.4)	361 (18.3)
Canine	33 (6.1)	58 (11.2)	55 (8.4)	18 (6.8)	164 (8.3)
Deer/Elk	37 (6.9)	33 (6.4)	61 (9.3)	30 (11.3)	161 (8.2)
Feline	0 (0)	0 (0)	3 (<1)	0 (0)	3 (<1)
Horse	6 (1.1)	0 (0)	0 (0)	0 (0)	6 (<1)
Human	1 (<1)	2 (<1)	2 (<1)	0 (0)	5 (<1)
Moose	10 (1.9)	22 (4.3)	5 (<1)	4 (1.5)	41 (2.1)
Rabbit	1 (<1)	1 (<1)	1 (<1)	0 (0)	3 (<1)
Raccoon	29 (5.4)	36 (7.0)	22 (3.4)	10 (3.8)	97 (4.9)
Rodent	97 (18.0)	59 (11.4)	118 (18.1)	60 (22.6)	334 (16.9)
Squirrel	2 (<1)	5 (1.0)	5 (<1)	0 (0)	12 (<1)
Unknown	62 (11.5)	30 (5.8)	33 (5.1)	13 (4.9)	138 (7.0)
Sum	540 (100)	516 (100)	653 (100)	266 (100)	1975 (100)

[1]Total is the total number of *E. coli* isolates ribotyped of each source.

[2]Rodent includes beavers and all other rodents.

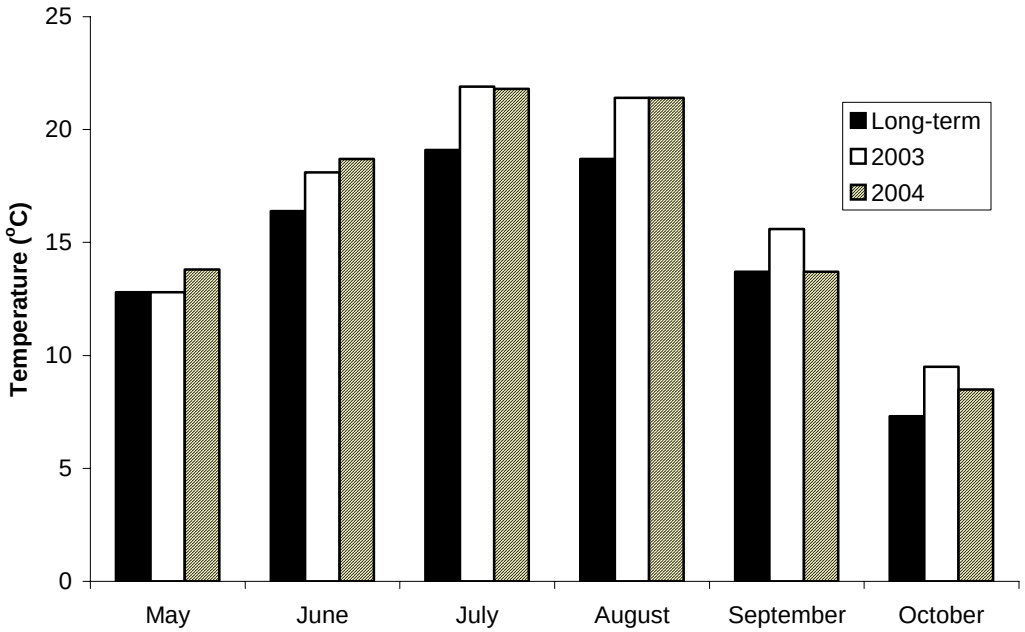

Figure 9. Comparison of long-term (1971-2000) daily average temperature (ºC) to 2003 and 2004 daily average temperatures.

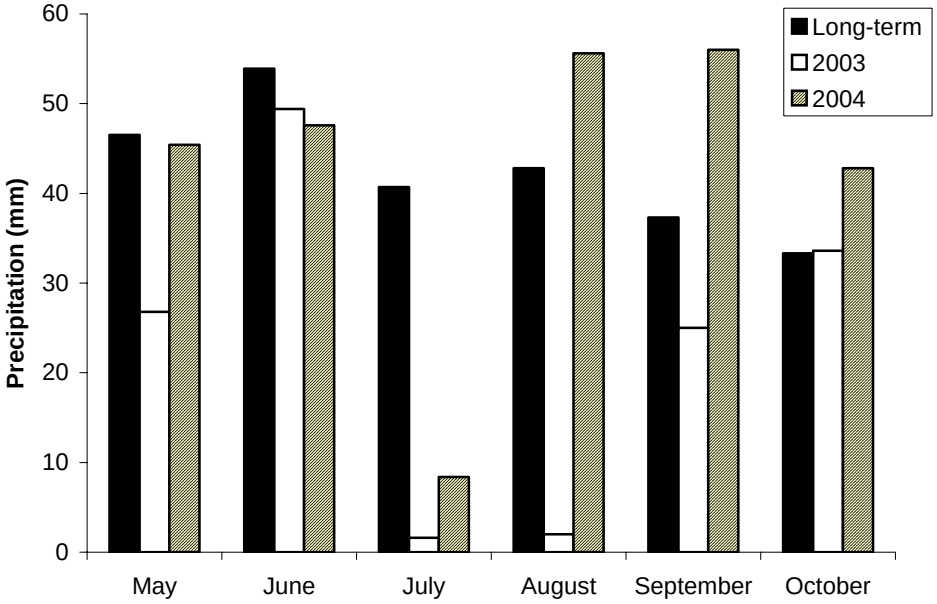

Figure 10. Comparison of long-term (1971-2000) monthly precipitation (mm) to 2003 and 2004 monthly precipitation.

Chapter 7: Conclusions – Summary and Synthesis

Research Objectives and Themes

Surface water from watersheds with many different user groups such as forestry, mining, agriculture, wildlife, urban development and recreation is used for drinking by many people around the world. Safe drinking water is essential to human health. *E. coli* is a bacterial indicator often used for assessing water quality. The objective of this study was to understand the sources and survival of *E. coli* in watersheds in BC. *Chapter 2* reviews the advantages and disadvantages of 7 methods that are currently being used in watersheds for BST studies. The survival of *E. coli* on land in beef cattle fecal pats is assessed in *Chapter 3*. The survival of beef cattle *E. coli* in stream water is examined in *Chapter 4*. In *Chapter 5*, the diurnal sources and concentrations of *E. coli* in 3 watersheds on 3 different days are reviewed. The sources and concentrations of *E. coli* within 4 watersheds over 2 years is studied and discussed in *Chapter 6*.

Summary of Major Findings

Bacterial Source Tracking Methods (Chapter 2)

There are many different molecular and biochemical methods and techniques that have been and are being developed for use in BST. Currently there is no standard method proposed for BST. Determining what method(s) to use in a given situation depends on a number of factors including: specific question to be answered, detail required and/or desired to answer the question, availability of resources (time and money), time constraints, and the ability to access a lab or facilities with the expertise to analyze the samples. Comparison studies are needed in order to determine which method works best for given situations. Standardization is also needed within methods for a clearer interpretation of comparison studies. Furthermore, more research is needed to

determine appropriate field protocol to collect samples for BST given the different methods available. The location and number of sites within a watershed, the timing and number of samples collected, the number of isolates identified and the analytical methods used also require further studies.

Survival of E. coli in Beef Cattle Fecal Pats (Chapter 3)

Often ranching and non-point source pollution by range cattle is cited as having a negative impact on water quality (Kauffman and Krueger 1984, Belsky et al. 1999). The survival of *E. coli* in beef cattle fecal pats was examined in order to characterize a rangeland situation where feces are surface-deposited on the landscape and *E. coli* was observed in its natural media. This experiment is unique in that it investigates the survival of natural populations of *E. coli* in beef cattle manure versus pure cultures inoculated into a medium. Even though making fecal pats is not the same as direct deposit by animals, uniformity of feces and replication was important in order to conduct a controlled experiment and minimize impacts of variables other than those under investigation.

Concentrations of *E. coli* remained high during the first 7 days of exposure to the hot, dry summer environment, and *E. coli* was found to be able to survive in the fecal pats for at least 45 days (end of controlled experiment). Shade was found to enhance the survival of *E. coli*. *E. coli* was observed to increase on Day 1 and Day 7, supporting other findings that suggest that *E. coli* can replicate in the environment (Gerba and McLeod 1976, Tassoula 1997, Byappanahalli and Fujioka 1998, Topp and Scott 2003, Topp et al. 2003, Unc and Goss 2003). Avery et al. (2004) found that *E. coli* could survive up to 162 days in winter months in feces deposited onto pastures, therefore,

season of deposition can impact the survival of *E. coli* in fecal pats. Observations from *E. coli* colonies plated during the experiment showed differing appearances indicating differing morphology. Further research is needed to determine if there is different survival, reproduction, and persistence of different strains of *E. coli* in feces and if these can be related to the differing phenotypes. Additional research is needed on the survival and behaviour of *E. coli* and other fecal bacteria and pathogens from different animal feces. Identifying similarities and differences in survival of pathogens of concern in a fecal matrix would also be beneficial in determining potential risk to human health within a watershed.

Grazing management plans can be developed using livestock management tools to reduce the risk of potential fecal contamination to water supplies. Results from this study suggest that *E. coli* in fecal pats under forested (shaded) situations would survive longer than in open grasslands and have the highest survival during the first week of exposure to the environment. Cattle feces distribution patterns are significantly associated with the location of livestock attractants, percent slope and aspect, hydrologic position, and season (Tate et al. 2003). Therefore, in locations where there are concerns with water quality due to fecal contamination, land managers could incorporate the information from this study to design grazing systems that minimizes the use in or around the riparian zone in the spring or fall as conditions are not as hot and dry as in the mid- to late summer.

This study did not investigate the transport potential of *E. coli* from fecal pats, which would be a very beneficial additional study. Fecal pats were observed to crust over and dry out rather quickly in the study, and it would be interesting to determine what

amount and intensity of precipitation would be needed to move the bacteria from fecal pats into soils or water sources from fecal pats of different age and crusting.

Survival of E. coli in stream water (Chapter 4)

Many of the studies that have looked at the survival of *E. coli* or fecal coliforms in water, have used pure cultures (McFeters and Stuart 1972, Bogosian et al. 1996, Darakas 2002), not *E. coli* in a natural fecal matrix. This experiment is unique in that it looks at the survival of natural populations of *E. coli* found in beef cattle manure in stream water. To understand the survival of *E. coli* in the watersheds, it was felt that it was important to look at the survival of *E. coli* mixed with cattle feces in stream water since it is more realistic to what happens in a rangeland environment.

Flint (1987) has suggested that competition with the natural microbial flora of the water, and temperature, are the main factors influencing the survival of bacteria in fresh water. This study was designed to investigate the survival of *E. coli* in beef cattle manure in stream water under different temperatures. *E. coli* concentrations remained higher in the coolest water treatment (6˚C) up to day 9 of the experiment, whereas the warmer treatments (20˚C and 26˚C) showed a 1.5-$\log_{10}$ decline in *E. coli* CFU g^{-1}. By day 16, all temperature treatments had a 2-$\log_{10}$ decrease in *E. coli,* which remained relatively constant to day 23. Flint (1987) observed a rapid decline of *E. coli* within 4 to 12 days in unfiltered and Whatman-filtered river water at similar temperatures to this study. Flint (1987) had much longer survival in water that was Millipore-filtered and autoclaved, indicating that competition with the natural microbial organisms in the water was the primary factor in the disappearance of introduced bacteria. Survival was much longer (23 days) in this study using unfiltered and 1 μm filtered stream water compared to the study

conducted by Flint (1987) using unfiltered and Whatman-filtered river water. Increased survival may have been associated with the organics and nutrients from the fecal material deposited into the water with the *E. coli*. Understanding the survival of *E. coli* in stream water is helpful in the prediction of how far bacteria can travel and survive in a given watershed when taking into account stream flow characteristics and the environment. More research is needed to test the survival of *E. coli* and fecal pathogens released with manure to a water body under fluctuating water temperatures, stream flows and solar exposure.

Diurnal variation in concentration and sources of E. coli (Chapter 5)

Non-point source pollution is a water quality concern, and is often difficult to quantify because sources are diffuse. The protocols for water quality monitoring programs often propose very few bacterial water quality samples to be taken before inferences and decisions are made. Sampling programs that detect and enumerate fecal bacteria, give an idea of concentration of fecal contamination at the time of sampling, but indicate very little about variability of concentrations of *E. coli* are over a sampling period and nothing about what the sources of contamination are. This study is unique in that concentrations of *E. coli* as well as sources of *E. coli* were analyzed every 15 minutes over a 24-hour period on 3 streams (each stream was analyzed over a different day). Results indicate that the concentration of *E. coli* varied throughout the day on each of the streams and therefore, the time of day, or number of samples taken can strongly influence the results of a monitoring program.

The sources of *E. coli* varied significantly with stream, although a wide range of *E. coli* sources were found in most of the samples taken throughout the day. The

majority of isolates sampled in this study, came from wildlife sources, especially canine, rodent, and avian which are very difficult to manage in a watershed.

Streams and the watersheds they drain are unique. Developing appropriate monitoring protocols for bacterial water quality is very challenging. Considerations include the timing and frequency of sample collections, location of sampling sites, number of samples collected, climate, and watershed characteristics. As demonstrated in this study, intensive sampling programs need to be conducted in order to characterize the *E. coli* concentrations in a stream prior to establishing a monitoring program. Sample frequency and number should be determined statistically based on an acceptable or established margin of error. Ribotyping is a very useful and beneficial tool in determining the sources of fecal contamination. However, more research is needed on the survival and transport of *E. coli*, and the relationship of *E. coli* to pathogens of concern.

Spatial and annual variability in concentrations and sources of E. coli in multiple watersheds in British Columbia (Chapter 6)

This research compliments the BST research presented in Chapter 5. In this study, 4812 isolates were classified, making this the largest source tracking case study to date. This study had a very low percent of unknowns (isolates not matched 100% to the library isolates) for both years (6.8% in 2003 and 7.0% in 2004) indicating a high degree of accuracy. Results of this experiment show that the concentration of fecal coliforms varied by year, month, and site, for each of the streams sampled. Higher fecal coliform numbers tended to be at the mid and lower elevation sites as compared to the headwater sites except for on S. Fortune Creek. Even though there was higher precipitation in 2004

versus 2003, only 2 of the 4 streams had higher fecal coliform counts indicating that the amount of precipitation in a year may only have a minor effect on fecal coliform counts and other factors may be equally or more important such as stream flow and disturbance of sediment, subsurface flows to the stream, and direct deposit by wildlife and livestock in the riparian zone.

As with the 24-hour study, the sources of *E. coli* varied significantly with stream, although a wide range of *E. coli* sources were found in most of the samples taken throughout the study. There were no noticeable differences between the main sources identified from one site to another within a watershed. The main source contributors tended to be similar at each site within the watersheds studied with only minor variations. This is the first study looking at the sources of *E. coli* in watersheds over more that one year, and it was shown that the sources of *E. coli* changed from one year to the next. These results suggest that it is important to look at more than one year of source tracking data to get an accurate picture of the dominant sources within a watershed. The majority of isolates classified in 2003 came from wildlife sources, especially deer/elk, avian, and canine, whereas the majority of isolates classified in 2004 came from avian, bovine and rodent sources. There were no distinctive differences between the main source contributors from one watershed to another even though there was different land-use in each watershed. Overall, wildlife was the largest contributor of *E. coli* to the watersheds in both 2003 (> 84 %) and 2004 (> 73%).

This study demonstrates that fecal pollution arises from multiple sources in watersheds. By positively identifying the sources of fecal pollution, land managers and stakeholders can develop watershed management plans to reduce fecal pollution by

livestock, humans and wildlife. Managing wildlife is more difficult than livestock, however it is a necessary step in reducing fecal bacteria and pathogen loading in drinking water watersheds.

Ribotyping proved to be a very useful tool in determining the sources of fecal contamination in a watershed, and would be a very useful tool when conducting a hazard analysis or risk assessment. As previously discussed, further improvements in both field and laboratory protocols are needed.

Summary

The Hazard Analysis of Critical Control Points (HACCP) model can be applied to watersheds for the protection of water quality (Barry et al. 1998). A multidisciplinary watershed team can be used to conduct a hazard analysis on any watershed and identify the critical control points in the collection, storage, and distribution of water (Barry et al. 1998). BST is a tool that is very useful for conducting a hazard analysis in a watershed. Risk assessment can be determined more accurately by identifying the sources of fecal contamination and sampling the feces from the main contributors of fecal pollution for pathogens of concern. A watershed team can use adaptive management (Elzinga et al. 1998), to develop strategies to try to reduce fecal contamination thereby reducing the risk to human health. This thesis provides valuable new knowledge to be built upon for future studies; although as identified, there are still large information gaps regarding *E. coli* survival, transport, and sources in the environment.

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Appendices

Appendix 1 – Photographs of Fecal Pat Experiment

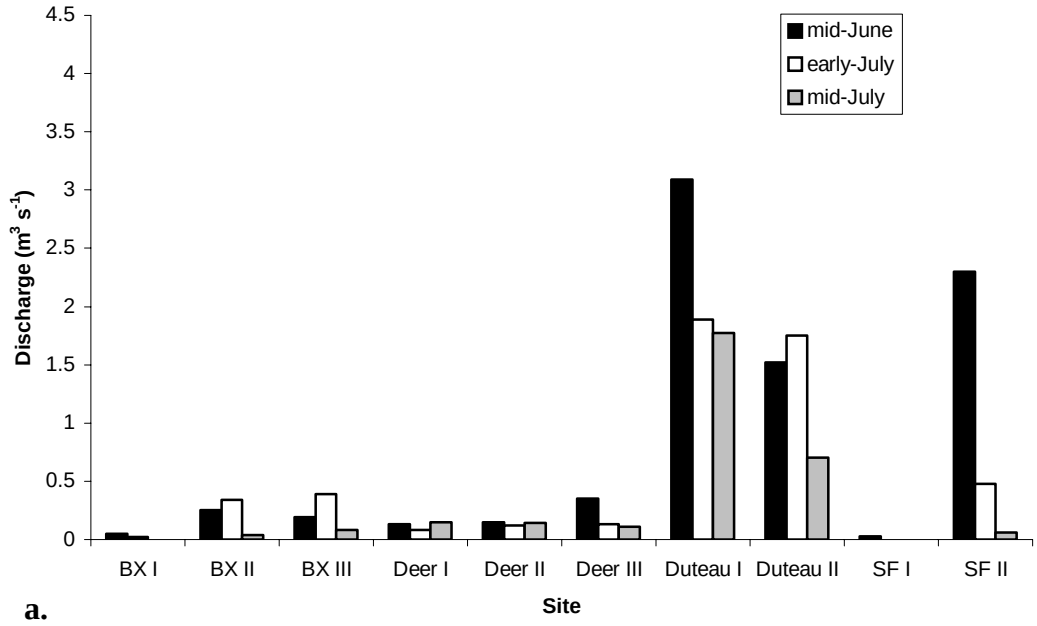
Step 1: Collecting cattle feces. (Top – Cindy Meays, bottom left – Terri France, bottom right – Joan)

Step 2: Blending manure (left – Charmaine Martens, right – Dr. Klaas Broersma)

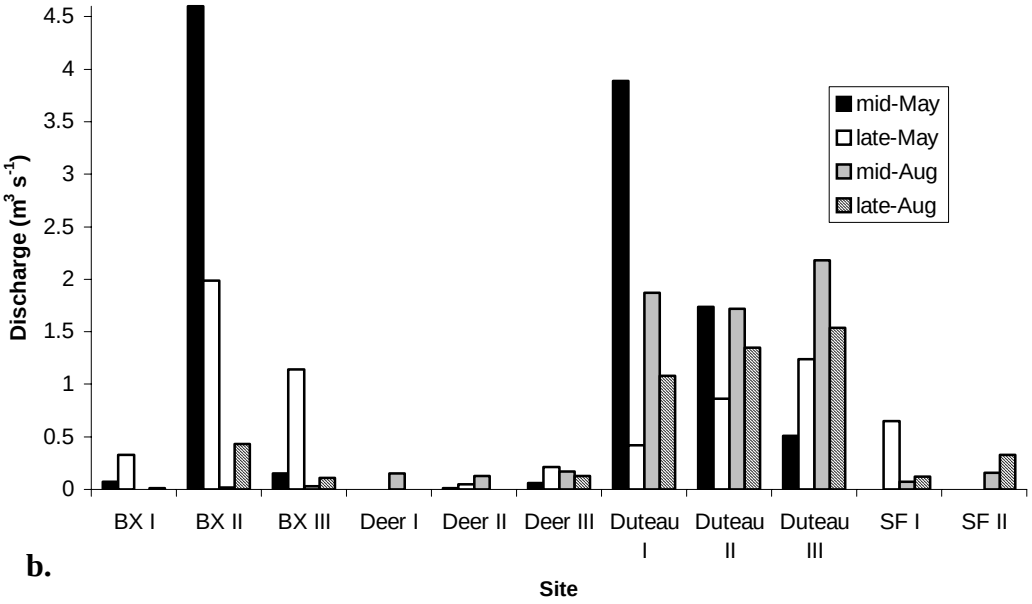
Step 3: Making fecal pats (Mavis Ulansky)

Step 4: Placement of fecal pats under 4 levels of shade treatment

Appendix 2. Discharge ($m^3 s^{-1}$) measurements for 2003 (a) and 2004 (b).



a.



b.

Note: For 2003, Duteau III was too high and too dangerous to enter. For 2004, S. Fortune I (SF I) and S. Fortune II (SF II) were too dangerous to enter in mid-May. Due to equipment failure, a limited number of stream flow measurements were collected.

Appendix 3. Water chemistry data collected for all streams in 2003 and 2004.

a. BX Creek water chemistry data 2003[1]

Parameter	June	July	August	September	October
pH[2]	8.0 ± 0.1	8.1 ± 0.1	8.1 ± 0.1	8.0 ± 0.1	8.0 ± 0.1
True Colour[3]	18 ± 26	5 ± 0	13 ± 18	5 ± 0	6 ± 2
Specific conductance[4]	183.3 ± 39.7	239.0 ± 60.4	294.0 ± 91.4	308.0 ± 104.0	330.5 ± 114.9
Nonfilterable residue[5]	4.3 ± 0.8	4.2 ± 0.4	4 ± 0	4.0 ± 0	4 ± 0
Turbidity[6]	1.4 ± 1.2	0.4 ± 0.3	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0
Dissolved Org. C[5]	2.1 ± 0.9	1.3 ± 0.5	1.7 ± 0.8	7.8 ± 0.5	1.7 ± 0.8
Total Organic C[5]	2.2 ± 1.0	1.4 ± 0.7	1.3 ± 0.7	1.3 ± 0.7	1.3 ± 0.7
Total Kjeldahl N[5]	0.07 ± 0.03	0.06 ± 0.03	0.06 ± 0.02	0.03 ± 0.01	0.05 ± 0.02
Total N[5]	0.09 ± 0.05	0.10 ± 0.06	0.11 ± 0.06	0.07 ± 0.05	0.08 ± 0.04
Total Organic N[5]	0.10 ± 0.01	0.10 ± 0	0.10 ± 0	0.10 ± 0	0.10 ± 0
Ammonia N[5]	0.005 ± 0	0.005 ± 0.001	0.006 ± 0.002	0.005 ± 0	0.005 ± 0.001
Dissolved Nitrate N[5]	0.03 ± 0.02	0.05 ± 0.03	0.06 ± 0.03	0.05 ± 0	0.04 ± 0.02
Nitrite N[5]	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Ortho-P[5]	0.005 ± 0	0.005 ± 0	0.006 ± 0.001	0.005 ± 0	0.005 ± 0
Total Dissolved P[5]	0.012 ± 0.010	0.005 ± 0	0.006 ± 0.002	0.005 ± 0	0.005 ± 0
Total P[5]	0.010 ± 0.007	0.005 ± 0	0.006 ± 0.002	0.008 ± 0.006	0.005 ± 0

[1]Data was collected from June to October 2003.

Units: [2]pH units, [3]Colour unit, [4]uS cm^{-1}, [5]mg L^{-1}, and [6]NTU

b. BX Creek water chemistry data 2004[7]

Parameter	May	June	July	August	September
pH[2]	7.6 ± 0.1	8.0 ± 0.1	8.1 ± 0.1	8.1 ± 0.1	8.1 ± 0.1
True Colour[3]	5 ± 0	6 ± 2	5 ± 0	14 ± 14	5 ± 0
Specific conductance[4]	176.0 ± 38.1	195.8 ± 49.6	271.0 ± 80.3	301.2 ± 94.3	257.8 ± 72.2
Nonfilterable residue[5]	4.0 ± 0	7.0 ± 6.0	4.0 ± 0	4.0 ± 0	4.0 ± 0
Turbidity[6]	0.95 ± 0.7	2.2 ± 3.0	0.6 ± 0.2	1.0 ± 0.7	1.1 ± 0.3
Dissolved Org. C[5]	3.23 ± 0.9	3.1 ± 2.0	2.2 ± 1.4	1.9 ± 0.6	1.7 ± 1.1
Total Organic C[5]	2.4 ± 1.0	2.7 ± 1.9	1.6 ± 0.9	1.7 ± 0.7	1.7 ± 1.1
Total Kjeldahl N[5]	0.07 ± 0.03	0.08 ± 0.05	0.06 ± 0.03	0.06 ± 0.02	0.07 ± 0.03
Total N[5]	0.12 ± 0.05	0.11 ± 0.07	0.11 ± 0.08	0.10 ± 0.05	0.09 ± 0.04
Total Organic N[5]	0.01 ± 0	0.11 ± 0.03	0.10 ± 0	0.10 ± 0	0.10 ± 0
Ammonia N[5]	0.005 ± 0	0.005 ± 0.001	0.007 ± 0.002	0.005 ± 0.001	0.005 ± 0
Dissolved Nitrate N[5]	0.04 ± 0.02	0.04 ± 0.02	0.06 ± 0.05	0.04 ± 0.03	0.03 ± 0.01
Nitrite N[5]	0.006 ± 0.001	0.005 ± 0	0.007 ± 0.002	0.005 ± 0.001	0.005 ± 0
Ortho-P[5]	0.005 ± 0.001	0.005 ± 0.001	0.006 ± 0.001	0.006 ± 0.002	0.005 ± 0
Total Dissolved P[5]	0.005 ± 0	0.005 ± 0	0.006 ± 0.001	0.005 ± 0	0.005 ± 0
Total P[5]	0.009 ± 0.001	0.009 ± 0.007	0.006 ± 0.001	0.005 ± 0	0.005 ± 0

[7]Data was collected from May to September 2004.

Units: [2]pH units, [3]Colour unit, [4]uS cm^{-1}, [5]mg L^{-1}, and [6]NTU

Appendix 3. Continued.

a. Deer Creek water chemistry data for 2003[1]

Parameter	June	July	August	September	October
pH[2]	7.5 ± 0.2	7.5 ± 0.2	7.5 ± 0.2	7.7 ± 0.3	7.8 ± 0.3
True Colour[3]	53 ± 18	62 ± 4	52 ± 8	39 ± 14	26 ± 20
Specific conductance[4]	58.2 ± 23.0	50.3 ± 11.5	51.2 ± 8.6	96.2 ± 62.8	156.0 ± 96.6
Nonfilterable residue[5]	4 ± 0	4.2 ± 0.4	4 ± 0	6 ± 5.5	4 ± 0
Turbidity[6]	1.3 ± 0.5	1.5 ± 0.9	1.7 ± 0.7	2.6 ± 1.7	1.0 ± 0.8
Dissolved Org. C[5]	9.4 ± 0.6	9.0 ± 1.5	11.0 ± 1.4	7.8 ± 2.6	7.5 ± 3.3
Total Organic C[5]	9.7 ± 0.5	9.5 ± 0.7	10.8 ± 1.3	8.3 ± 2.4	7.1 ± 3.4
Total Kjeldahl N[5]	0.33 ± 0.05	0.33 ± 0.03	0.38 ± 0.05	0.24 ± 0.18	0.27 ± 0.15
Total N[5]	0.34 ± 0.04	0.34 ± 0.03	0.39 ± 0.04	0.32 ± 0.18	0.36 ± 0.10
Total Organic N[5]	0.33 ± 0.05	0.33 ± 0.03	0.37 ± 0.05	0.26 ± 0.15	0.27 ± 0.15
Ammonia N[5]	0.006 ± 0.002	0.005 ± 0.001	0.006 ± 0.001	0.005 ± 0	0.007 ± 0.002
Dissolved Nitrate N[5]	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.08 ± 0.13	0.10 ± 0.12
Nitrite N[5]	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Ortho-P[5]	0.009 ± 0.005	0.007 ± 0.003	0.007 ± 0.002	0.006 ± 0.002	0.008 ± 0.004
Total Dissolved P[5]	0.017 ± 0.009	0.012 ± 0.003	0.014 ± 0.003	0.011 ± 0.003	0.014 ± 0.010
Total P[5]	0.019 ± 0.008	0.015 ± 0.002	0.020 ± 0.005	0.016 ± 0.003	0.019 ± 0.009

[1]Data was collected from June to October 2003.

Units: [2]pH units, [3]Colour unit, [4]uS cm^{-1}, [5]mg L^{-1}, and [6]NTU

b. Deer Creek water chemistry data for 2004[7].

Parameter	May	June	July	August	September
pH[2]	7.3 ± 0.2	7.7 ± 0.3	7.6 ± 0.3	7.6 ± 0.2	7.8 ± 0.3
True Colour[3]	57 ± 12	59 ± 9	57 ± 10	56 ± 21	37 ± 24
Specific conductance[4]	73.3 ± 40.1	72.9 ± 38.1	56.7 ± 18.7	70.7 ± 30.8	118.0 ± 72.6
Nonfilterable residue[5]	4 ± 0	4 ± 0	4 ± 0	4.3 ± 0.8	4.0 ± 0
Turbidity[6]	1.6 ± 0.7	1.4 ± 0.6	2.0 ± 0.7	2.3 ± 1.3	2.4 ± 1.8
Dissolved Org. C[5]	14.7 ± 3.3	12.6 ± 2.0	12.0 ± 2.2	10.9 ± 2.0	8.7 ± 4.0
Total Organic C[5]	13.2 ± 1.2	11.9 ± 1.7	11.3 ± 1.6	10.6 ± 2.1	8.8 ± 3.7
Total Kjeldahl N[5]	0.46 ± 0.13	0.38 ± 0.08	0.36 ± 0.05	0.44 ± 0.12	0.37 ± 0.16
Total N[5]	0.47 ± 0.12	0.39 ± 0.07	0.39 ± 0.06	0.49 ± 0.09	0.45 ± 0.05
Total Organic N[5]	0.45 ± 0.12	0.38 ± 0.07	0.35 ± 0.05	0.43 ± 0.12	0.36 ± 0.16
Ammonia N[5]	0.010 ± 0.007	0.008 ± 0.004	0.009 ± 0.003	0.008 ±.004	0.006 ±.001
Dissolved Nitrate N[5]	0.02 ± 0	0.02 ± 0.003	0.03 ± 0.02	0.05 ± 0.06	0.09 ± 0.11
Nitrite N[5]	0.005 ± 0.001	0.005 ± 0	0.007 ± 0.002	0.006 ± 0.001	0.005 ± 0
Ortho-P[5]	0.007 ± 0.004	0.010 ± 0.005	0.007 ± 0.002	0.010 ± 0.005	0.007 ± 0.003
Total Dissolved P[5]	0.019 ± 0.004	0.016 ± 0.004	0.014 ± 0.002	0.015 ± 0.008	0.017 ± 0.007
Total P[5]	0.459 ± 0.746	0.022 ± 0.004	0.021 ± 0.004	0.024 ± 0.010	0.022 ± 0.008

[1]Data was collected from June to October 2003.

Units: [2]pH units, [3]Colour unit, [4]uS cm^{-1}, [5]mg L^{-1}, and [6]NTU

Appendix 3. Continued.

a. Duteau Creek water chemistry data for 2003[1]

Parameter	June	July	August	September	October
pH[2]	7.5 ± 0.1	7.4 ± 0.1	7.3 ± 0.1	7.3 ± 0.1	7.3 ± 0.1
True Colour[3]	43 ± 5	53 ± 5	50 ± 9	38 ± 4	40 ± 6
Specific conductance[4]	39.8 ± 6.6	33.7 ± 2.3	36.0 ± 2.2	34.5 ± 2.3	37.8 ± 4.9
Nonfilterable residue[5]	4 ± 0	6.8 ± 4.4	4 ± 0	4.2 ± 0.4	4 ± 0
Turbidity[6]	1.5 ± 0.3	2.4 ± 0.8	1.6 ± 0.3	1.8 ± 0.4	1.3 ± 0.1
Dissolved Org. C[5]	7.8 ± 0.8	7.9 ± 0.8	9.5 ± 1.1	7.8 ± 0.5	8.5 ± 1.0
Total Organic C[5]	8.1 ± 0.7	7.6 ± 0.7	8.7 ± 0.6	8.0 ± 0.3	7.9 ± 0.7
Total Kjeldahl N[5]	0.27 ± 0.03	0.26 ± 0.03	0.35 ± 0.04	0.26 ± 0.04	0.29 ± 0.04
Total N[5]	0.30 ± 0.03	0.28 ± 0.02	0.36 ± 0.04	0.29 ± 0.03	0.30 ± 0.04
Total Organic N[5]	0.27 ± 0.03	0.25 ± 0.04	0.34 ± 0.04	0.26 ± 0.04	0.28 ± 0.05
Ammonia N[5]	0.006 ± 0.001	0.014 ± 0.010	0.007 ± 0.003	0.005 ± 0.001	0.006 ± 0.001
Dissolved Nitrate N[5]	0.02 ± 0	0.03 ± 0.02	0.02 ± 0	0.03 ± 0.008	0.03 ± 0.01
Nitrite N[5]	0.015 ± 0.011	0.005 ± 0	0.005 ± 0	0.011 ± 0.015	0.005 ± 0
Ortho-P[5]	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Total Dissolved P[5]	0.008 ± 0.003	0.010 ± 0.003	0.012 ± 0.003	0.007 ± 0.001	0.010 ± 0.004
Total P[5]	0.017 ± 0.006	0.015 ± 0.007	0.019 ± 0.004	0.016 ± 0.004	0.015 ± 0.004

[1]Data was collected from June to October 2003.

Units: [2]pH units, [3]Colour unit, [4]uS cm^{-1}, [5]mg L^{-1}, and [6]NTU

b. Duteau Creek water chemistry data for 2004[7].

Parameter	May	June	July	August	September
pH[2]	7.3 ± 0.1	7.4 ± 0.1	7.4 ± 0.1	7.3 ± 0.1	7.4 ± 0.1
True Colour[3]	17 ± 3	39 ± 3	43 ± 5	60 ± 11	43 ± 5
Specific conductance[4]	56.3 ± 14.6	41.7 ± 5.4	38.0 ± 3.2	40.5 ± 2.2	43.2 ± 7.8
Nonfilterable residue[5]	4 ± 0	5.6 ± 2.9	4.5 ± 1.2	4.0 ± 0	4.0 ± 0
Turbidity[6]	1.3 ± 0.2	2.2 ± 1.5	1.6 ± 0.3	2.2 ± 0.9	2.2 ± 0.7
Dissolved Org. C[5]	9.3 ± 0.6	9.9 ± 0.9	10.6 ± 2.0	9.8 ± 1.0	8.4 ± 0.8
Total Organic C[5]	9.3 ± 0.4	9.1 ± 0.3	9.8 ± 0.9	9.3 ± 0.4	8.4 ± 0.9
Total Kjeldahl N[5]	0.30 ± 0.02	0.32 ± 0.03	0.31 ± 0.03	0.38 ± 0.03	0.33 ± 0.05
Total N[5]	0.31 ± 0.02	0.39 ± 0.07	0.33 ± 0.02	0.41 ± 0.02	0.36 ± 0.03
Total Organic N[5]	0.30 ± 0.02	0.32 ± 0.03	0.30 ± 0.03	0.37 ± 0.02	0.32 ± 0.04
Ammonia N[5]	0.005 ± 0	0.005 ± 0	0.008 ± 0.004	0.010 ± 0.007	0.010 ± 0.004
Dissolved Nitrate N[5]	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.03 ± 0.01	0.03 ± 0.01
Nitrite N[5]	0.006 ± 0.001	0.005 ± 0	0.007 ± 0.002	0.006 ± 0.001	0.005 ± 0
Ortho-P[5]	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.007 ± 0.001	0.005 ± 0
Total Dissolved P[5]	0.010 ± 0.002	0.007 ± 0.001	0.009 ± 0.002	0.014 ± 0.005	0.008 ± 0.001
Total P[5]	0.015 ± 0.001	0.021 ± 0.008	0.019 ± 0.004	0.026 ± 0.013	0.017 ± 0.002

[7]Data was collected from May to September 2004.

Units: [2]pH units, [3]Colour unit, [4]uS cm^{-1}, [5]mg L^{-1}, and [6]NTU

Appendix 3. Continued.

a. S. Fortune Creek water chemistry data for 2003[1].

Parameter	June	July	August	September	October
pH[2]	7.7 ± 0.3	7.8 ± 0.3	7.6 ± 0.5	7.7 ± 0.4	7.7 ± 0.3
True Colour[3]	14 ± 5	19 ± 13	20 ± 18	11 ± 8	13 ± 9
Specific conductance[4]	81.3 ± 41.9	123.0 ± 65.3	125.0 ± 91.8	121.8 ± 77.9	136.0 ± 93.7
Nonfilterable residue[5]	4.0 ± 0	4.0 ± 0	4.0 ± 0	4.0 ± 0	4.0 ± 0
Turbidity[6]	0.5 ± 0.1	1.7 ± 2.8	1.0 ± 0.9	1.0 ± 1.0	1.5 ± 1.9
Dissolved Org. C[5]	4.4 ± 1.3	1.8 ± 1.5	3.8 ± 3.0	3.2 ± 2.4	3.7 ± 2.7
Total Organic C[5]	4.7 ± 1.6	3.6 ± 2.8	3.7 ± 3.0	3.3 ± 2.8	3.6 ± 3.0
Total Kjeldahl N[5]	0.14 ± 0.06	0.11 ± 0.09	0.14 ± 0.12	0.14 ± 0.12	0.17 ± 0.16
Total N[5]	0.14 ± 0.05	0.16 ± 0.11	0.18 ± 0.09	0.16 ± 0.11	0.20 ± 0.14
Total Organic N[5]	0.15 ± 0.05	0.14 ± 0.04	0.17 ± 0.08	0.17 ± 0.09	0.20 ± 0.12
Ammonia N[5]	0.005 ± 0	0.009 ± 0.007	0.006 ± 0.001	0.005 ± 0	0.007 ± 0.005
Dissolved Nitrate N[5]	0.02 ± 0	0.04 ± 0.03	0.045 ± 0.03	0.04 ± 0.03	0.04 ± 0.02
Nitrite N[5]	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Ortho-P[5]	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0	0.005 ± 0
Total Dissolved P[5]	0.007 ± 0.003	0.005 ± 0	0.006 ± 0.002	0.005 ± 0	0.008 ± 0.005
Total P[5]	0.005 ± 0	0.005 ± 0	0.009 ± 0.006	0.007 ± 0.002	0.010 ± 0.005

[1]Data was collected from June to October 2003.

Units: [2]pH units, [3]Colour unit, [4]uS cm^{-1}, [5]mg L^{-1}, and [6]NTU

b. S. Fortune Creek water chemistry data for 2004[7]

Parameter	May	June	July	August	September
pH[2]	7.3 ± 0.3	7.8 ± 0.2	7.9 ± 0.3	7.7 ± 0.5	7.8 ± 0.3
True Colour[3]	5 ± 0	7 ± 3	10 ± 7	16 ± 17	9 ± 5
Specific conductance[4]	81.5 ± 41.7	92.5 ± 49.8	131.3 ± 72.4	124.3 ± 84.6	142.0 ± 54.5
Nonfilterable residue[5]	4 ± 0	4.0 ± 0	6.5 ± 5.0	4.3 ± 0.5	13.0 ± 14.9
Turbidity[6]	0.5 ± 0.2	0.5 ± 0.2	5.6 ± 9.8	1.0 ± 0.7	9.5 ± 10.9
Dissolved Org. C[5]	5.9 ± 0.8	4.2 ± 1.3	3.3 ± 2.2	3.8 ± 2.7	2.8 ± 1.7
Total Organic C[5]	4.7 ± 0.5	3.9 ± 1.2	3.1 ± 2.3	3.2 ± 2.5	3.3 ± 2.6
Total Kjeldahl N[5]	0.16 ± 0.09	0.10 ± 0.05	0.13 ± 0.11	0.15 ± 0.14	0.18 ± 0.14
Total N[5]	0.17 ± 0.08	0.11 ± 0.05	0.16 ± 0.13	0.18 ± 0.12	0.20 ± 0.14
Total Organic N[5]	0.016 ± 0.08	0.12 ± 0.02	0.15 ± 0.09	0.18 ± 0.10	0.18 ± 0.11
Ammonia N[5]	0.005 ± 0	0.006 ± 0.003	0.008 ± 0.003	0.006 ± 0.002	0.013 ± 0.010
Dissolved Nitrate N[5]	0.02 ± 0	0.02 ± 0	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.01
Nitrite N[5]	0.006 ± 0.001	0.005 ± 0	0.006 ± 0.002	0.007 ± 0.003	0.005 ± 0
Ortho-P[5]	0.005 ± 0	0.005 ± 0	0.007 ± 0.002	0.005 ± 0	0.005 ± 0
Total Dissolved P[5]	0.005 ± 0	0.006 ± 0.002	0.005 ± 0	0.005 ± 0	0.005 ± 0
Total P[5]	0.007 ± 0.003	0.005 ± 0	0.011 ± 0.011	0.012 ± 0.008	0.017 ± 0.014

[1]Data was collected from June to October 2003.

Units: [2]pH units, [3]Colour unit, [4]uS cm^{-1}, [5]mg L^{-1}, and [6]NTU

Appendix 4. Total fecal coliform (FC) counts for each site on each stream for 2003 and 2004.

Creek	Year	Site	FC
BX	2003	I	86
		II	1567
		III	1050
	2004	I	52
		II	813
		III	1365
Deer	2003	I	74
		II	4600
		III	1413
	2004	I	80
		II	2618
		III	1530
Duteau	2003	I	283
		II	1839
		III	1662
	2004	I	149
		II	1950
		III	2037
S. Fortune	2003	I	87
		II	52
	2004	I	129
		II	465

Appendix 5. Test of significance (P-values) for differences in fecal coliform (FC) counts between sites on each stream for 2003 and 2004.

Creek	Year	Site comparison	P-value
BX	2003	I - II	< 0.0001
		I - III	< 0.0001
		II - III	< 0.0001
	2004	I - II	< 0.0001
		I - III	< 0.0001
		II - III	< 0.0001
Deer	2003	I - II	< 0.0001
		I - III	< 0.0001
		II - III	< 0.0001
	2004	I - II	< 0.0001
		I - III	< 0.0001
		II - III	< 0.0001
Duteau	2003	I - II	< 0.0001
		I - III	< 0.0001
		II - III	0.003
	2004	I - II	< 0.0001
		I - III	< 0.0001
		II - III	0.17
S. Fortune	2003	I - II	0.003
	2004	I - II	< 0.0001

Appendix 6. Test of significance (P-values) for differences in fecal coliform (FC) counts between years for each site on each stream for 2003 and 2004.

Creek	Site	Year comparison	P-value
BX	I	2003 - 2004	0.004
	II	2003 - 2004	< 0.0001
	III	2003 - 2004	< 0.0001
Deer	I	2003 - 2004	0.63
	II	2003 - 2004	< 0.0001
	III	2003 - 2004	0.03
Duteau	I	2003 - 2004	< 0.0001
	II	2003 - 2004	0.07
	III	2003 - 2004	< 0.0001
S. Fortune	I	2003 - 2004	0.005
	II	2003 - 2004	< 0.0001

Appendix 7. Total fecal coliform (FC) counts on each stream for June through September[1] 2003 and 2004.

Creek	Year	Month	FC
BX	2003	June	778
		July	446
		August	561
		September	918
	2004	June	232
		July	590
		August	1078
		September	330
Deer	2003	June	426
		July	1522
		August	1700
		September	2439
	2004	June	852
		July	739
		August	569
		September	2068
Duteau	2003	June	1465
		July	642
		August	1070
		September	607
	2004	June	2922
		July	566
		August	274
		September	374
S. Fortune	2003	June	35
		July	62
		August	24
		September	18
	2004	June	56
		July	258
		August	99
		September	181

[1] Note analysis was conducted on data from June through September for each year since only one sampling occurred in October (2003) and May (2004).

Appendix 8. Test of significance (P-values) for differences in total fecal coliform (FC) counts between months[1], on each stream for 2003 and 2004.

Creek	Month	Year comparison	P-value
BX	June	2003 - 2004	< 0.0001
	July	2003 - 2004	< 0.0001
	August	2003 - 2004	< 0.0001
	September	2003 - 2004	< 0.0001
Deer	June	2003 - 2004	< 0.0001
	July	2003 - 2004	< 0.0001
	August	2003 - 2004	< 0.0001
	September	2003 - 2004	< 0.0001
Duteau	June	2003 - 2004	< 0.0001
	July	2003 - 2004	0.03
	August	2003 - 2004	< 0.0001
	September	2003 - 2004	< 0.0001
S. Fortune	June	2003 - 2004	0.03
	July	2003 - 2004	< 0.0001
	August	2003 - 2004	< 0.0001
	September	2003 - 2004	< 0.0001

[1] Note analysis was conducted on data from June through September for each year since only one sampling occurred in October (2003) and May (2004).

Appendix 9. Total fecal coliform (FC) counts on each stream[1] for each year.

Year	Creek	FC
2003	BX	1136
	Deer	1487
	Duteau	1945
	S. Fortune	139
2004	BX	1417
	Deer	1610
	Duteau	2186
	S. Fortune	594

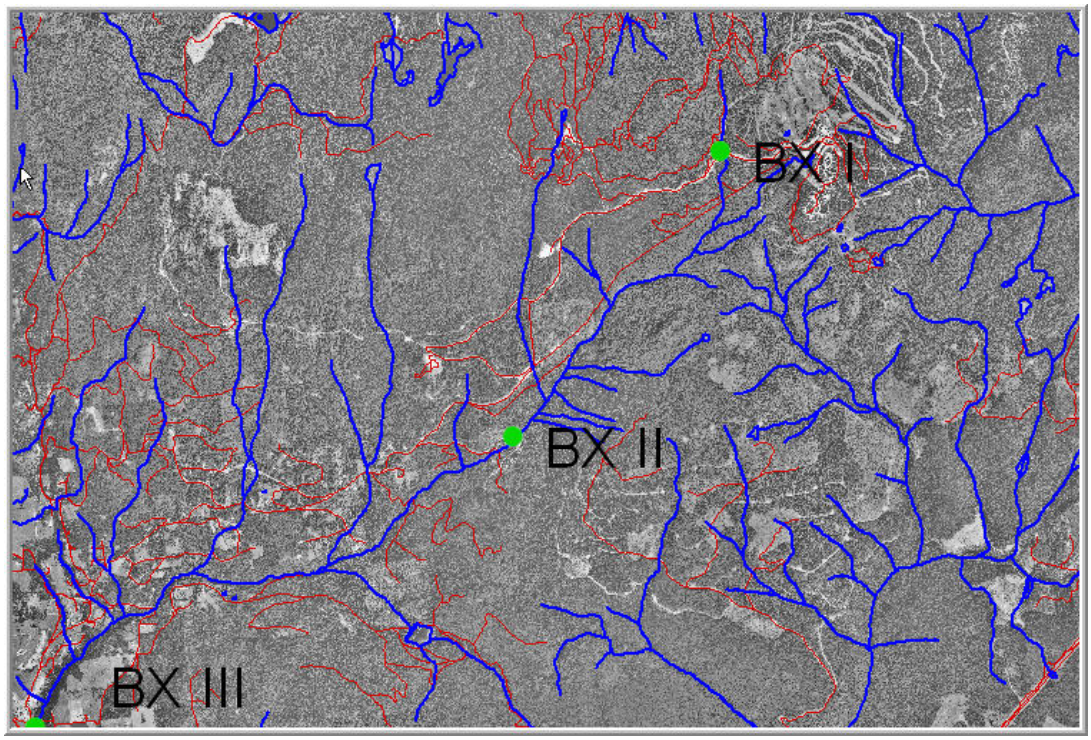
[1] Note analysis was conducted over sites I and III for each stream since S. Fortune only had 2 sites.

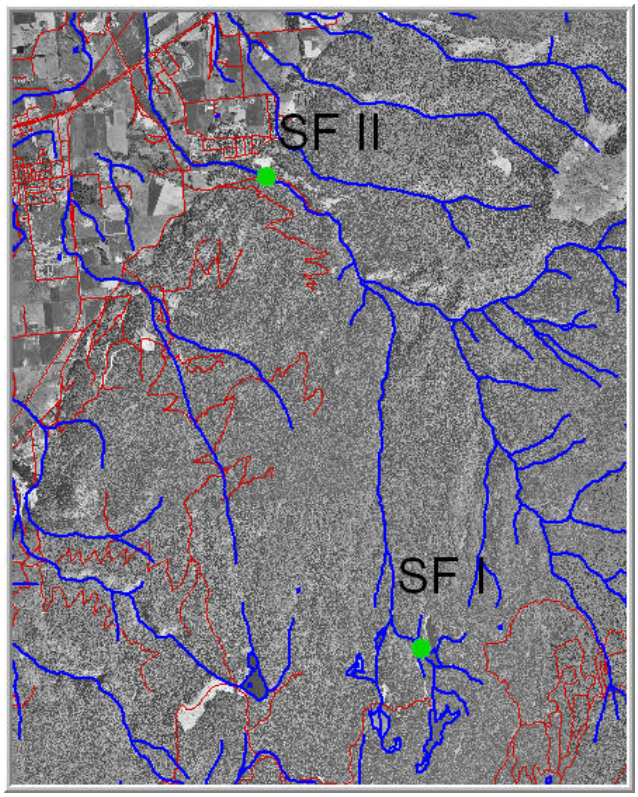
Appendix 10. Test of significance (P-values) for differences in total fecal coliform (FC) counts between each stream[1] for each year.

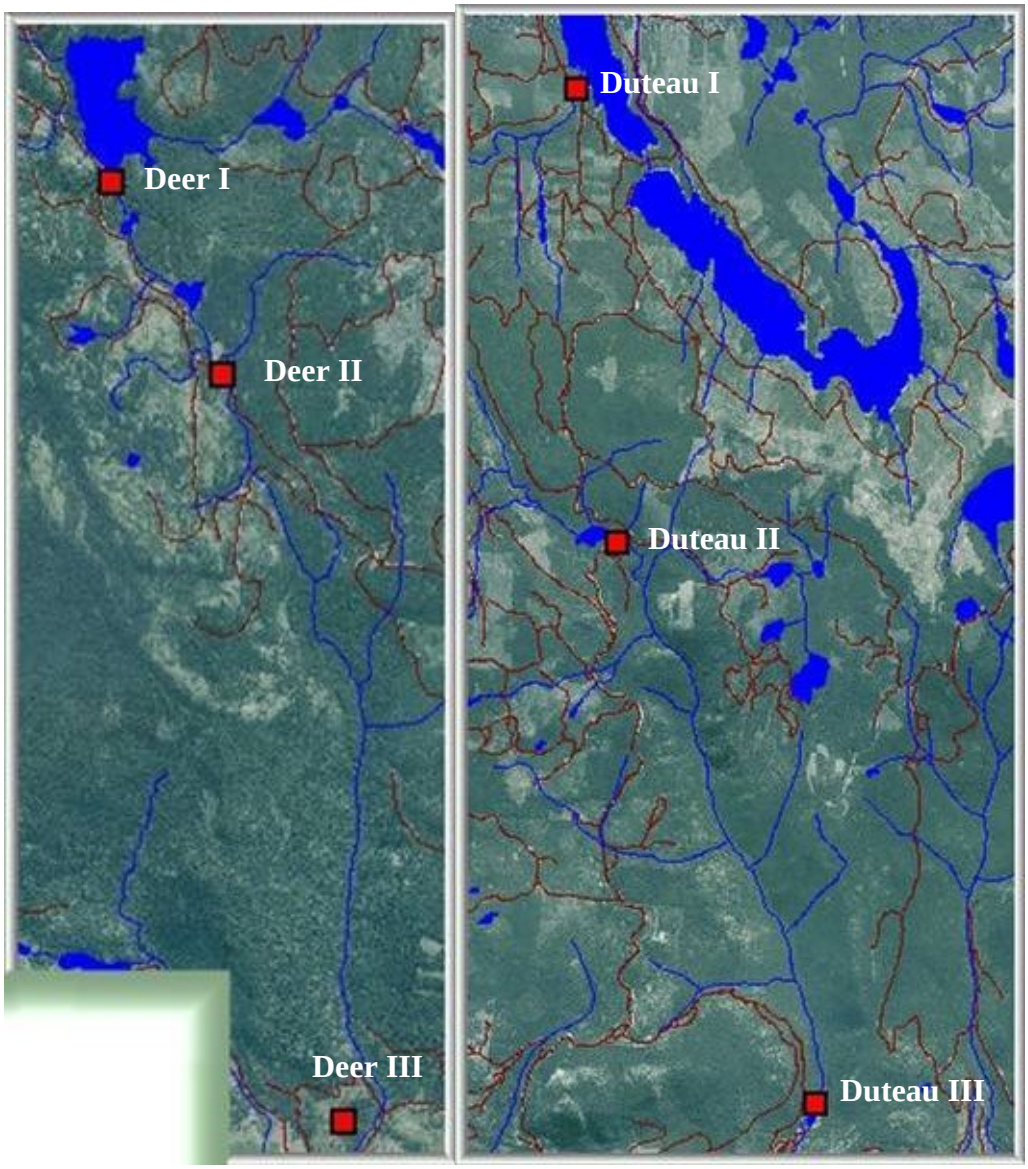
Year	Stream comparison	P-value
2003	BX - Deer	< 0.0001
	BX - Duteau	< 0.0001
	BX – S. Fortune	< 0.0001
	Deer – Duteau	< 0.0001
	Deer – S. Fortune	< 0.0001
	Duteau – S. Fortune	< 0.0001
2004	BX - Deer	0.0005
	BX - Duteau	< 0.0001
	BX – S. Fortune	< 0.0001
	Deer – Duteau	< 0.0001
	Deer – S. Fortune	< 0.0001
	Duteau – S. Fortune	< 0.0001

[1] Note analysis was conducted over sites I and III for each stream since S. Fortune only had 2 sites.

Appendix 11 – Maps of BX, S. Fortune, Deer, and Duteau watersheds.






Deer I
Deer II
Deer III
Duteau I
Duteau II
Duteau III