From Nuisance to Resource: Understanding Microbial Sources of Contamination in Urban Stormwater-Impacted Bodies of Water Intended for Water Reuse Activities

by

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Abstract:

Harvesting stormwater provides the province of Alberta, Canada, with a strategy to address the growing demands on water resources due to climate change and projected population growth. However, stormwater reuse poses a variety of challenges due to the potential of this source to be of low water quality, and to be contaminated with human and animal feces, and thus enteric bacterial pathogens including *Campylobacter* spp., *Salmonella* spp., and pathogenic *E. coli.* Storm events are correlated with an increased prevalence of disease, likely due to the mobilization of pathogens in the environment, leading to increased exposure and transmission risks. The contamination of water with human and/or animal excreta possesses significant risks to human health - albeit the risks associated with pathogens found in sewage are greater than those associated with animal wastes. Several recent studies have demonstrated that human feces are commonly found in urban stormwater systems, and therefore, urban stormwater risks associated with its use must be better understood in terms of contamination sources. Consequently, an objective of this thesis was to identify sources of contamination and select enteric bacterial pathogens, due to consequences associated with illness, present in various urban stormwater-impacted bodies of water in Calgary and Airdrie, Alberta, Canada.

Throughout the 2017 sampling season (i.e., May – September) 700 samples were collected from various stormwater-impacted bodies of water in Alberta, Canada. *Bacteriodes*-specific markers were used to identify sources of contamination (i.e., Human, Dog, Muskrat, Ruminant, Birds, and Canadian Goose) and pathogens present (i.e., *Arcobacter* spp.*, Campylobacter* spp.*, Salmonella* spp.*,* and shiga-toxin producing *E. coli* [STEC]) through qPCR. Culture-based methods for *Campylobacter* spp. and *Arcobacter* spp. were used on select stormwater samples to further determine the risks. Routine testing of fecal indicator bacteria (FIB) using culture-based methods (i.e., coliforms, thermotolerant coliforms, *E. coli* and

Enterococcus spp.) and molecular-based methods (e.g., qPCR *Enterococcus* spp.) was done to assess overall microbial water quality and for comparing stormwater quality against existing water quality standards.

This thesis research study will help to bring about a better understanding as to the risks from pathogens in the stormwater-impacted bodies of water in Alberta and aid in the development of governmental regulations for water reuse (e.g., baselines, treatment, and long-term municipal planning). Trends from this research show that the urban stormwater ponds face poor water quality, frequently contaminated with human fecal contamination, and the presence of pathogens. The USEPA guidelines for recreational water were violated in 20% of water samples for *E. coli* (i.e., running geometric mean) and in 17% of samples for *Enterococcus* spp. (i.e., STV). Microbial source tracking results reflected that there were two dominant sources of fecal pollution in the urban stormwater ponds: human and seagull (i.e., HF183 in 28% of samples and LeeSg in 9% of samples, respectively). The most dominant pathogen present was *A. butzleri* in 25% of stormwater samples. In order to determine the pathogenic nature of *A. butzleri*, a virulence gene screen was performed on nine putative virulence genes. The results from this additional testing indicate that the *A. butzleri* present in the urban stormwater ponds may be pathogenic.

Dedication:

This thesis is dedicated to my parents (Dave and Jackie) and sister (Rachel) in the United States. They helped to keep me upright when I would fall down, provided an endless amount of encouragement, and taught me to work hard for the things I aspire to achieve.

In addition, I dedicate this thesis to my Aunt Ruth in Nevada. There is no one in the world as patient, kind, and helpful. She has spent countless days and nights providing me guidance throughout the writing of this document. I cannot thank you enough.

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1 Introduction

1.1 Background

Freshwater is a finite resource. Countries all over the world are struggling to meet the growing demands on freshwater resources. Several countries, (e.g., Australia, Israel, and the United States) are investigating ways to address freshwater shortages, including the reuse of wastewater and stormwater as alternative water resources. In Canada, portions of the western prairie provinces are semi-arid and face higher risks of water scarcity issues than other provinces (Schindler & Donahue, 2006). Compounding this problem is the increase in Alberta's population, growth in the agriculture industry, and the large oil and gas industry, all of which contribute to an increase in freshwater use. To meet these growing demands for freshwater, industries, municipalities and governmental agencies are working together to investigate alternative modes of water reuse.

Using recycled water has a multitude of environmental benefits. These benefits encompass maintaining current water resources, curtailing the demand for drinking water, and helping to protect ecosystems by reducing the amount of water redirected from them (Chandrasena, *et al*., 2016; Makepeace, *et al*., 1995; Petterson, *et al*., 2016). However, studies have found that the presence of fecal contamination in stormwater-impacted bodies of water negatively impacts public health due to the presence of pathogens in human and animal feces (Sauer, *et al*., 2011; Converse, *et al*., 2011; Newton, *et al*., 2013; Chase, *et al*., 2012; Sidhu, *et al*., 2012). Consequently, this thesis research aimed to provide: a) baseline microbial water quality information about urban stormwater-impacted bodies of water in Alberta, Canada; b) information regarding the primary sources of fecal pollution impacting these stormwater

collection systems; and c) details about the dominant enteric bacterial pathogens present in stormwater and that may affect human health. This research will provide important requisite information for developing quantitative microbial health risk assessments (QMRAs) about stormwater, which in turn are going to be useful for the potential derivation of future public health standards and regulations regarding stormwater use in Alberta, Canada.

1.2 Stormwater

There are several types of alternative waters that can be reused, including rainwater, wastewater, and stormwater. Wastewater is water produced from the treatment of sewage. Rainwater is precipitation that does not touch the ground and is collected from roofs or impermeable surfaces above ground. Stormwater is precipitation collected from ground surfaces. Stormwater is often collected in retention ponds or directly discharged to rivers.

Stormwater has been typically seen as a nuisance that needs to be managed; however, it is now being increasingly viewed as an important alternative water resource that can be used safely for water-fit-for-purpose activities. Water-fit-for-purpose refers to water of sufficient quality for safely meeting the public health risks associated with end use of the reclaimed water (Chhipi-Shrestha, *et al*., 2017). Intended end-use activity includes using stormwater as irrigation water for crops, community gardens, recreational parks, sports fields, and golf courses (Begum, *et al*., 2008; Chong, *et al*., 2013; Nnadi, *et al*., 2015; Ahmed, *et al*., 2011) as well as non-irrigation related activities (i.e., toilet/urinal flushing, washing of equipment, aesthetic features [fountains], etc.). The approach of water-fit-for-purpose can consider three main objectives: the intended end use, economic viability, and environmental sustainability (Chhipi-Shrestha, *et al*., 2017). Collecting and processing stormwater helps to create sustainable environments (Begum, *et al*., 2008). Australia is considered a leader in harvesting stormwater and rainwater for potable

(drinking water) and non-potable (non-drinking water) purposes: 44% for irrigation water, 15% for toilet flushing, 15% for outdoor uses (i.e., car washing and water features), 8% for firefighting, along with 8% for potable use (Hatt & Deletic, 2005). In most countries though, stormwater is underutilized as a water resource (Liu, *et al*., 2015; Shannak, *et al*., 2014; Al-Salaymeh, *et al*., 2011).

The original and primary function of stormwater collection was to control urban flooding and reduce biological, physical and chemical contaminant hazards in urban areas prior to the discharge of excess water into other receiving water bodies (e.g., rivers, lakes, and oceans) (Begum, *et al*., 2008; Sidhu, *et al*., 2012). These urban environment catchment areas can be a critical source of pollution for three main reasons. Firstly, compared to natural environments, urban environments may have increased pollutant loading due to industrial, commercial, and residential activities (Li, *et al*., 2015). Secondly, the increase in impervious surfaces allows for pollutant build-up, which then becomes part of the stormwater runoff during storm events (Monaghan, *et al*., 2016; Begum, *et al*., 2008; Chandrasena, *et al*., 2016; Vogel & Moore, 2016; Wijesiri, *et al*., 2016). Therefore, stormwater has the ability to rapidly move accumulating pollutants into receiving bodies of water, and potentially impair them (Vogel & Moore, 2016; Wijesiri, *et al*., 2016; Zhoa & Li, 2013; Makepeake, *et al*., 1995). Lastly, some urban drainage designs allow for the mixing of raw sewage with stormwater during heavy precipitation events (i.e., combined sewer overflows [CSOs]). Even in separated systems (i.e., storm drainage separate from sanitary), there is a potential for raw sewage to get into stormwater systems.

Stormwater is not always treated before entering receiving waters, and should it be impacted with sewage, it may impair the quality of receiving bodies of water and cause a public health risk (Panasiuk, *et al*., 2015). Exposure to raw sewage (or water contaminated with raw

sewage) is associated with viral, bacterial, protozoan, and helminthic diseases, including salmonellosis, cholera, hepatitis A, as well as other diarrheal diseases (Lam, *et al*., 2015; Dickin, *et al*., 2016; Shakir, *et al*., 2017). Raw sewage can enter stormwater through failing infrastructure (e.g., broken sewer pipes and leakage), illicit cross-connections, defective sewage systems, weather-related flooding events, and general water runoff (Hughes, *et al*., 2017; Panasiuk, *et al*., 2015). Many studies have demonstrated that stormwater is often contaminated with raw human sewage (Sauer, *et al*., 2011; Converse, *et* al., 2011; Newton, *et al*., 2013; Chase, *et al*., 2012; Sidhu, et *al*., 2012; Tang, *et al*., 2013; Steele, *et al*., 2018; Hughes, *et al*., 2017; Shanks *et al*., 2013), which can lead to increased risks of waterborne outbreaks of disease. For example, an outbreak of *Cryptosporidium* in Sweden in 2010-2011 was linked to sewage contamination in storm sewer systems, where the stormwater had been released into the local drinking water supply, Storsjon Lake (Panasiuk, *et al*., 2015).

Additional concerns associated with stormwater reuse include aging infrastructure, lack of specific pathogen-related regulatory standards in North America, and public perceptions about the quality and overall safety of water reuse. More specifically, stormwater reuse faces several challenges associated with the stormwater infrastructure problems, which are time-consuming to investigate and expensive to fix (Begum, *et al*., 2008).

1.3 Acute Microbial Hazards of Stormwater

Microbes represent the primary acute health hazard associated with stormwater (Lim, *et al*., 2015); and as such this thesis focused on a detailed characterization of the microbial quality of stormwater in the urban municipalities of Calgary and Airdrie, Alberta, Canada. Microbial hazards include bacterial pathogens, parasites, protozoa, helminths, and viruses, with viruses signifying the primary threat to public health (Lim, *et al*., 2015; Scallen, *et al*., 2011). More

specifically, norovirus and adenovirus are often studied as these are key contributors to human illness (Scallen, *et al*., 2011). Although viruses are shed in high numbers and cause infections at low doses, and therefore drive most human health risk assessment models, infections from enteric bacterial pathogens are also important and can have more important long-term clinical sequelae associated with infection. Parasites, as protozoans and helminths, can persist in water and the environment for long periods of time; have been shown to be important causes of waterborne disease; and include protozoans, as *Cryptosporidium* and *Giardia*, and helminths, as *Ascaris* spp.

1.4 Microbial Water Quality Indicators

There are several factors to consider in assessing microbial contamination of stormwater, including the process by which stormwater becomes contaminated, the duration to which stormwater is exposed to contaminants, and the overall levels of contamination (Erikson, *et al*., 2007). Therefore, many governmental agencies or departments, as the United States Environmental Protection Agency (USEPA) or Health Canada recommend the use of microbial fecal indicator bacteria (FIB) (e.g., *Enterococcus* spp., thermotolerant coliforms, and *E. coli*) to help determine water quality. FIB are enteric bacteria found in the digestive tract of humans and animals and excreted in the feces, and therefore their presence in stormwater is indicative of fecal contamination. By association, the presence of FIB implies the potential presence of fecal-orally transmitted pathogens.

The 1972 Clean Water Act has defined a pathogen indicator as "a substance that indicates the potential for human disease" (EPA, 2012). In this context, there are several attributes than ideal FIB would possess, such as: a) are present whenever pathogens are present (i.e., acting as a surrogate for pathogens); b) occur in greater numbers than the pathogens, albeit still correlate

with their presence; c) have no "after-growth" in the environment; d) survive/persist greater than or equal to pathogens; and e) have constant characteristics (Bonde, 1966). In addition, it is important that FIB indicator occurrence and prevalence correlate with human health outcomes, as done through epidemiological investigations (EPA, 2012). Finally, laboratory methods for FIB assessment should be relatively cost-effective when compared to pathogen-specific alternatives and easier to measure than microbial pathogens. Most pathogen-specific detection methods can be expensive, challenging, and laborious (Wade, *et al*., 2006; Steele, *et al*., 2018; Wade, *et al*., 2003).

1.4.1 Enterococcus spp.

The genus *Enterococcus* was created in 1984 in order to accommodate a fecal-specific species (i.e., group D streptococci) from the *Streptococcus* genus (Bartram & Rees, 2000; Lebreton, *et al*., 2014). Classic biochemical definitions for enterococci include the designation as Gram positive bacteria able to grow between 10˚C and 45 ˚C and at elevated pH (9.6), resist to temperatures of 60 ˚C for 30 minutes; grow in the presence of 6.5% sodium chloride, and have the ability to reduce 0.01% methylene blue (Bartram & Rees, 2000). Species within the *Enterococcus* genus may occur within the human and animal gastrointestinal tracts (Bartram & Rees, 2000), in addition to fermented food and dairy (Giraffa, 2006), and environments, including soil and water (Bartram & Reese, 2000).

A significant amount of literature focuses on two key species: *E. faecalis* and *E. faecium,* which are often found in human and animal feces in large quantities (Bartram & Rees, 2000; Boehm & Sassoubre, 2014). Furthermore, Layton *et al*. (2010) reported that Enterococci have been detected in high concentrations (i.e., 10^4-10^6 bacteria/gram wet weight) in human feces. In addition to being present in high numbers, Leclerc *et al*. (2001) noted in their study that *Enterococcus* spp. was identified in 100% of human fecal samples. Furthermore, Layton *et al*.

(2010) identified *E. faecium* in 100% of human fecal and sewage samples; and found *E. faecalis* and *E. faecium* to be the dominant species of *Enterococcus* spp. in these samples (Layton *et al*., 2010). Therefore, the majority of research has focused on developing selective media to enrich for these two specific populations, as they could be used to assess water quality. It is important to note that other species of *Enterococcus* spp. have been identified in feces (i.e., *E. gallinarum*, *E. avium, E.hirae*), and may also be isolated from these media. *E. hirae* has been associated with animal microflora (Tannock & Cook, 2002), *E. avium* has been associated with gull and dog fecal material (Layton, *et al*., 2010), and *E. gallinarum* has been identified in human sewage and dog fecal material (Layton, *et al*., 2010). Enterococci fulfill many of the aforementioned criteria to make a suitable FIB, while also not being commonly detected in unpolluted waters (Ashbolt, *et al*., 2001).

Several studies have shown a relationship between illness and enterococci in fresh or marine waters throughout the world (Cabelli, *et al*., 1982; Boehm & Soller, 2011; Wade, *et al*., 2003; Wade, *et al*., 2006; Wiedenmann, *et al*., 2006). When high levels of *Enterococcus* spp. are found in fresh or marine water, it has been associated with adverse health outcomes in humans including enteric, skin, eye, and ear infections (Cabelli, *et al*., 1982). Increased levels of *Enterococcus* spp. can indicate the presence of pathogens, including norovirus, *Campylobacter* spp., and *Salmonella* spp. (Arnold, *et al*., 2013). A statistically significant correlation has been found between *Enterococcus* spp. measured through molecular-based methods and gastrointestinal illness in swimmers in water contaminated with human sewage (Fewtrell & Kay, 2015; Wade, *et al*., 2010; Colford, *et al*., 2012; Yau, *et al*., 2014; Wade, *et al*., 2003). These studies, and the criteria that were based off of them, may still be relevant for stormwater-impacted bodies of water for several reasons, which will be discussed in Chapter 6.

Overall, enterococci are considered a good indicator for enteric bacterial pathogens (EPA, 2012). However, they are less effective as an indicator for viruses and protozoa as several studies have not been able to show a relationship between enterococci concentrations and enteric viruses or protozoa (Jiang, *et al*., 2001). Although *Enterococcus* spp. may act as a good water quality indicator, they may also occur in environmental forms. Some species such as *E. haemoperoxidus*, *E. moraviensis*, and *E. aquirmarinus*, appear to members of the natural microbiota of aquatic environments (Byappanahalli, *et al*., 2012).

1.4.2 Thermotolerant Coliforms and Total Coliforms

Other FIB include the thermotolerant coliforms and total coliform groups. Total coliforms refer to a group of gram-negative, rod-shaped, non-spore forming, oxidase-negative bacteria. They are facultatively anaerobic and produce acid and gas from the fermentation of lactose sugar with ß-galactosidase within 24 hrs. In addition, total coliforms are capable of growth at 37˚C, while thermotolerant coliforms are capable of growth at 44-45˚C. Thermotolerant coliforms are also known as fecal coliforms.

Coliforms are a diverse grouping of bacteria from many different bacteria under the Enterobacteriaceae family. The coliform group is now commonly defined by ortho-nitrophenyl-ß-galacotoside (ONPG) activity within the Enterobacteriaceae; and utilizing this definition, it consists of 19 genera and 80 species (Leclerc, *et al*., 2001). There are four main members of the coliform group: *Klebsiella, Escherichia, Enterobacter*, and *Citrobacter* (Leclerc, *et al*., 2001). The *Klebseilla* genus includes the species *K. pneumoniae* and *K. oxytoca*. The *Enterobacter* genus includes two species: *E. aerogenes* and *E. cloacae*. *Citrobacter* contains three species: *C. freundii, C. koseri*, and *C. amalonaticus*. The *Escherihcia* genus includes the species *E. coli*.

Coliform bacteria may be found in many different sources, including feces and/or the intestines of warm-blooded animals, aquatic sources, or associated with plants. Some coliform

bacteria (e.g., *E. coli, K. oxytoca*, and *K. pneumoniae*) are abundant in the feces of warm-blooded animals, and were some of the first water quality indicators utilized. There are various classification schemes for identifying coliforms, with the earliest occurring in the beginning of the 20th century. One of these assays was developed by MacConkey in 1909, which recognized 128 different coliform types. As the need for a simple and relatively rapid method to determine water quality intensified, the Multiple-Tube Fermentation Test (known as the Most Probable Number Procedure) was invented (Ashbolt, *et al*., 2001). This technique was one of the first generally accepted methods for determining the levels of coliforms in water samples, even though further testing was required to determine coliform type (e.g., thermotolerant) (Ashbolt, *et al*., 2001).

Thru the 1950's, the use of a membrane filtration method became more widespread to detect coliforms; and in the 1960's, the U.S. Public Health Service recommended the use of fecal coliform bacteria as a FIB (EPA, 2012). Over the next fifteen years, the allowable levels of total coliforms and thermotolerant coliforms (i.e., fecal coliforms) were adjusted several times in the United States and later on dropped in favor of *Enterococcus* and *E. coli* as FIB (EPA, 2012). However, the presence of non-fecal bacteria within the coliform group and the ability of thermotolerant coliforms to grow in the environment were concerning. Furthermore, there had been reports of outbreaks of disease in the absence of coliforms (Smith & Rose, 1998). These findings, paired with the fact that coliform bacteria are not exclusively of fecal origin, question the utility of using these indicators broadly for water quality assessment (Hachich, *et al*., 2012).

Coliforms may serve as an adequate indicator for assessing the water quality of treated drinking water. USEPA's 2018 Edition of the Drinking Water Standards states that the maximum contaminant level goal (MCLG) for total coliforms is zero and the maximum contaminant level

(MCL) is 5.0%, and therefore no more than 5.0% of samples may be total coliform-positive in a month. For each sample in which total coliforms are detected, there must be additional testing for thermotolerant coliforms, as no thermotolerant coliforms are allowed in drinking water.

1.4.3 *E. coli*

Another commonly used bacterial indicator is *E. coli*. These bacteria are rod-shaped, non-spore forming Gram-negative bacilli from the Enterobacteriaceae family. Optimal growth temperature for *E. coli* is 37˚C, though it can survive from 2.5˚C - 45˚C. *E. coli* is a type of fecal coliform that can produce indole from tryptophan. The optimal pH for *E. coli* is neutral; however, it can survive from pH 4.5-9. Many strains of *E. coli* are motile via peritrichous flagella.

E. coli is generally considered a good indicator for fecal contamination of water due to their abundance in feces (i.e., 10^9 cells per gram), and often occurring at greater concentrations in the feces than *Enterococcus* spp. (Edberg, *et al*., 2000; Environmental Protection Agency, 2012). Studies have found *E. coli* to be present in 94% of human fecal samples (Leclerc, *et al*., 2001). Although some *E. coli* strains are considered pathogens, and grouped according to pathotypes, most *E. coli* are commensal residents of the gastrointestinal tract of warm-blooded animals. The classification of *E. coli* pathotypes is based on virulence factors (e.g., shiga-toxin), serotype (e.g., O157), and clinical symptoms (e.g., hemolytic uremic syndrome (HUS)) (Odonkor & Ampofo, 2013).

E. coli has been used as an indicator for fecal contamination for several decades now, as its use became more widespread in the 1980s due to the ability of improved detection methods, and the lack of specificity of other FIB (i.e., thermotolerant coliforms). In addition, USEPA has recommended that *E. coli* or *Enterococcus* spp. replace the use of fecal coliforms as water quality indicators. *E. coli* is considered a more specific fecal indicator than coliforms or

thermotolerant coliforms, given its restriction to a single genera of bacteria (Environmental Protection Agency, 2012). *E. coli* is considered both a coliform and a fecal coliform, but *E. coli* can be distinguished from total and fecal coliforms based on beta-D-glucuronidase activity (Bitton, 2005). In addition, studies have shown that *E. coli* may be a better indicator for disease risk than fecal coliforms (Odonkor & Ampofo, 2013).

E. coli meets many of the aforementioned criteria of a good FIB. Several studies have found *E. coli* concentrations in fresh waters to be correlated with gastrointestinal illness in swimmers (Wade, *et al*., 2003; Marion, *et al*., 2010; Wade, *et al*., 2003). In addition, studies on bodies of water impacted by both point-source and non-point source pollution have been successful in using *E. coli* as an indicator for fecal pollution. Furthermore, studies have found *E. coli* and enteric bacterial pathogens to have similar survival rates in water (Chandran & Hatha, 2005; Rhodes & Kator, 1988). However, similar to *Enterococcus*, *E. coli* is not as effective for predicting human enteric viruses and protozoa, as it has a decreased survival time in comparison. *E. coli* also possesses similar challenges as an indicator like *Enterococcus* spp., in that both can occur in environmental reservoirs.

Some studies suggested that *E. coli* may be able to grow in the aquatic environment (e.g., beach sand) (Hartz, *et al*., 2008; Kon, *et al*., 2007), which in turn may reduce the effectiveness of this indicator in assessing water safety (Hartz, *et al*., 2008). Even though *E. coli* is defined as a single species, sharing many of the same biochemical characteristics (e.g., beta-D-glucuronidase activity), they share only 10% of their pan-genome among individual members (Lukjancenko, *et al*., 2010). Some researchers have described host specific strains of *E. coli* for humans (Clermont, *et al*., 2008), rodents (Kosey, *et al*., 2000), and even wastewater (Zhi, *et al*., 2016)

among others. In particular, Zhi *et al*. (2016) described a niche-specific strain of *E. coli* found in wastewater treatment plants.

In summary, there are many challenges associated with the use of FIB as indicators for water quality. FIB are not always representative of other pathogens in the water as they can be derived from sources besides feces, such as environmental reservoirs as was previously mentioned. Moreover, FIB can serve as poor substitutes for testing some microbial pathogens (e.g., enteric viruses and parasitic protozoa), since they can be more environmentally resistant than FIB. A study by Schriewer *et al*. (2010) found that even when FIB were within USEPA recommended guidelines, there was still the potential for the presence of pathogens. Therefore, in our study, other methods were used in addition to FIB to assess the water quality of urban stormwater-impacted bodies of water.

1.4.4 Assessing Water Quality and Safety

USEPA guidelines allow for the use of molecular- and/or culture-based methods to assess water quality (EPA, 2012). Historically, most water quality analyses focused on the use of culture-based methods, such as most probable number in standardized broth cultures (e.g., lauryl tryptone broth [LTB) (Rice, *et al*., 2017), colony counts from membrane filtration on selective agar (e.g., mFC agar), and defined substrate methods employing presence/absence testing and most probable number (MPN) formats (e.g., Colilert®). It was only as recent as 2012 that molecular-based methods were approved by a regulatory agency (i.e., USEPA) for detecting *Enterococcus* spp. (Environmental Protection Agency, 2012). One significant benefit of using molecular-based methods is the sample processing speed. Culture-based methods currently take at least 18 hours to process water samples and report results, leading to potential danger from a public health standpoint; whereas sample processing with molecular-based methods has the potential to be completed and reported faster, as within five hours depending upon sampling

turnaround time (Noble, *et al*., 2010). The development of molecular-based quantitative polymerase chain reaction (qPCR) technologies for *Enterococcus* spp. quantification has enabled a more rapid measurement of water quality over greater expanses of beaches, thereby providing more public health management flexibility (e.g., as to the need or urgency for beach closure) and with it, greater potential protection to beach-going populations (McQuaig, *et al*., 2006; Noble & Fuhram, 2001). Another advantage to utilizing a molecular-based method for *Enterococcus* spp. is that a DNA extraction step is performed allowing additional molecular tests to be performed on the same sample. Although considered to be highly sensitive tests, the sensitivity of molecular tests such as PCR can be compromised due to the small analytical volumes used.

Water quality is often evaluated using a variety of FIB targets, largely based on the regulatory jurisdiction having authority. For example, federal agencies such as USEPA can set nation-wide standards for which individual states can adopt these standards or achieve higher standards. Health Canada sets national guidelines for which individual provinces can adapt these guidelines or set their own standards. An example of FIB standards used for regulatory/guidance purposes is provided in Table 1-1.

Table 1-1: An overview of water quality standards/guidelines for select water quality indicators for the USEPA, Health Canada, and Alberta Environment and Parks.

Overview of Water Quality Standards/Guidelines				
Agency	**FIB**	**Type of Water**	**Standard**	**Estimated Illness Rate**
US EPA	*E. coli* (culture-based methods)	Recreational	STV: 410 CFU/100mL GM: 126 CFU/100mL BAV: 235 CFU/100mL	36 per 1000 primary contact recreators
			STV: 320 CFU/100mL GM: 100 CFU/100mL BAV: 190 CFU/100mL	32 per 1000 primary contact recreators
	Enterococcus spp. (culture-based methods)	Recreational	STV: 130 CFU/100mL GM: 35 CFU/100mL BAV: 70 CFU/100mL	36 per 1000 primary contact recreators
			STV: 110 CFU/100mL GM: 30 CFU/100mL BAV: 60 CFU/100mL	32 per 1000 primary contact recreators
	Enterococcus (molecular-based methods)	Recreational	STV: 2000 CCE/100mL GM: 470 CCE/100mL BAV: 1000 CCE/100mL	36 per 1000 primary contact recreators
			STV: 1280 CCE/100mL GM: 300 CCE/100mL BAV: 640 CCE/100mL	32 per 1000 primary contact recreators
Health Canada	*E. coli* (culture-based methods)	Recreational	5-sample GM: $\leq$ 200 *E. coli*/100mL STV: $\leq$ 400 *E. coli*/100mL	10-20 illnesses per 1000 swimmers
	Enterococcus spp. (culture-based methods)	Recreational	5- sample GM: $\leq$ 35 *Enterococci*/100mL STV: $\leq$ 70 *Enterococci*/100mL	10-20 illnesses per 1000 swimmers
Alberta Environment and Parks	*E. coli* (culture-based methods)	Surface Water	STV: $\leq$320 CFU/100mL GM: $\leq$100 CFU/100mL	N/A
	Thermotolerant coliforms (culture-based methods)	Municipal, stormwater, wastewater	STV: $\leq$400 CFU/100mL	N/A

Two different criteria are commonly used to determine water quality based on FIB: a) the statistical threshold value (STV), which is based off of a single sample, and b) a multi-sample running geometric mean (GM). Each numeric concentration threshold provides its own benefits and limits. The GM is a running temporal association, based on the *n*th root of a product of *n* numbers, and often used setting microbial standards due to non-normal distribution of microbes often found in the environment. It can provide information on chronic contamination problems. Of note, is that a GM is less susceptible to outliers than an arithmetic mean. Consequently, since a GM normalizes data, it is not an ideal approach for assessing potential peak risks in contamination. The STV represents a single point in time measurement. It approximates the 90^{th} percentile of the water quality and represents a "do not exceed" value. In relation to the GM, the STV should not be exceeded by more than 10% of samples that are used to calculate the GM. In the case of USEPA's 2012 Recreational Water Quality Criteria, an additional target was also considered, and referred to as the beach action value (BAV). The BAV, which can be based on the 5-sample running GM, estimates the 75^{th} percentile of water quality. Its purpose is for notification; and it is considered a more conservative tool for beach management (i.e., react before the STV is violated).

In the early 2000's, USEPA conducted a series of research studies referred to as the National Epidemiological and Environmental Assessment of Recreational Water (NEEAR) studies to determine if current recreational water quality criteria and methods were effective in protecting public health (EPA, 2005); and to further refine USEPA water quality guidelines (EPA, 2012). The majority of recreational water bodies in these studies were beaches that were primarily impacted by wastewater treatment plants-with one site that was primarily influenced by urban runoff. These studies showed a relationship between FIB and human health; and following

these studies, USEPA concluded that the 1986 criteria for fecal coliforms, along with the current criteria for *E. coli* and *Enterococcus* spp. through culture-based methods were still valid. Even so, these indicators, which are used for regulatory purposes, provide little or no information on what sources of fecal pollution are contributing to the contaminant burden.

1.5 Microbial Source Tracking

The field of microbial source tracking aims to identify the specific host sources of fecal pollution impacting water quality (e.g., human, cattle, bird, etc.). Microbial source tracking tools are focused on genotypic and phenotypic differences in microbial populations found in the gut of different animals (Curtis & Trapp, 2016). There are a variety of techniques for microbial source tracking, including determining the antimicrobial resistance patterns of target bacteria; analyzing ratios of fecal coliforms to fecal streptococci; ribotyping; and utilizing mitochondrial DNA methods (Wuertz, *et al*., 2011). Each of these methods has different limits of detection and environmental persistence, which mean that when comparing methods, the results may differ (Wuertz, *et al*., 2011; Curtis & Trapp, 2016).

Microbial source tracking techniques fall into two categories: library independent and library dependent. The library dependent microbial source tracking method compares an environmental sample (e.g., water) to a library of "known source profiles" or bacterial strains (Wuertz, *et al*., 2011; Field, *et al*., 2003). Techniques for the library dependent method include culture-based methods and a combination of culture-based methods with a genetic marker (Wuertz, *et al*., 2011; Field, *et al*., 2003). Examples of culture-based methods include determining the fecal coliform to fecal streptococci ratio in order to indicate if human contamination is present (i.e., if ratio is >1, then assume animal fecal contamination); and

ascertaining the ratio of atypical colonies to typical colonies of total coliforms (Wuertz, *et al*., 2011; Field, *et al*., 2003). These methods are time-consuming, labor-intensive, and call for a sizable number of bacterial strains. In contrast, genetic-based methods involve the ideology that a particular host or environment would have a similar genetic fingerprints to each other (Scott, *et al*., 2002), which will be discussed in the following paragraph.

The library independent method of microbial source tracking does not rely on building a library of bacterial strains from a particular watershed (Field, *et al*., 2003). Many molecular microbial source tracking techniques have emerged over the last two decades, many of which are based on Polymerase Chain Reaction (PCR) methods targeting DNA sequences unique to certain bacteria found in various animals. The *Bacteroides-Prevotella* group is a common target for microbial source tracking assays. Other targets include *Escherichia*, the genera *Catellicoccus*, and members of the order *Bacteroidales* (Wuertz, *et al*., 2011). The PCR-based methods provide a rapid means for identifying potential sources of fecal pollution in the environment. However, there is some uncertainty associated with the use of these types of tools, which are similar to that of FIB, as little is known about the persistence of these microbial signatures in the environment (Wuertz, *et al*, 2011; Harwood, *et al*., 2014; Weidhass, *et al*., 2015). *Bacteroidales* are gram-negative, obligate anaerobes, non-spore forming, and rod-shaped bacteria, and within this order is the genus *Bacteroides* (Harwood, *et al*., 2014; Weidhass, *et* al., 2015). *Bacteroides* are bacteria that are commonly found in the intestine of warm-blooded animals and constitute a large portion of their gut microbiota (e.g., human, dog, etc.) (Weidhass, *et al*., 2015; Bower, *et al*., 2005; Shanks, *et al*., 2009). Several *Bacteroides* spp. have strong host or group specificities as indicated by several studies (Kildcare, *et al*., 2007; Layton, *et al*., 2006). Therefore, the 16S

rRNA genes of host-specific *Bacteroides* spp. can be used as markers for ascertaining human or animal fecal sources of pollution in water (Kildcare, *et al*., 2007; Layton, *et al*., 2006).

Table 1-2: Information on commonly used microbial source tracking targets including target, assay, target species, target locus, cross reaction, and reference.

Target	Assay	Target Species	Target Locus	Cross Reaction	Reference
Human fecal pollution					
Human	HF183	*Bacteroides dorei*	*16S*	Dogs, chickens, pigs, ducks, mongoose, feline	(Haugland, *et al*., 2010)
Human	HumM2	?	alpha-1 2-mannosidase homolog	Dog, chicken	(Shanks, *et al*., 2009)
Sewage	Human Polyomaviruses (i.e., HPyV)	JC virus (PCR) BK virus (qPCR)	None identified	None identified	(McQuaig, *et al*., 2006; McQuaig, *et al*., 2009)
Human	Human Adenovirus	Adenovirus type 40 and 41	Hexon	None identified	(Biofill-Mas, *et al*., 2000; Pina, *et al*., 2009)
Human	BacHum	*Bacteroidales*	*16S*	Dog, chicken	(Kildcare, *et al*., 2007)
Human	BacH	*Bacteroidetes*	*16s*	Cat	(Reischer, *et al*., 2007)
Human	*Cryptosporidium hominis*	*Cryptosporidium hominis*	*18s*	None identified	(Reucker, *et al*., 2011)
Human	Polyomavridae family	BKPyV	*VP*	None identified	(Hundesa, *et al*., 2006)
Human	Pepper Mild Mottle Virus	PMMoV	*126k gene coding for a subunit of the RNA polymerase complex*	Seagull, cow, chicken, goose	(Rasario, *et al*., 2009; Hamza, *et al*., 2011)
Ruminant					
Cattle, goats, sheep, deer	Rum2Bac	*Bacteroides* spp.	*16S*	Septage	(Mieszkin, *et al*., 2010)

Cow, deer, goat, bison, caribou	BacR	*Bacteroides* spp.	*16S*	Chicken, Duck	(Reischer, *et al*., 2006)
Bovine	Bovine adenvirus	*BAdV*	*hexon*	None identified	(Hundesa, *et al*., 2006)
Birds					
Gull	LeeSg	*Catellicoccus marimammaliu m*	*16S*	Chicken	(Lee, *et al*., 2013)
Gull	Gull2	*Catellicoccus marimammaliu m*	*16S*	None identified	(Lu, *et al*., 2008)
Canada Goose	CGO1	*Bacteroides* spp.	*16S*	None identified	(Fremaux, *et al*., 2010)
Dog					
Dog	BacCan SYBR	*Bacteroides* spp.	*16S*	Sewage, chicken, ruminant, duck	(Kildcare, *et al*., 2007)
Dog	Dog3	*Bacteroides* spp.	long chain fatty acid - CoA ligase	sewage	(Green, *et al*., 2014)

Table 1-2 lists commonly used microbial source tracking markers for PCR or qPCR, including target, target species, target locus, and any cross reactions that have been identified. As such, many researchers have sought to develop a human-specific marker to be used for microbial source tracking because of the health risks associated with human sewage (Harwood, *et al*., 2014) (Table 1-2), and as a result, there exists an array of PCR markers that aim to identify human sewage by *Bacteroides* (Table 1-2). In addition to markers that identify fecal sources by *Bacteroides* spp., other target species have been used (e.g., *Catellicoccus marimammalium*, adenovirus, etc.) (Table 1-2). Furthermore, qPCR markers are beneficial to researchers because they can provide information on the extent to which fecal pollution occurs, based on the abundance of qPCR marker detects.

Many source tracking markers that cross-react with species for which they are not intended to identify, as the bacterial species is not specific to a single host (Shanks, *et al*., 2009). The human fecal HF183 marker, which targets *Bacteroides dorei*, was developed after the realization that *Bacteroides dorei* was consistently found across human subjects based on human fecal samples (Sinigalliano, *et al*., 2013; Steele, *et al*., 2018; Bower, *et al*., 2005). However, HF183 is known to cross-react with fecal DNA from other animals, including dogs, pigs, chickens or ducks (McGinnis, *et al*., 2016; Shanks, *et al*., 2009; Green, *et al*., 2014) (Table 1-2), albeit the levels observed in these animals is far less than that observed in humans, and consequently, the concentrations observed in a water source may still be indicative of human contamination. The use of multiple markers allows researchers to lessen or negate the idea that results may be due to cross-reaction.

A multitude of studies have identified human fecal contamination in stormwater by using microbial source tracking markers (Sauer, *et al*., 2011; Converse, *et al*., 2011; Newton, *et al*.,

2013; Chase, *et al*., 2012). Many different types of animal fecal pollution impacts have also been identified in stormwater-impacted bodies of water (Fremaux, *et al*., 2010; Gorham & Lee, 2016; Rutledge, *et al*., 2006; Staley, *et al*., 2016; Ervin, *et al*., 2014; Converse, *et al*., 2012; Goodwin, *et al*., 2016; Lu, *et* al., 2011), including dog fecal pollution (Sauer, *et al*., 2011; Shanks, *et al*., 2009; Staley, *et al*., 2016; Green, *et al*., 2014) and ruminant fecal pollution (Gilbert, *et al*., 2014; Staley, *et al*., 2013; Raith, *et al*., 2013). Although exposure to human fecal material is the greatest driver of human illness, it should be noted that animal waste has the potential to carry zoonotic pathogens, therefore causing disease in humans (Ervin, *et al*., 2014; Harwood, *et al*., 2014). In addition, Soller *et al*. (2010) stated that overall human health risk (measured in Disability Adjusted Life Years [DALY]) associated with *Campylobacter* spp. from bovine fecal sources due to water exposure, is similar to the health risks associated with human enteric viruses found in recreational waters impacted by human sewage. Ascertaining the source of contamination will allow for a better assessment of risk associated with stormwater-impacted bodies of water. Finally, the use of quantitative methods allows for an estimation of fecal concentrations, which can be used for bacterial loading models (Shanks, *et al*., 2008).

1.6 Enteric Bacterial Pathogens

Infections in humans from enteric pathogens occur mainly through direct or indirect contact with feces from an infected source through food or water, or through the fecal-oral route. Enteric bacterial pathogens can enter a stormwater body through a variety of pathways, including contamination from a sewage system, direct defecation from the host, or runoff from nearby land (Toze, 2005). In addition, *Salmonella* spp., *Campylobacter* spp., STEC, and *Arcobacter* spp. are zoonotic pathogens, the type and amount of each pathogen present in the water can vary

depending on the animal host species, as well as the animal's age, the number of animals in the area, the distance from water, land management, and the time of year (McBride, *et al*., 2013). Enteric bacterial pathogens were chosen for several reasons, due to: their burden of disease; their prevalence in waterborne outbreaks; their use as surrogates or reference pathogens in quantitative microbial risk assessments; their relatively low infectious dose and associated health risks; and their association with human fecal contamination or sewage.

Campylobacter spp. are a zoonotic pathogen, and in a number of industrialized countries, it has been implicated in the majority of cases of diarrhea, with *C. jejuni* being the species identified in 90% of cases of diarrhea (Vandenberg, *et al*., 2004). Furthermore, about 20% of infections from *C. jejuni* are from a non-food source (e.g., contaminated water) (Clark, *et al*., 2003). *Campylobacter* spp. does not thrive outside the host environment (Whiley, *et al*., 2013). Common hosts for *Campylobacter* spp. include household pets, cattle, rodents, and birds. Roughly seven million cases of illness due to *Campylobacter* spp. occur annually in the U.S. at a cost of $1.2-6 billion (Clark, *et al*., 2003). *Campylobacter* spp., are rod-shaped, gram-negative bacterium in the same family as *Arcobacter* spp. *Campylobacter* spp. grow in 5-10% O_2 and at 30-45˚C, though will no longer grow at 21% O_2 (i.e., air) and 25 ˚C. The *Campylobacter* genus includes 17 species and six subspecies (Moore, *et al*., 2001). The two most commonly reported species in human illnesses are *C. jejuni* subspecies *jejuni* and *C. coli* (Moore, *et al*., 2001).

The *Arcobacter* genus was created in 1991 in an effort to accommodate aero-tolerant *Campylobacter* spp. (Vandenberg, *et al*., 2004). *Arcobacter* spp. are gram-negative, curved bacteria (Van Driessche & Houf, 2008). Currently, there are 22 species (Van Driessche & Houf, 2008). Potential sources of *Arcobacter* spp. include humans, birds, and livestock. This genus has been characterized as a potential food or waterborne pathogen; and has been implicated in

causing human disease, with such symptoms as bacteremia, diarrhea, and gastroenteritis from three species: *A. butzleri, A. cryaerophilus*, and *A. skirrowii* (Kayman, *et al*., 2012). *A. butzleri* contains the ability to survive and grow in the environment. Further, it can survive at lower temperatures (15-30˚C) than *Campylobacter* spp.; and can grow in the presence of oxygen (Wesley, *et al*., 2000; Van Driessche & Houf, 2008). *Arcobacter* spp. are considered a zoonotic pathogen, and one study identified it as the fourth most frequent bacteria isolated from humans with acute enteric disease (Levican, *et al*., 2013).

Salmonella spp. are a ubiquitous rod-shaped, facultative anaerobic, gram-negative bacterium in the Enterobacteriaceae family. There are two subspecies of *Salmonella* spp.*: S. bongori*, which typically found in cold-blooded animals, and *S. enterica*; and both have the ability to cause illness in humans. A common host for *Salmonella* spp. is birds, which tend to spread the disease through their feces since *Salmonella* spp. resides in their intestine (Krometis, *et al*., 2010).In addition, more than 2500 serotypes exist for *S. enterica* (Helke, *et al*., 2016). *Salmonella* spp. is an extremely resilient bacterium that can survive for weeks in dry conditions or for several months in water (Moore, *et al*., 2003). *Salmonella* spp. causes roughly 93.8 million illnesses and 155,000 deaths worldwide annually (Chia, *et al*., 2012). In the U.S., *Salmonella* caused an estimated one million cases of illness and 380 deaths from 2002-2008 (Chia, *et al*., 2012). *Salmonella* spp. contain the *Salmonella* pathogenicity island (SPI), which comprises many of *Salmonella*'s virulence factors, including a type III secretory system, acid resistance, oxygen resistance, and the ability to invade epithelial cells (i.e., *invA* gene) (Galan, *et al*., 1992). The *invA* gene is commonly used to identify *Salmonella* spp. by way of qPCR because the *invA* gene is distinctive to *Salmonella* spp. (Rahn, et al., 1992; Bulte & Jakob, 1995).

E. coli has caused over 2.8 million cases of acute illness in 21 countries annually (Majowicz, *et al*., 2014). There are various pathotypes of *E. coli* that can cause diarrhea, including enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), and enteroaggregative *E. coli* (EAEC) (Table 1-3). Of particular concern are the EHEC, primarily characterized by the presence and production of shigatoxin. One such set of virulence genes are the shiga-toxins, which constitute a family of genetically-related cytotoxins found in *E. coli* or *Shigella* (Lee, *et al*., 2010). There are two forms of shigatoxin - shigatoxin 1 and shigatoxin 2. They are 55% homologous to each other (Nadya, *et al*., 2016). In order to be classified as STEC, the pathogen must contain the presence of shigatoxin 1, shigatoxin 2 or both (Smith, *et al*., 2007). Currently, over 500 serotypes of *E. coli* that produce shigatoxin have been discovered (Kruger & Lucchesi, 2015), but disease in humans is most often associated with the O157 serogroup (~50% of EHEC infections) as well as six other non-O157 serogroup (i.e., O26, O45, O103, O111, O121, O145 [also known as the 'Big-Six']). These serotypes are linked to the majority of cases of bloody diarrhea, watery diarrhea, and hemolytic uremic syndrome (HUS) (Nadya, *et al*., 2016). In addition, the infectious dose associated with STEC (i.e., certain *E. coli* O157 strains) is very low (i.e., 10-100 cells) compared to other *E. coli* pathotypes (Johnson, *et al*., 2006). The most recognized and studied shigatoxin-producing *E. coli* is the serotype *E. coli* O157. It has been identified in multiple outbreaks worldwide, including the United States, Scotland, and England (Rangel, *et al*., 2005; Adams, *et a*l., 2016). Further, STEC strain *E. coli* O157 has been found in other farm animals in addition to cattle, which creates a greater health risk to humans (Dombek, *et al*., 2000; Perera, *et al*., 2015). Approximately 50% percent of all HUS cases in North America are due to non-O157:H7 *E. coli* (Luna-Gierke, *et al*., 2014), although 80-90% of clinical cases of

enterohemoragic *E. coli* are due to STEC. STEC can enter a water source from contaminated human or cattle feces (Dombek, *et al*., 2000). Furthermore, roughly 9% of waterborne outbreaks between 1982 and 2002 in the United States were due to *E. coli* O157:H7 (Cooley, *et al*., 2013).

Overall symptoms associated with an enteric bacterial infection include abdominal cramping and diarrhea due to gastrointestinal distress, fever, nausea, and dehydration. If severe enough, death is a possibility (Clark, *et al*., 2003). As previously mentioned, enteric bacterial pathogens can cause significant morbidity and even mortality. Infections due to these enteric bacterial pathogens are most severe among the at-risk populations, which include young children, elderly, and those who have a suppressed immune system (Kolling, *et al*., 2012). There are rare instances in which *C. jejuni* infection may result in extra-intestinal issues, including severe arthritis, Guillain-Barre syndrome, and bacteremia (Altekruse, *et al*., 1999). Infection due to STEC can cause a variety of outcomes, from mild abdominal discomfort to death, HUS, or end-stage renal failure (Smith, *et al*., 2007; Couturier, *et al*., 2011; Nadya, *et al*., 2016; Majowicz, *et al*., 2014). HUS has been associated with roughly 3500 *E. coli* cases a year in 21 countries (Majowicz, *et al*., 2014). Infections from *Salmonella* spp. can include enteric fevers, which are systemic infections that can be life threatening (Giannella, 1996). As *Arcobacter* spp. is newly reclassified genus, the effects of its infection are still under investigation. However, most gastrointestinal infections are generally considered self-limiting, with treatment consisting of drinking fluids and electrolyte replacements.

Table 1-3: This tables provides the virulence factors, essential virulence determinants, illness and infectious dose of a subset of the major pathotypes of E. coli involved in Diarrheal Infections.

Subset of Major Pathotypes of *E. coli* involved in Diarrheal Infections					
Pathotype	Virulence Factors:	Essential virulence determinant(s):	Illness:	Infectious Dose (cells)	References:
EPEC	T3SS[2], BFP[1], LEE[6], Porcine-associated adhesion; outer membrane adhesive protein (intimin), T3 protein secreted effectors	LEE[6]; intimin; EspB	diarrhea; associated with premature death in children	10^6	(Robines-Browne, *et al*., 2016; Schmid & Frank, 2007)
EHEC	T3SS[2]; *E. coli* factor for adherence; BFP[1]; LEE[6]; *stx*	Phage-encoded shiga toxin that causes HUS or Haemorrhagic colitis	Haemorrhagic colitis; HUS; diarrhea	<10-100	(Robines-Browne, *et al*., 2016; Schmid & Frank, 2007)
ETEC	Fimbria; porcine associated adhesion; LT[3]; ST[4]; colonization factors	LT[3] or ST[4] plus colonization factors	"traveler's diarrhea"; leading cause of diarrhea in children in developing countries	10^8-10^9	(Robines-Browne, *et al*., 2016; Gama, *et al*., 2012)
EAEC	pAA; aggregative adhesion	unknown	"traveler's diarrhea"	~10^{10}	(Robines-Browne, *et al*., 2016; Nataro, *et al*., 1998)
EIEC/Shigella	T3SS[2]; T3 protein secreted effectors; pINV[5]; adhesions, secreted toxins	pINV[5]	Bacillary dysentery; diarrhea	~10	(Robines-Browne, *et al*., 2016; Schmid & Frank, 2007)

1 Bundle-forming pilus (BFP)

2 Type 3 secretion system (T3SS)
3 Heat liable toxin (LT)
4 Heat stable toxin (ST)
5 Virulence plasmid of enteroinvasive E. coli and Shigella (pINV)
6 Locus of enterocyte effacement pathogenicity island (LEE)

1.6.1 Waterborne Outbreaks

There is an abundance of literature on waterborne disease outbreaks, the scope of which, is well beyond the intent of this literature review. Water acts as a vehicle for transmission of enteric pathogens and exposure pathways are diverse. Several review articles highlighting the relationship between contaminated water and outbreaks of illness have been written, and include exposure pathways associated with contaminated drinking water (Craun *et al*., 2005; Schuster, *et al*., 2005; Ashbolt, 2004), contaminated recreational bodies of water (Perkins & Trimmerier, 2017; Hlavsa, *et al*., 2015; Craun, *et al*., 2005; Domenech-Sanchez, *et al*., 2008; EPA, 2009), small community non-drinking water systems (Pons, *et al*., 2015), groundwater (Hynds, *et al*., 2014), and contaminated irrigation water (Markland, *et al*., 2017; Steele & Odumeru, 2004).

However, there is currently limited information as to the critical role of enteric bacterial pathogens in outbreaks associated with stormwater reuse. A study of the effects of extreme precipitation and emergency room visits associated with gastrointestinal illness over a four year period in the state of Massachusetts, United States, found that areas where CSOs impacted drinking water had higher rates of emergency room visits than non-CSO-impacted areas and where CSOs impacted recreational water (Jagai, *et al*., 2015). In addition, the US EPA has initiated a National Compliance Initiative (NCI) to keep raw sewage and contaminated stormwater out of bodies of water, by addressing-CSOs, sanitary sewer overflows, and municipal storm sewer system violations. Furthermore, the regulatory agency notes that one way to address these problems is through the use of green infrastructure (e.g., green roofs, permeable pavements, etc.) (Environmental Protection Agency, 2018). A study by Campos *et al*. (2016) looked at human norovirus and *E. coli* in three different effluents of water treatment systems (i.e., primary, secondary, tertiary), with the primary systems including storm tank overflows.

They found that the concentrations of norovirus and *E. coli* in untreated sewage were the same as with storm tank overflows. Moreover, they found that norovirus outbreaks in the population were consistent with the occurrence of norovirus in the community (Campos, *et al*., 2016). In addition, there was an outbreak of *Cryptosporidium hominis* in 27,000 residents of Östersund, in Sweden due to the presence of *Cryptosporidium* oocysts in their drinking water. This outbreak was due to insufficient microbial barriers in their water treatment system, in which the stream closest to wastewater treatment plant-Östersund had high levels of oocysts due to wastewater leaking into the storm water system from an apartment building (Widerstrom, *et al*., 2014).

1.7 Potential Factors affecting Stormwater Contamination in the Urban Environment

In general, urban environments may experience poor water quality because of an increase in impervious surfaces, non-point sources of pollution (e.g., run-off containing fecal material), and point sources of pollution, (e.g., human sewage from a leaking pipe) (Chen, *et al*., 2016; Begum, *et al*., 2008). Several studies have demonstrated that land use plays in an important role in determining the source and degree to which fecal contamination occurs in stormwater, in addition to overall water quality (Tiefenthaler, *et al*., 2011; Sajjad, *et al*., 2015; Mallin, *et al*., 2008). How land use is developed within the urban landscape (i.e., transportation, industrial, commercial, and residential) can further impact water quality, due to the specific set of pollutants associated with land use. For example, a multitude of studies have found agricultural areas to be associated with poor water quality, ruminant fecal contamination, and enteric bacterial pathogens (Won, *et al*., 2013; Raith, *et al*., 2013; Mallin, *et al*., 2008). Furthermore, stormwater ponds located in parks or residential sections have an increased likelihood for canine fecal

contamination and have found to be key contributors to FIB concentrations (Sauer, *et al*., 2011; Shanks, *et al*., 2009; Staley, *et al*., 2016; Green, *et al*., 2014).

Weather events (e.g., precipitation, snowmelt, or drought) may affect the levels and/or sources of contamination in urban stormwater ponds. Studies have shown that bacterial concentrations are higher after wet weather events, as extreme precipitation is associated with microbial fate and transport (Krometis, *et al*., 2010; Noble, *et al*., 2003; Steele, *et al*., 2018). In addition, storm events may trigger an increase in sewage entering stormwater channels due to wastewater treatment facilities exceeding their capacity (Chong, *et al*., 2013). However, other studies have found conflicting results. A study performed by Topalcengiz *et al*. (2017) did not find a strong correlation between precipitation and microbial water quality, which may be due to precipitation diluting runoff. Concerns regarding stormwater-impacted bodies of water are increased by the association found between storm events and microbial inputs into bodies of water, and the correlation between extreme storm events and waterborne disease outbreaks (Curriero, *et al*., 2001).

1.8 Research Rationale and Objectives

Underlying and shaping this research thesis is the recognition that there is an important knowledge gap about bacterial pathogens, microbial sources of pollution, and general microbial water quality in urban stormwater ponds, and subsequently the potential human health impact of using these alternative water sources. The goal of this thesis research was to fill these knowledge gaps by studying microbial water quality, bacterial pathogen occurrence and sources of pollution in stormwater and stormwater-impacted bodies of water in southern Alberta. The specific objectives for this research project are highlighted below.

1.8.1 Objective 1: Measure the bacterial water quality of urban stormwater ponds and stormwater-impacted rivers, thereby identifying the water quality characteristics of stormwater.

Bacterial water quality was determined by utilizing traditional water quality indicators as outlined in USEPA's Recreational Water Quality Criteria (2012), Health Canada's Guidelines for Recreational Water Quality (2012), and the Alberta Environment and Parks' Recreational Water Quality Standard. Our hypothesis was that urban stormwater and receiving bodies of water (ponds and rivers) would experience frequent violations of microbial water quality guidelines and potentially correlated with rain events.

1.8.2 Objective 2: Ascertain the microbial sources of fecal pollution present in urban stormwater ponds.

Microbial sources of pollution were determined by using qPCR microbial source tracking markers. The premise was that a diverse range of microbial sources of fecal pollution would be found in the stormwater samples. Further, it was hypothesized that some ponds could be more heavily impacted by bird fecal pollution and others dominated by human fecal pollution, depending upon geography and anthropogenic activity.

1.8.3 Objective 3: Establish the presence of enteric pathogens in urban stormwater ponds.

This research set out to determine the occurrence and concentration of specific enteric bacterial pathogens (*Salmonella* spp., *Campylobacter* spp., enterohaemorrhagic *E. coli* (STEC), and *Arcobacter butzleri*) in stormwater and water bodies impacted by stormwater using molecular-based and culture-based methods of detection. It was hypothesized that the presence

of these enteric bacterial pathogens would be routinely found in urban stormwater ponds, and that the microbial sources of pollution may relate to the presence of a specific enteric pathogens (e.g., birds and *Salmonella* spp).

1.9 Thesis Organization

Each of the previously mentioned objectives will be discussed in this thesis as follows: Chapter 2 describes the materials and methods utilized throughout the thesis; Chapter 3 presents and discusses the findings of bacterial water quality in the urban stormwater ponds and stormwater-impacted rivers; Chapter 4 presents and discusses the findings of microbial sources of pollution in the urban stormwater ponds and stormwater-impacted rivers; Chapter 5 presents and discusses the findings of enteric pathogens in the urban stormwater ponds and with a specific focus on *A. butzleri* due to its high prevalence of occurrence within stormwater ponds; and Chapter 6 highlights the key findings of this thesis, provides a discussion of all results presented, and discusses the strengths and limitations of the thesis research study. The ultimate goal of this thesis is to support the safe reuse of stormwater in society.

2 Research Methods

2.1 Stormwater Sampling

2.1.1 Stormwater Pond Sampling

To determine the microbial quality of stormwater, the sources of fecal contamination, and the pathogens present, stormwater samples were collected semi-weekly over 20 weeks, with an additional sample on the 21st week. Sampling began as soon as stormwater ponds were fully thawed (i.e., May 9th, 2017), and ended just before freezing (September 25th, 2017). These ponds were chosen for sampling due to the interest in them for reuse. Samples were collected at three stormwater ponds in Calgary, Alberta, Canada: McCall Lake, Country Hills Stormwater Facility, and Inverness Stormpond. At each pond, we sampled four (i.e., McCall Lake and Inverness) or five (i.e., Country Hills) locations (Table 2-1, Figure 2-1, Figure 2-2, Figure 2-3). Each site was sampled 41 times.

Table 2-1: GPS coordinates of all sampling sites in the three Calgary stormwater ponds (i.e., McCall Lake, Country Hills Stormwater Facility, and Inverness Stormpond).

		GPS Coordinates by Sampling Sites in Urban Stormwater Ponds
Pond	Sampling Site	GPS coordinates
McCall Lake	ML2	51˚ 5’ 8” N 114˚ 1’ 37” W
	PR60	51˚ 4’ 55” N 114˚ 1’ 32” W
	ML1	51˚ 5’ 1” N 114˚ 1’ 27” W
	Inlet 3/4	51˚ 5’ 4” N 114˚ 1’ 38” W
Country Hills	WP31A	51˚ 9’ 26” N 114˚ 3’ 22” W
	WP31B	51˚ 9’ 24” N 114˚ 3’ 22” W
	WP31C	51˚ 9’ 35” N 114˚ 3’ 25” W
	WP31D	51˚ 9’ 35” N 114˚ 3’ 31” W
	WP31E	51˚ 9’ 35” N 114˚ 3’ 27” W
Inverness	Outfalls/Inlet	50˚ 54’ 41” N 113˚ 57’ 28” W
	WP26B	50˚ 54’ 41” N 113˚ 57’ 55” W
	WP26C	50˚ 54’ 36” N 113˚ 57’ 55” W
	WP26D	50˚ 54’ 36” N 113˚ 57’ 53” W

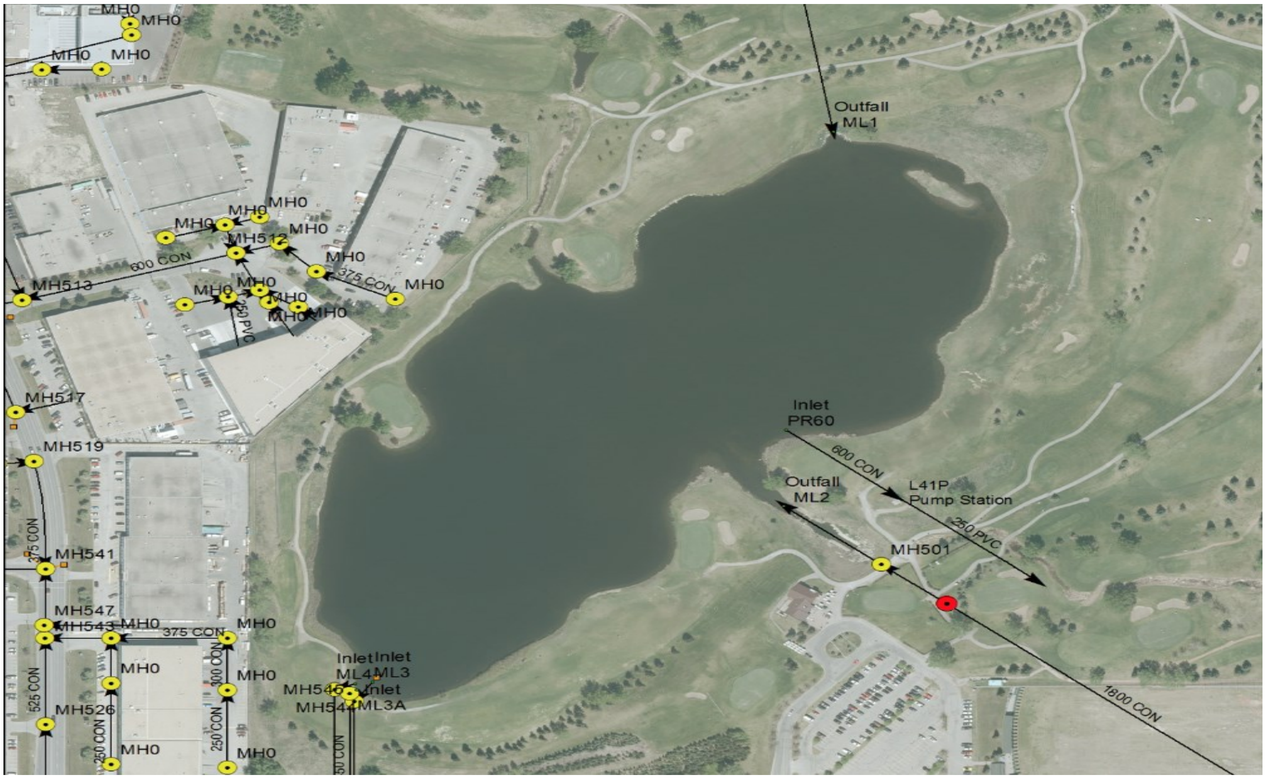

Figure 2-1: Arial photo of McCall Lake. The yellow circles represent storm manholes, the orange squares represent catch basins, the black arrows indicate the direction which storm drains flow, and the black lines are storm pipes [provided by The City of Calgary].

Figure 2-2: Arial photo of Country Hill Stormwater Facility. The yellow circles represent storm manholes, the orange squares represent catch basins, the black arrows indicate the direction which storm drains flow, the black lines are storm pipes, and the blue lines are culverts [provided by The City of Calgary].

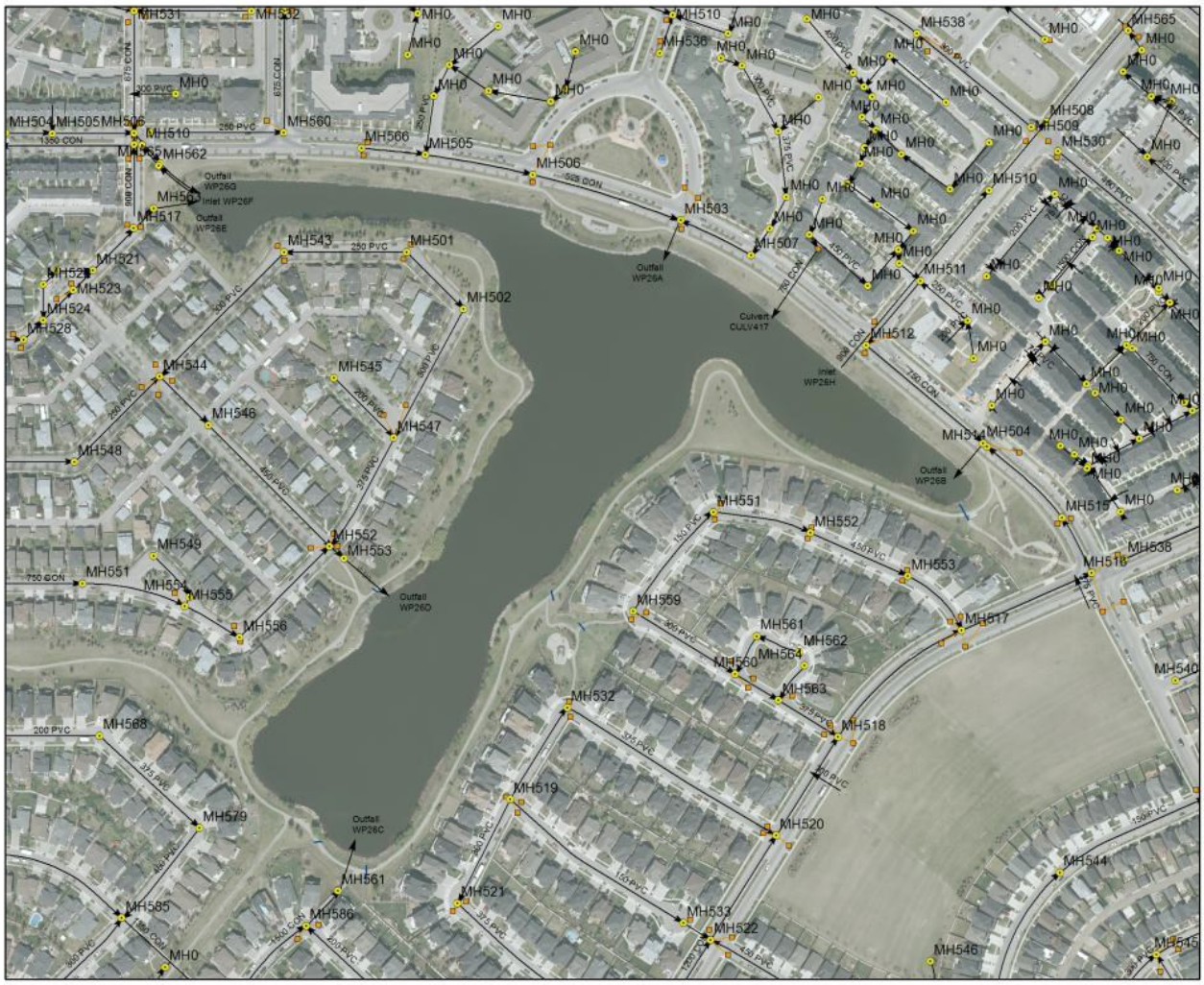

Figure 2-3: Arial photo of Inverness Storm Pond. The yellow circles represent storm manholes, the orange squares represent catch basins, the black arrows indicate the direction which storm drains flow, and the black lines are storm pipes [provided by The City of Calgary].

2.1.2 Stormwater-Impacted River Sampling

Synoptic sampling was carried out on the Nose Creek in Airdrie, Alberta, Canada, following rain events (i.e., enough rain so storm drains had a base flow). Five rain events occurred over the course of the study period: May 25, June 9, August 14, September 13, and September 21, 2017. There were 11 sampling locations along the Nose Creek. The Nose Creek sampling sites 25756, 25804, 25807, 25811, 25814, 25847, and 25855 were sampled five times each. The Nose Creek sampling sites 25793 and 25804 were sampled four times, and sites 25841 and 25817 were only sampled three times, resulting in 49 samples from the Nose Creek.

The Elbow River, which runs through the City of Calgary, begins west of Calgary in the Rocky Mountains, and provides drinking water to city residents. The river then continues eastward where it merges with the Bow River in downtown Calgary. For the purposes of this thesis research study, the Elbow River samples came from the area between the Glenmore Dam and downstream to the Bow River. This section of the river contains 88 stormwater outfalls and 13 sanitary sewer crossings beneath the river, and this waterway is utilized for summer recreational activities (e.g., swimming, canoeing, tubing, fishing, etc.). For the purposes of our study, ten sampling sites, which coincided with recreational access points along the river, were studied. Each of the ten sites along the Elbow River was sampled once a week 13 times from June 5th to August 28th in 2017.

In addition, a rural Alberta river, and one not heavily impacted by urban stormwater, was chosen for this study; and used as a water quality comparator against the urban impacted rivers (i.e., the Elbow River and the Nose Creek). This rural river is commonly used for recreational purposes. Water samples were collected from three sites on a weekly basis and processed using

the same method as stormwater samples, as discussed below. For these samples, sampling began May 30, 2017 and ended September 25, 2017.

2.1.3 Sampling Method

During sampling, 500 mL of water was collected into two sterile 250 mL Nalgene polyethylene bottles (Nalgene, Rochester, NY, USA) (Systems Plus, Baden, Ontario, Canada) by hand using sterile gloves. Following collection, samples were put on ice, taken to the Provincial Laboratory for Public Health (Calgary, AB, Canada) to begin processing for routine bacterial water quality indicators. Some samples were also sent for pathogen analysis to the University of Alberta (Edmonton, AB, Canada) within 24 hours.

2.2 Culture-based Methods

2.2.1 Bacterial Water Quality Indicators

Traditional water quality indicators were analyzed using defined substrate culture methods (IDEXX, Westbrook, ME, USA) for *E. coli* and total coliform detection using Colilert Quantitray-2000®. All samples were analyzed according to guidelines from the manufacturer. The Quantitrays® (IDEXX, Westbrook, ME, USA) were sealed and incubated for 24 hours at 35˚C. Following incubation, most probable numbers (MPN) were determined by scoring the number of positive wells (Colilert® – yellow wells = total coliforms, yellow and fluorescent = *E. coli*) and transforming results into quantitative estimates using IDEXX MPN charts. If the Quantitrays® were positive for *E. coli*, they were set aside for further analysis, as described in Section 2.3.2.

Enumeration of fecal coliform bacteria was performed by the Provincial Laboratory in Calgary, Alberta, Canada, in which water (10 mL) was filtered onto a 15x16 mm membrane that was placed on a Membrane Fecal Coliform (mFC) plate (Dalynn Biological, Calgary, AB, Canada), then incubated at 44.5 ˚C for 24 hours. Fecal coliform bacteria were enumerated according to standard operating procedures for the Provincial Lab of Public Health and in alignment with current practices of recreational water assessment in the province of Alberta, Canada.

2.2.2 Most Probable Number (MPN)-qPCR Assay for Detection of *Campylobacter* spp. and *Arcobacter butzleri* in Water Samples

Sample Preparation. A most probable number (MPN)-qPCR assay, as described by Banting *et al*. (2016), was used to detect *Campylobacter* spp., and modified slightly for detecting *A. butzleri* from stormwater samples. For each sampling, 400 ml were split into two 250 ml bottles and centrifuged (Thermo Scientific, Waltham, MA, USA) in a Sorval RC-58 Refrigerated Superspeed Centrifuge (Thermo Scientific, Waltham, MA, USA) at 20°C for 20 minutes. After centrifugation, pellets were suspended in Bolton Broth (BB) with BB supplements (Thermofisher, Ontario, Canada) to achieve a final volume of 4 mL. The sample was then divided into three 1-mL aliquots, for which one aliquot was added to a 1 mL deep-well in a 96-well MPN plate (Greiner BioOne, Monroe, NC, USA). In these plates, samples were serially diluted to 10^{-3} in BB (except for the first experiment on *A. butzleri,* which was diluted to 10^{-7}). Plates were then incubated at 42°C for 48 hours for *Campylobacter* spp., and 30°C for 48 hours for *A. butzleri* in microaerophilic containers with microaerophilic packs and a damp cloth

(AnaeroPack-MicoAero, Thermo Fisher Scientific, Waltham, MA, USA) [hereinafter, "microaerophilic conditions"].

qPCR and MPN Determination. Following incubation, 50 µl from each MPN well were transferred to replicate 96-well plates, with wells containing 90 µl of skim milk:glycerol solution (1:1) and microbes cryopreserved at -80°C, for further testing. In addition, 100 µl from the original MPN plate was transferred into a separate plate, where it was diluted 1:10 in sterile water and heated for 10 minutes at 95°C. One hundred copies of an internal amplification control plasmid were added to every *A. butzleri* reaction to test for inhibition (see Section 2.3.3). Inhibition occurred if the cycle threshold (C_T) values of the samples varied by $\geq$3 C_T. To be considered positive, wells had to have a C_T value of $\leq$35 and no inhibition. Enumeration was based on qPCR results, with the use of three-tube MPN tables to ascertain the final MPN/300 mL. Full descriptions of all qPCR methods are outlined in Section 2.3.3 below.

2.2.3 *Arcobacter butzleri* in Isolation

In order to obtain *A. butzleri* isolates from stormwater samples, 500 µL from the MPN plate culture was added to 500 µL of a 50% skim milk 50% glycerol mix and frozen at -80°C from *A. butzleri* positive wells (i.e., HSP60 qPCR with CT value <25). On a later date, these samples were thawed and streaked onto BB with supplement plates and grown at 30°C for 48 hours in microaerophilic conditions with a damp cloth. Subsequently, individual colonies were picked and placed into culture test tubes (Fisherbrand, Fisher Scientific, NH, USA) containing 2 mL of BB with supplements and grown in microaerophilic conditions at 30°C for 48 hours. After growth, 100 µL were pipetted into 96-well plates and boiled for 10 min at 95°C to lyse cells for

qPCR. The identity of the isolates was confirmed by HSP60 qPCR with the addition of an IAC, as previously described.

2.3 Molecular-based Methods

2.3.1 DNA Preparation of Stormwater Collected Samples

Immediately upon receipt of stormwater samples in the laboratory, 20 mL of water was filtered onto 0.4 micron polycarbonate filters by EPA method 1611 (Environmental Protection Agency, 2012) by the Provincial Laboratory in Calgary. Filters were then stored at -80˚C until they were shipped to Edmonton, Alberta, Canada on dry ice. To analyze the microbial sources of contamination, levels of *Enterococcus* spp., and the presence of pathogens, DNA was extracted from samples according to EPA Method 1611, the process of which is briefly described below.

Controls. Calibrator samples were prepared and analyzed in advance of the sampling season, then stored at -80˚C. An *Enterococcus faecalis* ATCC 29212 culture was obtained from the Provincial Laboratory (Edmonton, AB, Canada), and grown for three hours at 37˚C. Following growth, 100 µL (1:250 dilution) was transferred from the test tube to the flask holding 250 µL of broth and grown for another two hours and subsequently centrifuged for three minutes at 21,100 x *g*. Following centrifugation, the supernatant was removed. The remaining pellet was washed twice, with 1mL of buffer for each round of centrifugation. The pellet was then washed and centrifuged at 21,100 x *g* twice. Finally, the pellet was re-suspended into a final volume of 8 mL with buffered water to achieve a 1:4 dilution of culture to buffered water. Calibrators were then enumerated using standard plate count (SPC) plates down to a 10^{-7} dilution in triplicate, and incubated at 32°C for 24 hours. The CFU/10 µL was calculated as follows: [three highest colony count (between 30 and 300) added together] / [the dilution factors of each (added together) =

CFU/100 µL divide by 10 to achieve CFU/10 µL. These calibrators were then extracted and analyzed per batch of extractions in triplicate. Calibrators were extracted on the same day as water samples, and were used as positive controls for the *Enterococcus* spp. assay (EPA Method 1611). To determine if there was inhibition in water samples, salmon DNA was utilized as a control (Environmental Protection Agency, 2012).

For other assays (e.g., microbial source tracking), in order to ensure amplification, plasmids or positive samples were used as positive controls. IAC was used as a control. DNA free water was used as a negative control, prepared in the same manner as water samples to ensure no carryover.

DNA extraction for qPCR. Within 12 hours of sampling, 20 mL of water was filtered through a 0.4 µm-pore-size polycarbonate filters (Microfunnel, Pall Life Sciences, Port Washington, NY, USA). However, EPA Method 1611(Environmental Protection Agency, 2012) protocol calls for 100 mL of water to be filtered, however, tests carried out previously in the laboratory demonstrated an increased extraction efficiency when 20 mL of water was filtered. Filters were carefully folded and placed into bead tubes (GeneRite, North Brunswick, NJ, USA) using sterile tweezers.

Bead tubes were stored at -80˚C prior to further processing, and were subsequently shipped by courier on dry ice to the University of Alberta (Edmonton, AB, Canada) for DNA extraction. At the University of Alberta, 600 µL of 0.2 µg/mL salmon DNA in tris-EDTA (AE) buffer (Qiagen, Hilden, Germany) was added to each bead tube. Tubes were then placed in a Bead Mill 24 (Fisher Scientific, Hampton, NH, USA) where cells were lysed for 60 seconds at a speed of 3.10 rotations/sec. at room temperature. Following bead beating, cells were immediately centrifuged at 21,100 X *g* to pellet debris and beads in a Sorvall Legend Micro 21 Centrifuge

(Thermo Scientific, Waltham, MA, USA). Supernatants were transferred to 1.5 mL microcentrifuge tubes, centrifuged again at 21,100 X *g* for five minutes, and 250 µL of the supernatant from these tubes were then collected (i.e., DNA extracts) into 1.5 mL microcentrifuge tubes and analyzed by qPCR.

2.3.2 Colilert® DNA Extraction

To determine the presence of virulence genes in *E. coli*, a different DNA extraction technique was used on water samples that were positive for *E. coli* using Quantitray® MPN enumeration. All *E. coli* positive wells in a Quantitray® had the 1 mL of liquid extracted using a Becton Dickinson 10 mL syringe with Luer-Lok tip (Becton Dickinson, Franklin Lakes, NJ, USA), and the liquid from wells pooled into one 50 mL conical tube (Thermo Scientific, Waltham, MA, USA) per sample. Each conical tube was vortexed and 1 mL was pipetted into a microcentrifuge tube. Tubes were centrifuged at 21,100 X *g* on a Sorvall Legend Micro 21 Centrifuge (Thermo Scientific, Waltham, MA, USA) for five minutes. Pellets were then suspended in H_2O in 96-well Greiner plates (Greiner Bio-One, Monroe, NC, USA) and boiled for ten minutes at 95°C to lyse cells for qPCR.

2.3.3 qPCR Experiments

qPCR assays included targets for bacterial indicators (*Enterococcus* spp. and *E. coli*), microbial source tracking markers (i.e., human, cattle, seagull, goose, dog, and muskrat), as well bacterial pathogen-related genes (i.e., *Campylobacter* spp., *Salmonella* spp., and *A. butzleri*) [Table 2-1]. Two markers were used for human contamination to ensure that the results were not due to cross-reaction with other fecal sources (e.g., dog, turkey, and chickens) (Green, *et al.*,

2014). Each marker has a different limit of detection as assayed previously in the laboratory (Table 2-1). Each assay target species, target locus, and primer/probe names and their corresponding sequences are specified (Table 2-2).

Amplification was performed on an Applied Biosystems TaqMan 7500 fast real-time PCR system (Applied Biosystems, Foster City, CA, USA). The reaction mixture was made to a final volume of 20 µL (Table 2-3). The calibrator control standards and plasmid controls were performed in triplicate. The negative controls, *Enterococcus* spp*.,* and salmon DNA PCR assays were performed in duplicate. Sample assays were only performed once. All reactions were carried out in a MicroAmp Fast Optical 96-Well Reaction Plate (Applied Biosystems, Foster City, CA, USA). Samples were pulsed down at maximum speed in a centrifuge prior to thermal cycling. Thermal cycling conditions were 50°C for two minutes; 95°C for 30 seconds (holding); followed by 45 cycles of 95°C for three seconds; and 60°C for 30 seconds for all assays, except for the muskrat marker MuBac. The annealing temperature of MuBac was 57°C. The threshold cycle (C_T) was set at 0.1 for Entero1 and Sketa, and 0.05 for all other targets. For further analysis, all C_T values were entered into Microsoft Excel.

DNA plasmid standards for each assay were developed previously in the laboratory. Briefly, DNA targets were PCR amplified, run on a 2% agarose gels and amplicons extracted by a QIAquick (Qiagen, Hilden, Germany) gel extraction kit. The products were cloned into pCR2.1-TOPO (Thermo Fisher Scientific, Waltham, MA, USA) per manufacturer's instructions using TOP10 F' *E. coli* competent cells. The plasmids were mini-prepped using QIAprep spin mini prep kit (Qiagen, Hilden, Germany). Plasmids were then quantitated using the Qubit 2.0 fluorimeter (Invitrogen Carlsbad, CA, USA) and diluted to 100,000 copies/µL stocks. Thus,

stocks of plasmid DNA were aliquoted and stored at -80°C. During each qPCR analysis, 10-fold serial dilutions were made from the known concentration plasmid for each individual target.

Table 2-2: qPCR assays including the target, assay name, target species, target locus, primer/probe name and sequence (5'-3') and reference.

Target	Assay	Target Species	Target Locus	Primer (forward [F] and reverse [R]/Probe name	Sequence (5'-3')	Reference
Microbial Source Tracking Targets						
Human	HF183	*Bacteroides dorei*	*16S*	HF183F BFDrev BFD-FAM	ATCATGAGTTCACATGTCCG CGTAGGAGTTTGGACCGTGT FAM-CTGAGAGGAAGGTCCCCCACATTGGA-TAMRA	(Haugland, *et al.*, 2010)
Human	HumM2	?	alpha-1 2-mannosidase homolog	HumM2-F HumM2-R HumM2-P	CGTCAGGTTTGTTTCGGTATTG TCATCACGTAACTTATTTATATG CATTAGC FAM-TATCGAAAATCTCACGGATTAAC TCTTGTGTACGC-TAMRA	(Shanks, *et al*., 2009)
Ruminant	Rum2Bac	Bacteroides spp.	*16S*	BacB2-590F Bac708Rm BacB2-626P	ACAGCCCGCGATTGATACTGGTAA CAATCGGAGTTCTTCGTGAT FAM-ATGAGGTGGATGGAATTCGTGGTGT-TAMRA	(Mieszkin, *et al*., 2010)
Gull	LeeSg	*Catellicoccus marimammalium*	*16S*	LeeSg-F LeeSg-R LeeSg-P	AGGTGCTAATACCGCATAATACAGAG GCCGTTACCTCACCGTCTA FAM-TTCTCTGTTGAAAGGCGCTT-NFQMGB	(Lee, *et al*., 2013)
Canada Goose	CGO1	Bacteroides spp.	*16S*	CGO1-F CGO1-R CGO1-P	GTAGGCCGTGTTTTAAGTCAGC AGTTCCGCCTGCCTTGTCTA FAM-CCGTGCCGTTATACTGAG ACACTTGAG	(Fremaux, *et al*., 2010)
Muskrat	MuBac	Bacteroides spp.	*16S*	MuBac-F MuBac-R MuBac-P	CTCTTTTGCCGGGGAG TTTACCGCTTGCTTGACG FAM-GAGTACCCGGAGAAAAAGCA-(BHQ-1)	(Marti, *et al*., 2011)

Dog	Dog3	Bacteroides spp.	long chain fatty acid - CoA ligase	Dog3-F Dog3-R Dog3-P	TTTTCAGCCCCGTTGTTTCG TGAGCGGGCATGGTCATATT FAM-AGTCTACGCGGGCGTACT-MGBNFQ	(Green, *et al*., 2014)
Enteric Bacterial Pathogen Targets						
Campylobacter spp.	Van Dyke	*C. jejuni, C. coli, C. lari*	*16S*	VD16S-F VD16S-R VD16S-P	CTGCTTAACACAAGTTGAGTAGG TTCCTTAGGTACCGTCAGAA FAM-CGCTCCGAAAAGTGTCATCCTCC-BHQ1	(Van Dyke, *et al*., 2010)
Arcobacter butlzeri	Abutz	*Arcobacter spp*.	Hsp60	Abutz-F Abutz-R Abutz-P	CTCTTCATTAAAAGAGATGTTAC CAATTTT CACCATCTACATCTTCWGCAATA ATTACT FAM- CTTCCTGATTGATTTACTGATT-NFQMGB	(de Boer, *et al.*, 2013)
Salmonella spp.	InvA	*Salmonella* spp.	InvA	Sal 1-F Sal 2-R Sal 3-P	GTGAAATTATCGCCACGTTCGGG CAA TCATCGCACCGTCAAAGGAACC GCCCGGTAAACAGATGAGTATT GA	(Moganedi, *et al*., 2009)
Shigatoxin 1	*stx*1	E. coli	*stx*1	*stx*1-F *stx*1-R *stx*1-P	CATCGCGAGTTGCCAGAAT GCGTAATCCCACGGACTCTTC FAM- CTGCCGGACACATAGAAGGAAA CTCATCA- TAMRA	(Chui, *et al.*, 2013)
Shigatoxin 2	*stx*2	E. coli	*stx*2	*stx*2-F *stx*2-R *stx*2-P	CCGGAATGCAAATCAGTC CAGTGACAAAACGCAGAACT FAM- ACTGAACTCCATTAACGCCAGAT ATGA-TAMRA	(Chui, *et al*., 2013)
Bacterial Water Quality Indicator Targets						
Enterococcus	Entero1	*Enterococcus* spp.	23S	Entero1-F Entero1-R Entero1-P	GAGAAATTCCAAACGAACTTG CAGTGCTCTACCTCCATCATT 6FAM- TGGTTCTCTCCGAAATAGCTTTA GGGCTA-TAMRA	EPA Method 1611, 2012
Controls						
Internal Controls	Sketa (Salmon DNA)	Oncorhynchus keta	rRNA ITS	Sketa-F Sketa-R Sketa-P	GGTTTCCGCAGCTGGG CCGAGCCGTCCTGGTC VIC-AGTCGCAGGCGGCCACCGT-TAMRA	EPA Method 1611, 2012

Internal Amplification Control	IAC	synthetic gene	randomized sequence	IAC-F IAC-R IAC-P	CTAACCTTCGTGATGAGCAATCG GATCAGCTACGTGAGGTCCTAC VIC- AGCTAGTCGATGCACTCCAGTCC TCCT-NFQMGB	(Deer, *et al*., 2010)
Negative Control	DNA Free Water					

Table 2-3: PCR reagent concentrations per 20 µL reaction used for Enterococcus, Sketa, and microbial source tracking marker assays.

Assay (According to Table 2.2)	**Reagents**
HF183, Dog3, InvA, Van Dyke, Entero1, stx1, stx2, IAC, Sketa (per reaction)	1x Taqman Advanced MM, 200 µg/ml bovine serum albumin (Sigma, St. Louis, Missouri, USA), 1 µM of each primer, 80 nM FAM labeled TaqMan probe (Applied Biosystems, Foster City, CA, USA) and 5 µL of stormwater sample or plasmid DNA standard for a total reaction volume of 20 µL.
MuBac, CGO1, LeeSg, HumM1, Rum2Bac, Abutz (per reaction)	1x Taqman Advanced MM, 200 µg/ml bovine serum albumin (Sigma, St. Louis, Missouri, USA), 300 nM of each primer, 100 nM FAM labeled TaqMan probe (Applied Biosystems, Foster City, CA, USA) and 5 µL of stormwater sample or plasmid DNA standard for a total reaction volume of 20 µL.

2.3.4 Enterobacterial Repetitive Intergenic Consensus-PCR (ERIC-PCR)

Enterobacterial Repetitive Intergenic Consensus - PCR (ERIC-PCR) was used to determine the genetic similarity of *Arcobacter butzleri* isolates in order to reduce clonal redundancy in specimen libraries obtained from stormwater. Cryopreserved *A. butzleri* isolates were thawed from 96-well Greiner plates, of which 100 µL was pipetted into a new 96-well plate and diluted 1:10 in nuclease-free water. Isolates were then transferred to a 96-well Greiner plate containing 25 µL of the reaction mixture (Table 2-6). Reactions were run on a 2720 Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA), with the following reaction conditions:

94°C for five minutes, then 40 cycles of 94°C for one minute, 25°C for one minute, 72°C for two minutes. The endpoint PCR product was diluted 1:10 in DNA dilution buffer, and run on QIAxcel Advanced (Qiagen, Hilden, Germany) capillary electrophoresis system with a DNA size marker of 2.5-100 kb (diluted 1:20 in DNA dilution buffer), and a DNA alignment marker of 5-15 kb.

Table 2-4: ERIC-PCR Primer sequences (i.e., Forward and Reverse) listed 5'-3' (Houf, et al, 2002).

ERIC-F	5'- AAGTAAGTGACTGGGGTGAGCG -3'
ERIC-R	5'- ATGTAAGCTCCTGGGGATTCAC -3'

Table 2-5: ERIC-PCR Reagents with concentrations listed per 25 µL reaction

Solution	**Reagents**
ERIC-PCR (per reaction)	1.25 µl ERIC-F, 1.25 µl ERIC-R, 2.5 µl 10x Buffer, 0.5 µl of 40 mM dNTP mixture, 2 µl of 40mM $MgCl_2$, 0.5 µl of 5U *Taq* Polymerase, 15.5 µl PCR water

2.3.5 *A. butzleri* virulence gene screen

A. butzleri positive samples from MPN plates were screened for two putative *A. butzleri* virulence genes: *cadF* and *ciaB*. All isolates that were positive for HSP60, *CadF*, or *CiaB* were further screened for virulence genes *hecA*, *cj1349*, *irgA, mnlV, pldA, tlyA*, and *hecB* (Table 2-6). All PCR reactions contained 2x Maxima Hot Start PCR MasterMix (Thermofisher, Waltham,

MA, USA), 10 µL of primers, 5 µL of template, and 5 µL of water to achieve a final volume of 25 µL. PCR was run on a 2720 Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) with 35 cycles of denaturation at 94°C for 20 seconds, 58°C for 45 seconds, and 72°C for 20 seconds, followed by a 4°C hold. After cycling, the reaction was heated to 94°C for four minutes. A 1% agarose gel with a Tris-acetate-EDTA buffer at 150 V for 45 minutes using a 100 base pair marker was used to analyze the PCR products. Each gel was stained with SYBR Safe DNA gel stain (Invitrogen, Carlsbad, CA, USA), and photographed using an ImageQuant Las 400 UV transilluminator (GE HealthCare Life Sciences, Little Chalfont, United Kingdom).

Table 2-6: Primer sequences for putative virulence factors for A. butzleri listed 5'- 3' (Douidah, et al., 2011).

Assay	Primer	Sequence 5'- 3'
irgA	irgA-F	TGC AGA GGA TAC TTG GAG CGT AAC T
	irgA-R	GTA TAA CCC CAT TGA TGA GGA GCA
cj1349	cj1349-F	CCA GAA ATC ACT GGC TTT TGA A
	cj1349-R	GGG CAT AAG TTA GAT GAG GTT CC
hecA	hecA-F	GTG GAA GTA CAA CGA TAG CAG GCT C
	hecA-R	GTC TGT TTT AGT TGC TCT GCA CTC
hecB	hecB-F	CTA AAC TCT ACA AAT CGT GC
	hecB-R	CTT TTG ACT GTT GAC CTC
mviN	mviN-F	TGC ACT TGT TGC AAA ACG GTG
	mviN-R	TGC TGA TGG AGC TTT TAC GCA AGC
pldA	pldA-F	TTG ACG AGA CAA TAA GTG CAG C
	pldA-R	CGT CTT TAT CTT TGC TTT CAG GGA
tlyA	tlyA-F	CAA AGT CGA AAC AAA GCG ACT G
	tlyA-R	TCC ACC AGT GCT ACT TCC TAT A
CiaB	CiaB-F	TGG GCA GAT GTG GAT AGA GCT TGG A
	CiaB-R	TAG TGC TGG TCG TCC CAC ATA AAG
CadF	CadF-F	TTA CTC CTA CAC CGT AGT
	CadF-R	AAA CTA TGC TAA CGC TGG TT

2.3.6 Data Analysis

Fecal indicator bacteria, microbial source tracking markers and select enteric bacterial pathogens were corrected to the number of copies/100 mL and reported accordingly. When nothing was detected, it was reported as "Not Detected" in the results; and "detected but not quantifiable" (DNQ) when the CT was less than 5 per reaction. Data from *E. coli*, *Enterococcus*, fecal coliforms, and all qPCR marker concentrations were $\log_{10}$ transformed prior to analysis because the Shapiro-Wilk test for normality indicated the data was non-normally distributed data. All statistical analyses were performed in Microsoft Excel 2016 and StataIC. Geometric means cannot handle values of zero or one, therefore "2" was added to each original value before log transformation. After geometric means were calculated, $\log_{10}$ of 2 was subtracted from each of the final geometric means calculated.

3 Water Quality Characteristics of Urban Stormwater-Impacted Bodies of Water

3.1 Introduction

Understanding water quality impacts associated with stormwater collection and discharge into receiving bodies of water in the urban environment (i.e., retention ponds and rivers) is critical, particularly in terms of our evolving perceptions that stormwater represents an important alternative water resource for ensuring future water security. This resource management approach, as opposed to the historical nuisance management framework, underscores the importance of research aimed at evaluating the microbial quality of stormwater and the potential public health impacts that might be associated with exposure to these alternative urban water resources. Stormwater use is viewed as a critically important constituent to water resource management in the City of Calgary in order to meet the growing and future water demands for the city (i.e., irrigation, evaporative cooling, toilet and urinal flushing, etc.). Historically, data pertaining to microbial water quality from storm ponds in the City of Calgary are scant, or in most cases non-existent, due to the fact that these parameters are not regulated for and are therefore not monitored. Consequently, we sought to intensively monitor bacteriological water quality in three stormwater ponds in Calgary, the data of which forms a significant part of this thesis chapter. The data is important in providing baseline information on microbial water quality, and a requisite for informing human health risks assessments about stormwater and the derivation of future public health standards and regulations regarding stormwater use in the province.

In order to broaden our understanding of stormwater impacts within urban environments, we also examined water quality within the Elbow River in Calgary, an urban watershed impacted

by up to 100 stormwater outlets discharging storm drainage directly into the river. Unlike stormwater ponds, the Elbow River is used extensively for recreational activities by the public (e.g., swimming, fishing, tubing, canoeing, etc.), yet little historical bacteriological monitoring of water quality has been performed along the river, up until the last few years. Frequent water quality violations in thermotolerant coliform levels have been observed in the river, leading Alberta Health Services and the City of Calgary to post hazard signs along the river to discourage the public from engaging in recreational activities in the urban reaches of the Elbow River watershed. Consequently, intensive microbial water quality monitoring in these urban reaches of the Elbow River was carried out, in order to characterize potential stormwater impacts on the river that might affect human health, and to better understand the current state of urban stormwater quality overall.

3.2 Results

The results of this chapter are divided into two major sections. The first section discusses microbial water quality within urban stormwater ponds in the City of Calgary (i.e., McCall Lake, Country Hill Stormwater Facility, and Inverness Storm Pond). Given the large amount of data collected, and the inability to consolidate all this information into a single thesis, we concentrated on highlighting some general observations about water quality within these three stormwater systems, though subsequently focusing our analysis on the spatiotemporal characteristics of bacteriological water quality in McCall Lake (the rationale of which is provided later). Where appropriate, comparisons to the other stormwater ponds have been made, along with an analysis of this data and a consolidation of this information in the Appendix of this thesis.

The second section of the results of this chapter addresses microbial water quality within the stormwater-impacted urban reaches of the Elbow River. In this analysis, ten sequential downstream sampling points below the Glenmore Dam (and up to confluency with the Bow River) were monitored for bacteriological water quality order to identify potential spatiotemporal impacts of stormwater on river water quality.

3.2.1 Stormwater Ponds

3.2.1.1 Overview of the Physical Characteristics of Stormwater Ponds

All three stormwater ponds (i.e., McCall Lake, Country Hills Stormwater Facility, and Inverness Stormpond) and their associated sampling locations were analyzed for a variety of drainage characteristics, including drainage catchment size, land use, overland drainage contributions, and hydrological location of inlets and outfalls (i.e., above grade, at grade, or below grade). All data was provided by the City of Calgary, and information relevant to this thesis has been compiled in Table-3-1. Additional information, including aerial and ground-based photographs of the urban stormwater ponds and sampling sites, is provided in Appendix 1. Not all inlets/outlets coming into, or leaving, the ponds were monitored for water quality, though sites were chosen to provide spatially-distributed water quality datasets across each pond.

The overall catchment size, and associated land uses, were collected as part of this study to better understand how drainage demographics may affect bacteriological quality (Chapter 3), sources of fecal pollution (Chapter 4), and pathogen occurrence (Chapter 5) in the stormwater ponds (e.g., Are sources of human fecal pollution contributing to poor bacteriological water quality primarily due to cross connections in residential areas?). The catchment size and land use patterns reflect stormwater collected from infrastructure drainage systems within the catchment

and not from overland drainage (i.e., run-off [discussed below]). Catchment size varied considerably across the three stormwater ponds, with McCall Lake representing the largest catchment [1830 ha] and the Country Hills Stormwater Facility representing the smallest catchment [267 ha] (Table-3-1). Land use characteristics within each of the catchments also varied, with the McCall Lake catchment having the lowest percentage associated with residential drainage at 20%, compared to Country Hills for which 68% of the catchment was designated as residential. By contrast, the McCall Lake catchment had the greatest percentage of land use associated with industrial activity (26.8%) compared with Country Hills for which none of the catchment area was designated as industrial. The Inverness Stormpond had the greatest percentage of drainage coming from permanent infrastructure or transportation corridors (26%) followed by McCall Lake (19.4%). All stormwater ponds had similar drainage contributions coming from parks and institutions (13% [Inverness] to 18% [Country Hills]). All three stormwater ponds in this study were considered as fully developed urban areas, with little or no space designated to future development (Table-3-1).

The overland drainage characteristics of each stormwater pond were also important factors to consider for water quality, since overland drainage represents the cumulative non-point sources coming into each stormwater pond from localized run-off, and for which land use characteristics may be important in understanding the factors influencing water quality (e.g., pet feces from adjacent residential backyards, off-leash dog parks, or bird feces from adjacent greenspaces). In some stormponds, overland drainage was collected by culverts, though the collection of this stormwater through city infrastructure represented natural downhill water flows into the ponds, and therefore was included in the overland drainage characteristics. In all three stormwater ponds, parks and institutions comprised the majority of overland drainage flows,

contributing 94% in McCall Lake and 55% in Inverness (Table-3-1). For McCall Lake, no overland drainage was associated with residential land use areas; whereas in Inverness, 45% of overland drainage areas were also coming from residential areas (Table-3-1).

Another important physical feature to consider regarding water quality was the hydrological positioning of the inlets/outlets within the stormwater ponds. Inlets/outlets were either submerged (below grade), at grade, or above grade (i.e., cascading). Grab sampling was carried out as close to the inlets/outlets as physically and safely as possible. Consequently, grab samples from submerged outlets did not completely reflect water quality within the drainage networks themselves, but rather they represented diluted volumes of storm flows coming into the pond. For purposes of this study, it is important to note that most of the samples were taken from inlets/outlets below grade [i.e., submerged] (Table-3-1). However, some caution is warranted with respect to interpretations about how this might affect water quality at these sites. In some cases, poor water quality coming from storm drains may be diluted by good quality pond water; and in other cases, good quality stormwater may be impacted by poor quality pond water (i.e., aquatic birds like seagulls and/or geese residing near the outfall). Only one sampling site was at equal hydrological grade (i.e., ML1 at McCall Lake, see photographs in Appendix 1) and only one was above grade (i.e., ML2 at McCall Lake, see photographs in Appendix 1). Samples obtained at the ML2 site at McCall Lake were collected directly from the cascading outlet, and were therefore more representative of drainage flows coming into the pond from the catchment.

Table-3-1: Drainage watershed characteristics for the urban stormwater ponds represented in the current study

Stormwater Pond Facility	Sampling Site	Hydrological positioning of inlet/outlet (above grade, below grade, or equal grade)	Catchment size in hectares (Overland Drainage size in Parentheses)	Land Use Characteristics for Catchment Area (Land Use Characteristics for Overland Drainage in parentheses)					
				Residential	***Industrial***	***Infrastructure/ Transportation***	***Parks and Institutions***	***Commercial***	***Future Development***
McCall Lake	ML2	Above	275.99	4	69	4	11	12	0
	PR60[a]	Below	-	-	-	-	-	-	-
	ML1	Equal	1464.35	37	20	23	10	7	3
	Inlet 3/4[a]	Below	-	-	-	-	-	-	
	Total		**1830 (89.09)**	**30 (0)**	**26.8 (6)**	**19.4 (0)**	**14 (94)**	**7.3 (0)**	**2.6 (0)**
Country Hills	WP31A	Below	38.96	95	0	0	5	0	0
	WP31B[a]	Below	-	-	-	-	-	-	-
	WP31C	Below	28.49	5	0	17	28	50	0
	WP31D	Below	172.28	75	0	1	15	8	0
	WP31E	Below	10.63	100	0	0	0	0	0
	Total		**267**	**68 (20)**	**0 (0)**	**3 (2)**	**18 (77)**	**10 (1)**	**0 (0)**
Inverness	Inlets/outlets	Below	31.54	76	0	1	9	14	0

	WP26B	Below	89.52	72	0	4	20	4	0
	WP26C	Below	257.99	52	0	40	8	0	0
	WP26D	Unknown	15.3	62	0	0	38	0	0
	Total		**415 (13.14)**	**57 (45)**	**5 (0)**	**26 (0)**	**13 (55)**	**3 (0)**	**0 (0)**

[a] Site is an inlet, defined as a structure for which stormwater leaves the stormpond (i.e., not for drainage into pond).
[b] Site represented by two outfalls (WP26G and WP26E) draining in close proximity to each other, and for which land use characteristics were averaged across the two sites, but for which overland drainage size was summated.

3.2.1.2 General Overview of Bacterial Water Quality in Urban Stormwater Ponds

A total of 533 samples were taken from the three urban stormwater ponds (i.e., McCall Lake, Country Hills, and Inverness) in Calgary, Alberta, Canada, from May 9th through September 25th, 2017. Samples from each of the three stormwater study ponds were taken biweekly for 20 weeks, and once a week for one week, resulting in 41 samples for each sampling site. Biweekly sampling was performed in order to better understand the temporal occurrence patterns of fecal indicators, sources of pollution and occurrence of pathogens in recipient stormwater collection systems.

A high-level descriptive overview of the bacteriological water quality in each of these ponds, and at each of the sites, is provided in Table-3-2, and is based on the percentage of samples violating water quality standards/guidelines, as evaluated against: the USEPA's recreational water quality guideline for *Enterococcus* by molecular methods (Environmental Protection Agency, 2012); and Alberta's former recreational water quality standards based on thermotolerant coliform concentrations (Table-3-2). A number of observations are worth noting from this high-level analysis.

Table-3-2: Microbial water quality in the stormwater ponds based on the percentage of sample failing existing standards of water quality in Calgary.

Stormwater Pond/Facility	**Sampling Site**	*Percent failure based on the USEPA Recreational Water Quality Standard (Enterococcus* >1280 CCE/100 mL)	*Percent failure based on USEPA Recreational Water Quality Standard*		*Percent failure based on the Alberta Recreational Water Quality Standard* (Thermotolerant Coliforms > 400 CFU/ 100 mL)
			E. coli > 126 CFU/100 mL based on the running geomean of five previous samples[a]	*E. coli* > 410 CFU/100 mL	
McCall Lake	ML2	53	65	32	39
	PR60	26	17	5	9
	ML1	20	10	7	12
	Inlet 3/4	17	22	5	5
	Total (n = 164)	**29**	**29**	**12**	**16**
Country Hills	WP31A	2	5	0	0
	WP31B	5	5	0	0
	WP31C	12	46	20	26
	WP31D	20	39	12	12
	WP31E	10	20	12	10
	Total (n= 205)	**10**	**23**	**9**	**10**
Inverness	Outfalls/Inlet	20	5	0	2
	WP26B	0	5	0	2
	WP26C	10	7	0	0
	WP26D	12	5	0	0
	Total (n = 164)	**10**	**5**	**0**	**1**
Total (n=533)		**17**	**20**	**7**	**7**

Firstly, considerable spatial variation was observed with respect to the frequency of water quality failures among the urban stormwater ponds, with McCall Lake appearing to be the most contaminated of the three storm ponds. This result was true regardless of the bacterial water quality indicator chosen for analysis (i.e., *Enterococcus*, *E. coli* or thermotolerant coliforms). Approximately 29% of all water samples taken at McCall Lake failed water quality guidelines for *Enterococcus* and/or *E. coli* at the recommended STV or geomean values set out in the guidance documents. Inverness Stormpond had the fewest water quality violations among the three ponds, also based on all bacteriological indicators examined, and therefore was considered to have the best water quality overall.

However, variation in bacteriological water quality was also observed among sampling sites within a single pond. The most contaminated site across all stormwater ponds examined was site ML2 at McCall Lake, with upwards of 65% of all samples failing the US EPAs Guidelines for Recreational Water Quality for *E. coli* geomean concentrations >126 CFU/100 mL (Table 3-2). This site had the poorest water quality irrespective of the bacterial indicator used in the analysis. It is important to note, however, that ML2 was an above-grade outfall (Table-3-1), thereby potentially explaining the more frequent bacteriological failures at this site as due to the fact that water samples were directly collected from the outfall and not after dilution into the pond. By comparison in a single pond, outfall ML1 in McCall Lake had far fewer water quality failures (based on all bacterial indicators) compared to ML2, with only 10% of samples violating US EPA's Guidelines for Recreational Water Quality for *E. coli* based on a geomean value >126 CFU/100 mL.

A second important observation was that the frequency of water quality failures was contingent upon which bacteriological indicator was used in the analysis. Overall, the geomean

criteria of >126 *E. coli*/100mL was the most frequently violated water quality standard when all water samples were amalgamated into the analysis (i.e., 20%, Table-3-2). This result was followed by *Enterococcu*s by molecular methods (17%), the single sample STV for *E. coli* at >410 CFU/100mL (7%), and lastly the thermotolerant coliform criteria of >400 CFU/100mL (7%). The greatest discrepancy between indicator failures was noted in the Inverness stormwater pond, where none of the water samples from any of the sites violated the single sample STV for *E. coli* of >410 CFU/100mL, though 10% of all samples violated the *Enterococcus* molecular standard (Table-3-2). The largest percentage variance between indicator violations was observed at ML2 site of McCall Lake, where 65% of samples violated the *E. coli* geomean of >126 CFU/100mL, but only 32% of these same samples violated the *E. coli* STV of *E. coli* of >410 CFU/100mL (Table-3-2).

3.2.1.3 Spatial and Temporal Variability of Bacterial Water Quality Indicators

Based on the variation in water quality violations among: a) the different stormwater ponds; and b) sites within a single stormwater pond [Table-3-2], we sought to examine the spatial and temporal characteristics of water quality in each of the stormwater ponds and at each of the sites within a single stormwater pond. Spatial and temporal variations in water quality were examined among the various bacterial indicators of water quality (i.e., *E. coli*, *Enterococcus*, and thermotolerant coliforms).

Spatial Variation in Microbial Water Quality Characteristics. Considerable spatial variation in water quality was observed among all stormwater ponds, and among each of the sampling sites in the individual ponds. Similar to what was noted above in terms of the percentage of bacteriological failures, the ML2 site at McCall Lake had the greatest median concentrations of

all bacterial indicators, and therefore the poorest water quality, across all three ponds and study sites in these ponds (compare Figure-3-1 [McCall Lake] to Appendix 3-1 [all other stormwater ponds and sites]). Median levels of *Enterococcus* at the ML2 site approximated 3.1 $\log_{10}$ CCE/100 mL, whereas at all other sampling sites in McCall Lake (i.e., ML1, Inlet 3/4, and PR60), the median occurrence was almost an order of magnitude lower (~ 2.3 $\log_{10}$ CCE/100 mL) [Figure-3-1]. This pattern was also reflected in the concentrations of *E. coli* levels between sampling sites within McCall Lake (Figure 3-2). Incidentally, ML2 also had the largest overall interquartile variation in the concentration of *Enterococcus* and *E. coli* during the study season (Figure-3-1), with *E. coli* concentrations varying by upwards of 2.5 $\log_{10}$ CFU/100 mL (Figure-3-1). Concentrations of thermotolerant coliforms were also high at this site and followed a similar trend to that of *Enterococcus* and *E. coli* (Appendix 3-1).

It is important to note that in most cases for *Enterococcus* and *E. coli* at sites other than ML2, there were several outliers in the data set (Figure-3-2 and Figure-3-3). In the context of this study, outliers were defined as a data point greater or less than 1.5*interquartile range (i.e., whiskers). Although outliers may reflect recent localized contamination events not necessarily reflective of overall water quality in the stormwater pond (e.g., aquatic birds in one area of the pond), their occurrence could also reflect the periods of peak contamination in stormwater ponds, and for which this effect may be contingent on temporal variables associated with water quality (e.g., first flush from storms, to be discussed later). Specifically, outliers for *Enterococcus* concentrations were represented by values higher than ~3.5 $\log_{10}$ for Inlet 3/4, ~3.75 $\log_{10}$ for ML1, and ~4 $\log_{10}$ for PR60. Similarly, outliers for *E. coli* concentrations occurred in Inlet 3/4 above ~2.5 $\log_{10}$ and in PR60 above ~3.25 $\log_{10}$. In some cases, the outliers were at an equal level of contamination of that observed in the ML2 range of values (i.e., 2-5 $\log_{10}$ CCE/100 mL

for *Enterococcus* concentrations and 1-3.5 $\log_{10}$ CFU/100mL for *E. coli* concentrations) (Figure-3-1). The single greatest concentration of *Enterococcus* observed during the study period was observed at site PR60. The greatest concentration of *E. coli* observed was at ML1. Consequently, although ML2 represented the most consistently contaminated sampling site at McCall Lake, the other sampling sites in the stormwater pond appeared to be at risk for significant levels of periodic bacterial contamination. This observation warranted a closer examination of the temporal variance of bacteriological water quality in each of the ponds and at each of the sites within the ponds.

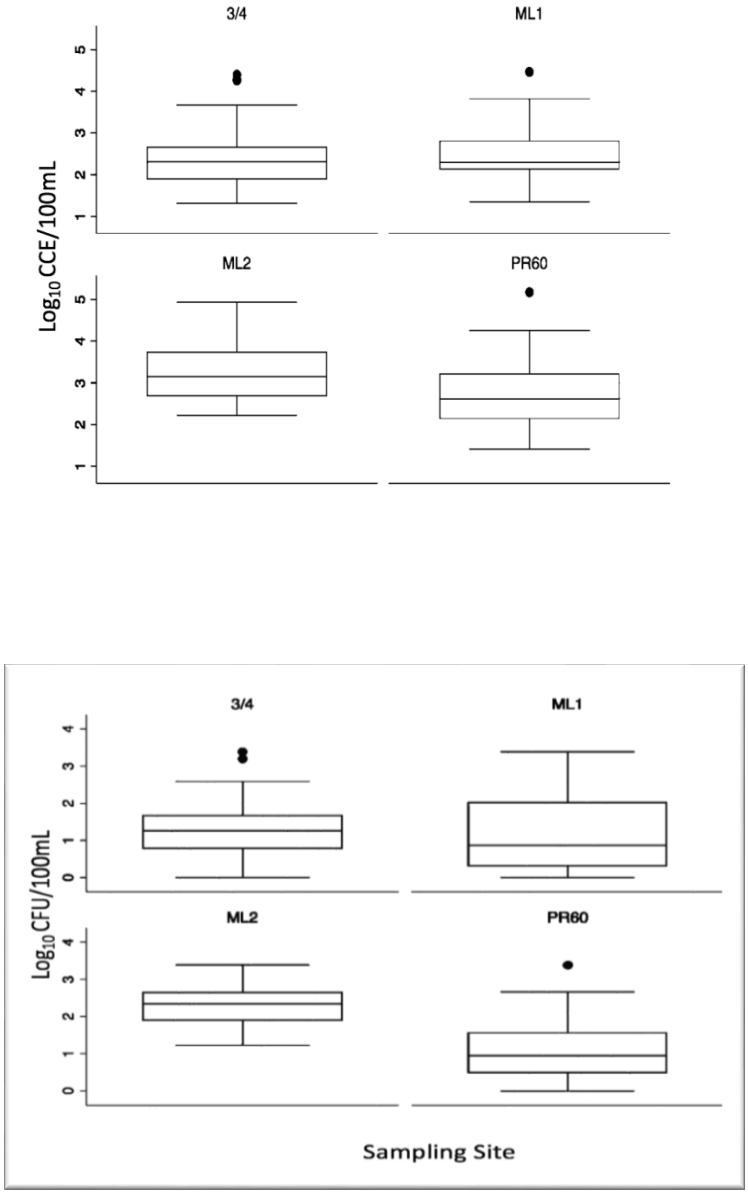

*Figure-3-1: Box and Whisker plot of Enterococcus log_{10} values (top) and E. coli log_{10} (bottom) values in McCall Lake over 21 weeks broken down by sampling site (i.e., ML2, ML1, Inlet ¾, and PR60). The outer edges of the box represent the 25th and 75th percentiles (i.e., interquartile range), and the line within the box represents the median. The location of median indicates the skew of the data. The whiskers represent the interquartile range*1.5. The outliers are determined by being greater or less than 1.5 times the upper of lower interquartile ranges as represented by circles.*

Temporal Variation in Microbial Water Quality Characteristics. Significant temporal fluctuations in bacteriological water quality were observed between the stormwater ponds, and among the sampling sites within a stormwater pond (Figure-3-2, Figure-3-3, and Appendix 3-1). In the context of McCall Lake, sampling site ML1 showed the greatest fluctuations of the microbial water quality indicator *E. coli* from one sampling date to the next (Figure-3-2 and Figure-3-3). For example, within one week, and in three consecutive samples from August 9th – 16th, water quality varied from below the statistical threshold value (STV) at 0.3 $\log_{10}$ CFU/100mL to above the STV at 3.5 $\log_{10}$ CFU/100mL then back below the STV at 1.7 $\log_{10}$ CFU/100mL (Figure-3-2). This high variability represented a significant fluctuation in water quality in a single week's time, raising potential concerns about the sampling frequency needed for water quality monitoring programs. In consideration of the STV threshold where we sampled biweekly, five water quality violations were due to extreme fluctuations in water quality indicators at ML1 (Figure-3-2). In fact, between any two sequential samples, variations in *E. coli* concentrations at ML1 could go from 0 $\log_{10}$ CFU/100mL to 4 $\log_{10}$ CFU/100mL (e.g., July 6th to July 10th). Although ML2 had a more consistent baseline contamination level (i.e., a higher median) than all other sites at McCall Lake, occasionally, water was of equally poor quality at some of the other sites, warranting a closer examination of the temporal patterns of occurrence associated with these failures across all sites in McCall Lake.

Interestingly, all of the sampling sites in McCall Lake failed the STV for *E. coli* on May 25th, July 10th, August 7th, and September 13th. Similarly, low values of *E. coli* were observed in all sampling sites at McCall Lake on May 16th, June 27th, July 6th, and September 5th, suggesting a variable linking contamination along all sampling sites. Since it is well-known that precipitation can lead to pathogen transport, we examined the amount of precipitation to see the

effects on the levels of fecal indicator bacteria. For simplicity, we evaluated potential relationships between antecedent rain (i.e., rain within 72 hours) and bacterial indicator values, noting that the highest values of antecedent rain occurred on May 25th, August 7th, and September 13th along with the highest values of bacterial indicators; whereas, the lowest values of antecedent rain occurred on May 16th, June 27th, July 6th, July 10th, and September 6th (i.e., no rain in the past 72 hours) and correlated with lower bacterial indicator values.

As with the *E. coli* results, there was considerable temporal variability for *Enterococcus* in each of the stormwater ponds and between each of the sampling sites at each stormwater pond (Appendix 3-1). *Enterococcus* levels at the McCall Lake sampling sites could be highly variable from week-to-week. A temporal change of ~2.5 $\log_{10}$ CCE/100mL with the resulting value being above ~4 $\log_{10}$ CCE/100mL was observed multiple times at each McCall Lake sampling site though usually on different sampling dates (e.g., ML2 on September 13th, ML1 on August 14th, PR60 on May 25th, and Inlet ¾ on July 17th) (Figure-3-3). This data overall suggested that microbial water quality indicators (i.e., *Enterococcus* and *E. coli*) could be highly variable (i.e., greater than 2.5 $\log_{10}$) in a relatively short period of time (i.e., two-to-five days).

Spatial-Temporal Variation in Microbial Water Quality Characteristics. Spatial-temporal variability in water quality was analyzed by the use of a 5-sample running geometric mean between stormwater ponds and among sampling sites within a single stormwater pond (Appendix 3-1). Similar to what has been stated above regarding the trend of higher levels of microbial water quality indicators, sampling site ML2 at McCall Lake also had the highest 5-sample running geometric mean during the 21-week sampling season (compare Figure-3-2 [McCall Lake] to Appendix 3-1). ML2 violated the 5-sample running geometric mean standard for *E. coli* (i.e., 2.1 $\log_{10}$ CFU/100mL) for all sampling dates, except for a three-week stretch of the 21-

week sampling season (i.e., June 15th through July 6th) (Figure-3-2). Further, within McCall Lake, all other sampling sites (i.e., ML1, Inlet ¾, and PR60) did not violate the 5-sample running geometric mean for *E. coli* during the entire 21-week sampling season (Figure-3-2). A comparable pattern was reflected in the concentration of *Enterococcus* between sampling sites at McCall Lake: ML2 however violated the 5-sample running geometric mean for *Enterococcus* throughout all 21 weeks of the sampling season, with the geometric mean being above the standard of 2.4 $\log_{10}$ CCE/100 mL (Figure-3-3). In contrast, at all other McCall Lake sampling sites (i.e., ML1, Inlet ¾, and PR60), the 5-sample running geometric mean for *Enterococcus* had less violations than ML2 (Figure-3-3). However, the 5-sample running geometric mean for *Enterococcus* reflected more water quality failures than for *E. coli*. Overall, this data suggested that ML2 had poorer water quality throughout the duration of the 21-week sampling season in comparison to the other McCall Lake sampling sites.

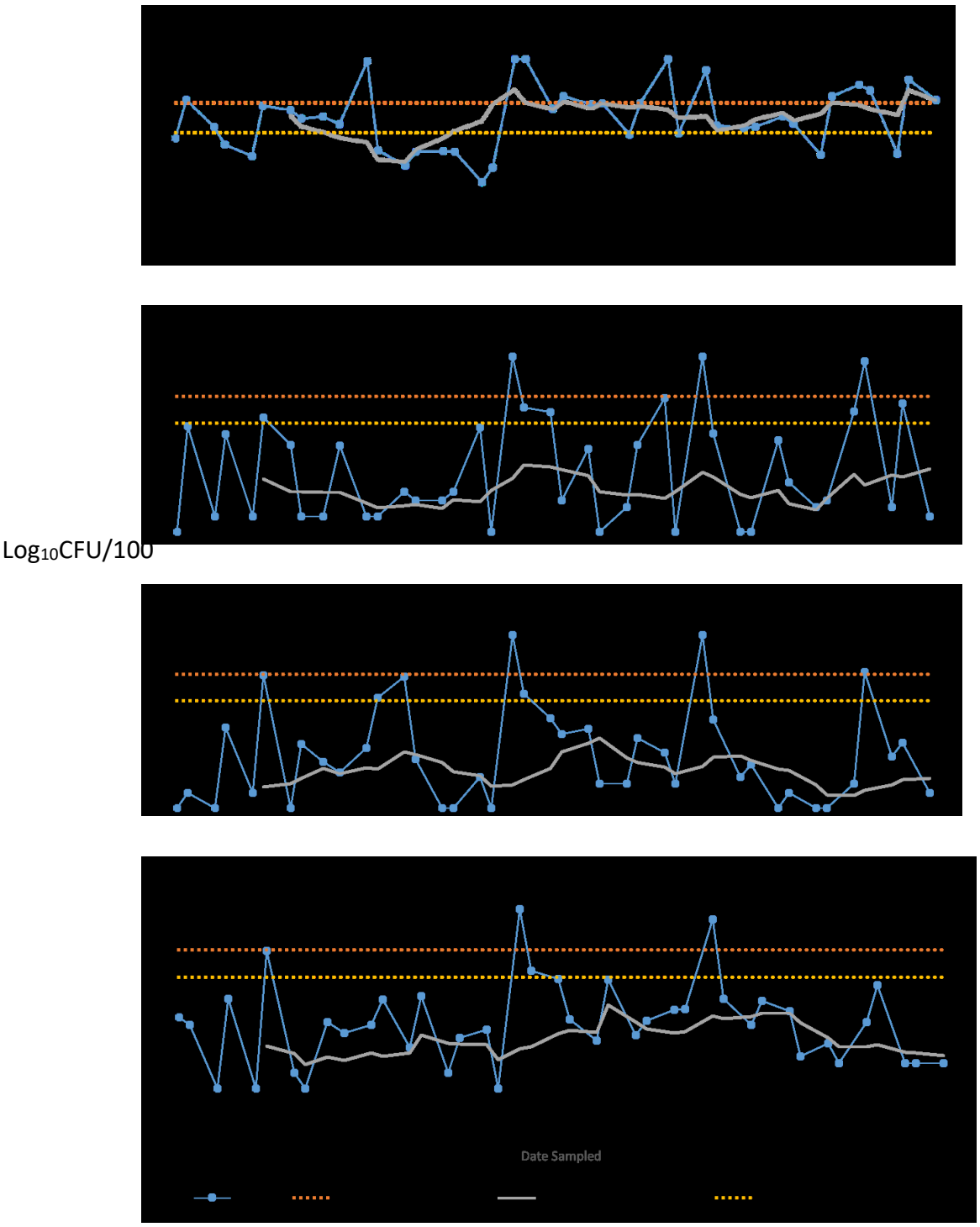

Figure-3-2: Temporal pattern of occurrence of E. coli log$_{10}$ concentrations at sampling site ML2 (top), ML1 (second from the top), PR60 (third from the top), and Inlet ¾ (bottom) in McCall Lake over 21-weeks. The US EPA Guidelines for Recreational Water Quality *geometric mean standard of >126 CFU/100mL (yellow dotted line) and single sample threshold value of >410 CFU/ 100mL (red dotted line) are also provided. The 5-sample running geometric mean of the water samples is in gray, and the individual water sample concentrations of E. coli are in blue.*

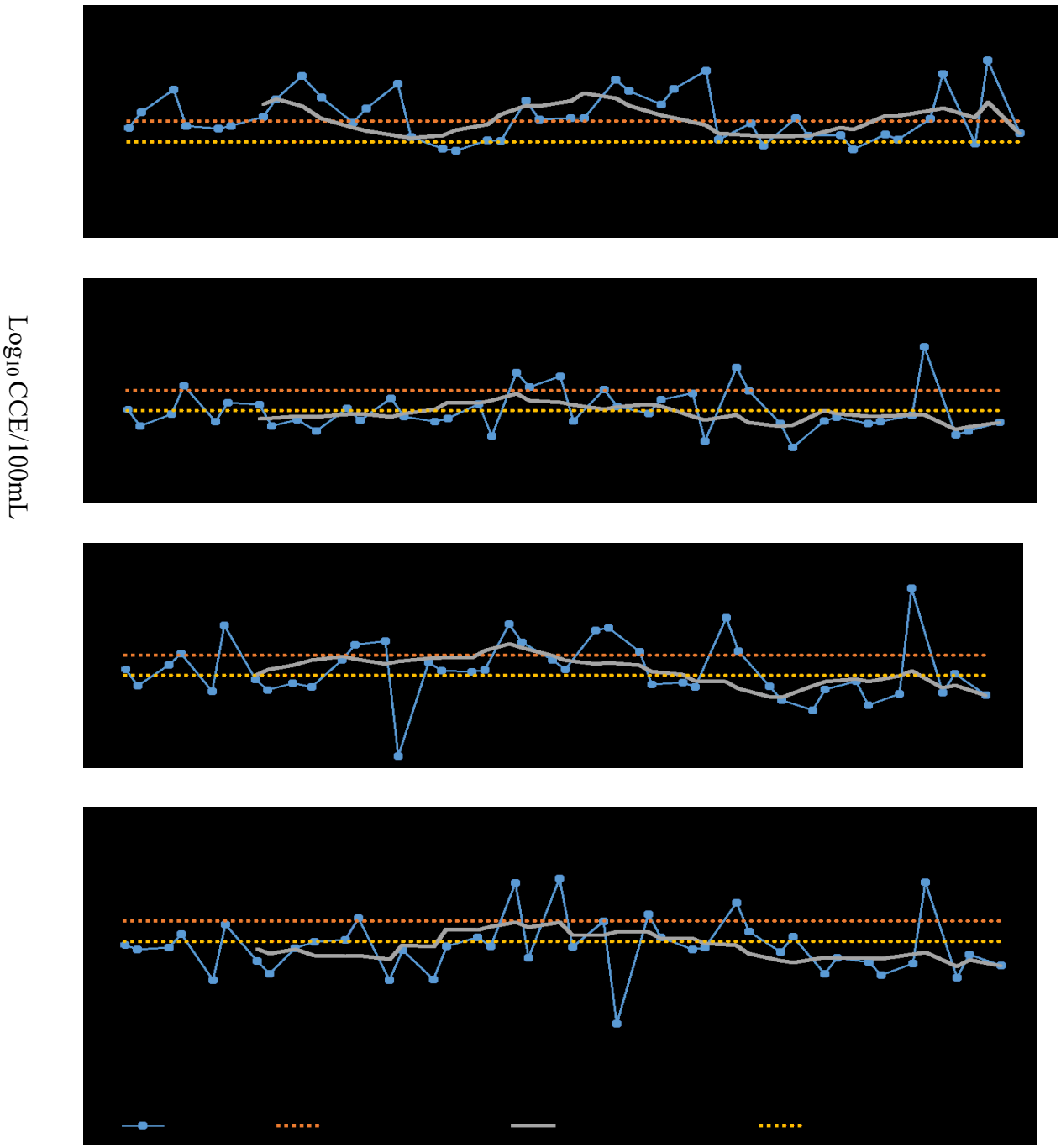

Figure-3-3: Temporal pattern of occurrence of Enterococcus log$_{10}$ concentrations at sampling site ML2 (top), ML1 (second from the top), PR60 (third from the top), and Inlet ¾ (bottom) located in McCall Lake over 21 weeks. The US EPA Guidelines for Recreational Water Quality *geometric mean standard of >300 CCE/100mL (yellow dotted line) and a single sample threshold value of >1280 CCE/ 100mL (red dotted line) are also provided. The 5-sample running geometric mean of the water samples is in gray, and the individual water sample concentrations of Enterococcus are in blue.*

3.2.2 Stormwater-Impacted Rivers

3.2.2.1 Stormwater Outfalls for the Elbow River and the Nose Creek

For the purposes of the thesis research study, the Elbow River samples came from the section of river between the Glenmore Dam and confluence with the Bow River. This waterway is utilized for summer recreational activities (e.g., swimming, canoeing, tubing, fishing, etc.), and the ten sampling sites coincided with recreational access points on the river (Figure 3-4). Water samples were collected directly from the main stem of the Elbow River. A rural river in Southern Alberta, and not impacted by stormwater, acted as a comparator to the Elbow river. This river is primarily impacted by wildlife and agriculture, and is also used for recreational activities. Like the Elbow River, samples were taken directly from the main stem of the river at accessible recreational points of access.

The Nose Creek is a small tributary draining into the Bow River. Ten sampling sites were sampled along the creek. Unlike the Elbow River samples, water samples collected from the Nose Creek study came directly from stormwater outfall samples collected during synoptic rain events, due to the lack of drainage during base flow conditions.

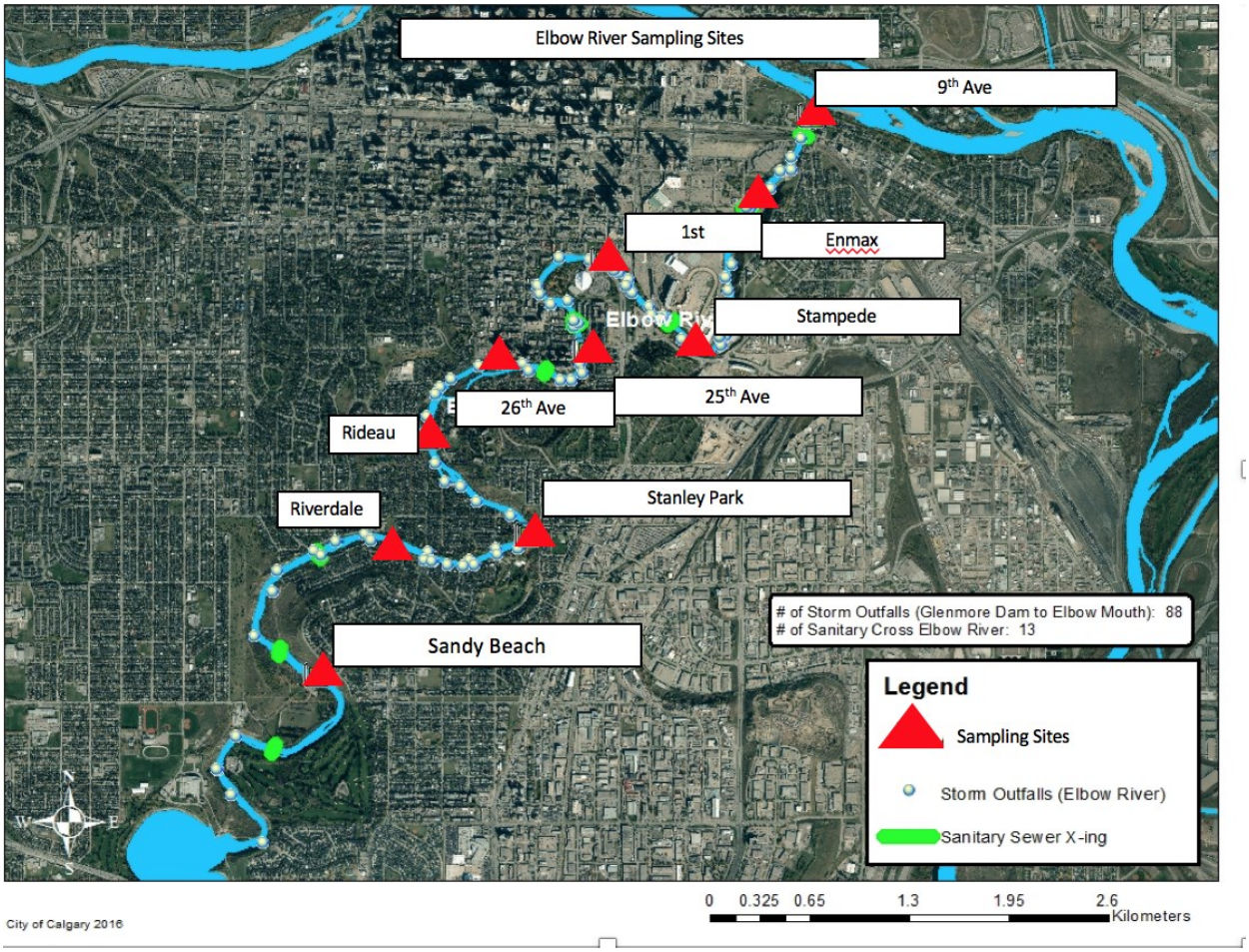

Figure 3-4: Map of the Elbow River sampling sites in Calgary Alberta, Canada (provided by the city of Calgary).

3.1.1.2 General Overview of Bacterial Water Quality in Urban Stormwater-Impacted Rivers

A total of 117 samples were taken from ten sampling sites along the Elbow River in Calgary. In addition, a total of 44 samples were taken from ten sampling sites along the Nose Creek in Airdrie. A high-level descriptive overview of bacteriological water quality at each of the sampling sites along the Elbow River and the Nose Creek is provided in 3-3 and Table 3-4. This overview revealed a number of noteworthy observations.

Foremost, substantial spatial variation existed among the sampling sites. Molecular-based methods for *Enterococcus* densities often exceeded the standard of 1280 CCE/100 mL, with 79% of samples failing in the Nose Creek stormwater samples in Airdrie. Four Nose Creek sampling sites had a 100% failure rate for *Enterococcus* STV. In the Elbow River, 21% of samples failed the Enterococcus STV of 1280 CCE/100 mL.

Additionally, thermotolerant coliforms from the Nose Creek sampling sites had a high failure rate, with 56% of samples exceeding the Alberta Recreational Water Quality Standard for thermotolerant coliforms of >400 CFU/100 mL in the Nose Creek. Three of the sampling sites out of ten (i.e., 25841, 25793, and 25804) were the only sampling sites at the Nose Creek to have had no failures in a single water quality standard.

In the Elbow River, eight sampling sites (i.e., Stanley Park, Rideau Pedestrian Bridge, 26th Ave SW, 25th Ave SW, 1 St SE, Stampede Grandstand, Enmax Park, and 9th Ave SE) failed the Alberta Recreational Water Quality Standard for thermotolerant coliforms of >400 CFU/100 mL. Four of the sampling sites (i.e., 26th Ave SW, 25th Ave SW, 1 St SE, and 9th Ave SE) had a failure rate of 23%.

Table 3-3: Microbial water quality in the Nose Creek based on the percentage of sample failing existing standards of water quality in Airdrie.

		Water Quality Standard/Guideline			
Stormwater-Impacted River	**Sampling Site**	*Percent failure based on the USEPA Recreational Water Quality Standard (Enterococcus* >1280 CCE/100 mL)	*Percent failure based on USEPA Recreational Water Quality Standard*		*Percent failure based on the Alberta Recreational Water Quality Standard* (Thermotolerant Coliforms > 400 CFU/ 100 mL)
			E. coli > 126 CFU/100 mL based on the running geomean of five previous samples[a]	*E. coli* > 410 CFU/100 mL	
Nose Creek	25756 N=5	80	100	40	80
	25793 N=4	50	25	0	0
	25804 N=4	50	0	0	0
	25807 N=5	100	80	40	80
	25811 N=5	80	80	60	80
	25814 N=5	100	100	60	80
	25817 N=3	100	100	66	33
	25841 N=3	33	0	0	0
	25847 N=5	100	80	40	60
	25855 N=5	80	100	80	100
Total (n=44)		**79**	**70**	**40**	**56**

Table 3-4: Microbial water quality in the Elbow River based on the percentage of samples failing existing standards of water quality in the Elbow River.

		Water Quality Standard/Guideline	
Stormwater-Impacted River	**Sampling Site**	*Percent failure based on the USEPA Recreational Water Quality Standard (Enterococcus* >1280 CCE/100 mL)	*Percent failure based on the Alberta Recreational Water Quality Standard* (Thermotolerant Coliforms > 400 CFU/ 100 mL)
Elbow River	Sandy Beach N=13	8	0
	Riverdale Pedestrian Bridge N=13	8	0
	Stanley Park N=13	15	8
	Rideau Pedestrian Bridge N=13	23	15
	26th AVE SW N=13	30	23
	25th AVE SW N=13	23	23
	1st ST SE N=13	23	23
	Stampede Grandstand N=13	38	8
	ENMAX Park N=13	15	15
	9th Ave SE N=13	30	23
Total (n=130)		**21**	**13**

3.2.2.2 Spatial and Temporal Variability of Bacterial Water Quality Indicators in Urban Stormwater-Impacted Rivers

Based on the variation in water quality violations observed in the Elbow River study we undertook an examination of the spatial and temporal characteristics of water quality at sites along the Elbow River. Spatial and temporal variations in water quality were examined using two bacterial indicators of water quality (i.e., *Enterococcus* and thermotolerant coliforms), reflective of the current regulations used for water quality in Alberta (i.e., thermotolerant coliforms by culture) and newly proposed guidelines for Alberta, similar to those developed and proposed by the USEPA (i.e., molecular *Enterococcus*).

Spatial Variation in Microbial Water Quality Characteristics. Considerable spatial variation in water quality was observed among the sampling sites in the Elbow River. Average concentrations of *Enterococcus* varied only slightly among the ten sampling sites along the Elbow River, with median values ranging from 2.5 $\log_{10}$ CCE/100mL to 3 $\log_{10}$ CCE/100mL (Figure 3-5). However, the site with the highest range of values was Stanley Park, with *Enterococcus* values ranging from 2.1 $\log_{10}$ CCE/100mL to 5.4 $\log_{10}$ CCE/100mL, and demonstrating how drastic water quality could vary within one sampling site.

It is important to note that four sampling sites (i.e., 1 Street SE, Riverdale Avenue Bridge, Sandy Beach, and Stanley Park) in the Elbow River had outliers in the data set (i.e., greater than 1.5*interquartile range, Figure 3-5). More specifically, the majority of these outliers occurred above ~ 4 $\log_{10}$ CCE/100mL. The single greatest concentration of *Enterococcus* noted during the study period was observed at Stanley Park (i.e., 5.4 $\log_{10}$ CCE/100mL) on July 10th. As represented by the outliers at the Elbow River sampling sites, bacterial water quality overall

appeared to be highly variable, and therefore at risk for significant levels of periodic bacterial contamination.

In contrast to the Elbow River, a box and whisker plot for the rural river control showed median values of ~2.2 $\log_{10}$ CCE/100mL at all three sampling sites tested. The highest range of values was observed at sampling site 'Rural River C', with *Enterococcus* values ranging from 1.6 to 2.6 $\log_{10}$ CCE/100mL. In addition, only two sampling sites had outliers (i.e., 'Rural River A' and 'Rural River B'), which occurred below the lower whisker. These ranges reflected the consistency of water quality in the rural river control. This observation justified a closer examination of the temporal variance of bacteriological water quality at each of the sites in the Elbow River and the rural river control.

For Nose Creek, median values of *Enterococcus* spp. ranged from 2.1- 4.6 $\log_{10}$ CCE/100mL (Table 3-5). Further, the highest range of values for *Enterococcus* spp. was at sampling site 25814, ranging from 2.1-4.9 $\log_{10}$ CCE/100mL, reflecting the wide range of values that could occur at one sampling site. Similar to *Enterococcus* spp., a wide range of values was observed among sampling sites for *E. coli*. At sampling site 25817, *E. coli* values ranged from 0.3-3.0 $\log_{10}$ CFU/100mL. However, it should be noted that there were only three samples taken from sampling site 25817.

Table 3-5: Range and median levels of Enterococcus spp. and E. coli at the Nose Creek sampling sites.

Nose Creek Sampling Site	*Range of Enterococcus* spp. concentrations (log_{10} CCE/100mL)	*Median Enterococcus* spp. concentration (log_{10} CCE/100mL)	*Range of E. coli* concentrations (log_{10} CFU/100mL)	*Median E. coli* concentrations (log_{10} CFU/100mL)
25756 (n=5)	3.0 - 5.8	3.6	2.2 - 3.1	2.5
25793 (n=4)	2.5 - 4.3	3.7	0.4 - 2.4	1.2
25804 (n=4)	2.0 - 3.4	2.1	0 - 0.4	0
25807 (n=5)	3.3 - 5.0	4.6	2.1 - 3.3	2.4
25811 (n=5)	3.0 - 4.7	3.4	1.5 - 3.3	2.8
25814 (n=5)	2.1 - 4.9	3.7	2.1 - 3.3	3.2
25817 (n=3)	3.6 - 4.8	3.6	0.3 - 3.0	2.3
25841 (n=3)	2.2 - 3.7	2.6	1.2 - 2.1	2.0
25847 (n=5)	3.0 - 4.0	3.6	1.9 - 2.8	2.1
25855 (n=5)	3.0 - 4.5	4.1	2.8 - 3.2	3.0

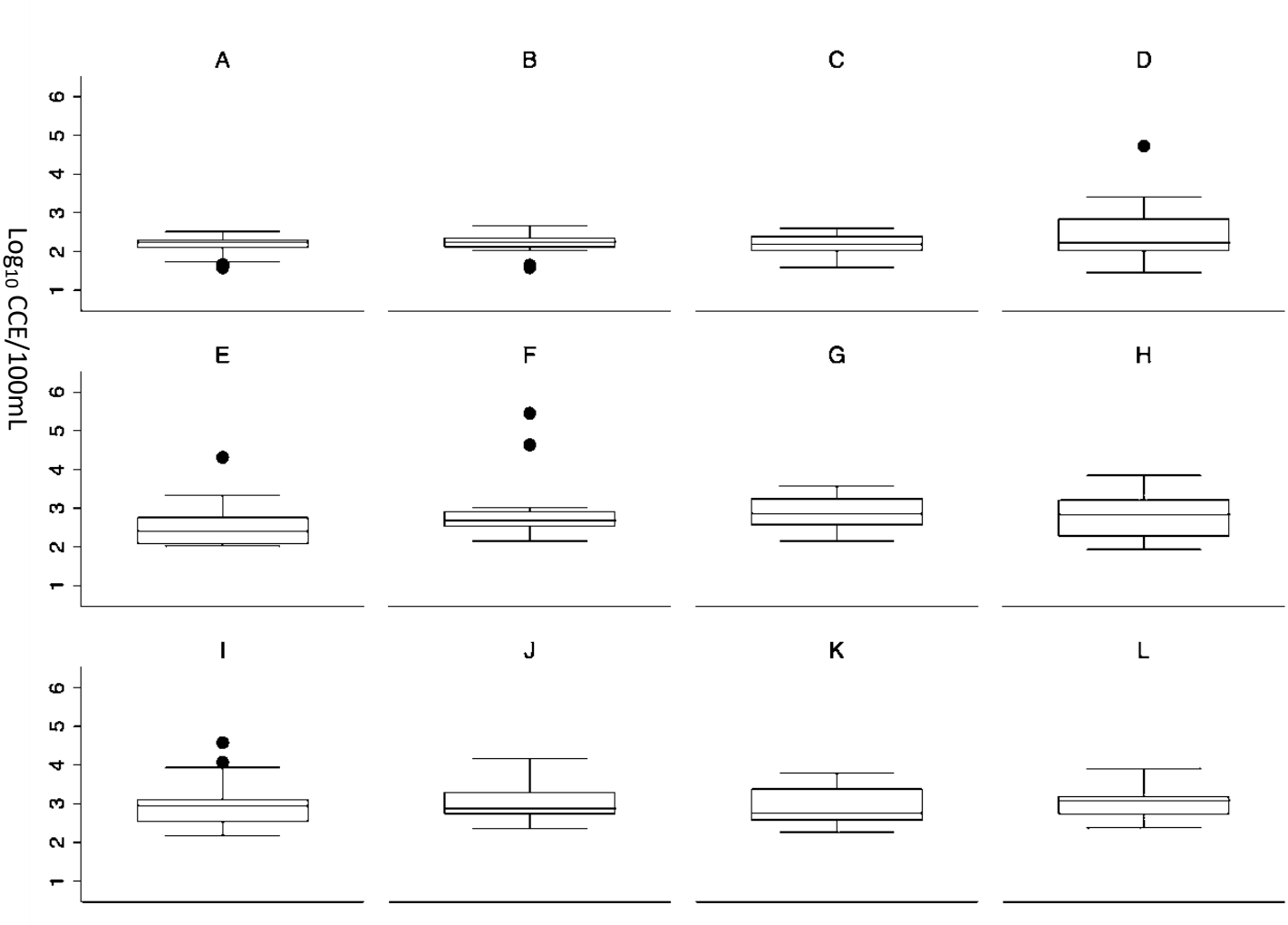

*Figure 3-5: Box and Whisker plot of Enterococcus spp. values in the Elbow River and Rural River broken down by sampling site. Three sampling sites in the rural river: "A" – Sampling site A, "B" – Sampling site B, and "C" – Sampling site C. Each site in the rural river had 18 data points for analysis. Ten sampling sites in the Elbow River: "D"- 1 St SE, "E"- 25th Ave Bridge, "F"- 26th Ave SW, "G"- 9th Ave, "H"- Enmax Park, "I"- Rideau Pedestrian Bridge, "J"- River Dale Avenue Bridge, "K"- Sandy Beach, and "L"- Stampede Grandstand, Stanley park. Each site in the Elbow River had 13 data points for analysis. The outer edges of the box represent the 25th and 75th percentiles (i.e., interquartile range), and the line within the box represents the median. The location of median indicates the skew of the data. The whiskers represent the interquartile range*1.5. The outliers are determined by being greater or less than 1.5 times the upper of lower interquartile ranges as represented by circles.*

Temporal Variation in Microbial Water Quality Characteristics. Noteworthy temporal fluctuations in bacteriological water quality were documented between sampling sites along the Elbow River (Figure 3-6, Appendix 3-1). In the context of the Elbow River, the temporal pattern of the microbial water quality indicator *Enterococcus* spp. was episodic. For example, within one month (i.e., three sampling dates, covering June 22nd through July 10th) at Stanley Park, concentrations of *Enterococcus* fluctuated from a low of 2.1 $\log_{10}$ CCE/100mL to 5.4 $\log_{10}$ CCE/100mL, and back down to 3.1 $\log_{10}$ CCE/100mL) (Figure 3-6). Furthermore, the data suggested that water quality could be highly variable from week-to-week. As an example, from July 4th to July 10th, *Enterococcus* spp. concentrations fluctuated at Sandy Beach from 2.1 $\log_{10}$ CCE/100mL to 4.7 $\log_{10}$ CCE/100mL (Figure 3-6). Interestingly, a sizable fluctuation of *Enterococcus* spp. was noted across all sampling sites in the Elbow River between these sequential sampling dates; however, the extent to which this result occurred varied between sampling sites. For example, Rideau Pedestrian Bridge saw an increase in *Enterococcus* spp. concentrations from 2.4 $\log_{10}$ CCE/100mL to 3.6 $\log_{10}$ CCE/100mL for the aforementioned sampling dates (Appendix 3-1). Though not as great as what was observed at Sandy Beach, these values suggested that a trend of changes in water quality could be occurring, as water flows down the Elbow River from Sandy Beach to Rideau Pedestrian Bridge.

Interestingly, the majority of the sampling sites in the Elbow River (i.e., Stanley Park, Enmax, 1 Street SE, Sandy Beach, River Dale Avenue Bridge, Rideau Pedestrian Bridge, and 25th Avenue SW) failed the STV for *Enterococcus* on July 12th. Furthermore, on August 14th, all ten sampling sites failed. In addition, on August 28th, all sampling sites had water quality above the standard (i.e., >1280 CCE/100mL). As was observed in stormwater ponds, the Elbow River appeared to show patterns of spatiotemporal consistency in bacterial indicators of water quality.

Two Elbow River sampling sites (i.e., Stanley Park and Sandy Beach) (Figure 3-6) were compared to a rural river control (Figure 3-7) throughout the sampling season. The Elbow River sampling sites were chosen for comparison due to one sampling site having the widest range of *Enterococcus* spp. concentrations (i.e., Stanley Park) (Figure 3-6), and the other, which was farthest upstream, being the least contaminated (i.e., Sandy Beach) (Figure 3-6). With respect to the rural river control, the most contaminated sampling site was chosen (i.e., 'Sample Site B') (Figure 3-7). *Enterococcus* spp. levels appeared to be significantly higher in the urban river (i.e., the Elbow River) impacted by stormwater than the rural river control, with the rural river control never exceeding the USEPA's Recreational Water Quality Standard for *Enterococcus* spp. at >1280 CCE/100mL (Figure 3-6 and Figure 3-7). This finding suggested that water quality of the rural river control was relatively more stable than the water quality of the urban stormwater-impacted Elbow River.

Spatial-Temporal Variation in Microbial Water Quality Characteristics. Spatial-temporal variability was analyzed by using a 5-sample running geometric mean between sampling sites within the Elbow River (Appendix 3-1). In general, microbial water quality was considered to be poor, with the running geometric mean at or above the recommended guidelines (i.e., >300 CCE/100mL) at the majority of the sampling sites (Figure 3-6). For example, at Stanley Park, the geometric mean of 300 CCE/100 mL (i.e., 2.4 $\log_{10}$) of *Enterococcus* spp. was violated for the entirety of the sampling season (Figure 3-14). A comparable pattern was observed at Sandy Beach, where the geometric mean violated the recreational water quality standards for the majority of the sampling season; though notably, the water quality met the recommended standards early in the sampling season (i.e., the first three weeks). In addition, the geometric means between the two sampling sites seemed to reflect each other, as both reached their highest

points on July 24th. This data overall suggested that water quality was consistently poor throughout the sampling season along the urban stormwater-impacted Elbow River.

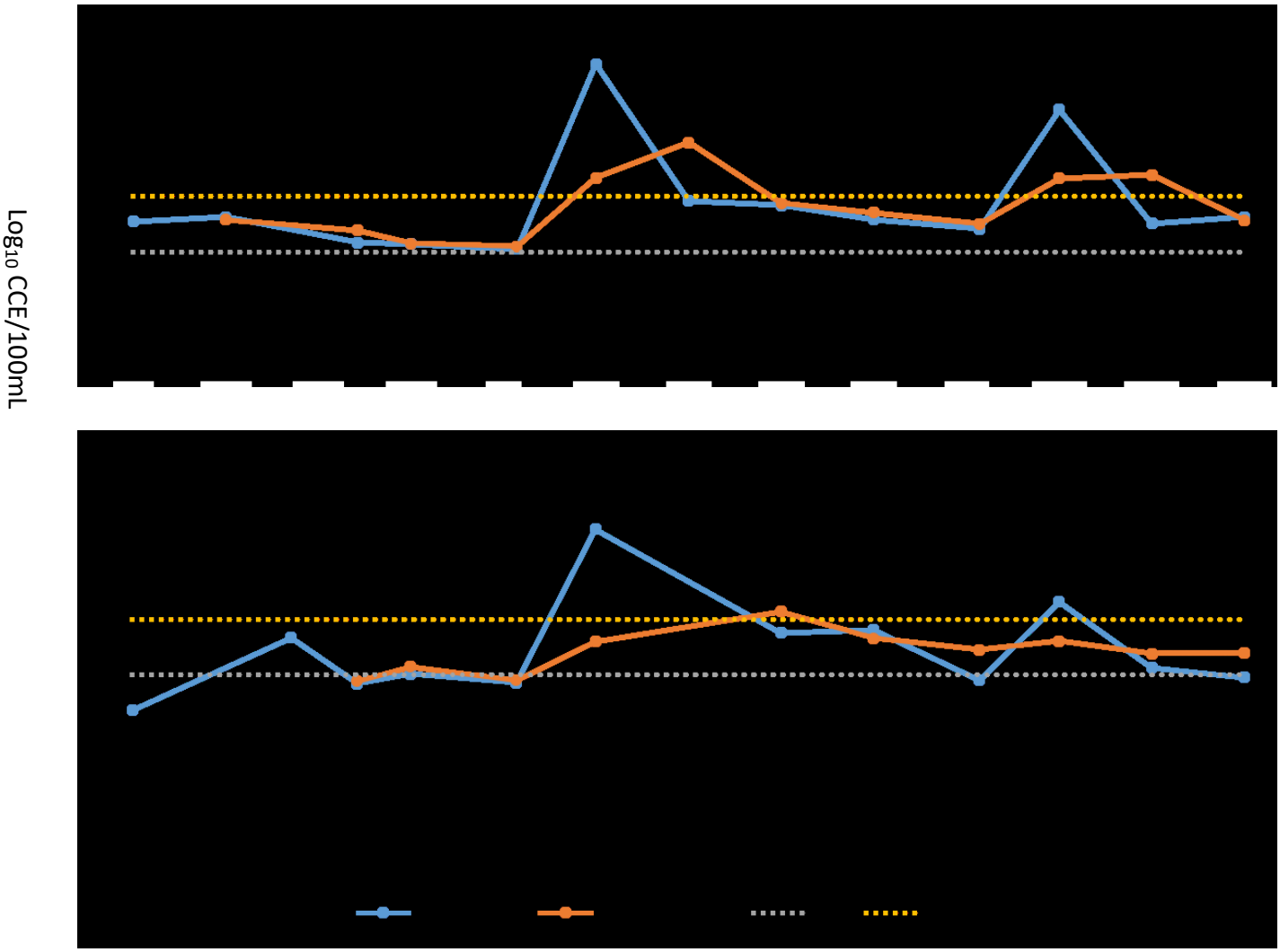

Figure 3-6: Temporal pattern of occurrence of Enterococcus spp. log_{10} CCE concentrations at sampling site Stanley Park (top) and Sandy Beach (bottom) located in the Elbow River. The USEPA Recreational Water Quality Standard geometric mean standard of >300 CCE/100mL is in yellow; the USEPA Recreational Water Quality Standard statistical threshold value of >1280 CCE/100mL is in red; the 5-sample running geometric mean of the water samples is in gray; and the single sample concentration of Enterococcus spp. are in blue.

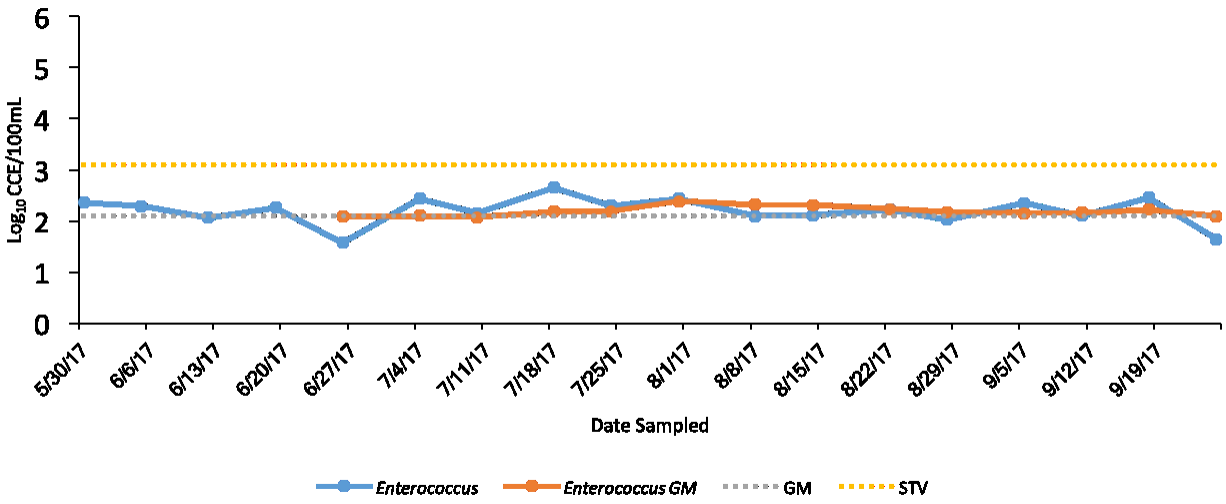

Figure 3-7: Temporal pattern of occurrence of Enterococcus spp. log_{10} CCE concentrations at a rural river control. The USEPA Recreational Water Quality Standard geometric mean standard of >300 CCE/100mL is in yellow; the USEPA Recreational Water Quality Standard statistical threshold value of >1280 CCE/100mL is in red; the 5-sample running geometric mean of the water samples is in gray; and the single sample concentration of Enterococcus spp. are in blue.

3.3 Discussion

Pathogens represent the most important acute risk to public health in the context of urban stormwater use. However, little is known about the microbial water quality in stormwater systems in many of the urban centers that are contemplating use of this alternative resource. Various research studies have demonstrated that stormwater systems can be significantly impacted fecal pollution, including human sewage (Sauer, *et al*., 2011; Converse, *et al*., 2011; Newton, *et al*., 2013; Chase, *et al*., 2012). Consequently, this portion of the thesis research examined microbial stormwater quality and impacts on receiving water bodies (retention ponds, rivers) in the municipalities of Calgary and Airdrie, Alberta, with the goal of using this information in the development of future strategies and approaches to the management of stormwater. Three different fecal indicator bacteria (FIB) were utilized and compared to water quality guidelines in this thesis: *Enterococcus* spp., *E. coli*, and thermotolerant coliforms. The most significant contribution of this research was the demonstration that stormwater can, at times, be of very poor microbial water quality, often failing existing water quality guidelines. In some ways, the results are not surprising, as the primary role of stormwater systems has been to reduce contaminant loading into surface waters. However, as society shifts its perceptions from viewing stormwater as a 'nuisance' towards stormwater as a 'resource', it is essential to understand that the microbial quality of this resource can, at times, be relatively poor and have the potential to inadvertently affect public health in the context of making use of this resource as an alternative water source.

Our findings with respect to stormwater quality are similar to that of other researchers (Kapoor, *et al*., 2016; Marsalek & Rochfort, 2004; Sauer, *et al*., 2011; Noble *et al.*, 2006). Noble *et al*. (2006) found a high occurrence (i.e., >50%) of water quality threshold failures according to

the USEPA regulation in stormwater drain-impacted areas along the Santa Monica Bay, in California, USA. In addition, Sauer *et al*. (2011) noted that two stormwater-impacted beaches in north central United States exceeded the USEPA's Recreational Water Quality Criteria for *E. coli* in 97% and 100% of samples; and Serrano & Delorezno (2008) found that stormwater pond samples exceeded standards for recreational water in 33% of samples for fecal coliform bacteria in a stormwater pond in Charleston, South Carolina, USA. Parker *et al*. (2010) found that 88% of stormwater samples from recreational beaches exceeded the single sample threshold set forth by the USEPA for *Enterococcus* spp. Therefore, our findings of poor quality stormwater are in line with other studies.

As previously noted in the results, not every sampling site that was impacted by stormwater, or every stormwater drain, had high FIB levels. In our study on the Elbow River, for example, Sandy Beach had the fewest water quality failures, and although it was the most upstream site in this system with the fewest number of cumulative stormwater drains impacting the site, there were several instances when water quality was poor, implying that Sandy Beach was at significant risk of fecal contamination at levels sufficient to violate recreational water quality guidelines on occasion. Other studies have found high levels of FIB in downstream waters in both urban and agriculturally-impacted environments (Petersen, *et al*., 2005; Sigua, *et al*., 2010; Crowther, *et al*., 2002). Petersen *et al*. (2005) studied the concentrations of *E. coli* in an urban stormwater-impacted river in Texas, USA, and found that the concentrations of *E. coli* increased from upstream to downstream. They also noticed this trend with fecal coliforms. The variability in the frequency of microbial water quality failures at different sites and at different times throughout the study period warranted a closer investigation into these spatial and temporal patterns.

In our study, considerable variation in stormwater quality was observed at each of the sampling sites in each of the urban stormwater ponds. At individual sampling sites, FIB concentrations in stormwater samples occurred over a wide range of values. *E. coli* values at sampling site ML2 at McCall Lake ranged from 17 CFU/100mL up to >2419.6 CFU/100mL (i.e., the detection limit of the assay), with many of the upper ranges detected far surpassing water quality guidelines. Liao *et al*. (2015) collected samples from an urban stormwater-impacted recreational creek in Blacksburg, Virginia, USA, during six summer storm events through auto-samplers. This study showed that *E. coli* levels could vary up to 2.5 orders of magnitude throughout the sampling season (i.e., from 10^2 up to $10^{4.5}$ $\log_{10}$ CFU/100mL) (Liao, *et al*., 2015). Similar to our findings, a study published by Marsalek & Rochfort (2004) found that *E. coli* counts in a Toronto, Canada, stormwater-impacted body of water ranged from 17 *E. coli* counts/100mL to up 5130 *E. coli* counts/100mL. Marsalek & Rochfort (2004) noted that *E. coli* counts in stormwater, which were not known to be impacted by combined sewer outfalls, would typically range from 10^3 to 10^4 units/100mL. However, in stormwater systems affected by combined sewer outfalls, higher levels of *E. coli* were observed (i.e., 10^6 units/100mL) likely due the presence of residual sewage and sewer sediments in the runoff (Marsalek & Rochfort, 2004). In North Carolina, USA, a study of stormwater runoff collected during storm events found levels of *E. coli* to be as high as 1.20×10^5 MPN/100mL during storm events, and baseflow concentrations to range from 10 to 4.78×10^2 MPN/100mL (Parker, *et al*., 2010). A study near Brisbane, Australia analyzing a stormwater pond intended for reuse activities (e.g., toilet-flushing, irrigation, etc.) found *E. coli* levels to range from 1.33×10^2 up to 1.07×10^4 CFU/100mL (Chong, *et al*., 2013).

This high variability of FIB was not limited to *E. coli* as an indicator, as it was also observed with *Enterococcus* spp. We found that *Enterococcus* spp. values ranged from 167 up to $1.8x10^5$ CCE/100mL in the Calgary urban stormwater ponds. The 2010 study by Wade *et al*., comparing sewage-impacted beaches in eastern United States (i.e., Mississippi, Alabama, and Rhode Island), found that *Enterococcus* spp. measured through molecular-based methods could range from 26 CCE/100mL up to $2.9x10^4$ CCE/100mL. In the study by Liao *et al*. (2015), the research showed *Enterococcus* spp. values ranging from 10^3 up to 10^5 $\log_{10}$ CCE/100mL through the duration of their sampling season. Similar to our findings, these studies demonstrate that the range of values in FIB can vary by several orders of magnitude due to changes in environmental variables (e.g., rainfall, snowmelt, etc.), non-point sources of pollution (e.g., seagull feces), or operational failures (e.g., broken sewer pipes). Further, such spatial variations can often be linked to contributing sources of pollution (Marsalek & Rochfort, 2004; Wan, *et al*., 2014). Therefore, the occurrence of such ranges of FIB levels at an individual sampling site can pose a complex challenge to the development of a stormwater sampling plan.

Understanding the spatial differences at stormwater sampling sites should allow us to better determine which sampling sites are best suited for stormwater reuse applications. Our study revealed that some urban stormwater ponds consistently failed water quality guidelines more often than other stormwater ponds: McCall Lake had more water quality guidelines failures than Inverness Stormpond and Country Hills Stormwater Facility, suggesting that the water quality is poor spatially. Sidhu *et al*. (2012) found that two of five urban stormwater ponds tested in Brisbane, Australia, had consistently poorer water quality, as the levels of *E. coli* and *Enterococcus* spp. were significantly higher than at the other sampling sites tested.

Further, the data in this thesis revealed that a specific sampling site within a stormwater pond may experience more consistent failures of water quality guidelines. Our results demonstrated that sampling site ML2 had the most failures of any sampling site at McCall Lake, suggesting that ML2 may be dealing with different sources of pollution. Serrano & DeLorenzo (2008) had similar findings, in that it was common to have more frequent water quality failures at a particular sampling site than others. Throughout their sampling season, they found higher levels of fecal coliform bacteria in the sampling site that drains the stormwater pond into a nearby estuary (i.e., 181 CFU/100mL at the estuary compared to 1519 CFU/100mL in the stormwater pond) (Serrano & DeLorenzo, 2008). A lack of consistency of FIB levels across sampling sites located within one body of water have been noted in previous studies (McCarthy, *et al*., 2007). Converse *et al*. (2011) suggested that different patterns of FIB and loading may be indicative of different sources of fecal pollution at each sampling site. These findings suggest that with respect to spatial characteristics, water quality can be highly variable even at one sampling site. Furthermore, several studies suggested that the spatial differences in water quality among sampling sites may be due to landscape characteristics, as well as catchment area and land use demographics (e.g., agriculture, residential, etc.) (Sidhu, *et al*., 2012; Converse, *et al*., 2011).

Peak concentrations of FIB greatly affect interpretations about water quality (Kapoor, *et al*., 2015). The occurrence of extremely high FIB levels in stormwater may be representative of times of peak fecal contamination, but also may imply a recent contamination event not reflective of overall water quality (e.g., a bird defecating at a sampling site just prior to a sample being taken). In the context of public health, a precautionary approach is warranted in terms of understanding the potential impacts associated with these 'outlier' events. In our study, the

highest value of *Enterococcus* spp. measured at any of the stormwater pond sampling sites was ML1 at McCall Lake, reaching a value of $1.5x10^5$ CCE/100mL based on molecular-based methods. By comparison, in the Wade *et al*. (2010) study, the highest concentration of *Enterococcus* spp. measured in recreational waters impacted by wastewater was $2.9x10^4$ CCE/100mL. Importantly, this study demonstrated a relationship between the levels of *Enterococcus* spp. and swimming-associated illness (e.g., diarrhea, rash, earache, gastrointestinal illness, etc.), supporting the correlation that the most common symptom related to high levels of FIB was gastrointestinal illness. They found that for every 1 $\log_{10}$ increase in the level of *Enterococcus* spp. sampled at 8 a.m. above the threshold of 1280 CCE/100mL., swimmers were 1.94 times as likely to get gastrointestinal illness (Wade, *et al*., 2010). Further studies reflected similar findings, including a study performed by Arnold *et al*. (2013), in which 21% of swimmers had diarrhea that could be attributed to swimming in waters beyond the USEPA guidelines for *Enterococcus* spp.

It should be noted that peaks in concentrations of FIB are not limited to stormwater-impacted recreational waters. Significant temporal variability was observed at some sampling sites in this thesis. ML1 at McCall Lake also had significant temporal variability in *E. coli* levels, with values ranging from not detected – 2419.6 (i.e., the detection limit of the assay). Chong *et al*. (2013) studied a stormwater pond near Brisbane, Australia, intended for water reuse (mentioned above) and detected the highest level of *Enterococcus* spp. (i.e., 3.11×10^4 CFU/100mL) and *E. coli* (i.e., 1.07×10^4) during a wet weather event. Such fluxes in water quality can change rapidly over time. Converse *et al*. (2011) found significant temporal variability when comparing *Enterococcus* spp. and *E. coli* concentrations in samples taken 24 hours apart in coastal stormwater outfalls in North Carolina, USA. Albeit, Parker *et al*. (2010)

found that there was little variability in the concentrations of *E. coli* and *Enterococcus* spp. when stormwater runoff samples were collected in North Carolina, USA, several hours apart. However, they observed considerable variability during the sampling season, as higher concentration of *E. coli* and *Enterococcus* spp. were measured in the summer and fall when warmer temperatures occurred (Parker, *et al*., 2010). As such, temporal fluxes in water quality may be attributed to short-term or longer term dynamics.

Short-term dynamics (e.g., rainfall, snowmelt, water treatment operational changes, etc.) can cause rapid changes in water quality (Besmer, *et al*., 2017). Rapid changes in water quality were observed in our research, as water quality in the urban stormwater ponds was found to be episodic, and in which at some sampling sites, FIB could vary 3-4 orders of magnitude in three days. The episodic nature of urban stormwater-impacted bodies of water has been noted in other studies (Tiefenthaler, *et al*., 2011; Lee, *et al*., 2002). Further, the effects of first flush (i.e., initial runoff from precipitation) may come into play on the amount of FIB that is measured at a sampling site (Lee, *et al*., 2002). First flush can be defined as the beginning time frame of stormwater runoff when the concentration of pollutants can be elevated in comparison to the concentrations found during the later stages of a storm. In this thesis, all water samples were collected through a routine grab sampling method using weekly/biweekly samples, and therefore the upper levels of FIB quantified in these samples may, or may not, exceed the first flush effect. In addition, grab samples may not be representative of an entire storm event (Converse, *et al*., 2011). This effect can be further explained as when 30-90% of the pollutants (e.g., FIB) are brought into the body of water in the first 50% of volume (i.e., rainfall) (Lee, *et al*., 2002; Bach, *et al*., 2010). However, the effects of first flush vary widely.

It is commonly found in the literature that FIB concentrations are higher throughout storm events (Converse, *et al*., 2011). McCarthy (2009) noted that the effects of first flush can be as high as 30% in a stormwater-impacted body of water. When analyzing first flush effects in a Melbourne, Australia catchment, Bach *et al*. (2010) observed its effects as measured by *E. coli*. They believed that this catchment experienced first flush due to septic cross-connections at that site (Bach, *et al*., 2010). Lee *et al*. (2002) observed first flush in two urban watersheds in Chongju, South Korea, by measuring the changes in suspended solids. Further, the amount and the intensity of rainfall have been found to influence first flush effects, along with the catchment area and the antecedent dry period (Besmer, *et al*., 2017; Lee, *et al*., 2002). Paule-Mercado *et al*. (2016) found a significant correlation between catchment area and FIB, since a larger catchment area would provide an opportunity for increased levels of FIB in the outfall. In our study, the largest catchment area was ML1, but did not have the most water quality failures of all outfalls. Semenza *et al*. (2012) suggested that contaminants brought in during the first initial rainfall of the season might be more significant than all subsequent rainfalls. Moreover, higher levels of FIB during first flush may also be due to residual human waste found in CSOs or cross-connected systems, or from movement of animal fecal waste into water from adjacent landscapes (Chong, *et al*., 2013; Sidhu, *et al*., 2012).

A further explanation for the variability observed in FIB levels may be the stirring up of sediment that occurs during rainfall, resulting in the resuspension of particle-associated bacteria that can occur with rainfall (Sidhu, *et al*., 2012). Sidhu *et al*. (2012) found that 15-30% of bacteria within a sample were attached to sediment particles, and could be resuspended during rainfall. Other factors affecting FIB concentrations related to precipitation effects include the intensity of the storm. Higher numbers of *Enterococcus* spp. and *E. coli* were detected following

the greatest recorded rainfall event at a site in Australia, in which 135 mm of rain, compared to 20 mm of rainfall on average, fell in two stormwater ponds in Australia (Sidhu, *et al*., 2012). At a North Carolina beach impacted by stormwater, researchers found that during their most intense storm event, FIB (i.e., *Enterococcus* spp. and *E. coli)* were several orders of magnitude greater when compared to all other storm events (Converse, *et al*., 2011). However, researchers have concluded that first flush may have only occurred in small subset of wet weather events (Bach, *et al*., 2010).

Conversely, some studies have shown that the precipitation from first flush showed no correlation between FIB numbers and the amount of precipitation and that storm events may actually dilute pollutants (Saget, *et al*., 1996; Parker, *et al*., 2010; McCarthy, 2009). McCarthy (2009) noted that some stormwater-impacted bodies of water experienced an "end flush" effect, when highly contaminated water enters the body of water at the end of a precipitation event. It is believed that "end flushes" could be due to the gradual saturation of soil, with groundwater interacting with leaky sanitary systems to move contaminated water into a stormwater system during high intensity storms (McCarthy, 2009). Converse *et al*. (2011) had a similar finding, in which the highest concentrations of FIB occurred at the end of storms in 23% of their samples. They also found that the highest concentration could occur in the middle of a storm, also occurring in 23% of their samples (Converse, *et al*., 2011). Surbeck *et al*. (2006) hypothesized that FIB would remain at high levels throughout the duration of a storm, as there is no wash-off when FIB are taken up by stormwater. However, the effect of wash-off can also be affected by population, with more densely populated areas experiencing less wash-off (Surbeck, *et al*., 2006). Thus, the phenomenon of first flush and the concept of pollutant buildup in arid urban environments (e.g., Calgary and Airdrie) require further research (Schiff, *et al*., 2016; Saget, *et*

al., 1996). Such research should include auto-samplers, which would entail taking samples throughout the duration of the storms, in order to determine the effects of first flush and contaminant loading in these systems.

Our study noted that two of the three highest concentrations of *E. coli* in Calgary urban stormwater ponds occurred when there had been greater than 10 mm of rain in the previous 72 hours. This may not hold true for all stormwater systems, as Converse *et al*. (2011) did not find a significant correlation between antecedent rainfall and *E. coli* and *Enterococcus* spp. concentrations. On the other hand, several studies have found that increased precipitation can negatively impact water quality through an increase in pathogenic microorganisms and FIB, an effect known as wet-weather loading (Semenza, *et al*., 2012; Chigbu, *et al*., 2004; Mallin, *et al*., 2001; DeLorenzo, *et al*., 2012). Some studies have demonstrated a relationship between antecedent rainfall and fecal coliforms and *Enterococcus* spp. concentrations in urban watersheds, and have shown that FIB may become more concentrated on land when there is no precipitation to wash them away (Kelsey, 2004; Hathway, *et al*., 2010). Mallin *et al*. (2008) found that antecedent rainfall in the past 72 hours significantly correlated to the levels of fecal coliforms in both rural and urban stormwater-impacted streams located in North Carolina, USA. They also tested rainfall for the previous 24- and 48-hour periods, which did not reveal any different results than the antecedent rain in the previous 72 hours. Further, the levels of fecal coliforms were significantly higher during wet-weather periods, when geometric mean counts were four-to-ten times higher (Mallin, *et al*., 2008). Converse (2009) found FIB loading during long storms (i.e., >12 hours in duration), ranged between 10^{11} to 10^{12} per storm, while other research studies documented FIB loading to be over 1000 times higher during precipitation than during baseflow conditions (Krometis, *et al*., 2007). The claim of higher levels of FIB during wet

weather events also occurred in the study by Chong *et al*. (2013), where much higher levels of *Enterococcus* spp. and *E. coli* were found in wet weather events than the dry weather events. Likewise, Sidhu *et al*. (2012) had tested five stormwater ponds intended for water reuse applications around Brisbane, Australia, and had similar findings. Sidhu *et al*. (2012) found *E. coli* concentrations to be higher during times of wet weather versus dry weather (i.e., 3.54 $\log_{10}$ CFU/100mL for wet as compared to 2.32 $\log_{10}$ CFU/100mL for dry), with similar findings for *Enterococcus* spp. (i.e., 3.35 $\log_{10}$ CCE/100mL for wet as compared to 2.43 $\log_{10}$ CCE/100mL for dry). So too, the effects of antecedent rainfall on FIB can be impacted by the physical makeup of the land (e.g., flat, hilly, etc.) and climate (e.g., temperature, sunlight, etc.), which can also influence the die-off rate of FIB (Hathway, *et al*., 2010).

Surrounding land use may contribute to the levels of microbial contamination as well (DeLorenzo, *et al*., 2012). In our study, the Calgary stormwater ponds, the Elbow River, and the Nose Creek were all located in urban environments. When comparing the Elbow River to a rural river, it was found that in the urban environment, the concentrations of *Enterococcus* spp. were several orders of magnitude higher. Urban environments represent a unique challenge to stormwater because there is the potential for an increase in stormwater runoff volume and pollutant loading, all due to the decrease in impervious surfaces (Vogel & Moore, 2016; Chow, *et al*., 2013). Also, studies on FIB have indicated that urban stormwater may be the leading cause of pollution in fresh water, thereby contributing to poor water quality (Mallin, *et al*., 2008; Smith & Perdek, 2004; Gasperi, *et al*., 2014). So too, studies have found a correlation between FIB and the degree of urbanization (Young & Thackston, 1999; Van Dolah, *et al*., 2008; Millin, *et al*., 2000; Duncan, 1999).

In fact, Young & Thankston (1999) found a positive association between the levels of FIB (i.e., fecal coliforms, *E. coli*, and fecal streptococci), and the extent of impermeable land, density of housing, population, and increased urban development overall in the Cumberland River region in Tennessee, USA. The 1999 Duncan review of several stormwater quality variables found that total coliforms, fecal coliforms and fecal streptococci were higher on average from high urban areas than from low urban areas, regardless of how the catchments were zoned (e.g., industrial, residential, agricultural, etc.). In addition, Duncan's study found that concentrations of stormwater quality variables tended to be similar between roads and high urban areas, whereas roofs and low urban areas had lower concentrations of water quality variables.

In contrast, Mallin *et al*. (2008) in their study in North Carolina, USA, found fecal coliforms correlated to the percent of urban development, and not to the percent of impervious land or overall watershed development. Paule *et al*. (2014) compared three different stormwater-impacted environments in South Korea (i.e., urban, agricultural, and mixed land use) by sampling the end of the drainage channels following storm events. In this study, they found the concentrations of FIB to be much higher at the urban sampling site than at the agricultural or industrial sites; and the agriculturally-impacted site to have the lowest FIB concentrations (Paule, *et al*., 2014). Further, for residentially-impacted urban stormwater sites, it has been found that higher density residential catchments have higher concentrations of FIB than lower density catchments (Chow, *et al*., 2013). Chow *et al*. (2013) found that in tropical urban catchments, areas with a higher percentage of commercial land use were more contaminated than residential areas; and it was hypothesized that this result was due to increased anthropogenic activities in the commercial catchment. Other studies have also supported this finding (Petersen, *et al*., 2005).

The rural river in our study was located in southern Alberta, Canada in a small agriculturally-driven community. Mallin *et al*. (2008) undertook a study comparing the effects of stormwater in urban, suburban, and rural environments as defined by the percent of impervious land coverage. They found the urban area to have the highest concentration of FIB most often (Mallin, *et al*., 2008). In contrast, Tiefenthaler *et al*. (2011) found the concentrations of *E. coli* to be higher in agricultural environments than urban, and believed that this was due to a local agriculture contamination source (i.e., horses). Even urban environments can experience localized contamination sources. For example, Ervin *et al*. (2014) studied an urban watershed located in Santa Barbara County, California, USA, for dog fecal pollution, detected it in 64% of samples in the surf zone. Sigua *et al*. (2010) did a comparison between livestock-based agriculture and agriculture without animals (e.g., crops); and found livestock-associated areas to have higher FIB levels.

In our study of the Calgary urban stormwater ponds, McCall Lake had the highest percentage of areas associated with industrial activity (i.e., 26%), in comparison to residential areas in which McCall Lake had 30% and Country Hills which was much higher at 68%. As previously stated, McCall Lake had the poorest water quality of all three Calgary stormwater ponds. In comparison, Converse *et al*. (2011) found that the site with lowest concentrations of *E. coli* and *Enterococcus* spp. was the most commercialized and least residential of all five sites tested. In addition, they found that the sampling sites with the largest drainage area had the highest concentrations of FIB (Converse, *et al*., 2011). That said, the findings vary in the literature. Tiefenthaler *et al*. (2011) studied eight different urban stormwater-impacted watersheds in or near Los Angeles, California, USA, and found that the mean *E. coli* concentrations were significantly higher in bodies of water as associated with recreational parks

compared to commercial, residential, industrial, and transportation. In our study, ML1 had the greatest percentage of land use associated with transportation (i.e., 23 ha), which includes roadways, light rail transit (i.e., LRT) systems, and airports. Sajjad *et al*. (2015) analyzed the effects of the LRT system on stormwater runoff. Their findings revealed that the LRT systems pollutant load was 2-to-9 times less than an adjacent road-bridge. That pollutant load was measured through temperature, pH, turbidity and conductivity (Sajjad, *et al*., 2015).

Findings overall have reflected that urban environments may have a negative impact on water quality, however, the effects of the specific land used in those areas varies between studies. Further, it is believed that catchment characteristics are one of the utmost important influences on urban stormwater quality (Liu, *et al*., 2013). Catchment characteristics include the percentage of land use for a variety of factors (e.g., transportation, residential, industrial, etc.), and can influence both point source and non-point sources of pollution (Petersen, *et al*., 2005). As stormwater flows through these areas, it can take up contaminants and transport them to a body of water, therefore affecting water quality (Paule, *et al*., 2014; Sidhu, *et al*., 2012).

An important element of this thesis is how the levels of fecal indicator bacteria affect health risk. As mentioned previously, Wade *et al*. (2010) demonstrated that the USEPA *Enterococcus* spp. guideline of 1280 CCE/100mL relates to illness in 36 per 1000 swimmers. However, the same epidemiological studies do not exist for alternative water reuse. In terms of this thesis, the reuse of urban stormwater also includes non-potable irrigation applications, as for crops, golf courses, and parks (Nnadi, *et al*., 2015; Lim, *et al*., 2015; McArdle, *et al*., 2011). Several studies have shown that stormwater can be treated for use for irrigation purposes; and a review of stormwater systems in Australia found that the most common use for reclaimed stormwater is for various types of urban irrigation (e.g., crop irrigation, athletic field irrigation,

and park irrigation) (Fletcher, *et al*., 2008; Mbanaso, *et al*., 2016; Rodriguez-Hernadez, *et al*., 2010; Coupe, *et al*., 2003; Nnadi, *et al*., 2013).

The consumption of food irrigated with contaminated water (e.g., crops) can transmit enteric pathogens. When comparing stormwater reuse activities, Lim *et al*. (2015) evaluated the presence of human viruses (i.e., norovirus and adenovirus) in stormwater for non-potable uses, including crop irrigation, toilet-flushing, and showering by utilizing quantitative microbial risk assessment (QMRA), revealing that food-crop irrigation had the highest annual viral infection risk. Furthermore, a 10-month study in Zaria, Nigeria, utilizing fecal coliform counts assessed the water quality of urban stormwater-impacted surface water that is used for crop irrigation. Twelve sampling sites were tested in total, located along various streams, dams, and rivers. The findings revealed that the sites more heavily impacted by urban stormwater recorded higher counts of fecal coliforms than those not as heavily impacted by urban stormwater. In that study, the mean fecal coliform counts (FCC) ranged from 2.0×10^1 FCC/100mL up to 7.8×10^5 FCC/100mL among all 12 sampling sites (Chigor, *et al*., 2012). For irrigation water, the USEPA and the U.S. Agency for International Development (USAID) recommend undetectable levels of fecal coliforms for raw foods. As such, none of the water samples tested in Zaria, Nigeria met the standards for unrestricted irrigation (Chigor, *et al*., 2012).

Significantly, a number of governmental agencies worldwide are developing guidelines for reclaimed stormwater for irrigation, using *E. coli* as the FIB due its high reporting in stormwater reuse literature. In Australia, for irrigating parks with open access, the New South Wales (NSW) Department of Environment and Conservation sets an *E. coli* guideline of <10 CFU/100mL, whereas controlled access would allow for a higher level of *E. coli* at <100 CFU/100mL (Environmental Guidelines, 2004). The Joint Research Centre (JRC) for the

European Commission has set guidelines for agricultural irrigation of raw foods, allowing <1 CFU/100mL for *E. coli*. The JRC allows for higher levels of *E. coli* in processed and non-food crops at 100 CFU/100mL and 1000 CFU/100mL, respectively (Environmental Guidelines, 2004)

Another potential end-use of stormwater is toilet- and urinal-flushing. The use of stormwater for this purpose would require a third pipe distribution system, which can incur cost as it is a separate system from drinking water and sanitary sewer lines (McArdle, *et al*., 2011; Lim, *et al*., 2015). The Chong *et al*. (2013) study focused on determining water quality in order to augment non-potable applications (e.g., showering, toilet-flushing, etc.). The *E. coli* and *Enterococcus* spp. ranges were well-above the NSW Department of Environment and Conservation *E. coli* guideline of ≤ 1 CFU/100mL of *E. coli* for toilet-flushing, which, along with showering, can transmit pathogens through the respiratory system to humans by the way of contaminated water (Lim, *et al*., 2015; Lundy, *et al*., 2017). Lim *et al*. (2015) found that toilet-flushing utilizing reclaimed stormwater had the lowest viral risk of infection (i.e., when compared to showering and crop irrigation) as determined by QMRA. In Australia, toilet-flushing uses approximately 12% of reclaimed stormwater; whereas, in the United States, it is much lower at approximately 3% because of public perception of potential exposure through this route (Lundy, *et al*., 2017). Still, there are parts of the world (e.g., China, Singapore, etc.) that already flush their toilets with reclaimed water (Leung, *et al*., 2012). At the same time, research is continuing to better understand stormwater contaminants in order to use stormwater for high value end uses (e.g., indirect potable reuse, direct potable augmentation of dams, hot water systems, etc.) (Chong, *et al*., 2013).

4 Sources of Fecal Pollution Impacting Stormwater Ponds and Stormwater-Impacted Rivers

4.1 Introduction

In the previous chapter, a general set of microbial water quality indicators (i.e., *Enterococcus* spp., *E. coli*, and fecal coliforms) were used to study the overall trend of water quality. This analysis revealed that stormwater ponds and stormwater-impacted urban rivers in southern Alberta, Canada, do not often meet existing guidelines or standards as laid out by USEPA, Health Canada, and Alberta Environments and Parks in terms of recreational water quality, surface water quality and/or irrigation water quality.

Previous studies have demonstrated that stormwater is often impacted by both human and animal sources of contamination (Sauer, *et al*., 2011; Converse, *et al*., 2011; Newton, *et al*., 2013; Chase, *et al*., 2012; Staley, *et al*., 2013; Sauer, *et al*., 2011; Templar, *et al*., 2016). From a public health viewpoint, contamination with human waste is of utmost concern, since water sources impacted by human waste are considered to be much greater risk to public health than those impacted by animal waste (Sauer, *et al*., 2011; Converse, *et al*., 2011; Newton, *et al*., 2013; Chase, *et al*., 2012). In most cases, traditional methods of microbial water quality analysis (i.e., culturable bacteria) do not account for the source of pollution contributing to the overall bacterial levels observed, and consequently risk can vary, based on the sources of pollution. For derivation of USEPA's Recreational Water Standards for *Enterococcus* using the molecular-based methods derived from the NEAAR (National Epidemiological and Environmental Assessment of Recreational Water) studies, recreational water study sites were selectively chosen based on the knowledge that municipal wastewater effluents impact these bodies of water, which points to human fecal contamination (Sauer, *et al.*, 2011; Converse, *et al*., 2011).

In our study, we utilized qPCR-based library-independent methods of microbial source tracking. These methods are based on the assumptions that specific animals (e.g., dogs) or group of animals (e.g., ruminants) carry sequences in their feces that are unique to them. Many of the microbial source tracking markers in our study target 16S rRNA sequences of *Bacteroides* spp. (except for one [i.e., LeeSg] which targets *Catellicoccus marimammalium*) commonly found in the gut of warm-blooded animals, and which display host-specificity or host preference. Furthermore, the application of these qPCR methods allowed us to simultaneously quantify the sources of fecal contamination. We used a "toolbox" approach in our study by utilizing seven different microbial source tracking markers (i.e., HF183 [human], HumM2 [human], Dog3 [dog], LeeSg [seagull], CGO1 [Canada goose], Rum2Bac [ruminant], and Mubac [muskrat]) to better understand where sources of FIB were originating and how these sources contributed to the burden of fecal contamination in stormwater systems (i.e., Chapter 3).

4.2 Results

Similar to what was presented in Chapter 3, the results of this chapter are divided into two major sections. The first section discusses the general sources of fecal pollution found in all three Calgary urban stormwater ponds (i.e., McCall Lake, Country Hill Stormwater Facility, and Inverness Stormpond) and the second discusses the tracking of fecal sources of pollution in the Elbow River, the Nose Creek and rural river systems.

4.1.1 General Overview of Microbial Sources of Fecal Pollution in Urban Stormwater Ponds

4.1.1.1 Dominant Sources of Fecal Pollution

A high-level descriptive overview of the frequency of occurrence of microbial source tracking markers in each of the Calgary stormwater ponds, and at each of the sites, is provided in

Table 4-1. Calgary stormwater ponds were mainly impacted by human and gull feces (Table 4-1). The human specific markers, HF183 and HumM2, were detected at 27% and 10%, of samples, respectively (Table 4-1). The gull specific marker (i.e., LeeSg) was found in 9% of samples (Table 4-1). Of these, the more dominant source of fecal pollution was from humans (Table 4-1). All other host-specific markers (i.e., dog, Canada geese, muskrat, and ruminants) were detected in $\leq$2% of pond samples (Table 4-1).

Both human fecal markers, HF183 and HumM2, were detected in every stormwater pond tested suggesting widespread sources of contamination in stormwater ponds. However, McCall Lake appeared to be the most heavily impacted by fecal pollution, and in particular, human fecal pollution. In McCall lake 39% of samples were positive for HF183 and 19% for HumM2 (Table 4-1). By comparison, in the Country Hills Stormwater Facility, 27% of samples were positive for HF183 and 9% of samples were positive for HumM2. In samples collected from the Inverness Stormpond, the human fecal marker HF183 was detected in 13% of samples and HumM2 was detected in 3% of samples.

Variation in human fecal contamination was observed among sampling sites within a single pond. The most human fecally-contaminated site across all stormwater ponds examined was the ML2 sampling site at McCall Lake, with approximately 93% of samples possessing HF183 and 59% of samples possessing HumM2 (Table 4-1). By comparison in McCall Lake, at sampling site Inlet 3/4, 12% of samples were positive for HF183 and 5% of samples were positive for HumM2.

The highest levels of gull fecal contamination was also observed at McCall Lake, with 15% of samples possessing the microbial source tracking marker LeeSg (Table 4-1). Spatial variability was evident when comparing the stormwater ponds, as 10% of samples were positive

for LeeSg in Country Hills Stormwater Facility, and only 4% of samples were positive for this same marker in the Inverness Stormpond.

Spatial variability also occurred between sampling sites within a pond for seagull fecal contamination (Table 4-1). At McCall Lake, 22% of samples were positive for contamination by seagull feces at ML2. In comparison, only 7% of samples were positive for seagull fecal contamination at Inlet ¾.

Markers of other sources of fecal contamination were found sporadically across the ponds and sites. At sampling site ML1 in, Canadian Goose (i.e., CGO1) was detected in 10% of samples (i.e., the most at any sampling site studied). The second highest frequency of Canadian Goose fecal material occurred within the same stormwater pond, at ML2 in 5% of samples. In comparison, within McCall Lake at Inlet ¾, Canadian Goose was not detected in any of our samples (Table 4-1). However, within an individual stormwater pond, the occurrence of Canadian Goose fecal contamination could vary.

Dog fecal pollution was relatively low in all stormwater ponds tested (i.e., 2% of samples) (Table 4-1). However, as was observed with the other markers, there was considerable spatial variability in the occurrence of dog fecal pollution within the stormwater ponds. For example, in McCall Lake at sampling site ML2, dog fecal pollution was detected in 7% of samples. In comparison, dog fecal pollution was never detected at ML1. A similar trend was noted in Country Hills Stormwater Facility, in which dog fecal pollution was detected in 7% of samples at sampling sites WP31A and WP31C, but never detected at WP31B or WP31E.

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Table 4-1: Occurrence of microbial source tracking markers in three Calgary stormwater ponds based on the percentage of samples for which each marker was detected.

		Frequency of Occurrence Based on the Percentage of Samples Positive for Microbial Source Tracking Markers in 533 Stormwater Samples						
Pond	Sampling Site	Human: HF183 [n=41 samples]	Human: HumM2 [n=41 samples]	Seagull: LeeSg [n=41 samples]	Canada goose: CGO1 [n=41 samples]	Dog: Dog3 [n=41 samples]	Ruminant: Rum2Bac [n=41 samples]	Muskrat: Mubac [n=41 samples]
McCall Lake	ML2	93	59	22	5	7	2	2
	PR60	32	5	15	2	2	2	0
	ML1	17	7	17	10	0	5	2
	Inlet 3/4	12	5	7	0	2	0	0
McCall Lake Total [N=164]		39	19	15	4	3	2	1
Country Hills	WP31A	10	2	5	0	7	0	0
	WP31B	23	0	5	0	0	5	0
	WP31C	19	7	17	0	7	2	5
	WP31D	41	22	12	0	2	7	2
	WP31E	32	7	10	2	0	0	0
Country Hills Total [N=205]		27	9	10	1	3	3	1
Inverness	Outfalls/Inlet	12	2	5	2	0	0	0
	WP26B	10	2	7	5	2	0	0
	WP26C	20	5	0	0	0	0	0
	WP26D	12	2	5	0	0	2	2
Inverness Total [N=164]		13	3	4	2	1	1	1
Total		27	10	9	2	2	2	1

4.2.1.1 Spatial and Temporal Variation of Human Fecal Contamination Indicators in McCall Lake

Based on the variation of human fecal contamination markers among: a) the different stormwater ponds, and b) sites within a single stormwater pond (Table 4-1), we examined the spatial and temporal characteristics of human fecal contamination in each of the stormwater ponds and at each of the sites within a single stormwater pond using detection of the two human markers (i.e., HF183 and HumM2).

Spatial Variation in Microbial Source Tracking markers for Human-Fecal Pollution. In congruence with finding that ML2 at McCall Lake was the most frequently contaminated site with human feces (Table 4-1), this site also had the greatest median concentration of the human fecal marker HF183 (i.e., 4.0 $\log_{10}$ copies/100 mL) observed across all three stormwater ponds and sampling sites in these ponds (compare Figure 4-1 [McCall Lake] to Appendix 4-1 [all other stormwater ponds and sites]). In comparison, all other McCall Lake sampling sites had a median concentration of HF183 at ~3.4 $\log_{10}$ copies/100 mL (i.e., close to the quantification limit of the assay) (Figure 4-1).

As was indicated in the previous chapter regarding bacterial indicators, outliers in the data may reflect localized contamination events/conditions (e.g., infrastructure failure, as a break in a sewer line) representing times of peak contamination in the urban stormwater ponds. Specifically, at ML2, there was a single outlier in the data set for HF183, represented by a value of 6.0 $\log_{10}$ copies/100 mL (Figure 4-1). However, although ML2 represented the most consistently contaminated sampling site with human fecal contamination at McCall Lake, all other sites in this thesis appeared to be at risk for human fecal contamination (Appendix 4-1).

A spatiotemporal pattern of contamination was noted regarding the detection of human fecal source tracking markers at McCall Lake. On at least three occasions, HF183 at McCall

Lake was detected concurrently at all sampling sites (i.e., PR60, ML2, ML1, and Inlet ¾). Thus, there may be a potential environmental variable that may be making these sites behave similarly or these results may be due to PR60 being located nearby to ML2 (i.e., around the corner).

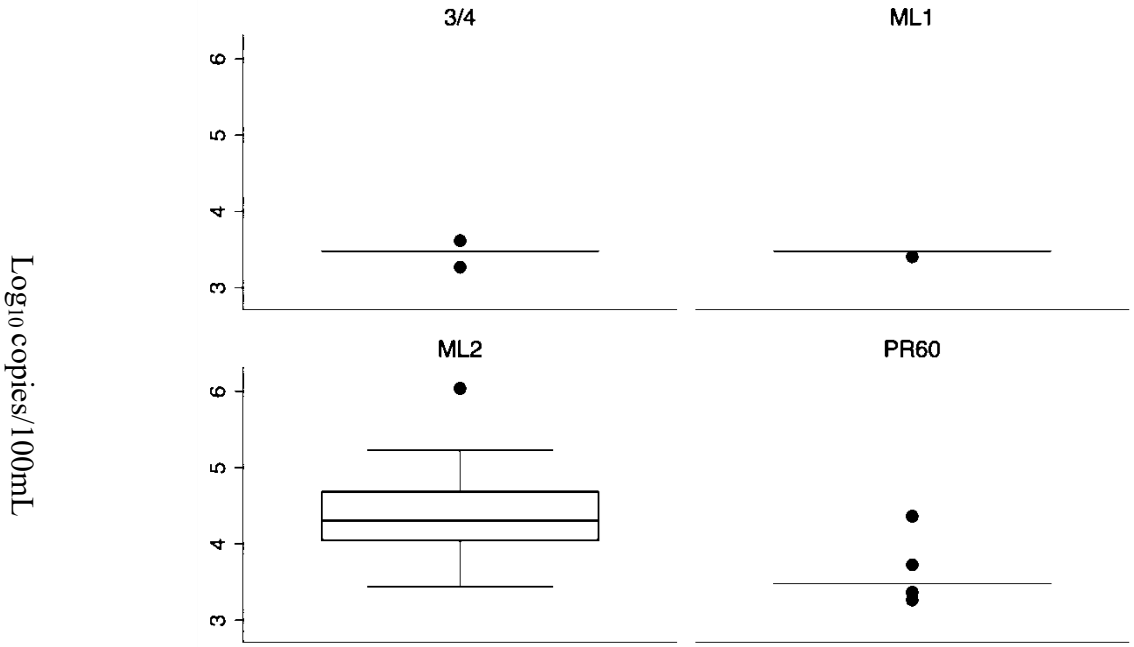

*Figure 4-1: Box and Whisker Plot of HF183 levels by sampling site in McCall Lake (ML2 n=38, ML1 n=6, PR60 n=13, Inlet ¾ n= 5). The outer edges of the box represent the 25th and 75th percentiles (i.e., interquartile range), and the line within the box represents the median. The location of median indicates the skew of the data. The whiskers represent the interquartile range*1.5. The outliers are determined by being greater or less than 1.5 times the upper of lower interquartile ranges as represented by circles.*

Temporal Variation in Microbial Source Tracking Markers for Human-Fecal Pollution.

Temporal fluctuations in human fecal pollution markers were noted between the stormwater ponds, and among the sampling sites within a stormwater pond (Figure 4-2 and Appendix 4-1). Of all stormwater pond sampling sites, ML2 at McCall Lake experienced the most consistent temporal pattern of human fecal contamination throughout the sampling season. For example, within the 41 sampling dates, over the 21-week sampling season, there were only two sampling dates in which we did not detect HF183 at ML2 (i.e., July 4th and August 28th) (Figure 4-2). However, there were other sampling dates when levels of HF183 decreased to a non-quantifiable level at ML2 (i.e., May 23rd, May 25th, August 8th, and August 14th). Interestingly, this pattern tended to occur after long weekends (i.e., holidays occurring on the following Mondays: May 22nd, July 3rd, August 7th, and September 3rd), and three of these long weekends corresponded to decreases in human fecal contamination markers on the following day of sampling (i.e., May 23rd, July 4th, and August 8th, which were Tuesdays). This suspicious temporal pattern of contamination suggested that the levels of human fecal contamination may have been related to industrial/commercial activities, as the levels of human fecal contamination decreased during times when industries/commercial premises may have been closed for the holidays (discussed further in the following section).

Human fecal contamination at the sampling sites was often highly variable between sequential sampling dates. For example, at Inlet PR60 in McCall Lake, within a two-week span, biweekly HF183 values fluctuated between undetectable levels (i.e., June 29th and July 6th) and 4.3 $\log_{10}$ copies/100mL (i.e., July 4th) and 3.5 $\log_{10}$ copies/100mL (i.e., July 10th). This high variability in human fecal contamination markers over sequential sampling dates, elicits potential

concerns regarding the sporadic nature of contamination and the stability of water quality in the urban stormwater ponds.

HumM2 was detected less frequently and at lower concentrations in all of the urban stormwater ponds tested. ML2 had the highest occurrence of HumM2 detections of all McCall Lake sampling sites, which corresponded with the findings with the human fecal contamination marker HF183 (Figure 4-2). Furthermore, Inlet ¾ had the lowest occurrence of HF183 in McCall Lake, and was also tied for the lowest occurrence of HumM2 in McCall Lake.

In addition, the data showed that detections of HumM2 did not always occur with detections of HF183. For example, on July 4th, at Inlet PR60, 4.3 $\log_{10}$ copies/100mL of HF183 was detected, while no HumM2 was detected. Conversely, HumM2 was detected on July 17th at Inlet ¾, whereas HF183 was not detected.

Variability was also observed with respect to the levels of human fecal contamination markers at sampling sites within a pond. The highest level of the human microbial source tracking markers detected at ML2 was 6.0 $\log_{10}$ copies/100mL for HF183 and 5.0 $\log_{10}$ copies/100mL for HumM2, both on September 13th. In comparison, the highest level detected at Inlet ¾ was 3.6 $\log_{10}$ copies/100mL for HF183 on July 10th, and 3.3 $\log_{10}$ copies/100mL for HumM2 on July 19th.

One explanation for the variation between these results may be antecedent rainfall (i.e., rainfall within the previous 72 hours). Thus, antecedent rainfall greater than 10 mm was also examined to see if it had any effect on the variability of human fecal pollution. Only three dates (i.e., May 25th, June 8th, and September 13th) had greater than 10 mm of rain. On September 13th, the highest values of HF183 (i.e., 6.0 $\log_{10}$ copies/100mL) and HumM2 (i.e., 5.0 $\log_{10}$

copies/100mL) were detected at ML2. The other two sampling dates (i.e., May 25th and June 8th) did not correspond to any apparent increases in human fecal contamination markers.

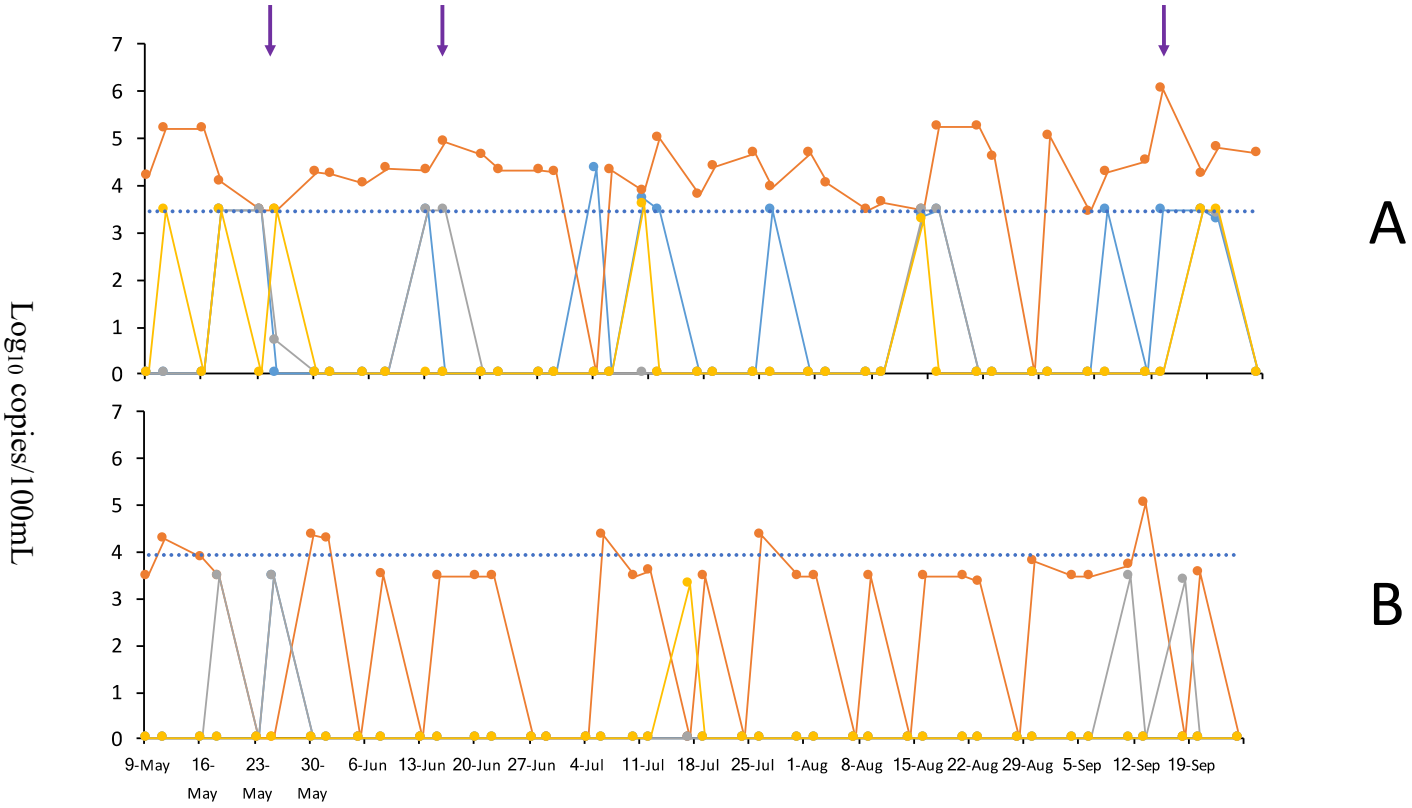

Figure 4-2: Temporal pattern of occurrence HF183 log$_{10}$ (A) and HumM2 (B) concentrations at all sampling sites in McCall Lake over the 21-week sampling season. Sampling site PR60 is in blue, ML2 in red, ML1 in gray, Inlet ¾ in yellow, and the limit of quantification$_{95}$ (LOQ$_{95}$) as a blue dotted line. The purple arrows represent greater than 10 mm of rain in the previous 72 hours.

4.2.1.2 Spatial and Temporal Variability of Seagull Contamination

Based on the variation of seagull fecal contamination markers among: a) the different stormwater ponds, and b) sites within a single stormwater pond (Table 4-1), we examined the spatial and temporal characteristics of seagull fecal contamination in each of the stormwater ponds and at each of the sites within a single stormwater pond. Spatial and temporal variations in seagull fecal contamination were examined utilizing the microbial source tracking marker LeeSg (Lee, *et al*, 2012).

Spatial Variation in Seagull Contamination. Spatial variation of seagull contamination was observed between the urban stormwater ponds (i.e., McCall Lake, Country Hills, and Inverness), and among each of the sampling sites within the individual urban stormwater ponds. Seagull fecal contamination was the second most common source of fecal contamination. The LeeSg gull fecal marker was detected at all sites but in only 9% of all samples. Analogous to what was noted above for human fecal contamination, McCall Lake had the highest occurrence of seagull fecal contamination of all urban stormwater ponds tested (compare [McCall Lake] to Appendix 4-1).

In the context of McCall Lake, seagull contamination was detected the most often at ML2, occurring in 22% of samples (Table 4-1). By comparison in McCall Lake, seagull contamination was the lowest at Inlet ¾, occurring in only 5% of samples. The highest level of seagull fecal contamination detected was 4.7 $\log_{10}$ copies/100 mL at Inlet ¾. Furthermore, the second highest level of LeeSg (i.e., 4.5 $\log_{10}$ copies/100 mL) in McCall Lake was also detected at Inlet ¾. In comparison, the highest level detected at ML2 was 4.1 $\log_{10}$ copies/100 mL.

Temporal Variation in Seagull Contamination. Temporal fluctuations in seagull fecal contamination were observed between the urban stormwater ponds, and at sampling sites within

an urban stormwater pond. In McCall Lake, seagull fecal contamination was considered to be a sporadic, highly variable, source of pollution. Seagull contamination was first noted in McCall Lake at the end of June, and tended to be episodic (Figure 4-3). For example, at ML2, seagull fecal contamination was detected on July 12th and at a level of 4.1 $\log_{10}$ copies/100 mL, and then it was not detected at quantifiable levels again until August 8th (i.e., 3.7 $\log_{10}$ copies/100 mL) (Figure 4-3). Although ML2 was most frequently positive for detection of seagull fecal contamination among all sampling sites, this pattern of sporadic, highly variable findings were also noted at the other McCall Lake sites (i.e., PR60, ML1, and Inlet ¾).

Overall, there were patterns of similarity in seagull contamination regarding temporal trends. These patterns were similar across the sampling sites in McCall Lake, which was noted for two key reasons. Firstly, there were three instances where seagull fecal contamination occurred concurrently at three or more McCall Lake sites (i.e., July 10th, August 14th, and September 13th). Secondly, on the aforementioned sampling dates, the levels of seagull fecal contamination detected were all within one order of magnitude of each other. These patterns suggested that a potential environmental variable linked the contamination along the sampling sites at McCall Lake.

One potential environmental variable examined was antecedent rainfall. Only three dates (i.e., May 25th, June 8th, and September 13th) had greater than 10 mm of rain. Seagull fecal contamination was detected on only one of the sampling dates (September 13th), though at three sampling sites on this date.

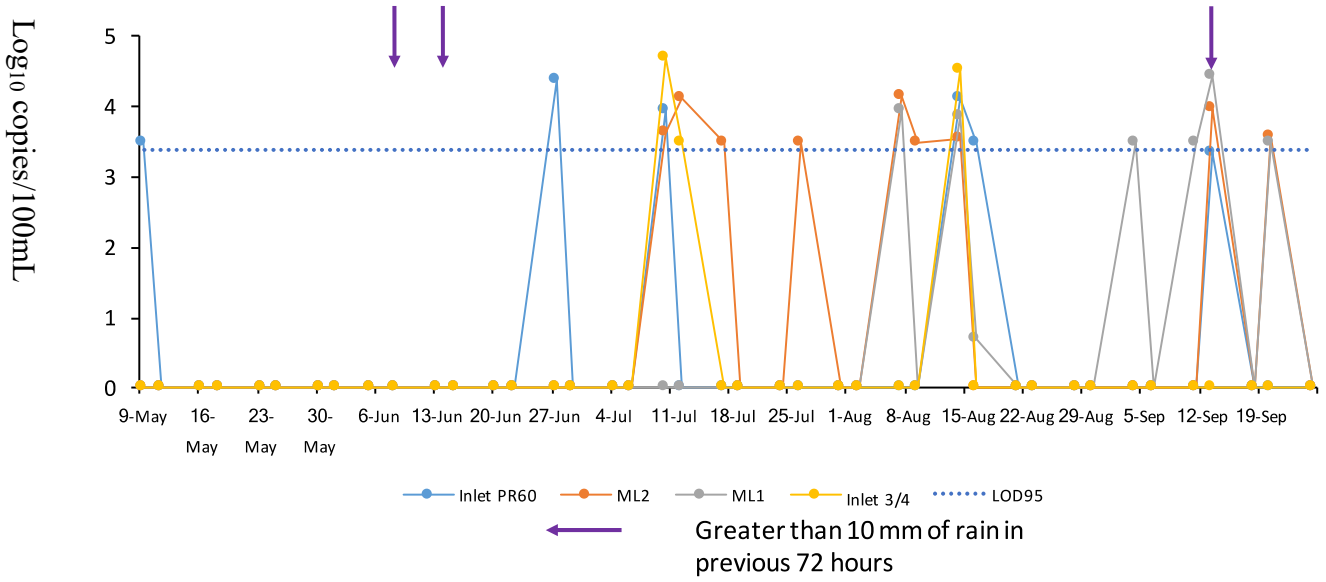

Figure 4-3: Temporal pattern of LeeSg contamination at all sampling sites in McCall Lake over 21 weeks. The blue line represents PR60, red line ML2, gray line ML1, yellow line Inlet ¾, and the blue dotted line is the LOQ_{95}. The purple arrows represent greater than 10 mm of rain in the previous 72 hours.

4.2.1.3 Temporal Patterns of Bacterial Water Quality Indicators and Microbial Sources of Fecal Pollution

In order to better understand how the occurrence of bacterial indicators of water quality related to source of pollution, we examined the patterns of occurrence between *E. coli* and *Enterococcus* spp. and the levels of human and seagull fecal markers at ML2 in McCall Lake (Figure 4-4). During the 21-week sampling season at ML2, there were two sampling dates (i.e., May 23rd and July 4th) when the levels of human fecal contamination decreased, as did the levels of *Enterococcus* spp. (Figure 4-4). On August 7th and August 14th, spikes in *Enterococcus* spp. occurred in the absence of high levels of human fecal contamination. On June 5th, high levels of *Enterococcus* spp. and *E. coli* occurred, while low levels of human fecal contamination were detected.

Temporal fluctuations of bacterial water quality indicators (i.e., *Enterococcus* spp. and *E. coli*) and the microbial source tracking marker for seagull fecal contamination (i.e., LeeSg) were also examined at McCall Lake sampling sites ML2 and ML1 (Figure 4-5). ML2 was chosen because it was the site most heavily impacted by seagull fecal contamination, and it was also impacted by human fecal contamination. Conversely, ML1 was chosen because it was not as heavily impacted by human fecal contamination, though it was the second most contaminated site with seagull contamination. During the 21-week sampling season at ML2, there were four sampling dates (i.e., July 12th, August 7th, August 14th, and September 13th) when the levels of seagull fecal contamination increased, as did the levels of *Enterococcus* spp. and *E. coli* concentrations (Figure 4-5), and at two of these dates (August 7th and August 14th) the spikes in *Enterococcus* spp. occurred in the absence of high levels of human fecal contamination. This led us to believe that seagull fecal contamination could be attributed to these spikes. For ML1, the

three sampling dates with detectable levels of seagull contamination (i.e., August 7th, August 14th, and September 13th) all corresponded to increases in *Enterococcus* spp. and *E. coli* concentrations.

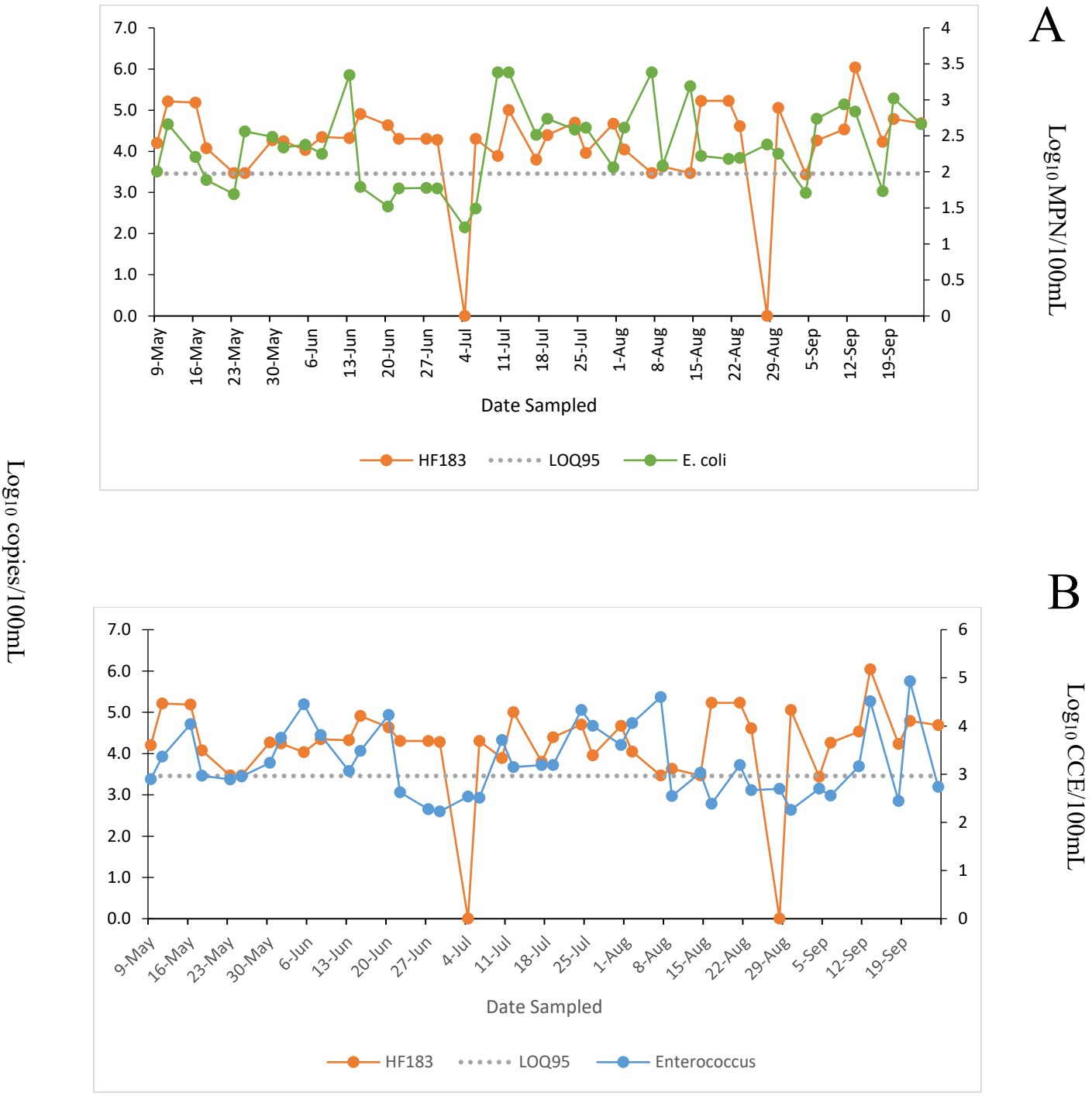

Figure 4-4 Temporal pattern of occurrence of E. coli log_{10} concentrations in green, HF183 log_{10} concentrations in red, and LOQ_{95} as a grey dotted line (A) and Enterococcus spp. log_{10} concentrations in blue, HF183 log_{10} concentrations in red, and LOQ_{95} as a grey dotted line (B) at ML2 in McCall Lake over 21 weeks. E. coli (A) and Enterococcus spp. (B) log_{10} concentrations are on the secondary Y-axis.

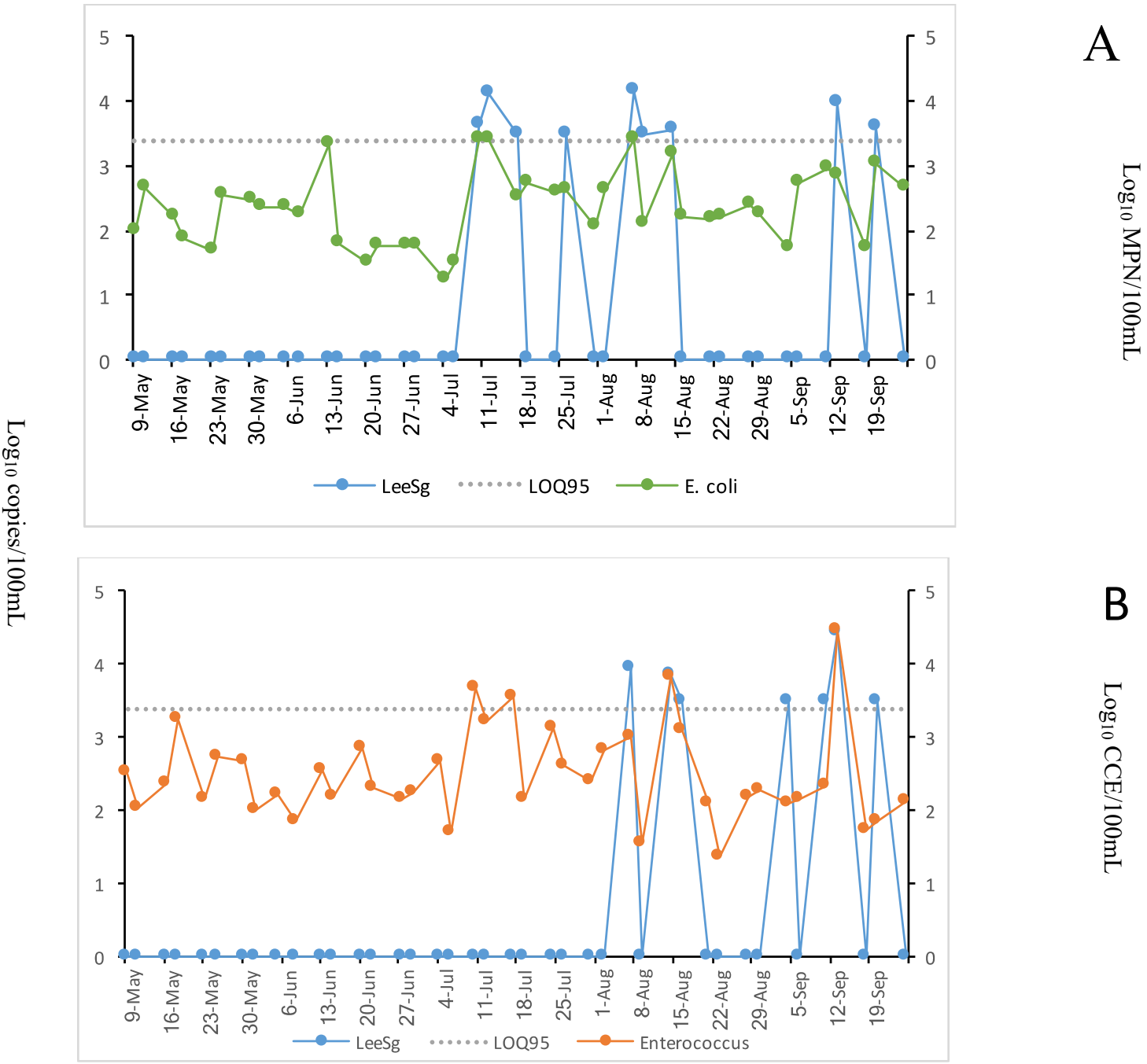

Figure 4-5: Temporal pattern of occurrence of E. coli log_{10} concentrations in green, LeeSg log_{10} concentrations in blue, and LOQ_{95} as a grey dotted line (A) and Enterococcus spp. log_{10} concentrations in orange, LeeSg log_{10} concentrations in blue, and LOQ_{95} as a grey dotted line (B) at ML1 in McCall Lake over 21 weeks. E. coli (A) and Enterococcus spp. (B) log_{10} concentrations are on the secondary Y-axis.

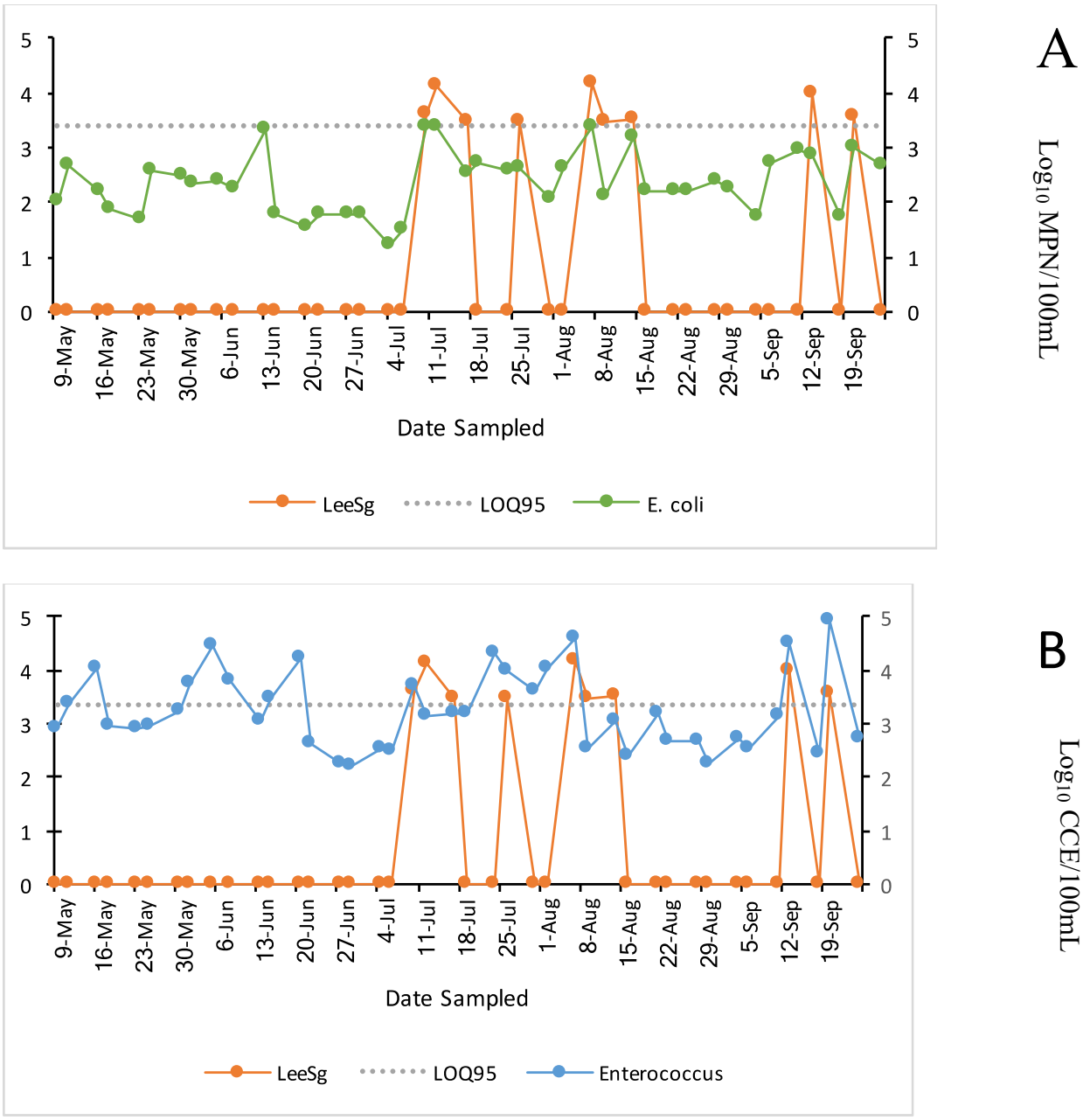

Figure 4-6 Temporal pattern of occurrence of E. coli log_{10} concentrations in green, LeeSg log_{10} concentrations in orange, and LOQ_{95} as a grey dotted line (A) and Enterococcus spp. log_{10} concentrations in blue, LeeSg log_{10} concentrations in orange, and LOQ_{95} as a grey dotted line (B) at ML2 in McCall Lake over 21 weeks. E. coli (A) and Enterococcus spp. (B) log_{10} concentrations are on the secondary Y-axis.

Due to the consistency in the high levels of human fecal contamination found at ML2, a series of special investigation samplings were initiated. Sequential upstream portions of the ML2 drainage network were sampled (Figure 4-6), to determine if contamination might have been caused by an illicit cross-connection from a commercial/industrial property into the drainage network. Since human fecal contamination was the primary contributor to ML2, HF183 and HumM2 were used to spatially track the source of fecal contamination in the drainage network (Table 4-2).

At each manhole tested, samples were taken from different drainage trunks (Figure 4-6-Figure 4-8). The results from the first special investigation revealed that human fecal contamination was found in manhole 501 and 537. Therefore, the second special investigation continued to track the contamination through the drainage network. For this sampling, samples were taken from the south and west drainage pipes leading into manhole 503, with the results showing that human fecal contamination was detected at manhole 503 in the south trunk and at 4.0 $\log_{10}$ copies/100mL, but not in the west trunk (Figure 4-7). In the final special investigation, human fecal contamination was only detected in manhole 531 south and 517 south, with the other five manholes tested not detecting any human fecal contamination. Thus, not all segments of the drainage network were positive for human fecal contamination (Table 4-2). This series of investigations identified a specific area of the city contributing human fecal pollution into the storm drains feeding into the ML2 site at McCall Lake (i.e., transparent red rectangle in Figure 4-8). It is interesting to note that our suspicions of the human fecal contamination coming from industrial/commercial properties (i.e., due to the absence of these markers after long-weekend holidays [see section above]), was validated by the special investigation studies. The area

identified as the contributing source of human fecal pollution at the ML2 site at McCall was indeed a commercial area of the city.

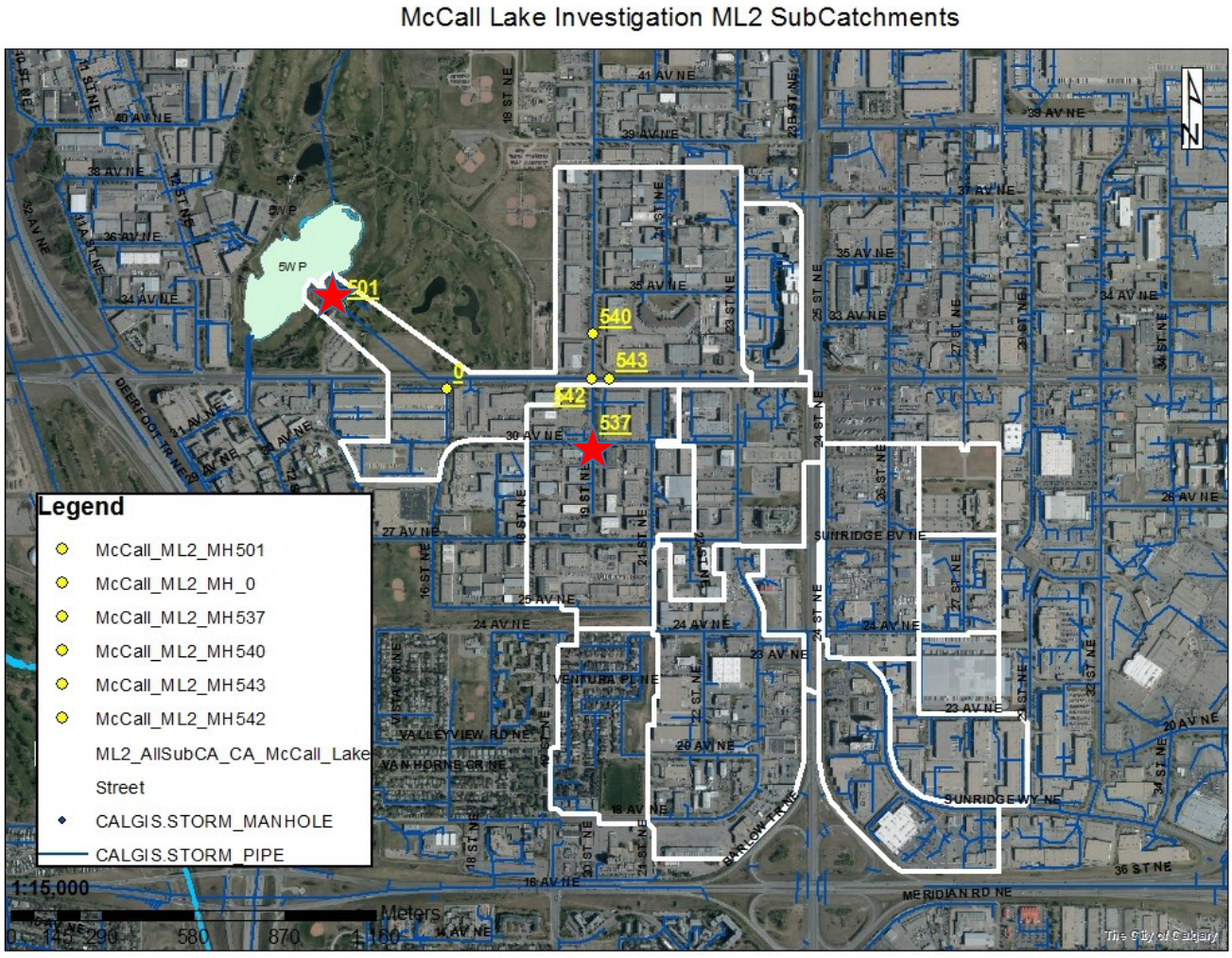

Figure 4-7:Map of the drainage network for ML2 from special investigations. The red stars indicate points where human fecal contamination was detected. Manhole 501 and manhole 537 were both tested in special investigation #1 and were positive.

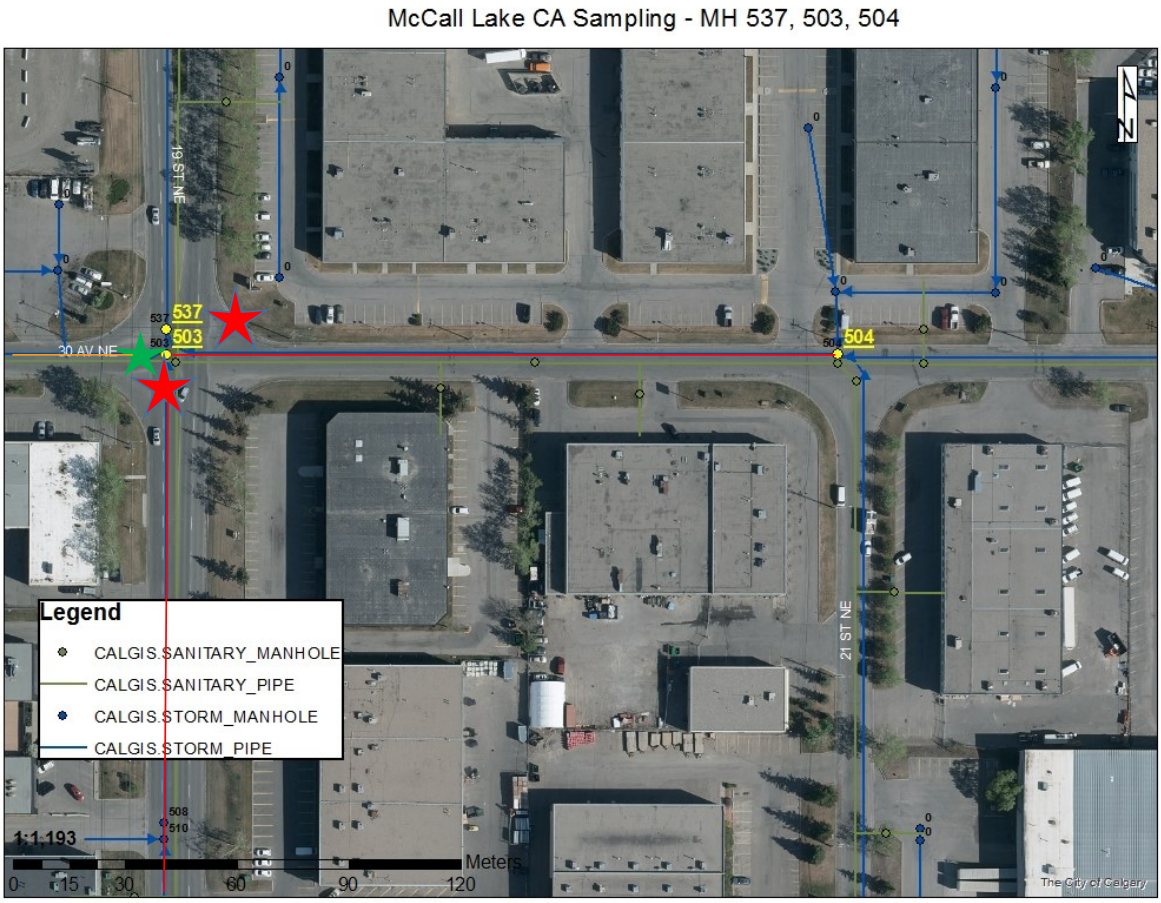

Figure 4-8: Map of the drainage network from special investigation #2. The red stars indicate that human fecal contamination was detected in manhole 503S and manhole 537E, and the green star indicates that no human fecal contamination was detected manhole 503W. The red line indicates portions of the drainage network that tested positive for human fecal contamination. The orange lines indicate segments of the drainage network that tested negative for human fecal contamination. The yellow numbers correspond to the names of manholes.

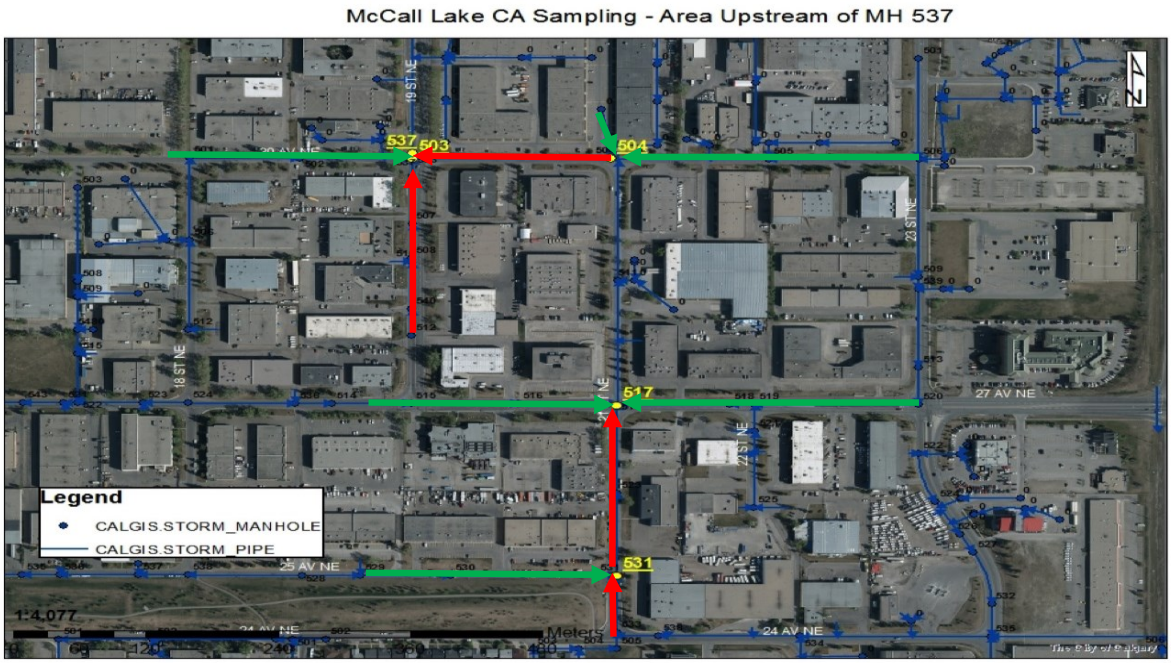

Figure 4-9: Map of drainage network that includes results from special investigations #2 and #3. The red arrows indicate segments of the drainage network, which tested positive for human fecal contamination. The green arrows indicate segments of the drainage network, which tested negative for human fecal contamination. The yellow numbers correspond to the names of manholes. The direction of the arrow is the flow of the drainage network.

Table 4-2: Microbial source tracking and FIB results from ML2 special investigations.

Special Investigation Number	**Microbial source tracking markers and FIB levels for McCall lake ML2 drainage network**			
	Location	HF183 Log_{10} CCE/100mL	HumM2 Log_{10} CCE/100mL	*Enterococcus* Log_{10} CCE/100mL
Special Investigation #1: June 8th, 2017	**MH501**	4.6	DNQ	4.8
	MH537	4.1	3.3	3.2
Special Investigation #2: July 16th, 2017	**MH537East**	3.9	DNQ	4.5
	MH503South	4.0	Not Detected	3.8
	MH503West	Not Detected	Not Detected	4.9
Special Investigation #3: September 8th, 2017	**MH503South**	Not Detected	Not Detected	4.6
	MH504North	Not Detected	Not Detected	3.0
	MH504East	Not Detected	Not Detected	2.4
	MH517East	Not Detected	Not Detected	3.1
	MH517South	3.4	Not Detected	3.1
	MH517West	Not Detected	Not Detected	3.2
	MH531South	2.9	DNQ	3.5
	MH531West	Not Detected	Not Detected	2.7

4.2.2 General Overview of Microbial Source Tracking Indicators in Urban Stormwater-Impacted Rivers used for Recreational Activity

A high-level descriptive overview was done on the microbial source tracking indicators in each of the sampling sites along the Elbow River, a rural river, and the Nose Creek (Table 4-3; and Table 4-4). Elbow River and the rural river sampling sites were tested for three microbial source tracking indicators (i.e., HF183, HumM2, and Rum2Bac). The Nose Creek sampling sites were tested for seven microbial source tracking indicators (i.e., HF183, HumM2, Rum2Bac, MuBac, LeeSg, CGO1, and Dog3).

The dominant source of contamination in the Elbow River was from humans (i.e., HF183 and HumM2) (Table 4-3). The human fecal marker HF183 was detected in 65% of samples from the Elbow River (Table 4-3). In comparison, HF183 was only detected in 4% of samples from a rural river. HumM2 was detected in 12% of samples in the Elbow River, and 0% of samples in the rural river.

Ruminant fecal contamination (i.e., Rum2Bac) was detected in less than 1% of samples in the Elbow River (Table 4-3). Ruminant fecal contamination was detected in only two Elbow River sampling sites, Rideau Pedestrian Bridge and 25th Avenue SW, in 15% and 8% of samples, respectively. By comparison, ruminant fecal contamination was detected in the rural river in 89% of samples.

There were two dominant sources of fecal pollution in the Nose Creek. The most dominant source of contamination was human fecal contamination with 57% of samples containing detectable levels of HF183 (Table 4-4). The second most dominant source of pollution was ruminants (i.e., Rum2Bac), which was detected in 34% of samples from the Nose Creek.

The Elbow River had considerable spatial variation with respect to the occurrence of source tracking markers at the sampling sites. HF183 marker was detected at every sampling site. However, the occurrence at which HF183 marker occurred varied between sampling sites. In particular, the 9th Avenue SE sampling site, located farthest downstream, experienced the most human fecal contamination with 85% of samples positive for HF183 and 30% positive for HumM2 (Table 4-3). This spatial variability became more evident when comparing 9th Avenue SE to the sampling site the furthest upstream (i.e., Sandy Beach), in which human fecal contamination was detected in 15% of samples by HF183 and 0% of samples by HumM2 (Table 4-3). These data revealed variability in the occurrence of human microbial source tracking markers particularly between the most upstream and downstream sampling sites. This finding reflected the cumulative abundance of storm drains (i.e., 99) impacting river water quality as the Elbow River flows from the Glenmore Reservoir to the confluence with the Bow River.

Spatial variability in human fecal contamination was also noted in the Nose Creek samples. At several sampling sites (i.e., 25807, 25811, 25814, and 25847), human fecal contamination (i.e., HF183) was detected in 80% of samples (Table 4-4). In contrast, at sampling site 25793, human fecal contamination (i.e., HF183 and HumM2) was never detected.

Spatial variability also occurred between sampling sites for ruminant fecal contamination (i.e., Rum2Bac). At four of the Nose Creek sampling sites (i.e., 25756, 25807, 25847, and 25855), ruminant fecal contamination was detected in 60% of samples (Table 4-4), whereas, at four other Nose Creek sampling sites (i.e., 25793, 25804, 25817, and 25841), no ruminant fecal contamination was detected.

Table 4-3: Occurrence of microbial source tracking markers in the Elbow River in Calgary, based on the percentage of samples that detected each microbial source tracking marker (i.e., HF183, HumM2, and Rum2Bac).

		Percent of Samples Possessing Microbial Source Tracking Markers in the Elbow River		
Sampling Location	**Sampling Site**	Human: HF183	Human: HumM2	Ruminant: Rum2Bac
Elbow River	Sandy Beach N=13	15	0	0
	Riverdale Pedestrian Bridge N=13	30	8	0
	Stanley Park N=13	85	23	0
	Rideau Pedestrian Bridge N=13	77	23	15
	26th AVE SW N=13	61	30	0
	25th AVE SW N=13	38	0	8
	1st ST SE N=13	46	23	0
	Stampede Grandstand N=13	70	30	0
	Enmax Park N=13	70	23	0
	9th Ave SE N=13	85	30	0
	Total Elbow River N=130	65	12	<1
Rural River	Rural River A N=18	5	0	83
	Rural River B N=18	0	0	88
	Rural River C N=18	5	0	94
	Total Rural River N=54	4	0	89

Table 4-4: Occurrence of microbial source tracking markers in the Nose Creek in Airdrie, based on the percentage of samples that detected each microbial source tracking marker (i.e., HF183, HumM2, LeeSg, CGO1, Dog3, Rum2Bac, and MuBac).

	Percent of samples possessing Microbial Source Tracking Markers in the Nose Creek						
	Human: HF183	Human: HumM2	Seagull: LeeSg	Canada Goose: CGO1	Dog: Dog3	Ruminant: Rum2Bac	Muskrat: MuBac
25756 N=5	60	20	20	0	40	60	0
25793 N=4	0	0	25	0	0	0	0
25804 N=4	25	0	0	0	0	0	0
25807 N=5	80	0	20	0	40	60	0
25811 N=5	80	40	40	20	20	40	0
25814 N=5	80	40	60	0	40	20	0
25817 N=3	66	0	0	0	0	0	0
25841 N=3	66	0	0	0	0	0	0
25847 N=5	80	0	40	0	0	60	0
25855 N=5	20	0	20	0	20	60	0
Total N=44	57	11	25	2	18	34	0

4.2.2.1 Spatial and Temporal Variation of Human Fecal Contamination Indicators in the Elbow River

Based on the variation in human fecal contamination between sampling sites within the Elbow River, we initiated an examination of the spatial and temporal characteristics of human fecal contamination at sites along the Elbow River. Spatial and temporal variations in human fecal contamination were examined using one microbial source tracking indicator (i.e., HF183).

Spatial Variation in Human Fecal Contamination. Considerable spatial variation with respect to the levels of human fecal contamination was observed among the sampling sites in the Elbow River. The highest level of human fecal contamination detected in the Elbow River was 5.0 $\log_{10}$ copies/100mL at Stanley Park. In comparison, two sampling sites (i.e., Sandy Beach and Riverdale Pedestrian Bridge) never reached quantifiable levels of human fecal contamination.

The site with the widest range of values was Stanley Park, ranging from not detected to 5.0 $\log_{10}$ copies/100mL (Table 4-5). Furthermore, this wide range of human fecal contamination showed how drastically human fecal contamination could vary within one sampling site. In comparison to Stanley Park, the values at Sandy Beach ranged from not detected to detected but not quantifiable (i.e., DNQ) (Table 4-5). The findings concerning spatial variation of human fecal contamination along the Elbow River overall, justified a closer examination of the temporal variance of human fecal contamination at sampling sites along the Elbow River.

Table 4-5: Range of HF183 values in the Elbow River sampling sites located in Calgary.

Elbow River: Range of HF183 values	
Elbow River Sampling Site	Human: Range of HF183 Log_{10} copies/100mL
Sandy Beach N=13	Not detected – DNQ
Riverdale Pedestrian Bridge N=13	Not detected – DNQ
Stanley Park N=13	Not detected – 5.0
Rideau Pedestrian Bridge N=13	Not detected – 4.3
26th AVE SW N=13	Not detected – 4.2
25th AVE SW N=13	Not detected – 3.8
1st ST SE N=13	Not detected – 4.4
Stampede Grandstand N=13	Not detected – 4.5
ENMAX Park N=13	Not detected – 3.9
9th Ave SE N=13	Not detected – 3.9

Temporal Variation in Human Fecal Contamination. Noteworthy temporal fluctuations in human fecal contamination were documented between sampling sites along the Elbow River. Human fecal contamination at the sites could be highly variable between sequential sampling dates, occurring once a week. For example, at Stanley Park, within a four-week span, concentrations of HF183 ranged from non-detectable on July 17th and July 31st, but for which in the intermittent weeks of July 24th and August 7th, levels of HF183 were > $3\log_{10}$ copies/100mL (Figure 4-9). This high variability between sequential sampling dates represented a significant fluctuation in human fecal contamination markers, eliciting potential concerns regarding the stability of water in urban stormwater-impacted rivers that are used for recreational activities. In comparison, Sandy Beach went through a 6-week span (i.e., July 24th through August 28th) without any detection of human fecal contamination (Figure 4-9).

On June 6th and June 12th, the only sampling site in which human fecal contamination was not detected was Sandy Beach, which is located the farthest upstream (Figure 4-9). This finding may suggest that the human fecal contamination occurred further downstream than Sandy Beach. A similar pattern was observed on July 24th and August 14th, in which all sampling sites had detection for human fecal contamination, except for Sandy Beach and Riverdale Avenue Bridge. Riverdale Avenue Bridge, located next to Sandy Beach, is the second most upstream sampling site (Figure 4-9). The next downstream sampling site is Stanley Park, and that sampling site did not contain the highest concentration of HF183 on the aforementioned dates (i.e., July 24th and August 14th), which might have been expected if the contamination event occurred between Riverdale Avenue Bridge and Stanley Park. These results suggested that the location of the sampling sites along the river may have an effect on the detections of human fecal contamination within the main stem of the river.

On July 10th, human fecal contamination was detected at all Elbow River sampling sites. In addition, the highest concentration of HF183 in the Elbow River (i.e., 5.0 $\log_{10}$ copies/100mL) was detected on July 10th (Figure 4-9). One explanation that was looked at was the variable of antecedent rainfall greater than 10 mm to see if it had any effect on the variability of human fecal pollution. However, there was no rainfall in the previous 72 hours on this sampling date.

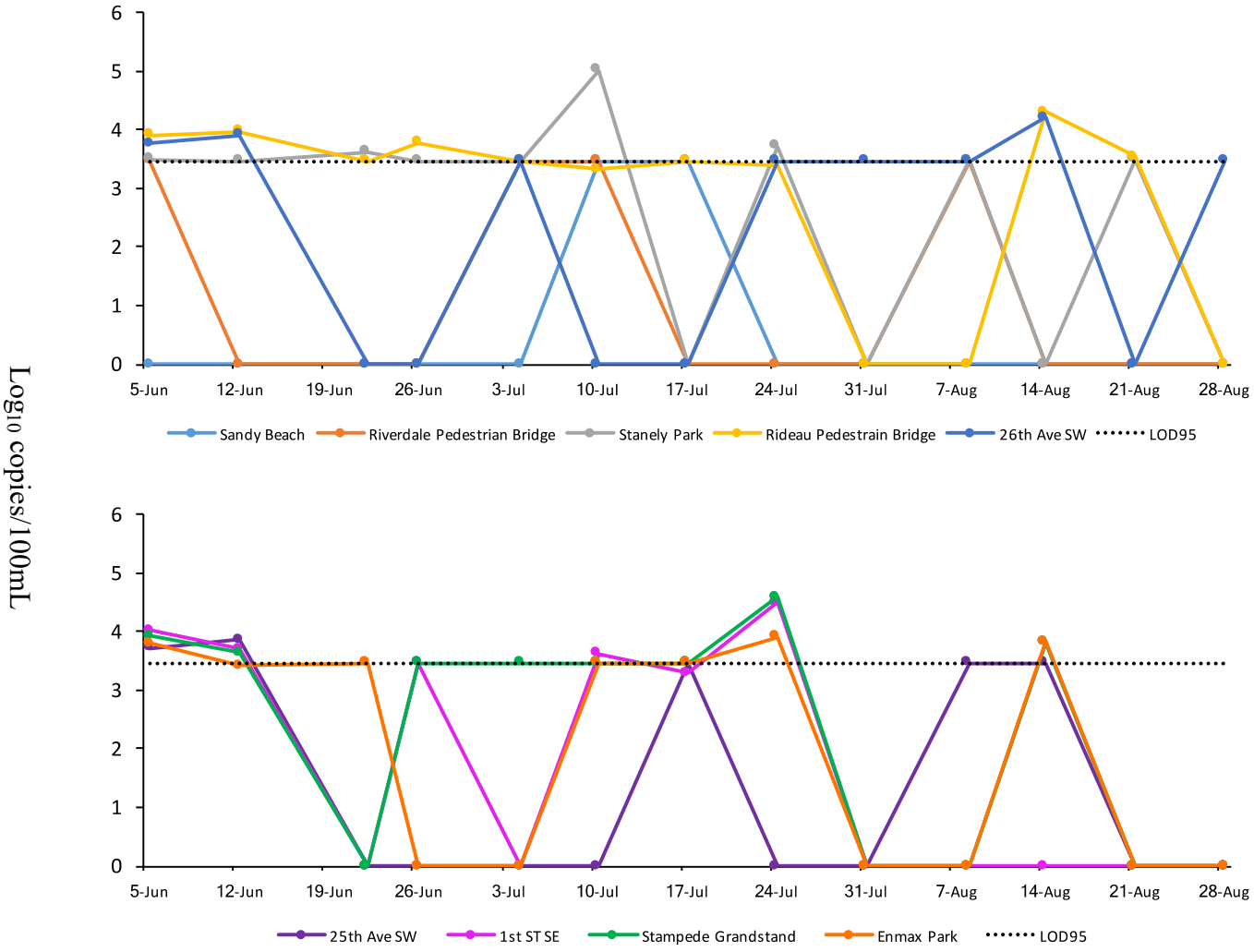

Figure 4-10:Temporal occurrence pattern of log_{10} HF183 concentrations at sampling sites along the Elbow River. Sandy Beach is in light blue, Riverdale Pedestrian Bridge in red, Stanley Park in gray, Rideau Pedestrian Bridge in yellow, 26th Ave SW in dark blue, 25th Ave SW in purple, 1st St SE in pink, Stampede Grandstand in green, Enmax Park in orange, and the LOQ_{95} as a black dotted line in both figures.

4.3 Discussion

Seven different microbial source tracking markers were utilized in this thesis to identify microbial sources of contamination impacting stormwater and receiving bodies of water in Calgary and Airdrie (i.e., HF183 [human], HumM2 [human], LeeSg [seagull], CGO1 [Canada Goose], Rum2Bac [ruminant], Dog3 [dog], and MuBac [muskrat]). The most significant contribution of this chapter is the evidence that stormwater can, at times, be contaminated with high levels of human fecal pollution. As society begins to make use of stormwater, it is essential to understand which microbial sources of pollution may be present and how they may impact public health through recreational and reuse activities.

Our findings with respect to human fecal pollution in stormwater-impacted bodies of water are similar to the findings of other researchers (Sauer, *et al*., 2011; Converse, *et al*., 2011; Newton, *et al*., 2013; Chase, *et al*., 2012). A 2013 study in Milwaukee, Wisconsin, USA, reflected that researchers considered human pollution to be a "chronic" problem in testing sites along Lake Michigan, impacted by stormwater outfalls (Newton *et al*., 2013). In a recreational stream in an urban environment in Hawaii, the human fecal pollution marker HF183 was detected in 83% of samples over a one-year period (Kirs, *et al*., 2016). Similarly, a study of two marine beaches impacted by storm drains or stormwater runoff in coastal California, USA, revealed that HF183 was detected in 27% of samples over the summer months (i.e., June to August) (McQuaig, *et al*., 2012). Sauer *et al*. (2011) detected the HF183 marker in every stormwater outfall tested (i.e., 45/45) in four urban watersheds located in Milwaukee, Wisconsin, USA, although none of the sampling sites were situated in a combined sewer outfall area. In total, they detected HF183 in 57% of samples over a four-year period (Sauer, *et al*., 2011).

Further, our study found that stormwater in urbanized centers in southern Alberta was also impacted by human fecal contamination. Silverman *et al.* (2013) looked at the presence of human fecal indicators (i.e., human norovirus GII, human adenovirus, and human *Bacteroidales*) in stream water impacted by stormwater and used for irrigation purposes, in Accra, Ghana. They detected human *Bacteroidales* in all but one sample (Silverman, *et al*., 2013). High levels of human *Bacteroides* spp. markers and enteric viruses have been identified in stormwater that may contain sewage effluent, which has been identified as a potential pathogen transmission route (Sauer, *et al*., 2011; Wade, *et al*., 2006).

Conversely, some studies have found stormwater-impacted bodies of water to not be heavily impacted by human fecal contamination. Staley *et al*. (2012) studied seven sampling sites in a stormwater-impacted freshwater lake in metropolitan Tampa, Florida, USA, and detected HF183 in only 10% of samples. In addition, there was one sampling site in which HF183 was never detected (Staley, *et al*., 2012). Surbeck *et al*. (2006) identified human fecal pollution by quantifying human adenovirus and human enterovirus in water samples from the Santa Ana River in California, USA, during storm events; and had only one positive sample. Therefore, the researchers above concluded that stormwater may not necessarily be heavily impacted by human sewage (Surbeck, *et al*., 2006), and implying that stormwater can be managed effectively to reduce risk. Our study demonstrated that some stormwater sites had relatively low concentrations of bacterial indicators and human fecal markers compared to other sites. Moreover, our special investigation study revealed that some stormwater drainage trunks were consistently free of human fecal contamination whereas others were not, suggesting that microbial source tracking tools can be used to effectively identify and mitigate problems associated with human fecal contamination within drainage networks (i.e., cross connections).

Different sources of fecal pollution pose varying threats to human health, albeit with human fecal pollution potentially causing the greatest risk to human health. The five nonhuman microbial source tracking markers tested in our study included targets for dogs, ruminants, Canada Geese, seagulls, and muskrats. Soller *et al*. (2010) compared recreational water contaminated with human fecal material (i.e., secondary disinfected wastewater effluent, primary wastewater effluent) and non-human fecal matter (i.e., cattle, pig, chicken, and gull feces) by calculating the estimated illness and infection risk through a QMRA-based approach. The Soller *et al*. (2010) analysis revealed that the risk of gastrointestinal illness associated with exposure to human fecal contamination may not be different than the risks associated with exposure to cattle fecal contamination, since their results reflected that the risks fell within a comparable range. This result is due to several reasons. Firstly, waters that were impacted by cattle fecal material contained potentially harmful levels of several zoonotic pathogens (i.e., *Campylobacter* spp., *Cryptosporidium*, *Giardia* spp., and *E. coli* O157:H7). Secondly, health outcomes associated with some of these pathogens, as shiga-toxin producing *E. coli* [STEC] (e.g., *E. coli* O157:H7), have the potential to be even more severe than other gastrointestinal illnesses. Soller *et al*. (2010) also studied recreational bodies of water impacted by gull, chicken, and pig feces. They found the risk of human illness to be lower than from human sewage-impacted water (i.e., median illness risk was found to be two or more orders of magnitude less) (Soller, *et al*., 2010). However, these other pollution sources should not be ignored, as there is the potential for new health risks to emerge. As such, non-human fecal waste still presents a significant risk to human health, as it can serve as reservoirs for zoonotic diseases (Staley, *et al*., 2013; Sauer, *et al*., 2011; Templar, *et al*., 2016).

In our study, seagull fecal contamination was the second most common source of fecal pollution identified. Other studies have also found seagulls to be a dominant source of fecal pollution (Araujo, *et al*., 2014; Converse, *et al*., 2012; Lee, *et al*., 2013; Ervin, *et al*., 2014). Many birds are carriers of human pathogens, such as bacteria (e.g., *Salmonella* spp. and *Campylobacter* spp.) (Araujo, *et al*., 2014) and viruses, (e.g., influenza) (Krauss, *et al*., 2007). Staley *et al*. (2016) found the seagull marker (i.e., LeeSg) to be more frequently detected in sampling sites along the Humber River in Toronto, Ontario, Canada, than in stormwater outfalls. Ervin *et al*. (2014) studied an urban watershed located in Santa Barbara County, California, USA, for gull fecal pollution, and found that 96% of samples had detectable levels in the surf zone, yet there was no correlation with FIB. In fact, in relation to FIB, a study estimated that seagulls can emit 4.8×10^9 *E. coli* and 2.1×10^8 *Enterococcus* spp. every day (Converse, et al., 2012). Further, at a beach in Racine, Wisconsin, USA, Converse *et al*. (2012) studied gull fecal pollution levels and the effects of gull removal on FIB (i.e., *E. coli* and *Enterococcus* spp.) levels, which were substantially reduced during a period of gull harassment. In addition, Goodwin *et al*. (2016) found that gulls were a primary pollutant source in a California watershed. Lu *et al*. (2011) studied over 1000 water samples from stormwater-impacted riverine and coastal locations from Toronto, Ottawa, and Hamilton, Ontario, Canada, with 58% of samples testing positive for gulls, whereby the highest mean concentration measured was at 6.1 $\log_{10}$ copies/100mL.

Bird fecal contamination is not limited to just seagulls. In our thesis, a microbial source tracking marker for Canada Geese (i.e., CGO1) was also utilized (Fremaux, *et al*, 2010). This marker was found to occur more predominantly in some sampling sites, such as ML1 at McCall Lake, where it was detected in 10% of samples. A study by Fremaux *et al*. (2010) detected the

CGO1 marker in 75 water samples collected from an urban lake located in Regina, Saskatchewan, Canada, over a three-month period (i.e., September to November). They detected the marker in 87% of samples, and in comparison, only detected a human fecal pollution marker (i.e., BacH) in 7% of samples (Fremaux, Boa, & Yost, 2010). Studies have confirmed that Canada Geese can serve as reservoirs for some human pathogens (i.e, *Campylobacter* spp., *Salmonella* spp., etc.) (Fremaux, *et al*., 2010; Gorham & Lee, 2016; Rutledge, *et al*., 2006). Vogt *et al*. (2018) studied fecal samples from Canada Geese in southern Ontario, Canada, for pathogens (i.e., *Campylobacter* spp., *Salmonella* spp., and *E. coli*) and antimicrobial resistant *E. coli*; and identified fecal isolates that were resistant to Category I (i.e., very high importance) antimicrobials, suggesting therefore that Canada Geese may spread pathogens and antimicrobial resistance in the environment. However, it is believed that birds feces may carry different human health risks than human fecal pollution (i.e., sewage), as bird feces may consist of species within a pathogenic genus that would not cause substantial risk to humans (Sinigalliano, *et al*., 2013).

Domestic pet feces (i.e., dogs) were detected in only 2% of samples from stormwater ponds in this thesis research. Domestic pets and wildlife have the potential, however, to be key contributors to FIB concentrations (Sauer, *et al*., 2011; Shanks, *et al*., 2009; Staley, *et al*., 2016; Green, *et al*., 2014). Green *et al*. (2014) identified canine feces as a significant pollutant to water bodies affected by urban runoff, noting the lack of official standards or guidelines concerning canine fecal waste, in spite of the zoonotic nature and threat of many pathogens associated with domestic pet feces. Staley *et al*. (2016) found that one stormwater outfall located along a river in Toronto was highly contaminated with dog feces, with 44% of samples testing positive for dog fecal markers. In addition, Converse *et al*. (2011) observed that domestic pets may contribute up to three times the amount of FIB than sewage from septic tanks during a storm event. Ervin *et al*.

(2014) in their study of an urban watershed in Santa Barbara County, California, USA, detected markers of dog fecal pollution in 64% of samples in the surf zone and in 45% of samples from lagoon. It was also noted that this watershed was frequently visited by dogs, and it was believed that the spatial differences might have been due to direct contamination or because of the tide exchange (Ervin, *et al*., 2014). In our study, three sampling sites in the Nose Creek (i.e., 25756, 25807, and 25814) detected the microbial source tracking marker for dogs in 40% of samples, albeit a relatively low number of samples were taken (i.e., 5 samples).

Ruminant fecal pollution also poses a potential danger to human health, in part due to the ability of these mammals to carry and transmit zoonotic pathogens (e.g., *E. coli* O157:H7, *Salmonella enterica*, etc.) in their feces (Gilbert, *et al*., 2014; Staley, *et al*., 2013; Raith, et al., 2013). The ruminant fecal marker (i.e., Rum2Bac) has been shown to be present in cattle, goats, sheep, and deer feces (Raith, *et al*., 2013). A key potential source of ruminant fecal pollution in bodies of water is manure, in which Canada alone produces 100 million metric tons of manure a year, and with the United States producing another 860 million (Lee et al., 2014). As ruminant animals are uncommon in an urban environment, it was not surprising that we only found this marker in 2% of samples in our study of the Calgary urban stormwater ponds. However, in a study of a river in Toronto, Ontario, Canada, the ruminant marker was detected in 63% of samples at one sampling site in the river (Won, *et al*., 2013). In our samples from a rural river in southern Alberta, 89% of water samples were positive for the ruminant marker overall, with 94% of samples being positive at one sampling site at the rural river.

Fecal pollution may enter bodies of water from point sources or non-point sources. Point sources of fecal pollution include combined sewer outflows (i.e., CSOs), animal feedlots, slaughterhouses, and on-site sewer systems, with CSOs and on-site sewer systems being more

common in urban environments; whereas, more often in rural areas, non-point source pollution can occur from distributed and diffuse surface runoff contaminated with fecal material from ruminants or birds, septic systems, landfills, and pastures (Sokolova, *et al*., 2012; Geonha & Hur, 2010). Non-point source pollution can be difficult to quantify due to the diffuse points of entry of contaminated water into a receiving water body. Studies have demonstrated an association at point source-impacted beaches with *Enterococcus* spp. levels and human health (i.e., Wade, *et al*., 2006), while the body of knowledge for non-point sources of pollution tends to be limited. Abdelzaher *et al*. (2011) looked at the health of swimmers at non-point source-impacted beaches and evaluated the presence of pathogens, microbial source tracking markers, indicators, and environmental variables. They found that the health of swimmers at non-point source-impacted beaches were affected more by the environmental variable of antecedent rainfall in the previous 24 hours than by the other variables tested (i.e., presence of pathogens and microbial source tracking markers) (Abdelzaher, *et al*., 2011).

It is important to note that the occurrence of high levels of microbial source tracking markers for human fecal pollution (i.e., HF183 and HumM2) in stormwater may be representative of times of peak contamination. Thus, in our study, there were several instances throughout the sampling period when high levels of HF183 were detected in McCall Lake, in particular. The highest level was 6.0 $\log_{10}$ copies/100mL (i.e., 1 x 10^6 copies/100mL) at sampling site ML2; and the highest level of HumM2 detected was 5.1 $\log_{10}$ copies/100mL (i.e., 1 x 10^5 copies/100mL), also at ML2, with both results occurring on the September 13th. In comparison, in prior research, the levels of HF183 detected in raw sewage ranged from 4.0 x 10^6 to 2.5 x 10^{10} copies/100mL (McQuaig, *et al*., 2012; Van De Werfhorst, *et al*., 2011). Shanks *et al*. (2013) tested 54 sewage samples, and the found the median concentration of HumM2 to be ~

2.8 $\log_{10}$ mean copy number/100mL, and the median of HF183 to be ~ 4.5 $\log_{10}$ mean copy number/100mL. Health outcomes related to exposure to untreated sewage are widely studied, since this exposure is considered to be a serious public health risk (Green *et al*., 2014; Shanks, *et al*., 2013; Boehm, *et al*., 2015). In their study concerning stormwater outfalls in Milwaukee, Wisconsin, USA, Sauer *et al*. (2011) revealed that over two-thirds of the stormwater outfalls had high levels of human fecal contamination (i.e., HF183) with a threshold set at 5.0 $\log_{10}$ copies/100mL. A study of the HumM2 marker by Shanks *et al*. (2009) showed that samples taken downstream from a wastewater discharge pipe generated positive results.

In our study at individual sampling sites within a pond, copies of HF183 in stormwater samples occurred over a wide range of values from undetectable to 6.0 $\log_{10}$ copies/100mL. In the literature, previous studies also observed considerable variation of human *Bacteroides* spp. markers in a particular outfall (Sauer *et al*., 2011; Sercu, *et al*., 2009). A study of CSOs in Philadelphia, Pennsylvania, USA, detected HF183 at the two sampling sites, ranging in concentrations from 1.3 x 10^1 to 8.2 x 10^6 copies/100mL at one sampling site, and 4.5 x 10^1 to 5.6 x 10^6 copies/100mL at the second sampling site, and in conjunction with high levels of FIB at both sampling sites (McGinnis, *et al*., 2016). Converse *et al*. (2011) found the levels of HF183 to vary widely between sampling sites within the Menomonee River in the urban area of Milwaukee, Wisconsin, USA, ranging from 993 to 4.1 x 10^6 copies/100mL. In the North Carolina, USA, study by Converse *et al*. (2011) involving five stormwater outfalls that impacted recreational beaches, their data showed that the mean concentrations of HF183 ranged from 10^2 to 10^5 copies/100mL. In addition, Templar *et al*. (2016) found that the upstream portions of the Menomonee River in Milwaukee, WI, USA, were not as heavily impacted by human fecal pollution as the downstream portions. Further, in the study of stream water in Accra, Ghana, the

levels of human *Bacteroides* spp. ranged from $10^{2.3}$ to $10^{6.7}$ gene copies/100mL (Silverman, *et al*., 2013).

A paper by Boehm *et al*. (2015) proposed that the qPCR marker concentration level exhibiting a health risk could be dependent on which human-specific microbial source tracking marker is used. Boehm and colleague's study suggested that when the concentration of HF183 reaches as high as 4200 copies/100 mL and the concentration of HumM2 reaches 2800 copies/100 mL, the median illness rate in the population approaches the 30 per 1000 individual's threshold of risk (Boehm, *et al*., 2015). Arnold *et al*. (2013) found that 21% of swimmers had diarrhea, which could be attributed to swimming in waters exceeding USEPA guidelines for *Enterococcus* spp.

In our study, considerable variation in the occurrence of microbial source tracking markers for fecal pollution was observed between the Calgary urban stormwater ponds, and sampling sites within a pond. In particular, there was considerable variation in the levels of human fecal pollution. Other studies observed similar findings as well (Staley, *et al*., 2016; Converse, *et al*., 2011; McQuaig, *et al*., 2012). McQuaig *et al*. (2012) studied five sampling sites along Doheny Beach in California, USA, and found one sampling site where HF183 was detected more frequently than at the other sampling sites, suggesting that spatial variability existed at the stormwater sampling sites. Converse *et al*. (2011), in their study in North Carolina, USA, found the concentrations of fecal *Bacteroides* spp. to be statistically different across five different sampling sites, even though the sites were geographically close to one another. The study on the Humber River in Toronto, Ontario, Canada, found the human fecal marker (i.e., HF183) was detected more frequently in the stormwater outfalls than at the sampling sites along the river (Staley, *et al*., 2016).

In our study, the microbial source tracking marker for seagulls (LeeSg) observed at sampling site ML2 in McCall Lake ranged from not detected to 4.2 $\log_{10}$ copies/100mL; and CGO1 ranged from not detected to 4.4 x 10^4 copies/100mL at sampling site ML1. Converse *et al*. (2012) observed concentrations of *Catellicoccus marimammalium* to range from 3.3 x 10^3 to 1.8 x 10^6 copies/100mL. In addition, a study in Regina, Saskatchewan, Canada found the range of levels of CGO1 to range from undetectable to 3.8 $\log_{10}$ copies/100mL (Tambalo, *et al*., 2012).

A wide range of concentrations of microbial source tracking markers was not limited to human fecal pollution. Such variability could be attributed to a variety of short-term or long-term environmental factors (e.g., temperature, exposure to sunlight, rainfall, etc.) (Sokolova, *et al*., 2012). Short-term dynamics, including rainfall, can trigger a cascade of events that can contribute rapid changes in the levels of human fecal contamination (e.g., increased runoff, sewage overflows, increased infiltration, and the resuspension of sediments) (Santiago-Rodriguez, *et al*., 2012). In our research, rapid changes in the levels of human fecal contamination were observed, as human fecal contamination could vary several orders of magnitude over a period of three days. Previous studies have demonstrated that rainfall leads to an increase in FIB levels, implying an increase in suspected pathogens in water (Santiago-Rodriguez, *et al*., 2012; Lee, *et al*., 2014; Teng, *et al*., 2012). An association between rainfall and waterborne disease outbreaks has been well-described (Lee, *et al*., 2014). Curriero *et al*. (2001) studied the association between outbreaks and extreme precipitation at the national level in the US, over a span of 46 years (i.e., 1948-1994). Their study revealed that 51% of drinking water-related outbreaks during this time were associated with extreme precipitation events. Although this study focused on drinking water-outbreaks, it is still relevant to our study due to the mechanisms (i.e., microbial fate and transport) of extreme precipitation effects on other types of

water (e.g., recreational and surface). Levy *et al*. (2016) conducted a systematic review on the relationship between diarrheal diseases and various environmental variables, including rainfall. They reviewed 31 articles on this matter, and found a significant positive association (i.e., 71%) between heavy rainfall and diarrhea. In addition, they found this trend to persist in studies that were nationwide (i.e., nationwide studies of Canada, United States, and the United Kingdom) (Levy, *et al*., 2016). Thomas *et al*. (2006) reviewed 92 waterborne outbreaks in Canada over a 26-year period (i.e., 1975-2001), and these researchers observed that when daily rainfall was greater than the 93rd percentile, it increased the odds for a waterborne outbreak. Herrador *et al*. (2016) conducted a matched-case control study, which instead of using a common threshold of extreme precipitation, utilized exceedance precipitation, which accounted for the variability between climates. Even so, their research revealed that in four Nordic countries (i.e., Denmark, Finland, Norway, and Sweden) there was an association between heavy precipitation in the week prior to a waterborne outbreak (Herrado, *et al*., 2016). Parker et al. (2010) found that rain events influence stormwater runoff, which have the potential to carry human fecal pollution. The North Carolina, USA, study by Converse *et al*. (2011) came to the conclusion that the highest concentration of fecal *Bacteroides* spp. was associated with increased rainfall. Other studies acknowledge that rainfall may be a factor in the levels or presence of human fecal pollution in bodies of water (Templar, *et al*., 2016; Marsalek & Rochfort, 2004).

Many previously published studies differ on what is the effect of rainfall on fecal *Bacteroides* spp. (Surbeck *et al*., 2006; Sauer *et al*., 2011). The 2016 study by Templar *et al*. in Wisconsin, USA, did not find a statistically significant difference between the concentrations of HF183 in wet versus dry weather events at the majority of sampling sites. Sauer *et al*. (2011), in their study of stormwater outfalls in Milwaukee, Wisconsin, USA, did not see an association

between rainfall and the levels of human *Bacteroides* spp. detected. As discussed in Chapter 3, inconsistent data in terms of fecal indicators was also observed for microbial source tracking markers. In contrast to the concept of first flush, Converse *et al*. (2011) found fecal *Bacteroides* spp. concentrations to be the highest in the last 24 hours of a storm event, opposite of what was observed with *Enterococcus* spp. and *E. coli*, implying that low concentrations of fecal *Bacteroides* spp. could be due to low flow later in the hydrograph (i.e., less diluted). However, the association of fecal *Bacteroides* spp. and rainfall was not as strong as *Enterococcus* spp. or *E. coli* and they noted that the concentrations of fecal *Bacteroides* spp. could vary significantly between storms (Converse, *et al*., 2011). Furthermore, several researchers have suggested that the higher levels of FIB during first flush may also be due to residual human waste found in CSOs or cross-connected systems (Chong, *et al*., 2013; Sidhu, *et al*., 2012).

Besides environmental variables, sources of contamination may be influenced by landscape characteristics. As previously mentioned, our study focused on urban environments, where human fecal pollution was the most frequently detected marker (i.e., 27% for HF183, 10% for HumM2 in the Calgary Stormwater Ponds, Table 4-1). By contrast, the ruminant marker was only detected in 2% of samples; and in the rural river, the ruminant marker was the most commonly detected microbial source of pollution, being detected in 88% of samples. Staley *et al*. (2013) studied the fecal sources of contamination in eight isolated human-made lakes in Florida. They characterized the lakes into three groups (i.e., undeveloped, cattle-grazing, and urban). They detected the general *Bacteroidales* marker in every sample, though they never detected the HF183 marker for human fecal pollution. In addition, they found the ruminant marker (i.e., ruminant *Bacteroidales* assay that utilized the CF128 forward with the Bac708 reverse primer) to be detected much more often in the lakes associated with cattle-grazing (i.e., six samples [which

equated to 57%]) than in the urban lakes (i.e., one sample) or in the undeveloped lakes (i.e., no samples) (Staley, *et al*., 2013). These results were in line with other studies (Stea, *et al*., 2015; Ridley, *et al*., 2014). Stea *et al*. (2015) detected general *Bacteroidales* spp. in 98% samples, but only detected the ruminant marker (i.e., BacR) in surface water samples taken from rural watersheds located in Nova Scotia, Canada. Evidence of human fecal pollution (i.e., HF183) was observed in only 10% of samples from both the rural and urban watersheds (Stea, *et al*., 2015). In a study of the Qu'Appelle River in Saskatchewan, Canada, impacted by cattle, agricultural fields, wastewater treatment plants, and an urban environment, researchers noted the highest concentrations of the ruminant marker at sites with nearby cattle-grazing fields during the irrigation water season (i.e. May-September) (Tambalo, *et al*., 2012). The researchers however did not find any differences with the human fecal pollution marker among the sampling sites (Tambalo, *et al*., 2012). Nonetheless, other studies have found human fecal pollution to be more common in an urban environment than in a rural area. The study of two urban stormwater-impacted estuaries (i.e., Kinnickinnic River and Menomonee River) in Milwaukee, Wisconsin, USA, revealed that samples from the Kinnickinnic River and downstream portion of the Menomonee River experienced chronic human fecal pollution (Templar, *et al*., 2016). Also, a study by Chase *et al*. (2012) of a river in Florida, USA, detected human fecal pollution in all sampling sites but two, and ruminant fecal contamination in sites more heavily impacted by agriculture. Furthermore, human fecal pollution was detected in 71% of samples at one site (Chase, *et al*., 2012).

As has been noted regarding the variation in occurrence of human fecal pollution in urban and rural environments, there have been studies noting the differences in the frequency of gull contamination in rural versus urban environments. Lu *et al*. (2011) found more gull fecal

pollution in urban lake watersheds and beaches of Ontario, Canada, than at others sites (e.g., creeks, rivers, municipal wastewater, and stormwater outfalls).

Drainage networks in the urban landscape can also be diverse, and as such, stormwater outfalls in the present study were categorized according to six different land use characteristics, as defined by zoning – residential; commercial; industrial; parks and institutions; major infrastructure and transportation; and future urban development (see Section 3.2.1). Mallin *et al*. (2001) found that coastal plan watersheds in North Carolina, USA containing industrial swine, agriculture, and poultry operations were correlated with increased fecal coliform counts in a stormwater-impacted stream following rainfall in the previous 24 hours, in comparison to areas without animal production. Furthermore, other studies have found that high concentrations of fecal coliform bacteria in stormwater runoff collected from streets in residential areas (Young & Thackston, 1999). The relationship between land use and fecal contamination was quite variable in our study, as the catchment area for each sewer outfall of McCall Lake consisted of different land uses. Sampling site ML2, with a catchment area comprising mostly of industrial land, was the outfall with the most frequent detections and the highest levels of human contamination, as opposed to its counterpart ML1, which was associated with a higher percentage of residential neighborhoods occupying a much larger catchment area. Moreover, it has been asserted in prior research that more commercialized areas release and distribute lower amounts of human fecal contamination than outfalls containing higher residential acreage in their catchment area (Converse, *et al*., 2011; Selvakumar & Borst, 2006). Notably, Converse *et al*. (2011) found that the lowest concentrations and loads of fecal *Bacteroides* spp. were associated with the most commercialized and least residential of all five sampling sites tested in North Carolina, USA. In our study, McCall Lake experienced the most human fecal contamination but also had land use

associated with higher amounts of industrial activities (i.e., 26%) and lower amounts of residential area (i.e., 30%) as compared to the other stormwater ponds tested. However, due to the mixed classification of land use in a watershed (e.g., McCall Lake having similar portions of industrial and residential classified land), it can be difficult to determine the relationship between type of contamination and land use.

As human fecal pollution was the most dominant marker detected in our study, it was important to ascertain the potential source of this contamination. Typical sources of human fecal pollution impacting stormwater systems in urbanized environments include cross-connections (residual/commercial), leaking sewage systems (due to aging infrastructure) and CSOs (Kapoor *et al*., 2015 Sokolova *et al*., 2012; Lipp, *et al*., 2001). Templar *et al*. (2016) found human fecal pollution in stormwater to be more common in older parts of the Milwaukee, Wisconsin, USA, metropolitan area, suggesting that aging infrastructure may have played a role in contaminating stormwater with human feces. In addition, sewage overflow in CSOs can further contribute to high levels of fecal bacteria to stormwater runoff (Kapoor, *et al*., 2015; Nishimyimana, *et al*., 2014; Surbeck, *et al*., 2006; McGinnis, *et al*., 2016). McGinnis *et al*. (2018) observed significant human fecal pollution in two recreational creeks in Philadelphia, Pennsylvania, USA, located downstream of multiple CSOs and in an area of the creeks not impacted by agriculture, wastewater treatment plants, or industry. Kapoor *et al*. (2015) demonstrated the detection of HF183 in 86% of samples in a non-recreational CSO-impacted watershed in Cincinnati, Ohio, USA, and found the range of HF183 levels to be 0 to 4.86 $\log_{10}$ copies/100mL, with a median of 3.52 $\log_{10}$ copies/100mL. Staley *et al*. (2016), detected human fecal contamination in all of their stormwater outfalls located in the Humber River in Toronto, Ontario, Canada, where HF183 was detected in 93% of samples at some of the outfalls. In fact, the lower portion of this river is

primarily impacted by CSOs (Staley, *et al*., 2016). Because the City of Calgary does not manage stormwater through CSOs infrastructure, these sources of pollution were ruled out. The dominant and consistent human fecal pollution signature from a single trunk of the stormwater drainage infrastructure and leading into a commercial/industrial area of the city, suggested that either a cross-connection or a leaky sanitary sewer were the likely sources of human sewage pollution.

The problem of the unusually high levels and persistent nature of the human sewage contamination in stormwater and stormwater-impacted bodies of water needs to be identified before a solution can be addressed (Newton, *et al*., 2013). Within our study, an "up the pipe" investigation was initiated at the ML2 sampling site in McCall Lake to pinpoint the causes of the high levels of human sewage contamination. Findings from three separate sub-investigations resulted in tracking the contamination through the ML2 drainage network. In this investigation a clear and directional contamination signature was observed in the drainage network, with HF183 consistently being detected in one of the drainage trunks and not in the others. Although the actual source of the contamination remains unknown at this point, it is suspected that an illicit cross-connection is the culprit. Our assumption is based on the overall levels of HF183 and the ability to track the human contamination signature into a commercial/industrial area of the city.

At this time, there is no bacterial qPCR marker that is 100% specific for human fecal contamination (Shanks, *et al*., 2009). Previous studies have found the human fecal pollution marker, HF183, to be present at low concentrations in feces from dogs, chickens, pigs, or ducks (McGinnis, *et al*., 2016; Staley, *et al*., 2012; Green, *et al*., 2014; Shanks, *et al*., 2009). Two prior independent studies on cross-reactivity with dogs have demonstrated reactivity rates less than 25% (Sauer, *et al*., 2011). Odagiri *et al*. (2015) in their study on sanitation in rural communities of India, found the mean level of HF183 in dog fecal samples to be 1.4 $\log_{10}$ gene copies per ng

of total DNA positive samples, when in comparison in human fecal samples the level was 2.3 $\log_{10}$ gene copies per ng of total DNA positive samples. In addition to noting cross reactivity of HF183 in dog fecal samples, Odagiri *et al*. (2015) also found that HumM2 cross reacted with dog, albeit at lower levels (i.e., 0.8 $\log_{10}$ gene copies per ng of total DNA positive samples). Therefore, these studies are important because they reflect that there is currently no assay (i.e., including BacHum, BacH, HF183 Syber, HF183 Taqman, HumM2) that can entirely differentiate between human and dog fecal samples (Odagiri, *et al*., 2015). In addition, all five of the aforementioned assays cross-reacted with chicken feces with mean levels ranging from 0.9 – 3.5 $\log_{10}$ gene copies per ng of total DNA positive samples (Odagiri, *et al*., 2015). However, Tambalo *et al*. (2012) did not find cross-reaction of HF183 with Canada geese, gulls, pigeons, moose, caribou or bison. Stea *et al*. (2015) reported 100% specificity and sensitivity to human feces in their study. For our study, in order to ensure that findings were not due to cross-reaction, a second human *Bacteroides* spp. marker, HumM2, was used. In addition, we tested a marker for dog fecal pollution (i.e., Dog3).

Notably, one of the limitations of microbial source tracking is the inability of the assay to distinguish between live and dead cells (Kapoor, *et al*., 2015). As such, the detection of genetic material cannot determine whether the fecal pollution is from a recent or past contamination event. A further important factor affecting the validity of the marker is its survival in the environment. A marker that survives too long might pose a challenge as to whether the contamination event is recent, whereas a marker that does not survive long enough might pose a risk to human health as it could signify that the water quality is acceptable when it is not. In addition, the survival of an organism can be effected by environmental factors (e.g., temperature, sunlight, etc.) which may increase the decay rate (Walters, *et al*., 2013; Bae & Wuertz, 2009;

Dick *et al*., 2010; Green, *et al*., 2014; Schulz & Childers, 2011). Even though HF183 does not persist long-term in the environment, it has, however, been detected for up to 24 days at 4°C (Seurinck *et al*., 2005).

Finally, public health officials are continuously faced with the daunting task of determining the best method for identifying threats to public health, efficiently, accurately, and safely. The combination of testing for FIB with microbial source tracking markers provides an approach that helps identify microbial sources of pollution. Lastly, by understanding the microbial sources of pollution, we would possibly have a clearer understanding of the presence of enteric bacterial pathogens present in stormwater-impacted bodies of water, which will be discussed in Chapter 5.

5 Enteric Bacterial Pathogens in Stormwater-Impacted Bodies of Water

5.1 Introduction

Although there is an abundance of literature on enteric bacterial pathogens in water systems, there is a lack of data on their presence in urban stormwater. Poor water quality within urban stormwater-impacted bodies of water represents a potentially important public health problem (see Chapter 3), as many of these water bodies are subjected to chronic issues with human and animal sources of fecal contamination (see Chapter 4), inferring the potential for zoonotic and anthropogenic enteric bacterial pathogens to be present. Pathogens as *Arcobacter butzleri* (Douidah, *et al*., 2012; Van Driessche & Houf, 2008; Levican, *et al*., 2013; Hafliger, Hubner, & Luthy, 2000; Craun, *et al*., 2005), *Campylobacter* spp. (Moore, *et al*., 2001; Clark, *et al*., 2003), *Salmonella* spp. (Krometis, *et al*., 2010), and Shiga-toxin producing *E. coli* (STEC) (Rangel, *et al*., 2005; Adams, *et al*., 2016), have all been implicated in waterborne outbreaks throughout the world. Rain events mobilize and transport fecal pathogen in the environment thereby increasing the effects of non-point and point sources of contamination, which in turn can augment the risk to public health (Staley, et al., 2018).

Stormwater-impacted bodies of water can serve as reservoirs for transmission for enteric bacterial pathogens by the fecal-oral route through ingestion of contaminated water during recreational activities (e.g., swallowing water while swimming); by way of contaminated irrigation water on food that is then eaten; or by accidental ingestion that occurs during irrigation. An outbreak of HUS associated with a recreational water body in Connecticut, USA, occurred where STEC was detected in a storm drain that emptied onto the beach (McCarthy *et al*., 2001). In a study of stormwater discharges and gastrointestinal illness following wet weather in California, USA, Soller *et al*. (2017) that wet weather exposure during surfing lead to higher

than average illness rates due to human enteric viruses. In addition, they observed *Campylobacter* spp. above the method detection limit in over half of their samples from stormwater discharges. Meng *et al*. (2018) identified *Campylobacter* spp. in stormwater constructed wetlands intended for reuse activities, and found the concentrations to be similar between wet and dry weather events. Furthermore, they found that log reduction targets for reuse activities were not being met (Meng, *et al*., 2018).

The objective of this portion of this thesis study was to identify and determine the prevalence of the enteric bacterial pathogens *A. butzleri*, *Campylobacter* spp., *Salmonella* spp., and STEC in the Calgary stormwater ponds and the Nose Creek stormwater outfalls studied in Chapters 3 and 4 of this thesis.

5.2 Results

5.2.1 Occurrence of Enteric Bacterial Pathogens in Stormwater Ponds and Stormwater-Impacted Rivers

A high-level descriptive overview of the frequency of several enteric bacterial pathogens (i.e., *A. butzleri*, *Campylobacter* spp., *Salmonella* spp., and STEC) in each of the Calgary urban stormwater ponds (i.e., McCall Lake, Country Hills Stormwater Facility, and Inverness Stormpond), and at each sampling site within the ponds is provided in Table 5-1. The most frequently detected bacterial pathogen found in stormwater ponds was *A. butzleri*, detected in 25% of samples (Table 5-1). The second most common pathogen detected was STEC, in 8% of samples, and followed by *Campylobacter* spp. (4%) and *Salmonella* spp. (1%).

As was observed with microbial fecal indicators (Chapter 3) and source tracking markers (Chapter 4), considerable spatial variation was observed with respect to the occurrence of enteric bacterial pathogens. *A. butzleri* was the pathogen most frequently detected in all stormwater

pond; however, the frequency of detection varied among the ponds. In McCall Lake, *A. butzleri* was detected in 38% of samples; whereas, in the Inverness Stormpond and Country Hills Stormwater Facility, *A. butzleri* was detected in 22% of samples (Table 5-1). In addition, *A. butzleri* contamination varied between sampling sites within a single urban stormwater pond. Interestingly, the Inverness Stormpond had the highest frequency of *A. butzleri*, with 49% of samples testing positive for *A. butzleri* at the site designated as 'Outfalls/Inlets'. *A. butzleri* occurrence at this sampling site exceeded the other sites at Inverness Stormpond (i.e., WP26B, WP26C, WP26D), where the frequency of occurrence of *A. butzleri* detections ranged from 10-17% (Table 5-1). In McCall Lake, *A. butzleri* was observed in 47% of samples at ML2. By comparison, Inlet 3/4 at McCall Lake had the lowest frequency of detection of *A. butzleri,* occurring in only 29% of samples (Table 5-1).

A similar pattern of spatial variation for urban stormwater ponds and sampling sites within a pond was noted for STEC, *Campylobacter* spp. and *Salmonella* spp. McCall Lake had the highest frequency of detection of STEC, occurring in 14% of samples; whereas, in the Country Hills Stormwater Facility and Inverness Stormpond, STEC was detected in 7% and 5% of samples, respectively. Within McCall Lake, STEC was detected in 15% of samples at sampling sites ML2 and PR60 (Table 5-1). *Campylobacter* spp. was detected most frequently in McCall Lake in 7% of samples, whereas *Campylobacter* spp. was only detected in 1% of samples at Inverness Stormpond. Within the Country Hills stormwater pond, *Campylobacter* spp. detection varied from 0% (not detected) at site WP31B to 10% at WP31D. *Salmonella* spp. was the least frequently detected enteric bacterial pathogen in our study, and with no spatial variability observed between the stormwater ponds (i.e., detection in only 1% of samples at each pond).

In addition to stormwater samples being taken from the Calgary stormwater ponds, water samples were also taken from the Nose Creek storm drains (Airdrie, Alberta, Canada) to assess enteric bacterial pathogen occurrence. Overall, in the Nose Creek storm drains, *A. butzleri* was detected in 27% of samples and STEC in 23% of samples (Table 5-2). *Campylobacter* spp. and *Salmonella* spp. were observed in only 2% and 0% of samples from the storm drain samples, respectively. Spatial variability was also observed at specific sampling sites (storm drains) discharging into the Nose Creek. *Campylobacte*r spp. was detected in 20% of samples at sampling site 25814. Spatial variability occurred between samplings sites for *A. butzleri*, as 66% of samples tested positive at site 25817, which was the highest frequency of occurrence for *A. butzleri* at any of the outfall tested. However, *A. butzleri* was not detected in any samples taken from site 25804. As for STEC, 40% of samples at three sampling sites (i.e., 25756, 25807, and 25855) were positive, whereas STEC was not detected in any samples taken from three other sampling sites (i.e., 25841, 25847, and 25793).

Table 5-1: Frequency of occurrence of samples positive based on pathogen-specific qPCR screening of stormwater ponds in Calgary, Alberta.

		Percent of samples positive for bacterial pathogens			
Pond	Sampling Site	*A. butzleri*: HSP60	*Campylobacter* spp.: VD16S	*Salmonella* spp.: InvA	*E. coli* shigatoxin: *stx*1 & *stx*2[a]
McCall Lake	ML2 n=41	47	7	4	15
	PR60 n=41	41	4	0	15
	ML1 n=41	34	7	0	12
	Inlet 3/4 n=41	29	7	0	10
	McCall Lake Total n=164	38	7	1	14
Country Hills	WP31A n=41	14	2	0	5
	WP31B n=41	34	0	0	7
	WP31C n=41	20	7	0	15
	WP31D n=41	20	10	2	5
	WP31E n=41	22	2	0	7
	Country Hills Total n=205	22	4	1	7
Inverness	Outfalls/Inlet n=41	49	2	2	5
	WP26B n=41	12	0	0	7
	WP26C n=41	10	0	0	5
	WP26D n=41	17	2	0	2
	Inverness Total n=164	22	1	1	5
Total n=533		25	4	1	8

a = presence/absence

Table 5-2: Frequency of occurrence of positive samples by pathogen-specific qPCR screening of stormwater samples flowing into the Nose Creek from Airdrie, Alberta.

	Percentage of Samples Positive for Enteric Bacterial Pathogen Markers			
Location	*A. butzleri:* HSP60	*Campylobacter spp.:* VD16S	*Salmonella spp.:* InvA	*E. coli*: Shigatoxin *stx*1 & *stx*2 [a]
25756 (n=5)	40	0	0	40
25793 (n=4)	50	0	0	0
25804 (n=4)	0	0	0	25
25807 (n=5)	20	0	0	40
25811 (n=5)	40	0	0	20
25814 (n=5)	40	20	0	20
25817 (n=3)	66	0	0	0
25841 (n=3)	33	0	0	0
25847 (n=5)	20	0	0	20
25855 (n=5)	20	0	0	40
Total n=44	27	2	0	23

a = test of presence/absence

5.2.2 Spatial and Temporal Variability of *A. butzleri* concentrations in Calgary Stormwater Ponds

Due to the high frequency of occurrence of *A. butzleri* in the Calgary stormwater ponds, further analysis was performed in order to better assess the concentration of the pathogen amongst: a) the different urban stormwater ponds; and b) sampling sites within a single urban stormwater pond (Table 5-1).

Spatial Variation of *A. butzleri.* Considerable spatial variation in the levels of *A. butzleri* was observed among all of the urban stormwater ponds, and among each of the sampling sites in the individual ponds. The highest concentration of *A. butzleri* was detected at Inverness Stormpond based on qPCR (5.0 $\log_{10}$ copies/100 mL at outfall WP26D) (Appendix 5-1). However, at McCall Lake, which had the highest prevalence of *A. butzleri*, the single greatest concentration of *A. butzleri* observed was 4.8 $\log_{10}$ copies/100 mL at Inlet PR60 (Figure 5-1), which occurred on June 13th when sites ML2 and Inlet ¾ had detectable but not quantifiable levels (i.e., DNQ [3.5 $\log_{10}$ copies/100mL]).

In addition, based on culture confirmation, the average highest MPN observed during the thesis research study was at sampling site ML2 which was 18 MPN/300 mL (Table 5-3). The single highest concentration of *A. butzleri* measured through culture-based methods occurred at sampling site ML2 on September 13th, in which 93 MPN/300mL was observed.

Temporal Variation of *A. butzleri*. To better understand temporal variation, we further examined patterns of occurrence based on molecular qPCR results. Notable temporal fluctuations in *A. butzleri* were observed between the urban stormwater ponds, and among the sampling sites within a pond (Figure 5-1, and Appendix). We found that at Inlet ¾, in McCall Lake, considerable temporal fluctuations were detected in the levels of *A. butzleri* between sequential sampling dates. Within a two-week time period (i.e., four sequential sampling dates, June 20th – June 29th), the concentration of *A. butzleri* varied from being not detected (i.e., below the limit of quantification of 3.5 $\log_{10}$ copies/100 mL) on June 20th, then spiking to 3.9 $\log_{10}$ copies/100 mL on June 22nd, to be not detected on June 27th, and spiking again to 4.3 $\log_{10}$ copies/100 mL on June 29th.

We tracked environmental variables that could contribute to temporal fluctuations in *A. butzleri* concentrations (e.g., antecedent rainfall data, temperature, etc.). Of note, we recorded three sampling dates that had rainfall greater than 10 mm (i.e., May 25th, June 8th, and September 13th, Figure 5-1). We noted that *A. butzleri* was detected at all McCall Lake sampling sites on several sampling dates, July 10th, August 14th, August 16th, September 13th, of which September 13th had significant rainfall (Figure 5-1). However, on another rainfall date (i.e., May 25th) *A. butzleri* was not observed at any of the sampling sites, and on June 8th, *A. butzleri* concentrations reached detectable levels only at the outfalls (i.e., ML1 and ML2).

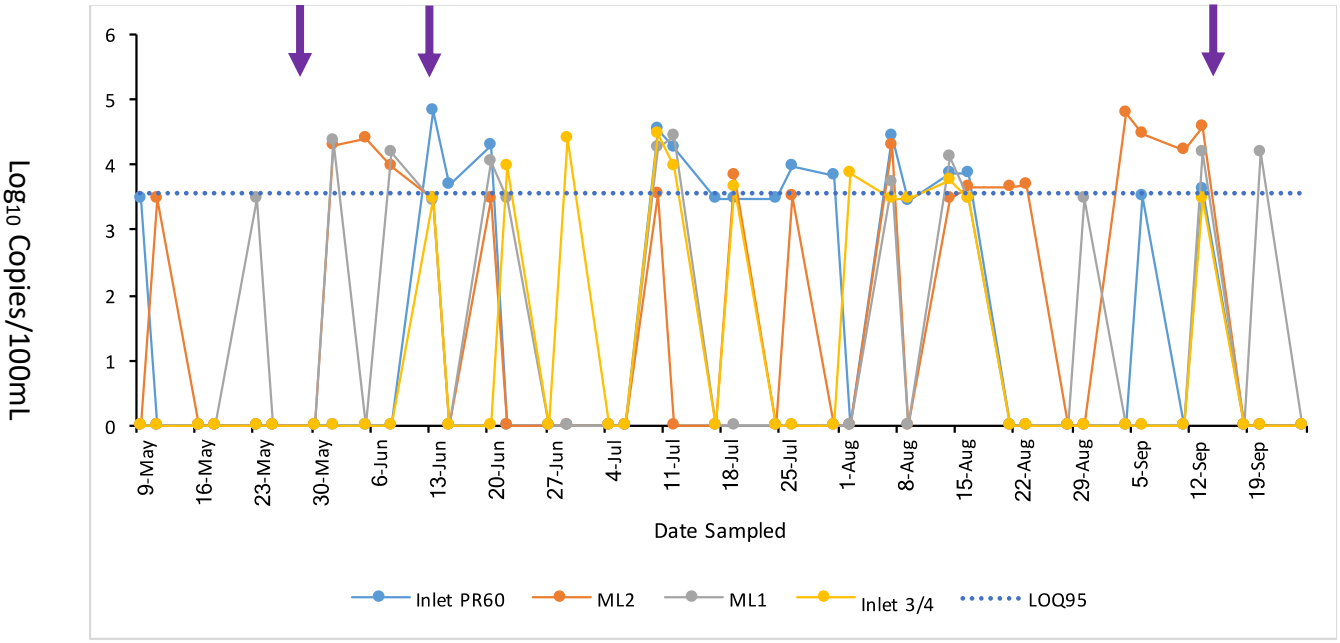

Figure 5-1: Levels of A. butzleri, represented as log_{10} copies/100mL, in all sampling sites at McCall Lake over 21 weeks. The blue line represents inlet PR60, the red line is ML2, gray line is ML1, yellow line is Inlet ¾, and the limit of quanitifcation$_{95}$ (LOQ_{95}) is a blue dotted line. Purple arrows denote sampling date with rainfall >10 mm in the previous 72 hours.

5.2.3 Culture and Molecular-based Method Comparison

MPN-qPCR assays were performed for *Campylobacter* spp. and *A. butzleri* on stormwater samples from all sampling sites in McCall Lake (i.e., ML2, ML1, Inlet ¾, PR60) on sampling dates starting from mid-August through the end of the sampling season (i.e., August 21st - September 25th). The assays were carried out on split samples in order to determine if molecular-based methods were comparable to results obtained by culture. Based on the limited number of samples collected for comparison (n-32), no *Campylobacter* spp. was detected by either method (i.e., MPN-qPCR assay or qPCR screen assay) (Table 5-4), suggesting that molecular screen testing and culture-based testing methods led to similar results.

However, this was not the case for *A. butzleri*, with 24 of 32 samples (75%) testing positive for *A. butzleri* by culture-based methods, but only 6 of these same samples were also positive by molecular-based methods [18.75%] (Table 5-5). Eighteen samples positive for *A. butzleri* by culture were negative by molecular-based screening methods (Table 5-5). These results suggest that the molecular screen results presented in Table 5-1 and Table 5-2 may underestimate the true occurrence of *A. butzleri* in stormwater samples, an effect possibly explained by the relatively low concentration of *A. butzleri* observed in stormwater samples (i.e., ~10^1bacteria /100mL, [Table 5-3]). Although molecular assays are highly sensitive, a major limitation rests in the overall sample volume examined during analysis, due to the extra processing steps that are required to prepare the template, and the small template volumes used during PCR amplification (i.e., 5.0 uL). When these volume corrections are taken into account, the PCR assay only examines the occurrence of a pathogen target within a 5.0 mL volume of the original stormwater sample. Consequently, in samples where only 10^1 *A. butzleri* /100mL exist

(or 0.1 bacteria/100mL) the likelihood of detecting this concentration by PCR is low, and particularly relevant for a single copy gene such as *hsp60.*

Table 5-3: Most Probable Number qPCR assay performed on McCall Lake sampling sites (i.e., ML1, ML2, PR60, Inlet ¾) on eight sampling dates from August 21st - September 25th, 2017 for A. butzleri with the most probable number (MPN) reflected as per 300mL.

	MPN *A. butzleri* **/300mL**			
Sampling Date	ML1	ML2	PR60	Inlet 3/4
August 21, 2017	Not detected	23	2.3	2.3
August 23, 2017	43	2.1	17	9.3
August 28, 2017	Not detected	9.3	2.3	18
August 30, 2017	4.3	2.3	2.1	2.3
September 6, 2017	2.3	9.3	2.3	Not detected
September 13, 2017	4.3	93	2.3	Not detected
September 20, 2017	0.4	0.9	Not detected	1.5
September 25, 2017	4.3	4.3	Not detected	0.4
Average	7.3	18	3.5	4.2

Table 5-4: Comparison of culture-based and molecular-based methods for Campylobacter spp. represented in a positive-negative two-by-two table.

		Molecular-based Methods for *Campylobacter* spp.	
		Positive	Negative
Culture-based methods for *Campylobacter* spp.	Positive	0	0
	Negative	0	32

Table 5-5: Comparison of culture-based and molecular-based methods for A. butzleri represented in a positive-negative two-by-two table.

		Molecular-based Methods for *A. butzleri*	
		Positive	Negative
Culture-based methods for *A. butzleri*	Positive	6	18
	Negative	0	8

5.2.4 Relationship between Microbial Sources of Fecal Contamination and *A. butzleri* in McCall Lake

Due to the prevalence and abundance of *A. butzleri* contamination, we sought to determine the potential sources of its contamination. Water samples were analyzed by identifying which microbial source tracking markers occurred most often with *A. butzleri* detections. We found that the most common source of pollution co-occurring with *A. butzleri* detection was human fecal pollution. The human marker HF183 was present in 43% of *A. butzleri* positive samples, while the human marker HumM2 was detected in 10% of *A. butzleri* positive samples (Table 5-6). The second most dominant source of fecal pollution was seagull (i.e., LeeSg), which corresponded to *A. butzleri* detection in 10% of *A. butzleri* stormwater positive samples. The only other markers found in conjunction with *A. butzleri* were for Canada geese (i.e., CGO1) and ruminants (i.e., Rum2Bac), which were detected in 2% and 1% of positive samples, respectively.

Human fecal contamination and *A. butzleri* co-occurred in many water samples throughout this study. Amongst the Calgary urban stormwater ponds, *A. butzleri* and human fecal contamination occurred most often in McCall Lake in 51% of aggregate samples from all sampling sites analyzed for HF183. In addition, high simultaneous occurrences of the two markers (i.e., HSP60 and HF183) occurred at individual sampling sites within a stormwater pond. Within McCall Lake at ML2, *A. butzleri* and HF183 co-occurred in 78% of *A. butzleri* positive samples, which was the highest simultaneous occurrence observed of any microbial source tracking marker. In addition, ML2 also had the highest simultaneous occurrence of HumM2 and *A. butzleri*, which occurred in 50% of all *A. butzleri* positive stormwater samples. In comparison, HF183 and HumM2 were only detected in 13% and 0% of samples at Inlet ¾. In order to better understand the co-occurrence of human fecal material and *A. butzleri*, temporal patterns of the qPCR markers were analyzed. During the 21-week sampling season at ML2, there

were six sampling dates (i.e., June 1st, June 8th, August 16th, August 21st, August 28th and September 13th) when human fecal contamination and *A. butzleri* both reached quantifiable levels (Figure 5-2). On five of those dates (i.e., June 1st, June 8th, August 16th, August 21st, August 28th) the only microbial source of fecal contamination detected was human. This finding suggests that human fecal contamination may be a factor contributing to *A. butzleri* loading. In comparison, at sampling site ML1, HF183 and *A. butzleri* were only quantified together once (i.e., September 20th).

Patterns of co-occurrence were not limited to human fecal contamination, as seagull fecal contamination also occurred simultaneously with *A. butzleri*. Between the three urban stormwater ponds tested, *A. butzleri* and seagull fecal contamination occurred most often in McCall Lake (i.e., 16% of samples). The most contaminated site across all stormwater ponds examined for seagull fecal contamination and *A. butzleri* was sampling site ML1 at McCall Lake, where 40% of samples detected seagull fecal contamination and *A. butzleri*. It should be noted that ML1 was not heavily impacted by human fecal contamination, though it was the second most contaminated site, with seagull contamination. In comparison, at sampling site ML2, seagull fecal contamination was detected with *A. butzleri* in 21% of samples.

In order to better understand the co-occurrence of bird fecal material (i.e., LeeSg and CGO1) and *A. butzleri*, temporal patterns of the qPCR markers were analyzed. This analysis revealed that there were three sampling dates at ML1 when *A. butzleri* was detected in conjunction with seagull fecal contamination LeeSg (i.e., August 7th, August 14th, and September 13th) (Figure 5-3). Furthermore, on August 7th and September 13th at sampling site ML1, no human fecal contamination was detected. In addition, there was one sampling date (i.e., June 20th) when the Canada Goose marker (i.e., CGO1) was detected along with *A. butzleri*. Also on

this date there was no human fecal contamination detected at ML1. This suggests that *A. butzleri* contamination may occur in the absence of human fecal contamination, and therefore may be influenced by another source such as bird fecal contamination. In comparison, although much more heavily contaminated with human fecal contamination, sampling site ML2 had three *A. butzleri* detections occurring with seagull contamination (i.e., July 10th, August 7th, and September 13th). On all of these dates human fecal contamination was also detected at sampling site ML2.

There were also several instances when *A. butzleri* was detected in the absence of human and animal microbial source tracking markers (Figure 5-2 and Figure 5-3). For example, at sampling site ML1 on July 10th and July 12th *A. butzleri* was detected, however, on these two dates no microbial source tracking markers were detected (Figure 5-2 and Figure 5-3). This finding suggests that there could be another source of *A. butzleri* in the urban stormwater ponds, or that *A. butzleri* may be more persistent in the environment than the markers used for microbial source tracking.

Table 5-6: Co-occurrence of microbial fecal source tracking markers and molecular-methods for A. butzleri in the Calgary urban stormwater ponds.

		Percentage of fecal marker samples positive among *A. butzleri* positive samples						
Pond	Sampling Site N=number of *A. butzleri* positive samples	Human: HF183	Human: HumM2	Seagull: LeeSg	Canada Goose: CG01	Dog: Dog3	Ruminant: Rum2Bac	Muskrat: MuBac
McCall Lake	ML2 N=14	78	50	21	0	0	0	0
	PR60 N=13	38	0	0	0	0	0	0
	ML1 N=10	60	10	40	2	0	10	0
	Inlet ¾ N=8	13	0	0	0	0	0	0
	McCall Lake Total N=45	51	18	16	0	0	2	0
Country Hills	WP31A N=4	0	0	0	0	0	0	0
	WP31B N=5	0	0	0	0	0	0	0
	WP31C N=7	57	28	0	14	0	0	0
	WP31D N=7	57	0	14	0	0	0	0
	WP31E N=6	66	0	33	16	0	0	0
	Country Hills Total N=29	41	7	10	7	0	0	0
Inverness	Outfalls/Inlet N=18	27	0	0	0	0	0	0
	WP26B N=0	0	0	0	0	0	0	0
	WP26C N=2	50	0	0	0	0	0	0
	WP26D N=2	0	0	0	0	0	0	0
	Inverness Total N=22	27	0	0	0	0	0	0
Total	N=96	43	10	10	2	0	1	0

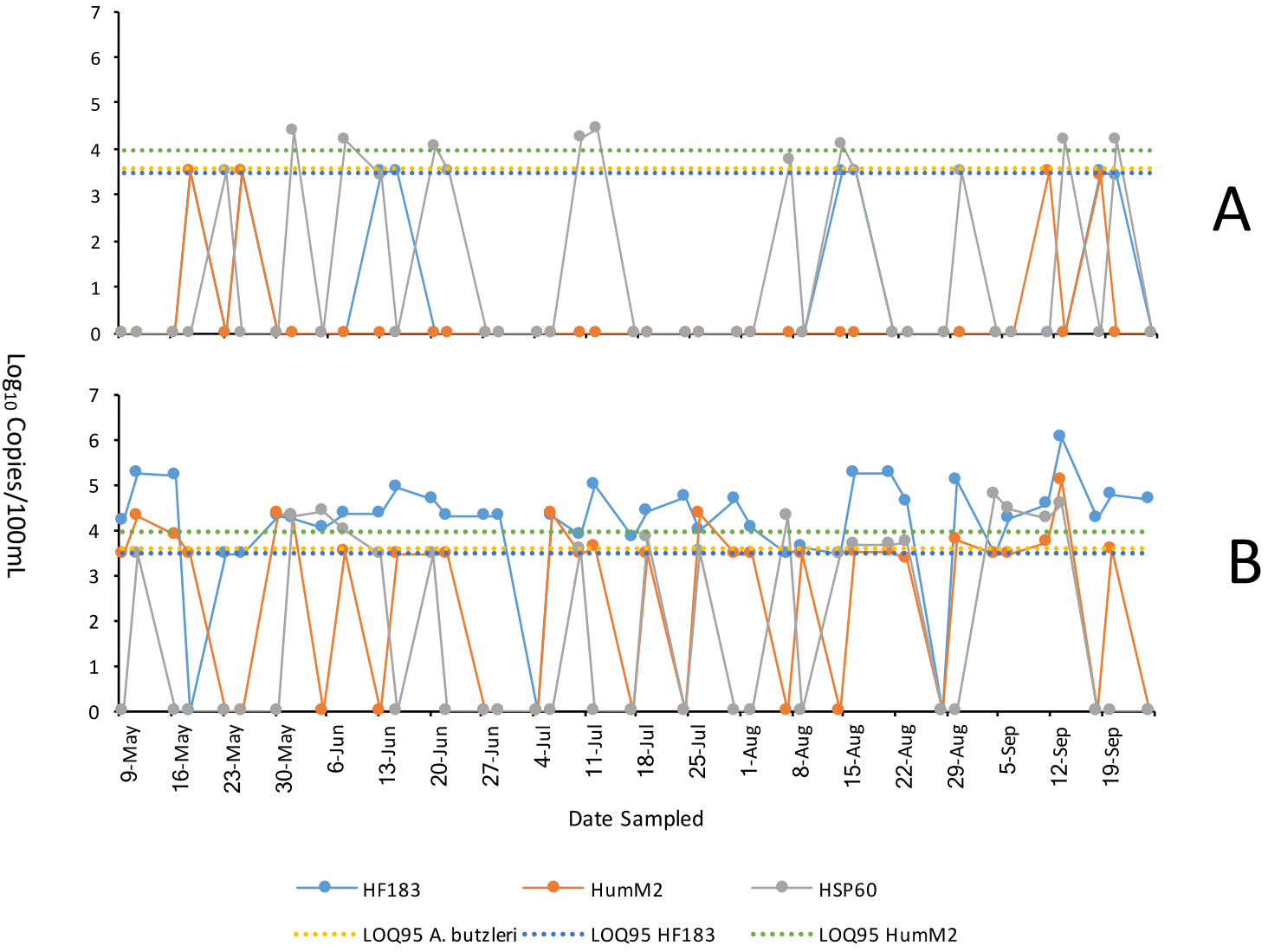

Figure 5-2: Association between A. butzleri and human microbial source tracking markers (HF183 and HumM2) at ML1 (A) and ML2 (B) sites over 21 weeks and represented on a scale of log_{10} copies/100mL. The gray line represents A. butzleri (HSP60), the blue line represents HF183, the red line represents HumM2, the yellow dotted line is the LOQ_{95} for A. butzleri, the blue dotted line is the LOQ_{95} for HF183, and the green dotted line is LOQ_{95} for HumM2.

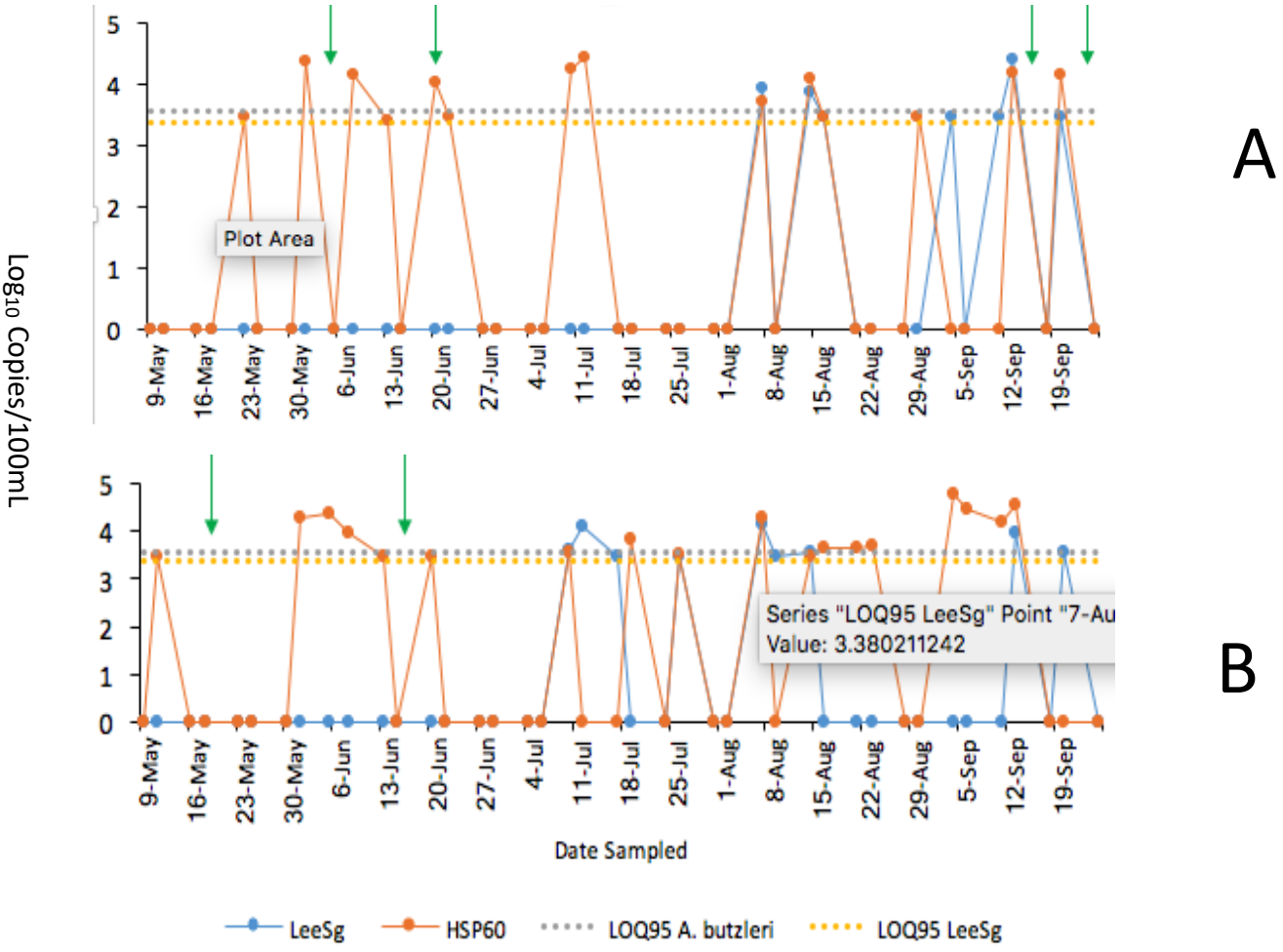

Figure 5-3: Association between A. butzleri and seagull fecal contamination (LeeSg) over 21 weeks at ML1 (A) and ML2 (B) sites and represented on a scale of log_{10} copies/100mL. The red lines represent A. butzleri (HSP60), the blue lines represent seagull marker (LeeSg), the gray dotted line is the LOQ_{95} of A. butzleri, and the yellow dotted line is LOQ_{95} for LeeSg. The green arrows represent sampling dates when the Canada goose marker (CGO1) was detected.

5.2.5 Virulence Gene Composition of *A. butzleri* isolates from McCall Lake

To determine the pathogenic potential of *A. butzleri* found in stormwater samples in McCall Lake, *A. butzleri* isolates were screened for: a) genetic variability through ERIC-PCR [a method of bacterial fingerprinting], and b) the presence of virulence genes characterized based on homologs of virulence genes found in *Campylobacter* spp. ERIC-PCR bacterial fingerprints were analyzed for similar DNA banding patterns by comparing all 85 *A. butzleri* stormwater isolates against each other (Figure 5-4). Genetic similarity was further assessed through the corresponding capillary electropherograms where peaks in relative fluorescence units and size were assessed against each of the *A. butzleri* stormwater positive isolates (Figure 5-5). Genetic similarity was based on visual assessment when banding patterns differed by more than two bands. For example, in Figures 5-4 and 5-5, two *A. butzleri* isolates (i.e., isolates D1 and D2) from the same water sample were deemed to be genetically similar based on their bacterial fingerprint and electropherogram, while a third isolate from the same water sample was deemed genetically distinct based on its bacterial fingerprint and electropherogram (i.e., isolate C12) (Figure 5-4, Figure 5-5).These analyses from ERIC – PCR reflected that only 12 *A. butzleri* isolates were genetically similar to others within the original collection of 85 isolated. Thus, a considerable number of genetically diverse *A. butzleri* stormwater isolates (73 in total) were collected from the McCall Lake stormwater pond alone.

All 73 genetically distinct isolates were screened for the virulence genes *ciaB* and *cadF* initially. One putative virulence marker, *ciaB,* was found in 100% (i.e., 73) of genetically distinct *A. butzleri* stormwater isolates. In addition, *cadF* was detected in 91% of genetically distinct *A. butzleri* stormwater isolates. Since all genetically distinct isolates reflected the presence of *cadF* or *ciaB*, further screening was initiated on an additional seven putative virulence genes (i.e.,

mviN, pldA, tlyA, irgA, hecA, hecB, and *cj1349*) on all 73 isolates. Three out-of-seven of these virulence genes (i.e., *cj1349, tlyA, pldA*) tested positive in 90% or more of the McCall Lake *A. butzleri* isolates (Figure 5-4). Finally, the frequency of occurrence of all virulence genes tested was at least 50%. Not only was a number of genetically distinct isolates collected from McCall Lake, many of the isolates were characterized as having multiple virulence genes (Figure 5-6). Importantly, 21 of the 73 *A. butzleri* isolates contained all 9 virulence genes, suggesting that these strains are likely pathogenic in humans.

A high-level descriptive overview of each of the isolates was performed, in order to see if there was a relationship between the presence of virulence markers and positive detections of the dominant sources of microbial fecal pollution (i.e., HF183, HumM2, and LeeSg) in those water samples. A number of noteworthy observations were revealed in this analysis.

There was little spatial variability with respect to the frequency of virulence marker detections between sampling sites at McCall Lake (i.e., ML2, ML1, PR60, and Inlet ¾). This result was true regardless of the virulence marker (i.e., *cadF, ciaB, cj1349, hecA, hecB, mniV, irgA, tlyA*, and *pldA* (Figure 5-7, Figure 5-8). However, notably, *cadF* was positive for 100% of samples at ML1 (Figure 5-8); and *pldA* was positive for 100% of samples at ML1 and PR60 (Figure 5-7, Figure 5-8).

There did not appear to be a consistent relationship between fecal source (i.e., human or seagull) and virulence markers. The median number of virulence genes associated with *A. butzleri* isolates obtained from water samples where the human fecal marker (i.e., HF183) was observed was 6.5. The median number of virulence genes associated with *A. butzleri* isolates obtained from water samples where the seagull marker (i.e., LeeSg) was observed was 8. Water samples taken at ML2 on August 22nd were positive for both microbial source tracking markers

for human fecal contamination (i.e., HF183 and HumM2), however *ciaB* was the only virulence marker that was found in every genetically unique isolate on that date. Further, one sample from ML2 on August 22nd contained two other virulence markers (i.e., *cadF* and *mniV*). In addition, a series of samples taken from ML1 on September 12th, in which a seagull signature (i.e., *LeeSg*) had been detected, tested positive for 7/9 of the virulence markers (i.e., *hecA, irgA, ciaB, cadF, cj1349, tlvA*, and *pldA*) (Figure 5-7, Figure 5-8). With that said, it needs to be noted that in many of the water samples in which potentially pathogenic *A. butzleri* were isolated, there was no corresponding microbial source tracking marker observed (i.e., human, dog, ruminant, seagull or Canada goose) (Figure 5-7, Figure 5-8), raising the possibility that: a) other fecal sources of pollution may be contributing to stormwater contamination and the presence of *A. butzleri*, and/or b) that environmental sources of *A. butzleri* may exist, as reported by others (Van Driessche & Houf, 2008; Wesley, *et al*., 2000; Van Driessche, *et al*., 2005).

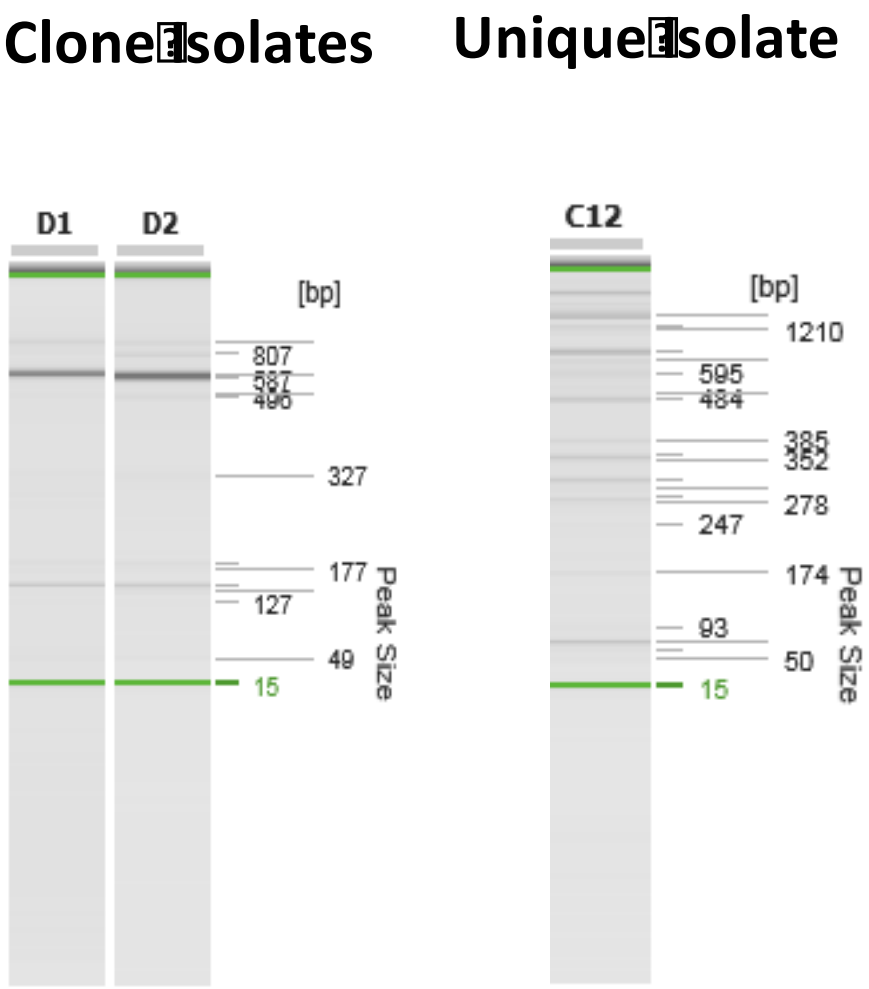

Figure 5-4: Comparison of ERIC-PCR gel images of samples taken from Inlet 3/4 at McCall Lake on August 8th. The two samples on the left (i.e., D1 and D2) were determined to be genetically similar, while the sample on the right was determined to be genetically unique (i.e., C12).

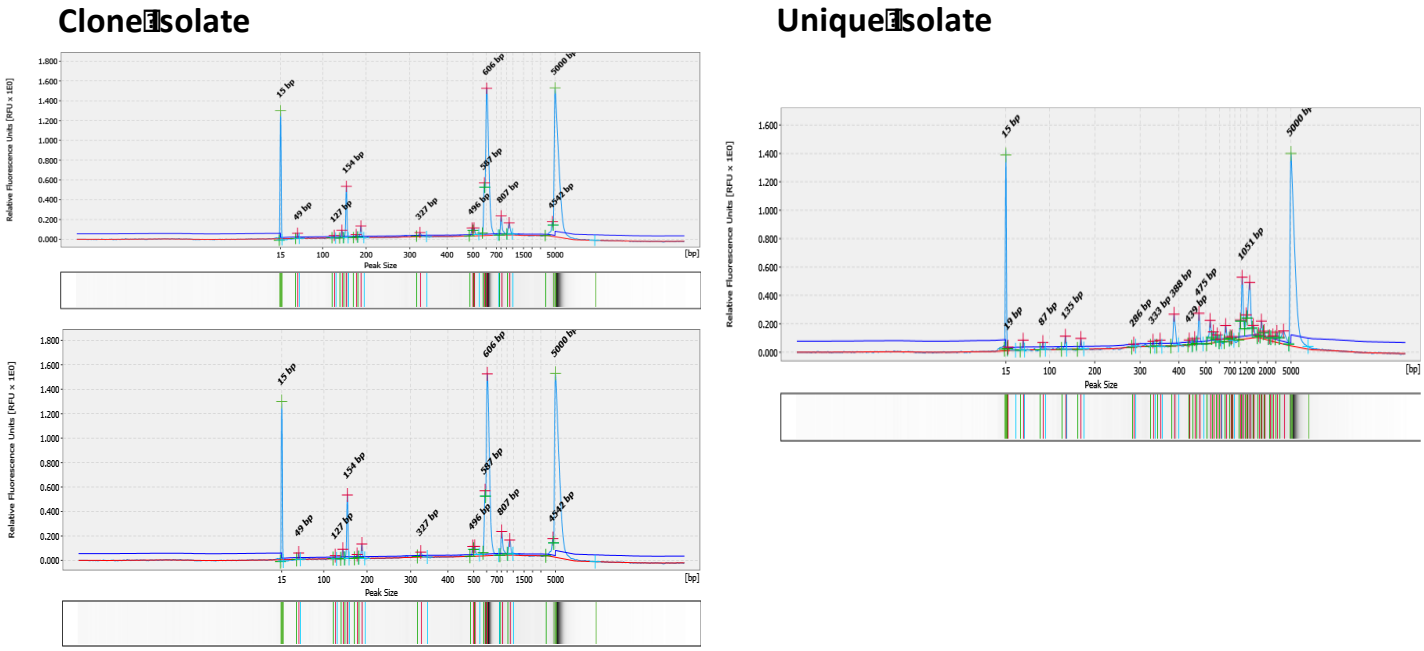

Figure 5-5: Comparison of ERIC-PCR electropherograms of two isolates determined to be clones (top, D1 and D2) and a unique isolate (bottom, C12). The peaks at 15 bp and 5000 bp are from size markers. Comparisons were made by looking at the peaks in the isolates. All samples were taken from Inlet 3/4 at McCall Lake on August 8th.

Table 5-7: Frequency of occurrence based on the percent of putative virulence markers positive in A. butzleri isolates from McCall Lake. A total of 73 unique isolates were tested for each virulence marker.

Virulence Markers	Percent of *A. butzleri* isolates possessing the virulence gene (N=73)
cadF	91
ciaB	100
cj1349	93
hecA	75
hecB	57
mniV	89
irgA	64
tlyA	90
pldA	90

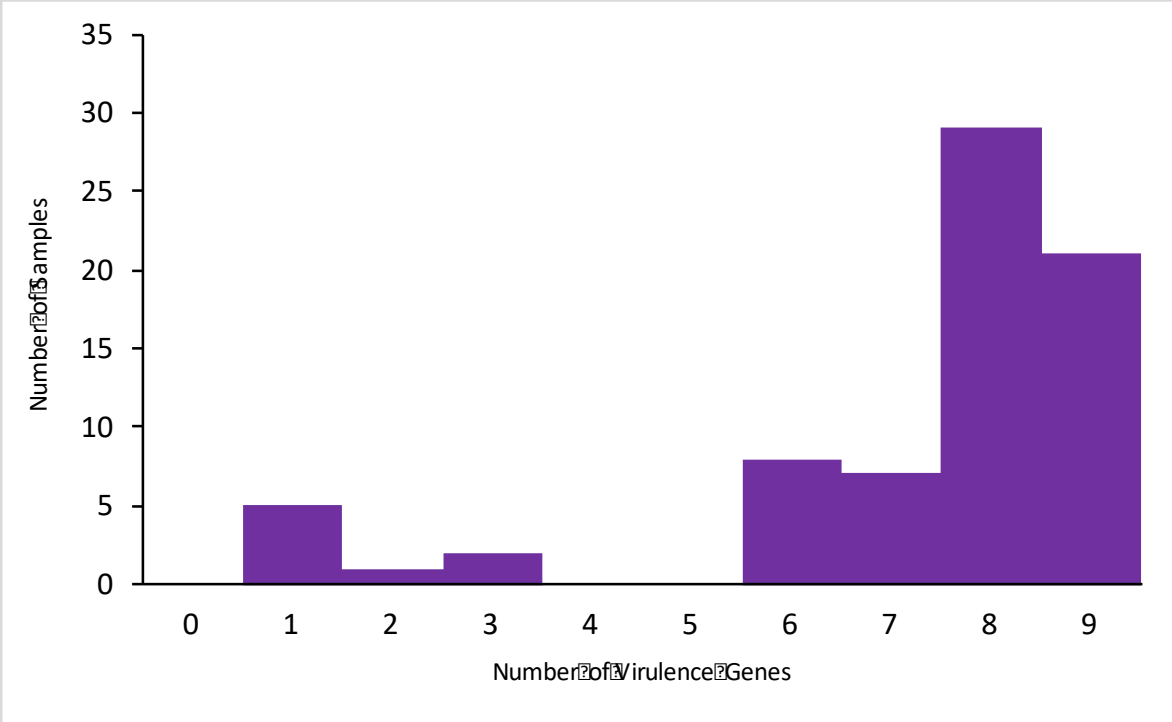

Figure 5-6: Histogram representing the number of virulence genes carried by genotypically-distinct A. butzleri isolates collected from stormwater (total n=73).

		Microbial Source Tracking			Virulence Genes								
Isolate ID	Sampling date	HF183	HumM2	LeeSg	HecA	IrgA	CiaB	CadF	Cj1349	mviN	HecB	TlvA	PldA
1	24-Aug												
2	24-Aug												
3	24-Aug												
4	24-Aug												
5	29-Aug												
6	31-Aug												
7	31-Aug												
8	18-Sep												
9	26-Sep												
10	26-Sep												
11	26-Sep												
12	26-Sep												

Inlet 3/4

Isolate ID	Sampling date	HF183	HumM2	LeeSg	HecA	IrgA	CiaB	CadF	Cj1349	mviN	HecB	TlvA	PldA
13	22-Aug												
14	22-Aug												
15	22-Aug												
16	29-Aug												
17	29-Aug												
18	29-Aug												
19	29-Aug												
20	29-Aug												
21	29-Aug												
22	5-Sep												
23	5-Sep												
24	12-Sep												
25	12-Sep												
26	12-Sep												

Inlet PR60

Isolate ID	Sampling date	HF183	HumM2	LeeSg	HecA	IrgA	CiaB	CadF	Cj1349	mviN	HecB	TlvA	PldA
58	22-Aug												
59	22-Aug												
60	22-Aug												
61	22-Aug												
62	22-Aug												
63	22-Aug												
64	5-Sep												
65	5-Sep												
66	5-Sep												
67	12-Sep												
68	12-Sep												
69	12-Sep												
70	12-Sep												
71	26-Sep												
72	26-Sep												
73	26-Sep												

Outfall ML2

Figure 5-7: Association between sampling location, date, select microbial source tracking markers (i.e., HF183, HumM2, LeeSg,) and virulence genes (i.e., cadF, ciaB, cj1349, hecA, hecB, mniV, irgA, tlyA, pldA) in A. butzleri isolates collected from representative stormwater samples (by date) at Inlet ¾ (top panel), Inlet PR60 (middle panel) and the ML2 Outfall (bottom panel) at McCall Lake. Targets that were not detected (microbial source tracking marker or virulence genes) are represented by white boxes, whereas detectable levels of the microbial source tracking markers are shown as blue boxes, and the presence of the virulence genes shown with yellow boxes.

		Microbial Source Tracking			Virulence Genes								
Isolate ID	Sampling date	HF183	HumM2	LeeSg	HecA	IrgA	CiaB	CadF	Cj1349	mviN	HecB	TlvA	PldA
27	24-Aug												
28	24-Aug												
29	24-Aug												
30	31-Aug												
31	31-Aug												
32	31-Aug												
33	31-Aug												
34	31-Aug												
35	31-Aug												
36	31-Aug												
37	31-Aug												
38	31-Aug												
39	31-Aug												
40	31-Aug												
41	31-Aug												
42	31-Aug												
43	12-Sep												
44	12-Sep												
45	12-Sep												
46	12-Sep												
47	12-Sep												
48	12-Sep												
49	12-Sep												
50	19-Sep												
51	19-Sep												
52	19-Sep												
53	19-Sep												
54	19-Sep												
55	26-Sep												
56	26-Sep												
57	26-Sep												

Figure 5-8: Association between sampling location, date, select microbial source tracking markers (i.e., HF183, HumM2, and LeeSg,) and virulence genes (i.e., cadF, ciaB, cj1349, hecA, hecB, mniV, irgA, tlyA, and pldA) in A. butzleri isolates collected from the representative stormwater samples (by date) at outfall ML1 in McCall Lake. Targets that were not detected (microbial source tracking marker or virulence genes) are represented by white boxes, whereas detectable levels of the microbial source tracking markers are shown as blue boxes, and the presence of the virulence genes shown with yellow boxes.

5.3 Discussion

The occurrence of waterborne enteric bacterial pathogens was investigated due to the often poor water quality and sources of fecal contamination in urban stormwater ponds and stormwater effluents in southern Alberta (i.e., Chapters 3 and 4). Fecal pollution from domestic animals (e.g., dogs), wild animals, birds (e.g., seagulls, Canada geese), as well as humans have the potential to carry zoonotic enteric bacterial pathogens. *Campylobacter* spp., *Salmonella* spp., *Arcobacter butzleri* and shiga-toxin producing *E. coli* (STEC) can enter stormwater through a variety of ways, including runoff due to increased rainfall, open defecation by the host, or failures in a drainage system (Tilburg, *et al*., 2015; Edge, *et al*., 2013). Therefore, this portion of the thesis research project set out to identify the presence of enteric bacterial pathogens in the Calgary urban stormwater ponds and the stormwater-impacted rivers in southern Alberta, Canada.

In our study, *Salmonella* spp. were detected in only 1% of samples from the Calgary urban stormwater ponds by molecular-based methods. In contrast to our study, Sidhu *et al*. (2012) detected *Salmonella* spp. in seven of 22 samples (i.e., 31.8%) tested from stormwater runoff in Brisbane, Australia through molecular-based methods. Steele *et al*. (2018) also used molecular-based methods to detect *Salmonella* spp. at a southern California beach, USA, however, they detected it in 25% of samples. Bradshaw *et al*. (2016) detected *Salmonella* spp. in 76% of samples from a stormwater-impacted watershed from the South Fork Broad River in Georgia, USA, through the use of an enrichment technique followed by qPCR against the *invA* gene. Ahmed *et al*. (2018) detected *Salmonella* spp. targeting the *invA* gene through microfluidic qPCR in storm drain outfalls in Tampa Bay, Florida, USA.

Many studies utilize culture-based methods for pathogen detection. Staley *et al*. (2012) found that *Salmonella* spp. was detected the least frequently of all pathogens tested in their study (i.e., *Salmonella* spp., *Cryptosporidium*, and *Giardia*), and for which 10% of their samples were positive from a stormwater-impacted freshwater lake located in Florida, USA, through culture-based methods. Jokinen *et al*. (2011) isolated *Salmonella* spp. from 29/342 water samples from the Oldman River watershed in Alberta, Canada, by culture-based methods. Other studies however have found a higher prevalence of *Salmonella* spp. Meinersmann *et al*. (2008) detected *Salmonella* spp. in 75% of water samples after a synoptic event from the stormwater-impacted Oconee River in Georgia, USA, by isolating *Salmonella* spp.

In our study, 8% of water samples from the Calgary urban stormwater ponds were contaminated with STEC and in samples that were taken directly from the storm drains in Airdrie, Canada, 23% of samples were positive for STEC. To the best of our knowledge, there is a scientific gap regarding the occurrence of STEC in stormwater-impacted bodies of water. There are only a few comparable studies to the one presented here. Bradshaw *et al*. (2016) detected STEC in 61% of water samples from a stormwater-impacted river in Georgia, USA, by enriching samples and then utilizing a qPCR target against the stx_2 gene. Other studies have demonstrated STEC to occur at higher rates (i.e., 9-35%) using culture-based methods in water types such as surface water (Nadya, *et al*., 2016; Johnson *et al*., 2014). In contrast, in the Oldman River watershed in Alberta, Canada, Jokinen *et al*. (2011) isolated *E. coli* O157:H7 in only 8/342 surface water samples through culture-based methods. McCarthy *et al*. (2001) identified STEC in a storm drain that emptied into a recreational body of water in Connecticut, USA, through culture-based methods.

There is limited research on detecting *Campylobacter* spp. in stormwater ponds through culture-based methods; however, studies on other types of water have been successful at detecting *Campylobacter* spp., albeit at varying levels through culture-based methods. In our study, *Campylobacter* spp. was detected in 0% of samples from the Calgary urban stormwater ponds based on our culture-based MPN-qPCR method. Meinersmann *et al*. (2008) isolated *Campylobacter* spp. from 12 of 83 sampling sites along the stormwater-impacted Oconee River in Georgia, USA, utilizing culture-based methods followed by multiplex PCR colony conformation. Bradshaw *et al*. (2016) utilized the same methods as Meinersmann, and detected *Campylobacter* spp. in 33% of water samples from the stormwater-impacted South Fork Broad River in Georgia, USA. In the Oldman River watershed located in Alberta, Canada, Jokinen *et al*. (2011) isolated *Campylobacter* spp. from 91/342 surface water samples by culture-based methods. When Murphy *et al*. (2017) studied sampling sites for the Australian Guidelines for Water Reuse (2009), the maximum concentration of *Campylobacter* spp. observed was 15 MPN/L. Rechenburg and Kistemann (2009) documented the concentrations of *Campylobacter* spp. to be greater than 1000 CFU/100mL in 31.5% of samples, with *Campylobacter* spp. being detected in 86% of samples using a semi-quantitative enrichment method. Moreover, they found that in raw sewage, the levels of *Campylobacter* spp. could range from 10 – $>10^6$ *Campylobacter* spp./100mL (Rechenburg & Kistemann, 2009). Henry *et al*. (2015) found concentrations as high as 65 MPN/L at an urban stormwater site through the use of an MPN-PCR, method with primers developed by Uyttendaele *et al*. (1994), in a river in Germany, which was influenced by combined sewer overflows (CSOs). Khan *et al*. (2013) detected levels of *Campylobacter* spp. to be as high as 10^5 MPN/100mL for samples taken near a wastewater discharge location through

isolation and subsequent verification through PCR amplification using the primers of Linton *et al.* (1996).

When examining occurrence of *Campylobacter* spp. by direct molecular methods, only 4% of samples were positive by qPCR, with levels ranging from not detected to 4.3 $\log_{10}$ copies/100mL, when using the primers of Van Dyke *et al*. (2010). Steele *et al*. (2018) utilized molecular-based methods and detected *Campylobacter* spp. in 100% of samples from a southern California beach, USA, using primers developed by LaGier *et al*. (2004) that detect *C. coli*, *C. lari*, and *C. jejuni* through a multiplex PCR assay. In addition, a study on stormwater samples in Brisbane, Australia, detected *Campylobacter* spp. in all samples tested from multiple sampling sites by molecular-based method using primers developed by Lund *et al*. (2004) (Sidhu, *et al*., 2012).

The large discrepancy between the rates of occurrences and levels of *Campylobacter* spp. among various studies could be due to a variety of reasons, however, the most prominent reason may relate to the specificity (i.e., cross-reactivity) of gene targets used in the molecular methods. Currently, there are a variety of PCR targets available to detect *Campylobacter* spp.; however, it has been established that detection rates can vary depending on which target and culture-method are utilized (Banting *et al*., 2016). Banting *et al*. (2016) found that in irrigation water samples the detections rates were: 0% using the de Boer Lv1-16s qPCR assay (de Boer, *et al*., 2013), 2.5% using the Van Dyke primers (Van Dyke, *et al*., 2010) and Jensen *glyA* qPCR assays (Jenson, *et al*., 2005), and 75% by the Linton 16S endpoint primers (Linton, *et al*., 1996). They further evaluated these primers, and found that the Linton 16S endpoint primers (Linton *et al*., 1996) cross react with *Arcobacter* spp., which may contribute to false positive results in some studies,

with the Van Dyke primers (Van Dyke *et al*., 2010) proving to be the most sensitive and specific of all primers tested (Banting *et al*., 2016).

Another potential variable leading to higher detections of *Campylobacter* spp., could include environmental variables such as rainfall and temperature. Research by Rechenburg and Kistemann (2009) found that rain events caused an increase in the median levels of *Campylobacter* spp. when using a semi-quantitative enrichment method, and believed that this outcome was due to runoff. Additionally, two outbreaks of *Campylobacter* spp. have been associated with heavy rain storm events (Clark *et al*., 2003). However, Meng *et al*. (2018) identified *Campylobacter* spp. through an MPN-qPCR method in stormwater wetlands in Australia and found the concentrations of *Campylobacter* spp. to be similar regardless of wet or dry weather. Wilkes *et al*. (2011) suggested that season and temperature could have an effect on *Campylobacter* spp. detections. Most cases of *Campylobacter* spp., infection occurs in the summer (Patrick, et al., 2004), yet *Campylobacter* spp. can be found in higher concentrations in the winter in water, which may be due to decreased daylight, leading to decreased levels of UV light rays and lower temperatures (Jones, 2001). However, this study may only be relevant at certain latitudes, where temperature declines but precipitation persists as rain instead of snow. Wilkes *et al*. (2011) undertook a five-year study, using bacterial subtyping, of the river basin eastern in Ontario, Canada, and found the ratio of detects to non-detects to be 1.1 and 0.24 when the temperature was above and below 8$^{\circ C}$, respectively. Furthermore, they found that the presence of more than two pathogens was more likely to occur when temperature of the water was less than 14$^{\circ C}$ (Wilkes, et al., 2011). Bradshaw *et al*. (2016) had similar findings for *Campylobacter* spp., in that higher detections were observed in colder weather through molecular-based methods.

To the best of our knowledge, this is the first report on the occurrence of *A. butzleri* in stormwater ponds. *A. butzleri* was the most common pathogen detected in the present study. Based on direct molecular testing, *A. butzleri* was detected in 25% of all water samples. However, utilizing an integrated cell culture molecular method (i.e., MPN-qPCR assay utilizing *hsp60* as our assay target) as many as 75% of stormwater samples were shown to be positive for *A. butzleri*. We detected *A. butzleri* on all sampling dates at site ML2 (i.e., 8/8 times) in comparison to the other McCall Lake sampling sites in which *A. butzleri* was detected 6/8 times. Currently, there is limited knowledge on *A. butzleri* in stormwater. However it has been detected in many different types of water, ranging from rivers and wells (Wesley, *et al*., 2000; Fong, *et al*., 2007; Van Driessche & Houf, 2008) to saltwater lakes and coastal seawater (Wesley, *et al*., 2000; Fong, *et al*., 2007; Van Driessche & Houf, 2008), and even drinking water reservoirs (Wesley, *et al*., 2000; Van Driessche & Houf, 2008). Banting *et al*. (2016) detected *A. butzleri* in 54% of irrigation water samples in Alberta, Canada, through an MPN-qPCR assay, using *hsp*60 as their target. In addition, Webb *et al*. (2016a) found that raw sewage had the highest density of *A. butzleri* in two wastewater treatment facilities in southwestern Alberta, Canada. Collado *et al*. (2010) tested 12 sampling sites along the Llobregat River in Catalonia, Spain; and at nine of the sampling sites, *Arcobacter* spp. was detected in 100% of samples, and at one site it was not detected in any of the samples. Collado *et al*. (2008), in their study of a fecally-contaminated freshwater stream, found the highest amount of *Arcobacter* spp. (i.e., 3.7×10^5 MPN/100mL) to be at the sampling location closest to the wastewater treatment.

At individual sampling sites, concentrations of pathogens in stormwater samples occurred over a narrow range of values through culture-based methods. In our study, *A. butzleri* measured through culture-based methods at ML2 ranged from 0.9-93 MPN/300mL. As previously

mentioned, there is limited research on the *Arcobacter spp*. detections through culture-based methods in stormwater; however, prior studies have found culturable levels of *A. butzleri* to be as high as 10^5 MPN/100mL in raw sewage (Banting *et al*., 2016), which is considerably higher than the levels detected in our study. The finding of *Arcobacter* spp. in sewage or water impacted by raw sewage is not uncommon (McLellan, *et al*., 2010; Collado, *et al*., 2008; Khan, *et al*., 2009). Collado *et al*. (2008), in their study in Spain, found the presence of *A. butzleri* in 58% of river water samples and in 100% of sewage samples. In addition, Merga *et al*. (2014) detected *A. butzleri* in 100% of domestic sewage samples in the United Kingdom. Furthermore, Collado *et al*. (2008, 2010) demonstrated that *A. butzleri* in urban sewage can survive treatment and therefore has the potential to be released into environmental bodies of water.

When direct molecular methods were used, *A. butzleri* was detected at levels as high as 4.7 $\log_{10}$ genome copies/100mL at sampling site ML2. Webb *et al*. (2016a) found the density of *Arcobacter* spp. to range from $10^{1.5}$ to 10^4 $\log_{10}$ genome copies mL^{-1} in treated sewage by molecular-based methods with primers developed by Webb *et al*. (2016b). Lee *et al*. (2012) found the levels of *A. butzleri* to range from 1 x $10^{2.7}$ to 1 x 10^5 gene copies/100mL at one Lake Erie beach over a 3-month period (i.e., July – August) through molecular-based methods using ArcoI and ArcoII primers developed by Bastyns *et al*. (1995). The occurrence of such ranges of enteric bacterial pathogens at individual sampling sites can pose a unique challenge to the development of a stormwater treatment facility and sampling plan.

Understanding the spatial differences at stormwater sampling sites could allow us to better determine which sampling sites would be best suited for stormwater reuse applications. Our study revealed that some urban stormwater ponds have more consistent pathogen detections than other stormwater ponds: McCall Lake had the most *A. butzleri* detections, in comparison to

Inverness Stormpond and Country Hills Stormwater Facility. Talay *et al*. (2016) tested 115 different water samples (i.e., sewage, rivers, spring water, and drinking water) by molecular-based methods from Izmir Turkey. They found that the prevalence of *Arcobacter* spp. was highest in river water (i.e., 52% of samples), and that all drinking water samples were negative (Talay, *et al*., 2016). Webb *et al*. (2016b) in their testing of two different wastewater treatment plants in southwestern Alberta, Canada, found higher densities of *A. butzleri* in the Lethbridge wastewater treatment plant than in the Fort Macleod treatment plant through molecular-based methods.

In addition, the data in our study revealed that a specific sampling site within a stormwater pond may experience more consistent *A. butzleri* contamination than other sampling sites. Our results demonstrated that one of the sampling sites in Inverness Stormpond (known as Outfalls/Inlet) had the most detections of *A. butzleri* of any sampling site in the Calgary urban stormwater ponds. At the same time, this site infrequently failed microbial water quality standards (i.e., only 2% of samples failed the current fecal coliform guidelines [Chapter 3]) and for which the human and seagull markers of pollution were also infrequently observed (i.e., only 12 % and 5% of samples contained HF183 and LeeSg markers, respectively). This data demonstrates that pathogen occurrence may not always correlate with poor microbial water quality or associate with certain markers of fecal contamination, a theme for discussion in Chapter 6 of this thesis.

However, the variation in *A. butzleri* is not limited to spatial differences as the prevalence of *A. butzleri* in the Calgary urban stormwater pond samples was 25% based on molecular methods and 75% based on culture-methods, utilizing *hsp60* as our target. This led us to believe that our molecular-methods for *A. butzleri* were relatively insensitive compared to our culture-

based methods, with molecular methods underestimating pathogen prevalence, which may be due to the amount of water used during each protocol (i.e., 300 mL used in culture methods compared to 20 mL in molecular methods).

However, differences in methods may not be only variable affecting *A. butzleri* occurrences and concentrations. A study by Fera *et al*. (2010) indicated that *Arcobacter* spp. may survive better at lower temperatures. Lee *et al*. (2012) found that *Arcobacter* spp. detections in recreational water was higher in September at Lake Erie in North America, and suggested that the levels show a negative correlation with the temperature of the water through molecular methods. Conversely, Webb *et al*. (2016b) reported lower densities of *A. butzleri* in December and March from samples from wastewater treatment plants located in southwestern Alberta, Canada, by molecular-based methods. However, some studies did not find a seasonal effect on enteric pathogens (Rechenburg & Kistemann, 2009). That said, environmental variables are not the only factor influencing enteric bacterial pathogens in stormwater-impacted bodies of water.

Studies have found an association between microbial source tracking markers for human fecal contamination and enteric bacterial pathogens (Sidhu, *et al*., 2012). In our study, at sampling site ML2, the microbial source tracking marker for human fecal pollution (i.e., HF183) was detected in 43% of samples in which *A. butzleri* was also detected, thereby implying that humans may be a potential source of *A. butzleri*, along with other enteric bacterial pathogens. In prior studies, *Arcobacter* spp. has been detected with human microbial source tracking markers or in human sewage (Lee, *et al*., 2012; Levican, *et al*., 2013; McLellan, *et al*., 2010; Fisher, *et al*., 2014). A study ensued in the United Kingdom of nine wastewater treatment facilities, where *Arcobacter* spp. was isolated from raw sewage samples (Merga, *et al*., 2014). Still, even following treatment, *Arcobacter* spp. has been detected in wastewater effluent, as Rodriguez-

Mazano *et al*. (2012) found the enteric bacterial pathogen at the tertiary reclaimed water section of a wastewater treatment plant, which could mean that *Arcobacter* spp. may have been discharged from the wastewater treatment plant. In their study of four Lake Erie beaches in North America, Lee *et al*. (2012) found that *Arcobacter* spp. levels correlated significantly with the human fecal contamination marker Hubac at beaches that were also highly contaminated with the enteric bacterial pathogen when using molecular methods. Furthermore, sewage-contaminated drinking water has been associated with outbreaks of *Arcobacter* spp. around the world, as in Switzerland, Uganda, and the United States (Levican, *et al*., 2013; Hafliger, Hubner, & Luthy, 2000; Craun, *et al*., 2005. The studies above suggest that human contamination may be a source of the pathogen.

Arcobacter spp. has been detected from human clinical fecal samples. Using molecular-based methods, carriage rates in a South African hospital were 13%, though only 3% in healthy children (Samie, *et al*., 2007). Further, in the Webb *et al*. (2016b) study of southwestern Alberta, Canada, which also used molecular-based methods, carriage rates were found to be 45.5% in healthy individuals, with the higher densities of *A. butzleri* in the individuals with diarrhea at 56.7%. Besides human fecal waste, animal fecal waste has the potential for being a source of enteric bacterial pathogens.

Birds (i.e., seagulls) were the second most dominant source of fecal pollution in our study, and the seagull marker (i.e., LeeSg) for fecal pollution was detected in combination with *A. butzleri* in 40% of samples at ML1. Wesley and Baetz (1999) carried out a study in which they were able to recover *Arcobacter* spp. from 15% of birds tested. Furthermore, poultry is a common source of *Arcobacter* spp. (Karadas, *et al*., 2013; Wesley & Baetz, 1999). Adjesiji *et al*. (2011) were able to isolate *Arcobacter* spp. in 1.3% of chicken feces from backyard flocks in

Nigeria. Notably, in a review article of pathogens associated with Canada geese feces, Gorham and Lee (2016) stated that Canada geese could be considered potentially carriers of *Arcobacter* spp.

Other animal sources of *Arcobacter* spp. include domestic animals, as dogs and pet reptiles (i.e., snakes and lizards) (Houf, *et al*., 2002; Gilbert, *et al*., 2014) as well as cattle. In our study, no water samples tested positive for co-occurrence of *A. butzleri* and dogs feces (based on the Dog3 marker). Patyal *et al*. (2011) did not detect *Arcobacter* spp. in any dog fecal samples when testing isolates by PCR, though Houf *et al*. (2008) were able to isolate *Arcobacter* spp. from seven different dogs out of 267 samples. In a review of the pathogenesis of *Arcobacter* spp., Ferreira *et al*. (2015) stated that the detections of *A. butzleri* in domestic cats and dogs may indicate that these animals could transmit the pathogen to humans.

In our study, *Arcobacter* spp. and the microbial source tracking marker for ruminant fecal contamination (i.e., Rum2Bac) occurred together in 10% of samples. Prior studies have found that cattle may serve as a potential reservoir for *Arcobacter* spp. In a study conducted by Wesley *et al*. (2000), they isolated *Arcobacter* spp. from 14.3% of feces in the dairy cattle tested. Van Driessche *et al*. (2005) found the prevalence of *Arcobacter* spp. in samples tested to range from 7.5% to 15% on dairy farms in Belgium and, more specifically, the occurrence of the enteric pathogen in dairy cattle ranged from 5.9% to 11% of samples. The highest prevalence of *Arcobacter* spp. was found in young cattle and calves, at 18.9% and 27.3% of samples, respectively (Van Driessche, *et al*., 2005). Although these studies highlight carriage rates in cattle, *Arcobacter* spp. is commonly found in other ruminants (e.g., deer) (Khoshbakht et al., 2015). The presence of wildlife, such as deer, in the urban environment in the northern climes

might explain the relationship between the occurrence of the ruminant marker Rum2Bac in the Calgary stormponds and occurrence of *A. butzleri* in a proportion of our samples.

Another potential source of *Arcobacter* spp. may be the environment itself, as we detected *A. butzleri* in the absence of microbial source tracking markers. *Arcobacter* spp. possesses several qualities that help it survive in the environment. This enteric pathogen has the ability to survive at lower temperatures than *Campylobacter* spp. (i.e., at 15-30°C); and to grow in the presence of oxygen (Wesley, *et al*., 2000; Van Driessche & Houf, 2008). Previous studies have found that warmer climates make *Arcobacter* spp. transmission easier (Wesley, *et al*., 2000). Furthermore, *Arcobacter* spp. has also been found to attach to pipe distribution systems, which then could lead to the potential spread of the bacteria (Assanta, *et al*., 2002). Banting *et al*. (2016) has suggested that due to higher frequency and concentration of *Arcobacter* spp. in irrigation water, the pathogen may have greater ability to survive and grow in the environment than *Campylobacter* spp. Furthermore, *Arcobacter* spp. is found in higher levels in wastewater than in human feces and therefore may have the ability to replicate in wastewater treatment plants or sewer systems (Shanks *et al*., 2013; McLellan *et al*., 2010). In addition, the molecular marker for *A. butzleri* may be more stable in the environment than the microbial source tracking markers.

Our study suggested that irrespective of the source of *A. butzleri,* the isolates observed in the stormwater appear to be potentially pathogenic. Currently, the pathogenicity and virulence of *Arcobacter* spp. are not yet fully understood and are still being explored (Banting & Figueras Salvat, 2017; Levican, *et al*., 2013). For our study, we tested the *A. butzleri* isolates from McCall Lake for nine putative virulence genes that have been previously reported in clinical isolates associated with clinical outbreak of *A. butzleri* (i.e., *ciaB, cadF, mviN, pldA, tlyA, irgA, hecA,*

hecB, and *cj1349*) (Levican, *et al*., 2013). In our study, *cadF* was positive in 91% of samples, and *ciaB* was positive in 100% of samples. Our results were similar to the findings in prior studies, in which 100% of *A. butzleri* samples were positive for *cadF* and *ciaB* in clinical samples (Levican, *et al*., 2013). Ferreira *et al*. (2015) found *cadF* and *ciaB* to be positive in 100% of samples they tested from human and non-human sources (i.e., slaughterhouse surface, poultry carcass, and poultry caecum). In *C. jejuni,* the role of *cadF* is adhesion to fibronectin; whereas ciaB functions as an invasion protein (Levican, *et al*., 2013; Ferreira, *et al*., 2015). Furthermore, *A. butzleri* is known to show the highest prevalence of virulence genes of all *Arcobacter* spp. (Levican, *et al*., 2016; Levican, *et al*., 2013; Douidah, *et al*., 2012; Karadas, *et al*., 2013). The seven other virulence genes tested in our study were: *mviN, pldA, tlyA, irgA, hecA, hecB*, and *cj1349*. Similar to *cadF* and *ciaB*, these genes were chosen since they are homologs to *Campylobacter* spp. In our study, *cj1349* was positive in 91% of samples tested; *mniV* in 89%; *pldA* in 90%; *tlyA* in 90%; *hecA* in 75%; and *hecB* in 57% of samples. In the Levican *et al*. (2013) study, the *cj1349* virulence gene was positive in 91% of samples tested, including the samples from human sewage; however, those sewage samples were negative for *irgA* and *hecA*. Levican *et al*. (2013) also found that strains from animal and human fecal sources were significantly more invasive than strains from seawater and piggery effluent. Previous studies have found that strains of a fecal origin possess more virulence genes than those from other origins, as in food (Levican, *et al*., 2013). Most significantly, *irgA* was far more common in sewage strains than in food strains, at 54.5% compared to 8% (Levican, *et al*., 2013). Other studies revealed detection rates of *hecB* ranging from 0-80%, with isolates from clams having the highest detection rate (Girbau, *et al*., 2015). In contrast, *IrgA* and *hecA* was found to have low detection rates at 15% and 13%, respectively, in the 2013 Karadas study. The role of cj1349 is

to encode for a protein that adheres to fibronectin, similar to *cadF* (Levican, *et al*., 2013; Douidah, *et al*., 2012; Karadas, *et al*., 2013). Douidah *et al*. (2012) found that this gene was present in 97.6% of *Arcobacter* spp. isolates tested, thereby suggesting that this virulence gene was conserved. The virulence factor *pldA* is a phospholipid-related gene; *mviN* is a known virulence factor; tlyA is a hemolysin; irgA encodes for an outer membrane protein which is regulated by iron; hecA is part of filamentous hemagglutinin; and hecB is a hemolysin activation protein (Karadas, *et al*., 2013; Douidah, *et al*., 2012). Although our study focused specifically on *A. butzleri*, other potentially pathogenic strains of *Arcobacter* include *A. cryaerophilus, A. skirrowii, A. trophiarum*, and *A. defluvii* (Levican, *et al*., 2013).

The data above has provided insights into the levels of enteric bacterial pathogens present in the Calgary urban stormwater ponds along with their virulence factors. Our study found that *A. butzleri* was the most common pathogen present; and a growing number of researchers are suggesting that the clinical prevalence of the *Arcobacter* species is probably underestimated since the species is not routinely tested for (Levican, *et al*., 2013; Douidah, *et al*., 2012) and primers that have been used to identify *Campylobacter* spp. cross react with *Arcobacter* spp. (Banting, *et al*., 2016). Further, our study reflected that the *A. butzleri* found in McCall Lake harbors many virulence genes regardless of source of fecal contamination, and should therefore be treated as pathogenic. The prevalence and levels of *A. butzleri* need to be taken into consideration when developing an urban stormwater sampling plan and stormwater treatment.

6 Discussion

6.1 Key Findings

The overall purpose of this study was to determine the bacteriological water quality, the microbial sources of fecal contamination, and the enteric bacterial pathogens in stormwater-impacted bodies of water in southern Alberta, Canada, intended for water reuse and/or recreational activities. This research had several key findings: a) the overall water quality of stormwater-impacted bodies of water often failed existing water quality guidelines established by the USEPA, Health Canada, and Alberta Environment and Parks for recreational and surface waters; b) the water quality was highly variable; c) there were two dominant sources of microbial fecal pollution (i.e., human and seagull) in the stormwater-impacted bodies of water; and d) of the four enteric bacterial pathogens tested (i.e., *Campylobacter* spp., *Salmonella* spp., shiga-toxin producing *E. coli* [STEC], and *Arcobacter butzleri*), the putative pathogen *A. butzleri* was the most dominant. Thus, this work aimed to reflect the intersectionality between poor water quality, the sources of contamination, and the pathogens present.

6.2 Overall Interpretation and Discussion

6.2.1 Water Quality in Urban Stormwater-Impacted Bodies of Water

The assessment of traditional water quality indicators in urban stormwater-impacted bodies of water is essential to better understand the risks associated with water reuse. In this study, when all of the stormwater pond samples were amalgamated into the analysis, 20% of samples failed the geomean criteria of >126 *E. coli*/100mL, 17% of samples exceeded the single sample statistical threshold value (STV) of 1280 CCE/100mL for *Enterococcus* spp. by molecular methods, 7% of samples failed the single sample STV for *E. coli* at >410 CFU/100mL, and 7% of samples exceeded the single sample guideline of 400 CFU/100mL for thermotolerant coliforms. This trend of high failure rates was not limited to the urban stormwater

ponds, as it was also observed with the stormwater-impacted rivers. When the findings from all of the sampling sites in the Elbow River were consolidated, 21% of samples exceeded the single sample STV of 1280 CCE/100mL for *Enterococcus* spp. by molecular methods, and 13% of samples exceeded the single sample guideline of 400 CFU/100mL for thermotolerant coliforms. Water samples from the Nose Creek storm drains often exceeded existing water quality guidelines, with 70% of water samples failing the geomean criteria of >126 CFU/100mL for *E. coli,* 79% of samples exceeding the single sample STV of 1280 CCE/100mL for *Enterococcus* spp. by molecular methods, 40% of samples failing the single sample STV for *E. coli* at >410 CFU/100mL, and 56% of samples exceeding the single sample guideline of 400 CFU/100mL for thermotolerant coliforms. These findings have revealed that the tested stormwater-impacted bodies of water in southern Alberta repeatedly failed existing water quality guidelines, which in turn filled the knowledge gap regarding the bacteriological quality of this potential resource. That said, it is recommended that the source of the poor water quality also be factored in when determining the risks associated with stormwater reuse.

This trend, of poor stormwater quality, persisted with the high frequency of occurrence of human fecal pollution with microbial source tracking markers (e.g., HF183 was detected in 27% of the urban stormwater pond samples; in 65% of the Elbow River samples; and in 57% of the Nose Creek storm drain samples). These findings indicated that detecting human fecal pollution in urban stormwater-impacted bodies of water may not be an isolated anomaly and is instead a widespread occurrence. Human fecal pollution may enter stormwater through a variety of ways: combined sewer outfalls, cross connections, and failing infrastructure. Although the majority of sampling sites were measurably effected by human fecal pollution, some locations were far more heavily impacted by fecal pollution. This finding was similar to other studies (Sauer, *et al*., 2011;

Converse, *et al*., 2011; Newton, *et al*., 2013; Chase, *et al*., 2012). Sampling site ML2, at McCall Lake, had the highest occurrence of human fecal microbial source tracking markers (i.e., in 93% of samples for HF183 and in 59% of samples for HumM2), with HF183 levels as high as 6.0 $\log_{10}$ copies/100 mL.

In order to determine why high levels and frequent detections of human fecal pollution were occurring at ML2, the drainage network of ML2 was sampled. These samples showed a specific path that the contamination may be taking (i.e., not all trunks were contaminated) and indicated that an industrial complex may be contributing to the human fecal contamination. This conclusion was further substantiated by the findings of decreased levels of human fecal contamination following long weekends, when the industrial complex would be closed, as discussed in Chapter 4. Therefore, it was concluded that there could be an infrastructure failure (e.g., pipe breakage, illicit cross-connection, general system leakage, etc.) near the industrial complex, which may be contributing to the extent of detection of human fecal pollution at ML2. However, the exact cause of human fecal pollution cannot be definitively determined without sending a robot or human into the drainage network so as to ascertain what kind of infrastructure failure is occurring. These findings reflect that human fecal contamination is an extensive problem for the urban stormwater-impacted bodies of water. This special investigation showed that the infrastructure contributing to stormwater-impacted bodies of water needs to be understood in order to get an appropriate assessment of risk.

Human fecal pollution is commonly found and has the potential to occur at high levels in the urban stormwater-impacted bodies of water and poses a great risk to human health because it carries a multitude of pathogens that drive human illness (e.g., viruses, bacteria, and protozoa). In this study, a select group of enteric bacterial pathogens were examined (i.e., *Salmonella* spp.,

Campylobacter spp., STEC, and *A. butzleri*); however, the risk of infection from waterborne pathogens is subject to a variety of factors (e.g., pathogen number, dispersion in water, infective dose, etc.). *A. butzleri* was the most often detected (i.e., in 27% of the urban stormwater pond samples; in 65% of the Elbow River samples; and in 57% of the Nose Creek storm drain samples).

In addition, the virulence gene screen reflected that a potentially virulent pathogen is present regardless of source of contamination. This suggests that *A. butzleri* be treated as a putative pathogen when assessing the risks associated with stormwater reuse. In summary, these findings highlight the presence of enteric bacterial pathogens in stormwater.

6.2.2 Development of a Stormwater Monitoring Program

The data from this thesis study has demonstrated that based on the existing water quality guidelines as specified by the USEPA, Health Canada, and Alberta Environment and Parks, the water quality in the stormwater-impacted bodies of water in Calgary and Airdrie, Alberta, Canada, is poor and highly variable. One goal of this thesis has been to provide information that will facilitate the development of a comprehensive stormwater monitoring program for the province of Alberta, Canada, which could in turn stipulate guidelines for a stormwater sampling protocol. Such a monitoring program would provide information on sampling location, how often sampling needs to occur, and which indicator is recommended for analysis.

There are a multitude of factors to take into account when determining which indicator organism is the most suited to evaluate the water quality of stormwater. In this study, a variation of the failure rates between indicators was observed. For example, with respect to the Calgary urban stormwater ponds, the guideline for STV for *Enterococcus* spp. was exceeded in 17% of samples, whereas the STV for *E. coli* was exceeded in 7% of samples and the STV for fecal

coliforms was exceeded in 7% of samples. Based on the variation of failure rates, which were dependent on the microbial water quality indicator used, the data reflected that some FIB may be less conservative than others. The USEPA 2012 Guidelines for Water Reuse only looked at one microbial indicator (i.e., fecal coliforms). Shahryari *et al*. (2014) compared microbial water quality indicators in drinking water samples and found that *E. coli* and total coliforms were the least frequently detected of the established FIB tested (i.e., total coliforms, fecal coliforms, *E. coli*, and fecal streptococci). Haugland *et al*. (2005) and Dufour *et al*. (1984) showed that *Enterococcus* spp. and *E. coli* are suitable for predicting gastrointestinal illness in freshwater. The results in this study reflected that the *Enterococcus* spp. molecular-based method guideline may be a more conservative FIB regarding public health, when measuring a single sample, as compared to the other FIB tested. For this reason, and the potential timeliness and ease of the method, *Enterococcus* spp. may be the best suited FIB for stormwater reuse guidelines, if only one indicator is used (Noble, *et al*., 2010). In addition, dependent on the sampling scheme, utilizing molecular-based methods can allow for a quicker turnaround time than culture-based methods, given five hours compared to at least 18 hours for culture-based methods (Wade, *et al*., 2010). Furthermore, two U.S. states (i.e., Texas and Virginia) use *Enterococcus* spp. to monitor reclaimed water (EPA, 2012).

An added benefit of utilizing the *Enterococcus* spp. molecular-based method is that the DNA extracted from each sample can be used for further tests, such as determining the source of pollution. For example, if a water sample was to fail the water quality guideline for *Enterococcus* spp., then a microbial source tracking assay for human fecal pollution could be run, which would provide a greater idea of the risk associated with using the water, as it is generally accepted that human fecal pollution poses a greater risk to human health than non-human fecal pollution

(Sauer, *et al*., 2011; Cao ,*et al*., 2017; Staley, *et al*., 2016). Furthermore, some researchers believe that host-specific Bacteroidales may serve as good indicators for fecal contamination or are better than current indicators (e.g., *E. coli*) (Dick & Field, 2004; Lamendella, *et al*., 2007). Moreover, given the expense and time-consuming nature of monitoring for individual pathogens (Noble, *et al*., 2010), it is recommended to use FIB in lieu of pathogen-specific monitoring for stormwater. That said, some U.S. states, including Florida, have set additional individual standards to monitor specific pathogens (i.e., *Giardia* and *Cryptosporidium)* (EPA, 2012).

It should be noted that the use of molecular-based methods (e.g., qPCR) may result in the overestimation of FIB, since the assay detects genetic material instead of only viable cells, as the culture-based assay does (Noble, *et al*., 2010). However, Noble *et al*. (2010) compared molecular and culture-based methods for *Enterococcus* spp. in recreational water. Their findings revealed that when comparing the *Enterococcus* spp. molecular-based method to a culture-based method as detected by Enterolert®, there was only a 6% disagreement rate with a higher rate of failures in the culture-based assay. Therefore, the other FIB tested by molecular-based methods (e.g., *E. coli*, fecal coliforms, etc.) may still be valid for food production or for monitoring other types of water, such as irrigation or marine waters. As such, this topic remains open to discussion.

Determining the proper location to sample is vital for effective monitoring. It is recommended that water be sampled at point-of-use. In the case of stormwater pond reuse, it is recommended to ascertain the quality of stormwater for fit-for-purpose activities in order to treat the stormwater most appropriately; and it is suggested that the water collection sampling site correspond to the location at which water is being pulled from the pond reuse applications. In the case of stormwater-impacted recreational bodies of water, the point-of-use would be where recreation is occurring. This is similar to the USEPA 2012 Water Reuse Guidelines which

recommend that reclaimed water be monitored at the water reclamation point. Even though results indicated spatial variability among sampling sites, there was consistency in the patterns that were observed, as all sites failed recreational water quality guidelines, in addition to human fecal contamination and *A. butzleri* being detected from water samples at all sampling sites except for one.

With respect to frequency of sampling, a multitude of environmental variables can affect the microbial quality of water (e.g., time of day, weather conditions, etc.). Several studies have documented that there can be a wide range in indicator organism density in recreational water samples impacted by stormwater across several days (Leecaster &Weisberg, 2001; Boehm, *et al*., 2002; Whitman & Nevers, 2004); as such, two days after sampling water quality may no longer correspond to the sampling results. For example, the results from sampling on a Monday may not be reflective of water quality on a Wednesday, given potential natural and human-made impacts. These studies reflect that more frequent monitoring, albeit costlier and time-consuming, can be advantageous, since increased monitoring is providing information on most up-to-date water quality, which would benefit public health. Health Canada and the USEPA recommend that bodies of water used for recreation (e.g., the Elbow River and the Nose Creek) are monitored at least once a week during the recreational season (e.g., May-September). In addition, the recommendation in the USEPA 2012 Recreational Water Quality Criteria contends that in the initials stages of monitoring a new location (i.e., the first 30 days), a larger data set is more beneficial in determining the water quality of the waterbody; and for more populated areas (e.g., beaches), more frequent testing is recommended. Furthermore, the USEPA recommends that monitoring following rain events should be considered, as precipitation may lead to pathogen

transport. In this study, the highest values of rainfall occurred with some of the highest levels of bacterial water quality indicators.

The 2009 Australian Guidelines for Water Recycling provided recommendations for managing the health and environmental risks of recycling rainwater and stormwater from urban environments through a risk-based approach. These guidelines noted that stormwater varied considerably between storm events. Therefore, it may be beneficial to request additional monitoring after heavy rain events (i.e., >10 mm). In addition, the guidelines with respect to irrigation include weekly monitoring of the disinfection system using *E. coli* and a 72-hour buffer between stormwater collection and water use for irrigation. The goal of this buffer time is intended to reduce the effects of first flush, during which time the buffer aims to equalize the levels of pathogens. However, there is no definitive answer on how often stormwater-impacted bodies of water should be sampled.

The development of a comprehensive monitoring program also necessitates a consideration of how often sampling should occur, which should take into account the aforementioned variables. For stormwater ponds and stormwater-impacted rivers, it is recommended that biweekly monitoring occur. This recommendation is due to the highly variable nature of microbial water quality indicators, microbial sources of fecal pollution, and enteric bacterial pathogens, as throughout this study there were temporal fluctuations at samplings sites for FIB, human fecal pollution, and *A. butzleri*. Variability is common in stormwater and it is suggested that this be taken into account when developing a monitoring plan (Austrailian Guidelines for Water Recycling, 2009). In addition, considerable temporal fluctuations were noted between sequential sampling dates. Therefore, more frequent monitoring would aid in capturing these fluctuations and may be protective of public health. For example,

should the water quality meet standard guidelines initially and then deteriorate, it is recommended that water use be curtailed until the next sampling date when the water quality guidelines are once again met. Furthermore, if a failure occurs during the first monitoring day of the week, but not on the second sampling date, more frequent monitoring may provide for increased opportunities for water reuse. Bi-weekly sampling may capture these changes in water quality that weekly sampling may not. Bi-weekly sampling could be further strengthened by the use of the appropriate numeric concentration threshold.

As specified in Chapter 3, two different analyses were used to evaluate water quality: STV, which is based off of a single sample, as well as a 5-sample running geometric mean (GM). Each statistical test revealed different key findings in the study. The GM provided information on chronic contamination issues, which was observed at sampling site ML2 at McCall Lake. That said, the GM does not capture the peaks in risk that the STV does. One example of this normalization would be if only a GM had been utilized to evaluate water quality at McCall Lake. In this case then, three of the four sampling sites (i.e., ML1, Inlet ¾, and PR60) would have reflected that they had good water quality. However, as already noted, each of these sampling sites had several instances where they violated the STV. In this study, the guideline with the most failures in urban stormwater ponds was the *E. coli* 5-sample running GM. Interestingly, both Texas and Virginia, USA, utilize *Enterococcus* spp. as a water quality indicator, and apply a GM for the period of a month or 30 days; and the state of Virginia provides a maximum allowable GM concentration for *Enterococcus* spp., similar to a STV for *Enterococcus* spp. (EPA, 2012).

The STV, representing a single point in time measurement, is a “do not exceed” value. In this study, the USEPA’s 2012 recreational water quality recommendation for *Enterococcus* by

the molecular-based method revealed the most water quality failures. This measurement allows researchers and public health officials to see the fluctuations and outliers that may indicate peak times of risk. For example, the highest value of *Enterococcus* spp. observed in the study was at sampling site PR60, which would not have violated the GM at that time.

By taking into consideration how the numeric concentration baseline operates for both the GM and STV and the benefits and challenges associated with each when determining water quality, it is recommended that a mixed-approach could be the most advantageous to understanding water quality in a stormwater-impacted body of water, using both the GM and STV during the initial monitoring program for determining if the body of water is appropriate for water reuse applications. The use of two numeric concentration baselines would allow for observation of the overall trends in water quality and the peak times of risk, which would in turn make for more effective evaluation of water quality in the short and long-term. Further, since the results in this study showed that water quality was highly variable, each system would need to be studied independently prior to reuse. After an initial season of monitoring with the two numeric concentration baselines, the subsequent seasons could be evaluated with only the STV. The reason for this approach is that the data demonstrated that the STV may be a more conservative measure of risk, as previously discussed.

An STV requires a "do not exceed value." The USEPA provides two different STVs based on the estimated levels of background illness in the population. The first criteria for *Enterococcus* spp. measured through molecular methods of 1280 CCE/100mL estimates a background level of illness in the population of 32 per 1000 people, and the second criteria of 2000 CCE/100mL estimates a background illness of 36 per 1000 people. As previously mentioned the use of *Enterococcus* spp. as the FIB allows for additional testing, such as the

microbial source of pollution. It is recommended that for a stormwater monitoring program there be additional testing for human and ruminant microbial source tracking markers (i.e., HF183 and Rum2Bac) when the levels of *Enterococcus* spp. exceed 1280 CCE/100mL. These two markers were chosen as fecal pollution from humans and ruminants pose the greatest risk to human health (Sauer, *et al*., 2011; Converse, *et al*., 2011; Newton, *et al*., 2013; Chase, *et al*., 2012; Soller, *et* al., 2010). If positive for either microbial source tracking marker and above 1280 CCE/100mL, it is suggested water reuse stop until the next sampling date. If negative for these two microbial source tracking markers and below 2000 CCE/100mL for *Enterococcu*s spp, water reuse can proceed until the next sampling date. If greater than 2000 CCE/mL it is suggested that all water reuse activities stop until the next sampling date.

6.3 Further Consideration of Study Strengths and Limitations

The main strengths of this study include: 1) the large sample size from the urban stormwater ponds in the City of Calgary with sampling taken biweekly over 21 weeks to allow for a significant analysis; 2) the testing of several different water quality markers to ensure accurate results; 3) the use of seven different microbial source tracking markers to alleviate concerns of cross-reaction or alternate sources of pollution; and 4) the employment of molecular- and culture- based methods to assure the accuracy of results.

There are limitations to the study, including: 1) the use of grab sampling, which may introduce bias into the sample set, as it only allowed for samples to be taken near the surface and within certain areas of the pond; 2) the sampling of urban stormwater ponds located specifically in southern Alberta, and the results of which may not extend to all environments; 3) the use of only a molecular-screen for some types of pathogens which does not determine if the bacteria are viable (e.g., *Salmonella* spp. and STEC), and 4) the lack of analysis of viruses, protozoa, and

chemicals in stormwater-impacted bodies of water intended for reuse applications to get a wider lens and a more complete understanding of the risks associated with water reuse. It is noteworthy that viruses are thought to be the primary driver of swimming-related illness in human fecal pollution-impacted bodies of water (Staley, *et al*., 2012). Furthermore, viruses are shed in high numbers and therefore drive most human health risk assessment models

A further limitation of this study is that no direct comparisons could be made between sampling site ML2 at McCall Lake and any of the other outfalls, due to placement. ML2 was the only outfall in this study that was fully above ground. As such, ML2 was the only sampling site in which samples were not diluted by pond water, as would occur in the partially submerged outfall (i.e., ML1 at McCall Lake) and the fully submerged outfalls (i.e., WP31A, WP31C, WP31D, WP31E at Country Hills; and outfalls/inlets WP26B, WP26C, WP26D at Inverness). For the most accurate assessment – and to safeguard the assessment – of the stormwater flowing into the McCall Lake stormwater pond, the placement of auto-samplers in the manholes upstream of the outfalls is recommended, which will allow for a direct comparison between stormwater pond outfalls, along with an analysis of the undiluted stormwater from the ML2 sampling site. Although these limitations are significant enough to mention, they should not take away from the results of this thesis research study.

6.4 Future Directions

The findings from the study help to lay a foundation for a variety of future projects. In terms of the high levels of human fecal contamination found in McCall Lake, research projects are ongoing to pinpoint the cause and thereby address it. It is suggested that additional research and statistical analysis be done to determine the effects that land use characteristics have on sources of contamination, in addition to hydrographical modeling on the potential water reuse

locations, and a risk assessment for reclaiming water from the urban stormwater ponds. Furthermore, there are still several key challenges facing stormwater reuse, including the lack of knowledge regarding pathogenic microorganisms in stormwater, the pathogen loading of the water source in order to determine effective treatment, and the ability of the previously mentioned systems to remove pathogens from stormwater, all of which could provide for the basis of future studies (Fletcher, *et al*., 2008; McCarthy, *et al*., 2007).

The results concluded that the tested stormwater-impacted bodies of water may not be considered a pristine resource that is readily usable. Therefore, a quantitative microbial risk assessment (QMRA) should be performed, which could help fill several knowledge gaps regarding stormwater reuse in Calgary and Airdrie. This knowledge gaps include a dose-response model for *A. butzleri*, estimated the risk from exposure to stormwater, and recommendations for stormwater treatment. Risk assessments are designed to aid in estimating the risk from exposure to the microorganisms identified in research studies (e.g., bacterial pathogens, viruses, etc.).

As this study reflected that stormwater is not a pristine resource, this suggests that treatment options be considered. There are several options for stormwater treatment, and can be dependent on the end-use goal of the water. Depending on the end-use for stormwater, treatment options can often include natural purification processes. The Water Environment & Reuse Foundation (WERF), with headquarters in Alexandria, Virginia, and Denver, Colorado, USA, has developed the Risk-Based Framework for the Development of Public Health Guidance for Decentralized Non-Potable Water Systems for stormwater reuse. This document contains various performance-based $\log_{10}$ reduction targets for the treatment of pathogens (e.g., the recommendation of natural and biological processes, filtration processes, and disinfection processes) (Sharvelle, *et al*.,

2017). Natural and biological treatment processes include a one $\log_{10}$ reduction of bacteria for stormwater being accrued into a stormwater pond; and involve the pollutant-removing mechanisms, taking retention time and exposure to sunlight into account (Fletcher, *et al*., 2008; Sharvelle, *et al*., 2017). Filtration processes include the use of microfilters, nanofilters, and reverse osmosis, all of which may be able to achieve a greater than six $\log_{10}$ reduction of bacteria (Sharvelle, *et al*., 2017). Furthermore, there are several management processes that could be utilized for stormwater treatment

Further, an unexpected result from the thesis study research has been the levels *of A. butzleri* detected in the urban stormwater ponds. *Arcobacter butzleri* is an emerging pathogen and due to its putative nature there are many potential research projects that could be conducted to further assess its true effects on human health. As mentioned previously in Chapter 5, the burden of *Arcobacter* spp. may be underestimated since primers that were published to be specific for *Campylobacter* spp. tend to cross-react with *Arcobacter* spp., thereby identifying *Campylobacter* spp. instead of *Arcobacter* spp. (Banting, *et al*., 2016). Therefore, studies that have utilized these primers may want to consider testing with an *Arcobacter* species-specific primer to determine if detections are truly *Campylobacter* spp. It would be beneficial to use an *Arcobacter* species-specific primer to see if there are potentially other species of *Arcobacter* present, in particular *A. cyraerophilus* which is associated with human sewage. During the thesis research study, a primer capable of identifying *A. butzleri* was used.

As part of this thesis research study, *A. butzleri* was isolated from stormwater samples at the end of the stormwater season in one of the urban stormwater ponds (i.e., McCall Lake). As shown in Chapter 5, the molecular and culture-based methods results for *A. butzleri* did not always align, as the culture-based methods tended to be more sensitive. A possible remedy could

involve conducting a short-term trial of culture-based methods to see if the results between molecular- and culture- based methods are comparable.

A further consideration could be to isolate *A. butzleri* from samples from the other stormwater-impacted bodies of water in order to ascertain how widespread the virulence genes may be. This approach could determine if the frequency of virulence genes detected in McCall Lake are representative of the *A. butzleri* population that may be in the southern Alberta, Canada, urban stormwater ponds and urban storm-impacted bodies of water as a whole. In addition, the virulence gene screen reflected that one microbial source of pollution did not contain more virulent *A. butzleri* than the other potential sources. However, continuing research in this area is suggested to determine if the dominant sources of microbial fecal pollution (i.e., human and seagull) contained virulent *Arcobacter* spp. by testing fecal samples from these sources. Moreover, as there is limited research on *Arcobacter* spp. the development of a dose-response model and fraction likely to be human infectious for *Arcobacter* spp. would be beneficial to further aid in the determination of risk. Further, evaluation of *Arcobacter* spp. environmental forms is necessary to evaluate the risk, as other bacteria (e.g., *Aeromonas hydrophila*) have been been found to have common environmental forms.

6.5 Conclusion

This thesis research study examined the water quality of the urban stormwater ponds and stormwater-impacted rivers in Calgary and Airdrie, Alberta, Canada, by ascertaining the levels of FIB, microbial sources of contamination, and pathogens present. This study has aimed to fill knowledge gaps regarding the water quality of the urban stormwater ponds and stormwater-impacted river in southern Alberta, and what is contributing to the poor water quality of these vital water resources. With the respect to the Calgary urban stormwater ponds and the Elbow River in

Calgary and the Nose Creek in Airdrie, human fecal contamination was found to be the most common source of fecal pollution. The most frequently detected enteric bacterial pathogen was *A. butzleri*, representing a significant health risk to public health. Further research is required on *A. butzleri* to fully understand its risks, such as determining the infectious dose, pathogenicity in other model organisms (e.g., mice), in addition to burden and likelihood of illness. These findings from this research study provides information regarding the bacterial water quality of urban stormwater-impacted bodies of water and provides a starting point for further studies for the fields of water microbiology and water reuse.

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Appendix

Figure 0-1: Photo of sampling site ML1 located at McCall Lake. ML1 is a partially submerged outlet.

Figure 0-2: Sampling Site ML2 located in McCall Lake. ML2 is an above-ground outfall.

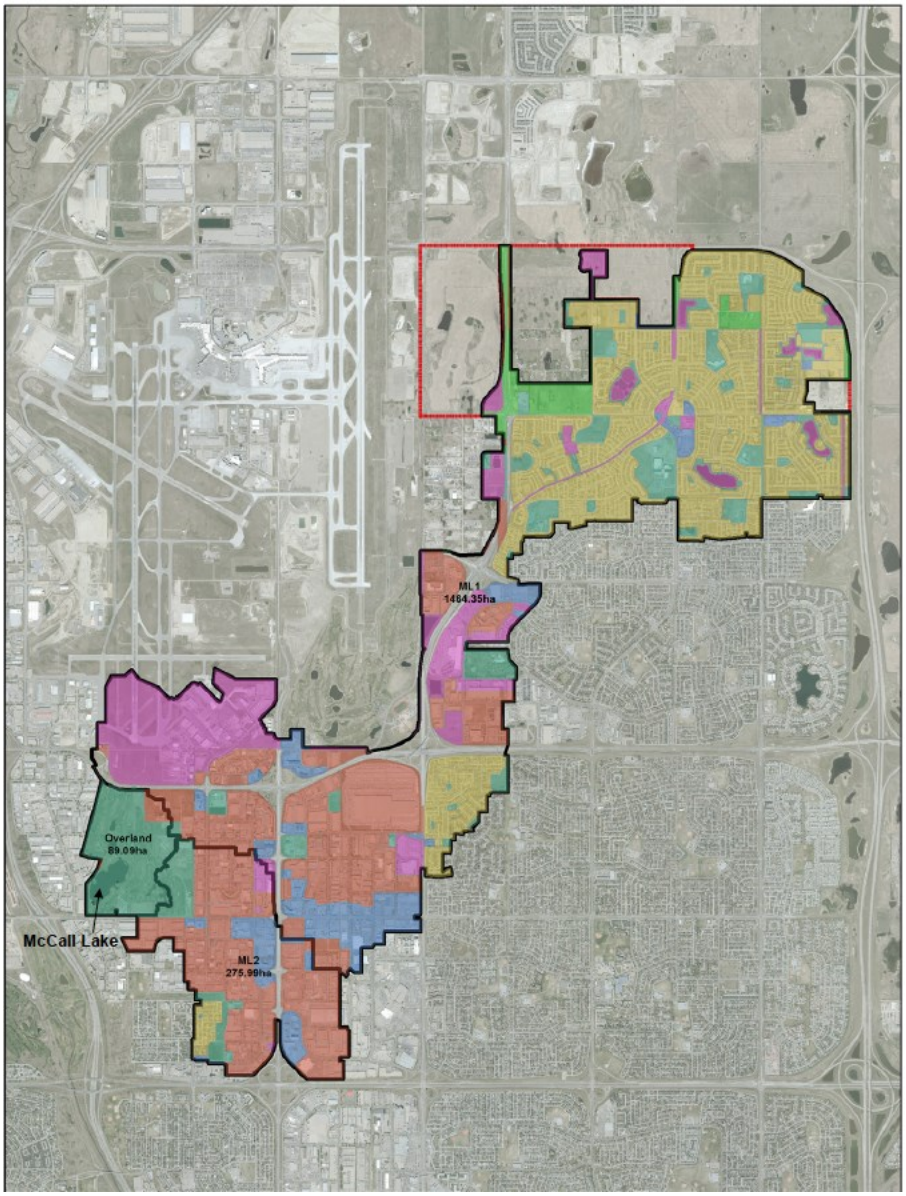


Figure 0-3: Catchment areas of outfalls ML1 and ML2 in McCall Lake. The black outline defines the McCall Lake sub catchment area and red outline defines the undeveloped area. Land use is color coded with blue representing commercial, lime green is future urban development, orange is industrial, pink is major infrastructure and transportation, teal green is parks and institutions, and yellow residential.

Figure 0-4: Sampling site WP31B located in Country Hills Stormwater Facility.

Figure 0-5: Sampling site WP31C located in Country Hills Stormwater Facility.

Figure 0-6: Sampling site WP31E located in Country Hill Stormwater Facility.

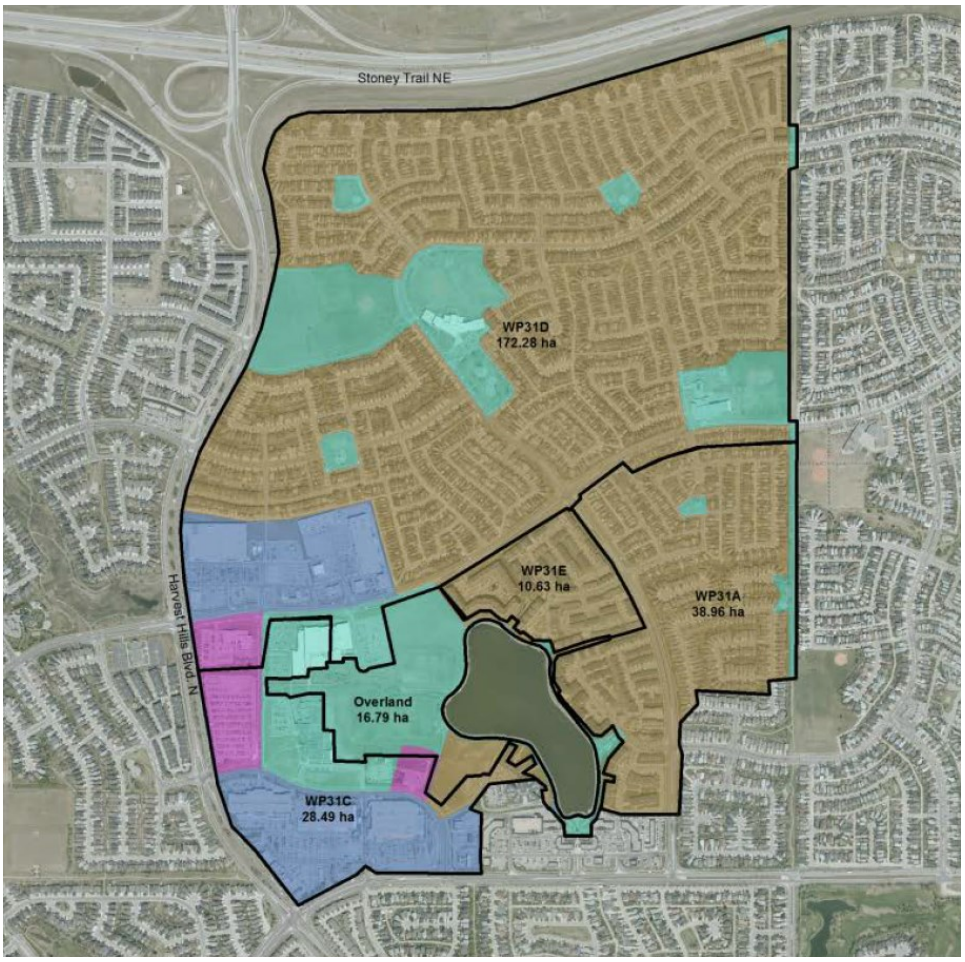


Figure 0-7: Catchment areas of outfalls WP31A, WP31C, WP31D, WP31E, and the overland drainage in Country Hills Stormwater Facility, of which WP31A, WP31C, WP31D and WP31E were sampled in this thesis. The black outline defines the Country Hills sub catchment area. Land use is color coded with blue representing commercial, pink is major infrastructure and transportation, teal green is parks and institutions, and yellow residential.

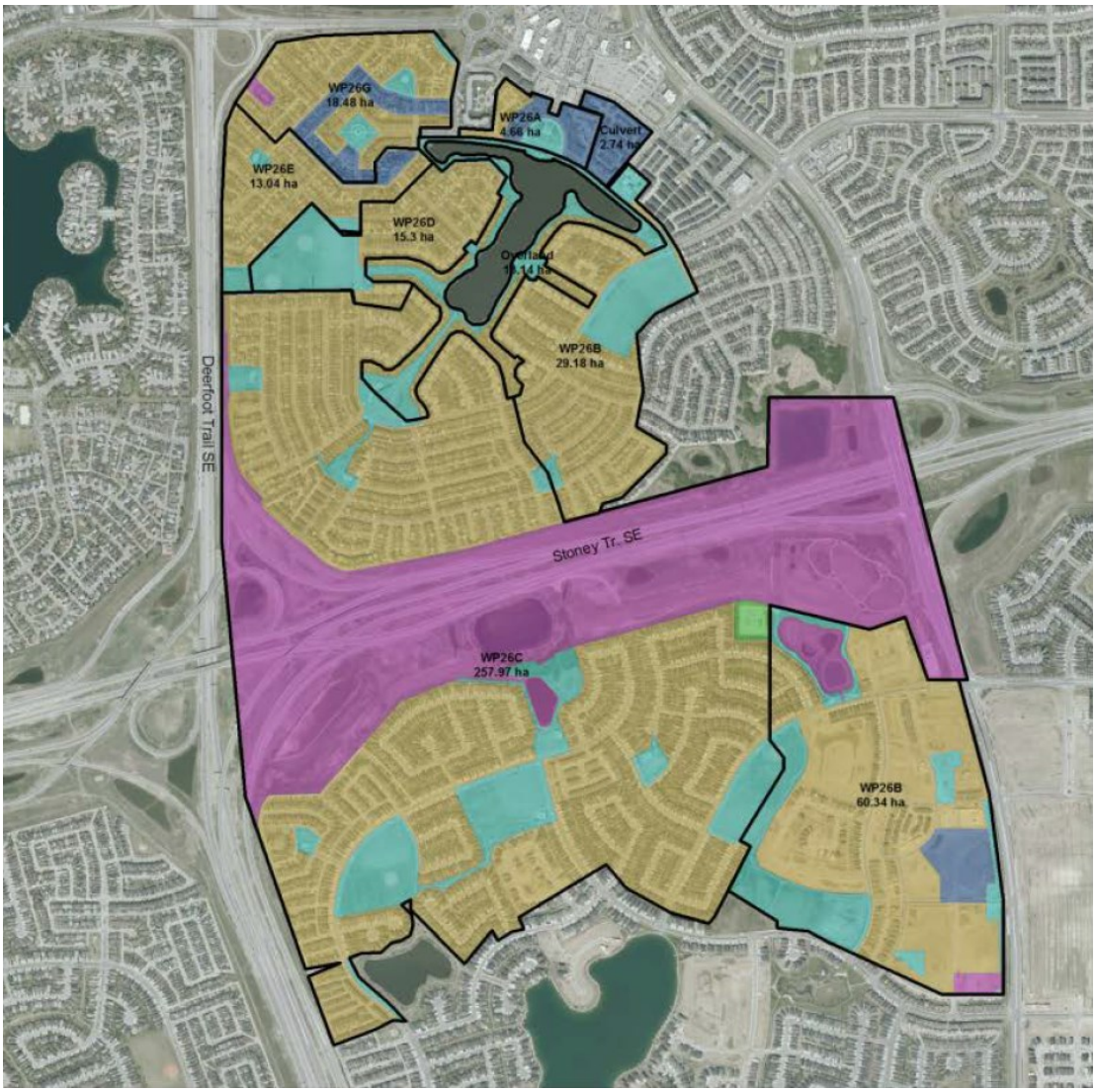


Figure 0-8: Catchment areas of outfalls WP26A, WP26B, WP26C, WP26D, WP26E, WP26G and the culvert in Inverness Stormpond, of which WP26B, WP26C and WP26D were sampled in this thesis. The black outline defines the Inverness Stormpond sub catchment area. Land use is color coded with blue representing commercial, lime green is future urban development, pink is major infrastructure and transportation, teal green is parks and institutions, and yellow residential.

Figure 0-9: Sampling site WP26D located in Inverness Stormpond.

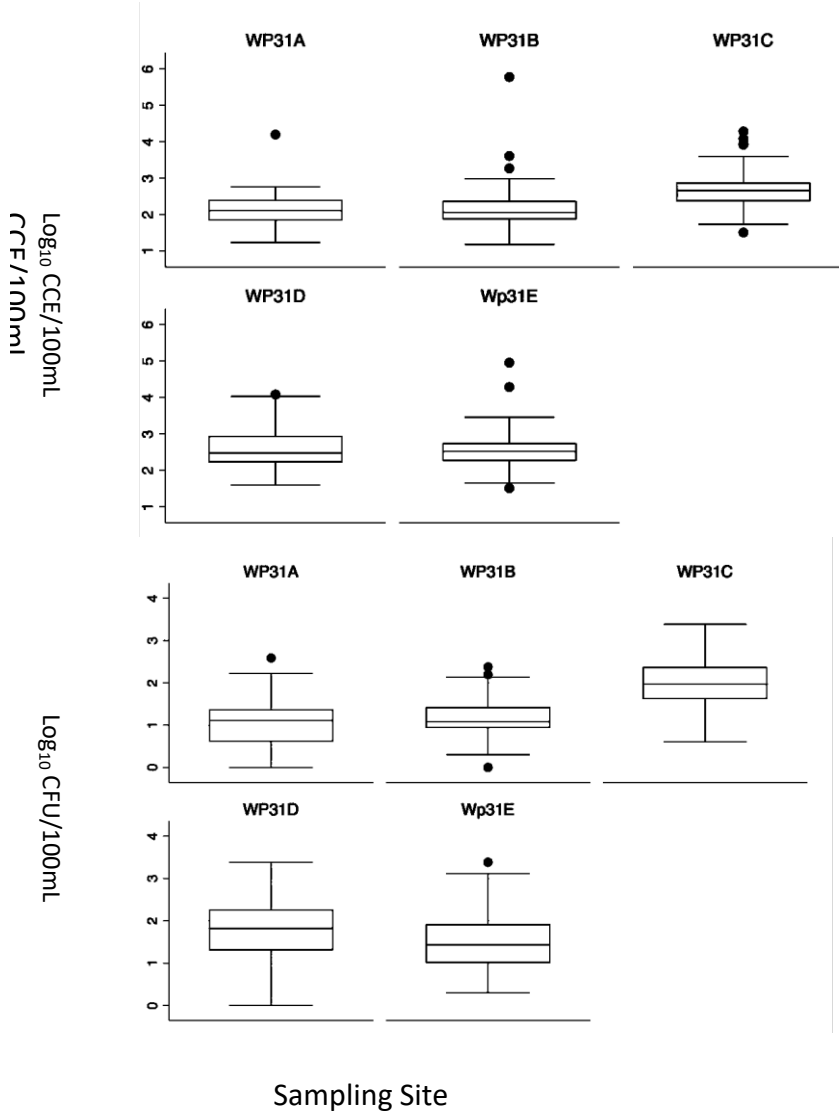


*Figure 0-10: Box and Whisker plot of E. coli and Enterococcus log_{10} values (top) and E. coli log_{10} (bottom) values in Country Hills Stormwater Facility over 21 weeks broken down by sampling site (i.e.,WP31A, WP31B, WP31C, WP31D, and WP31E). The outer edges of the box represent the 25th and 75th percentiles (i.e., interquartile range), and the line within the box represents the median. The location of median indicates the skew of the data. The whiskers represent the interquartile range*1.5. The outliers are determined by being greater or less than 1.5 times the upper of lower interquartile ranges as represented by circles.*

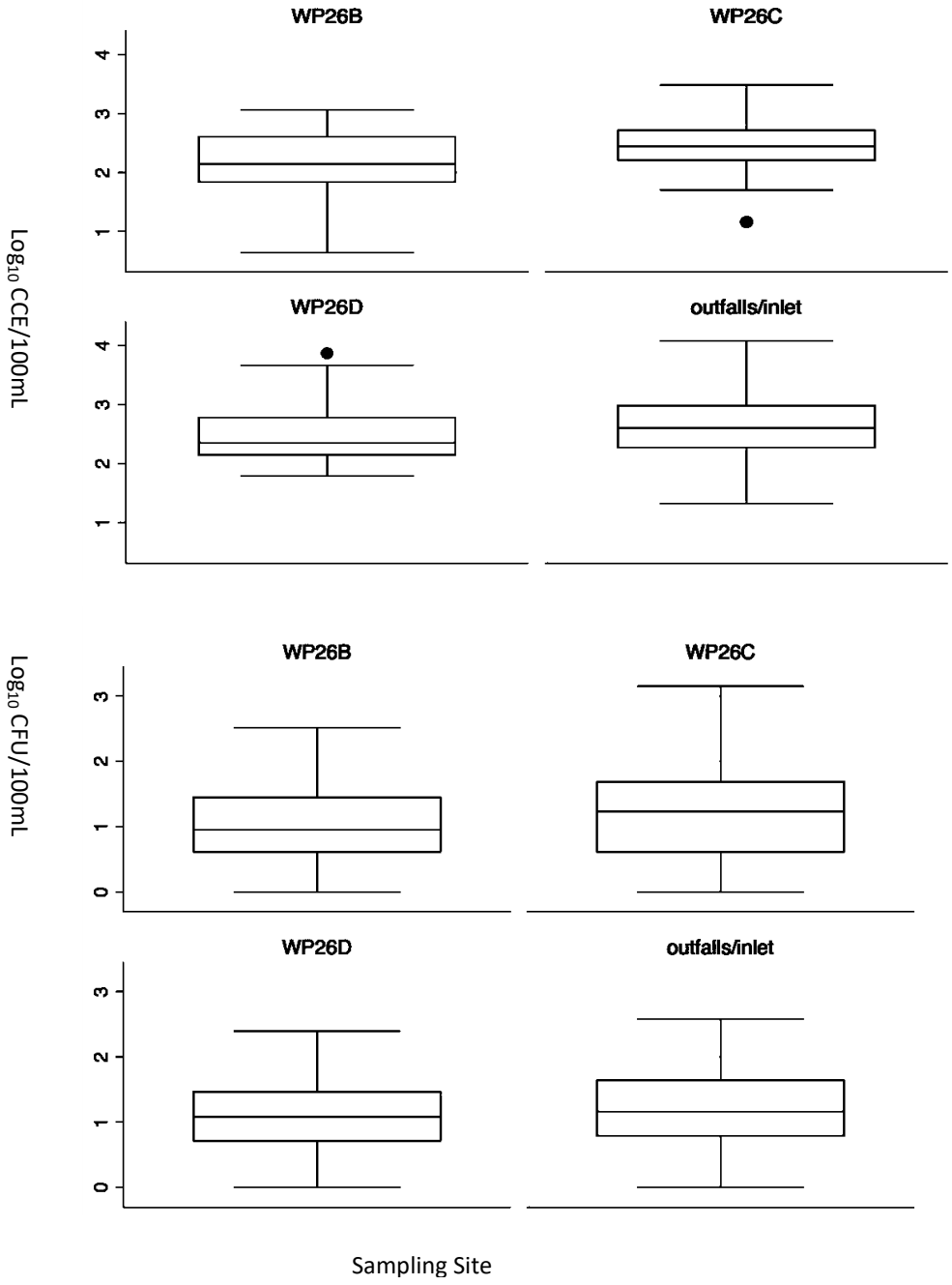


*Figure 0-11: Box and Whisker plot of Enterococcus log_{10} values (top) and E. coli log_{10} (bottom) values in Inverness Storm Pond over 21 weeks broken down by sampling site (i.e.,WP26B, WP26C, SP26D, and outfalls/inlet). The outer edges of the box represent the 25th and 75th percentiles (i.e., interquartile range), and the line within the box represents the median. The location of median indicates the skew of the data. The whiskers represent the interquartile range*1.5. The outliers are determined by being greater or less than 1.5 times the upper of lower interquartile ranges as represented by circles.*

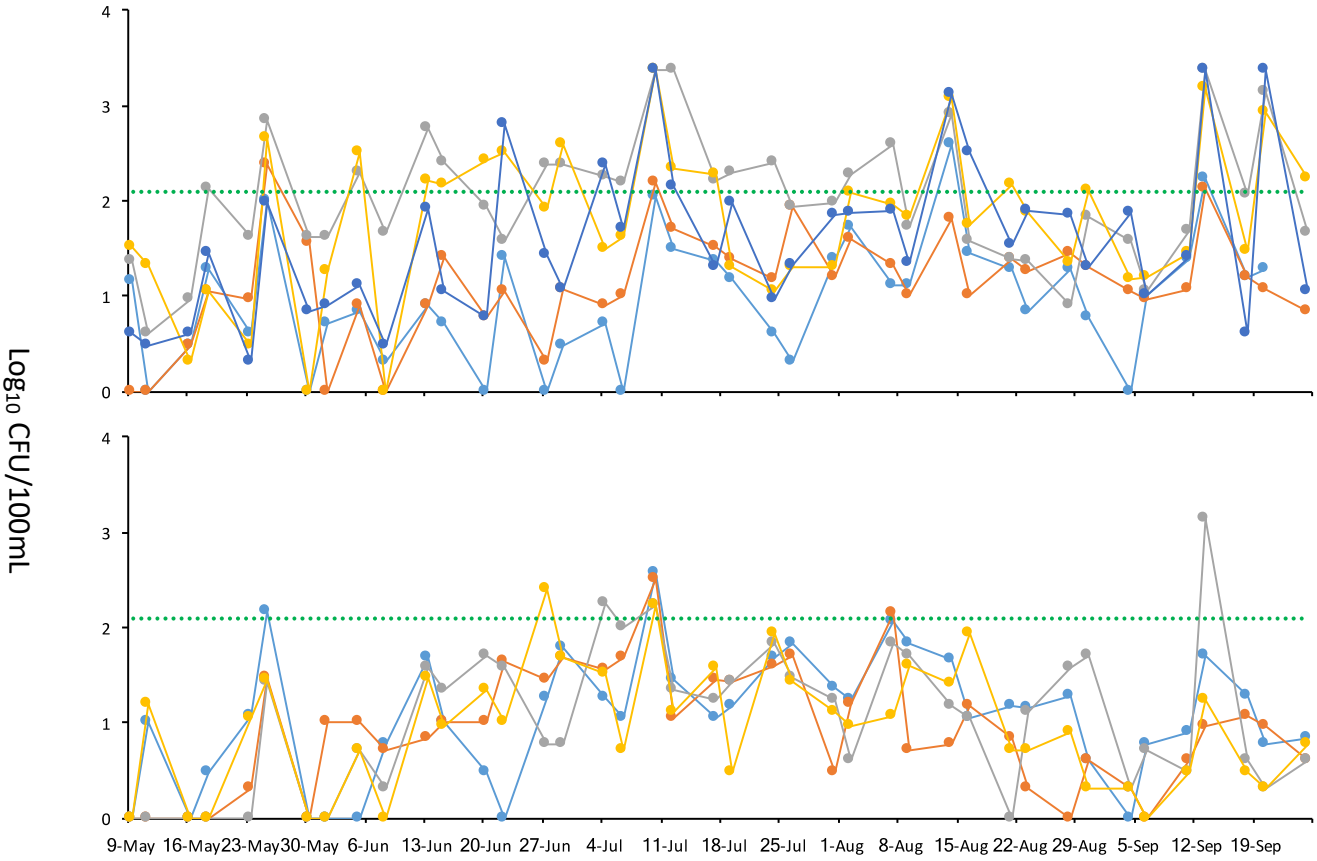


Figure 0-12: Temporal pattern of occurrence of E. coli log_{10} concentrations at Country Hills Stormwater Facility at all sampling sites (i.e., WP31A in blue, WP31B in red, WP31C in grey, WP31D in yellow, WP31E in blue) and Inverness Stormpond at all sampling sites (i.e., outfalls/inlet in blue, WP26B in red, WP26C in grey, WP26D in yellow) over 21-weeks. The US EPA Recreational Water Quality Guidelines have a single sample threshold value of >410 CFU/ 100mL (green dotted line) is also provided.

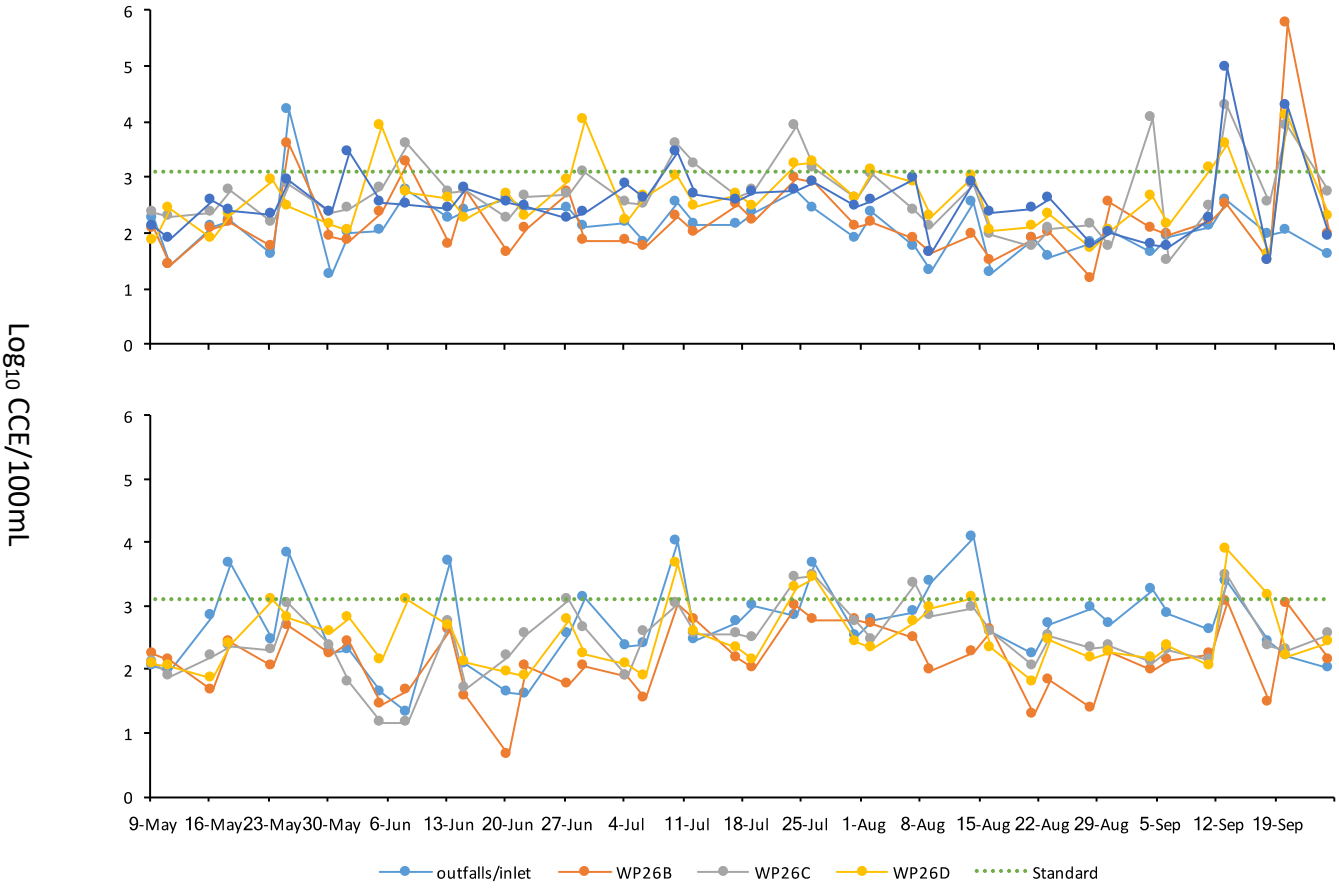
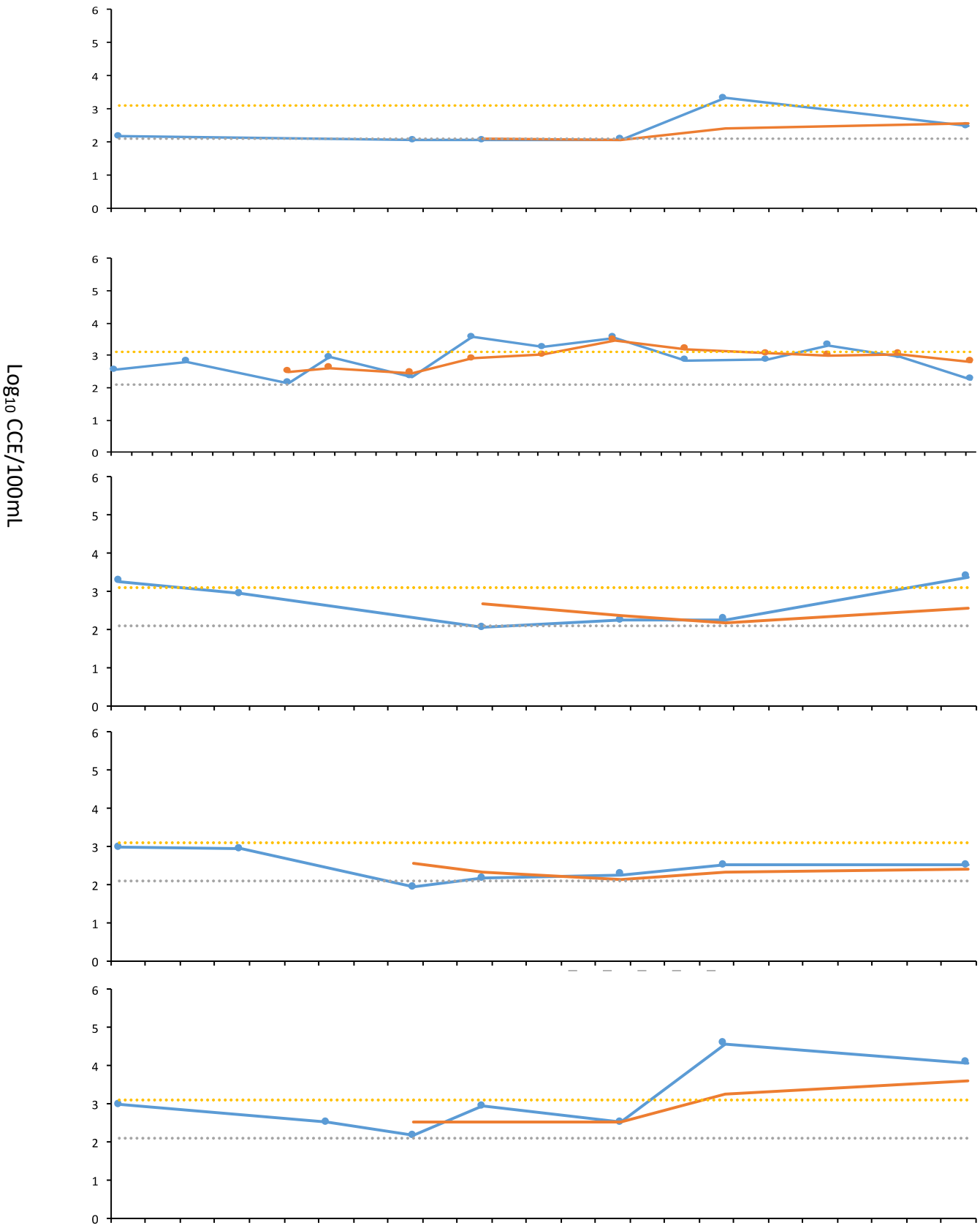


Figure 0-13: Temporal pattern of occurrence of Enterococcus log$_{10}$ concentrations at Country Hills Stormwater Facility at all sampling sites (i.e., WP31A in blue, WP31B in red, WP31C in grey, WP31D in yellow, WP31E in blue) and Inverness Storm Pond at all sampling sites (i.e., outfalls/inlet in blue, WP26B in red, WP26C in grey, WP26D in yellow) over 21-weeks. The US EPA's Recreational Water Quality Guideline single sample threshold value of >1280 CCE/ 100mL (green dotted line) is also provided.


Log10 CCE/100mL
0
1
2
3
4
5
6

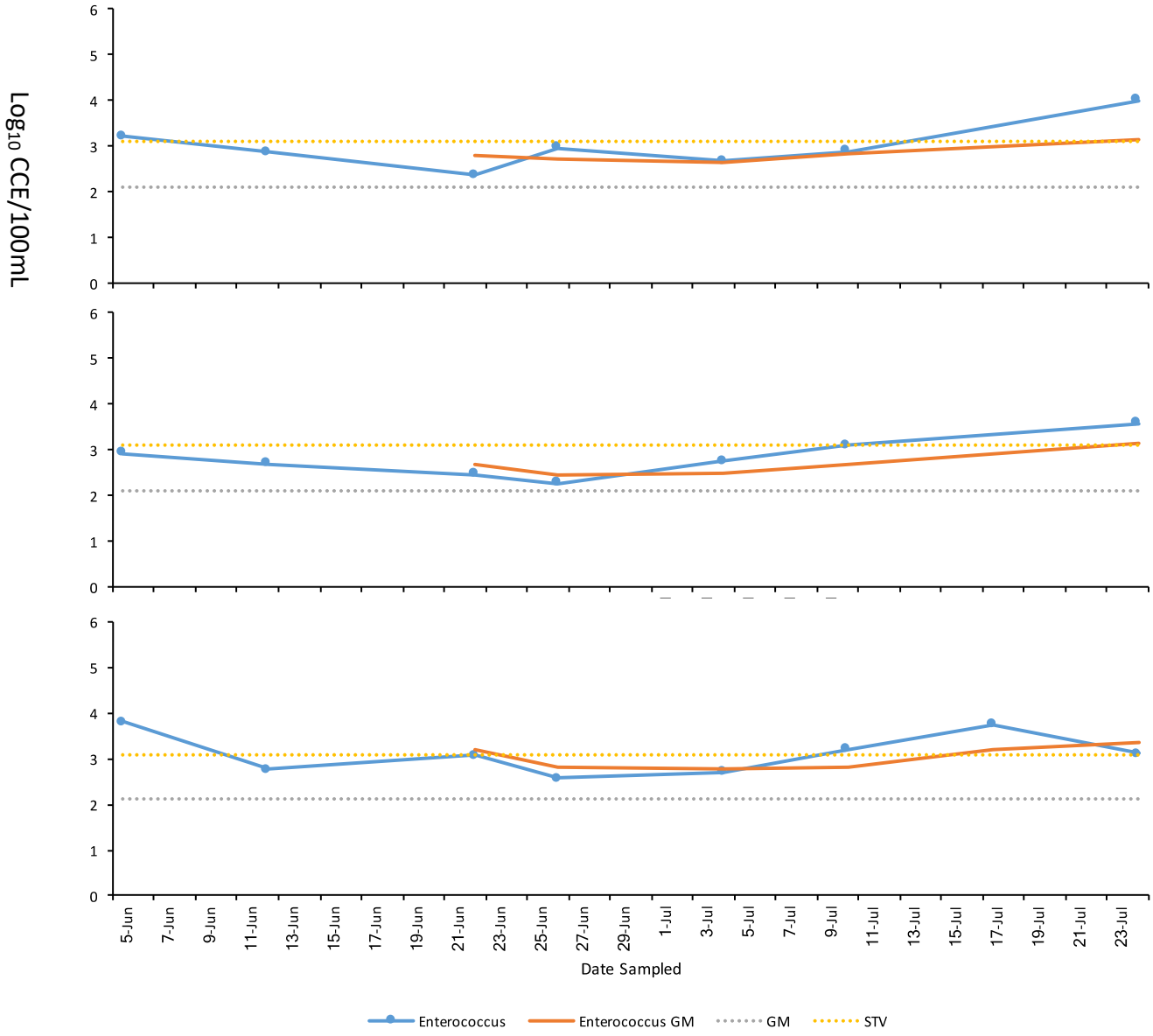


Figure 0-14: Temporal pattern of occurrence of Enterococcus log_{10} concentrations Elbow River at all sampling sites in the following order: Riverdale, Rideau, 26th Ave, 25th Ave, 1st Street, Stampede, Enmax Park, and 9th Ave over 21-weeks. The US EPA's Guideline for Recreational Water Quality Guideline for a single sample threshold value of >1280 CCE/ 100mL (yellow dotted line) and the GM of >300 CCE/100mL (grey dotted line) is provided. The 5-sample running geometric mean of the water samples is in red, the individual water sample concentrations of Enterococcus are in blue.

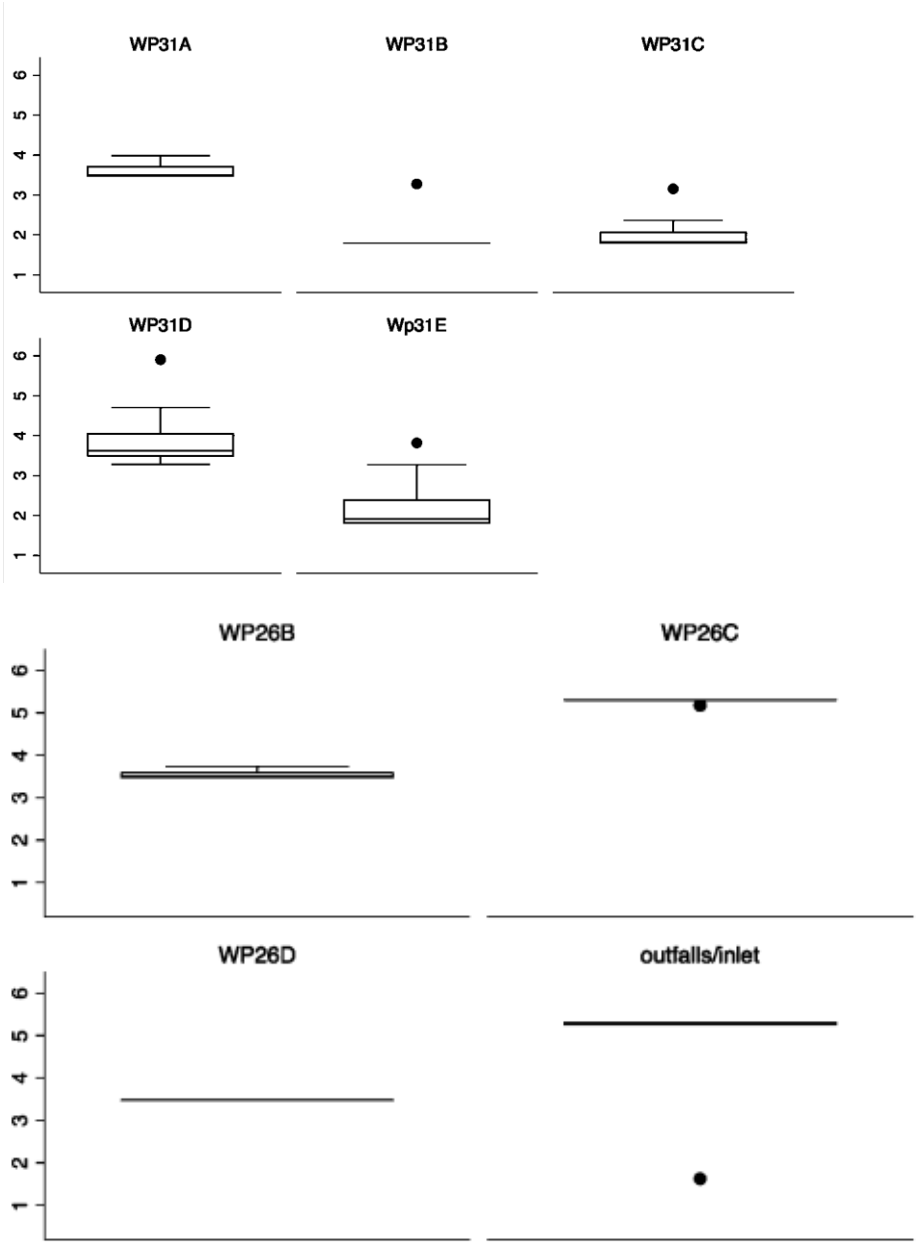


*Figure 0-15:Box and Whisker Plot of HF183 levels by sampling site in Country Hills (upper) (WP31A n=4, WP31B n=7, WP31C n=11, WP31D n= 17, WP31E n=13) and Inverness (lower)(WP26B n=4, WP26C n= 7, WP26D n=4, outfalls/inlet n=6). The outer edges of the box represent the 25th and 75th percentiles (i.e., interquartile range), the line within the box represents the median. The location of median indicates the skew of the data. The whiskers represent the interquartile range*1.5. The outliers are determined by being greater or less than 1.5 times the upper of lower interquartile ranges as represented by circles.*

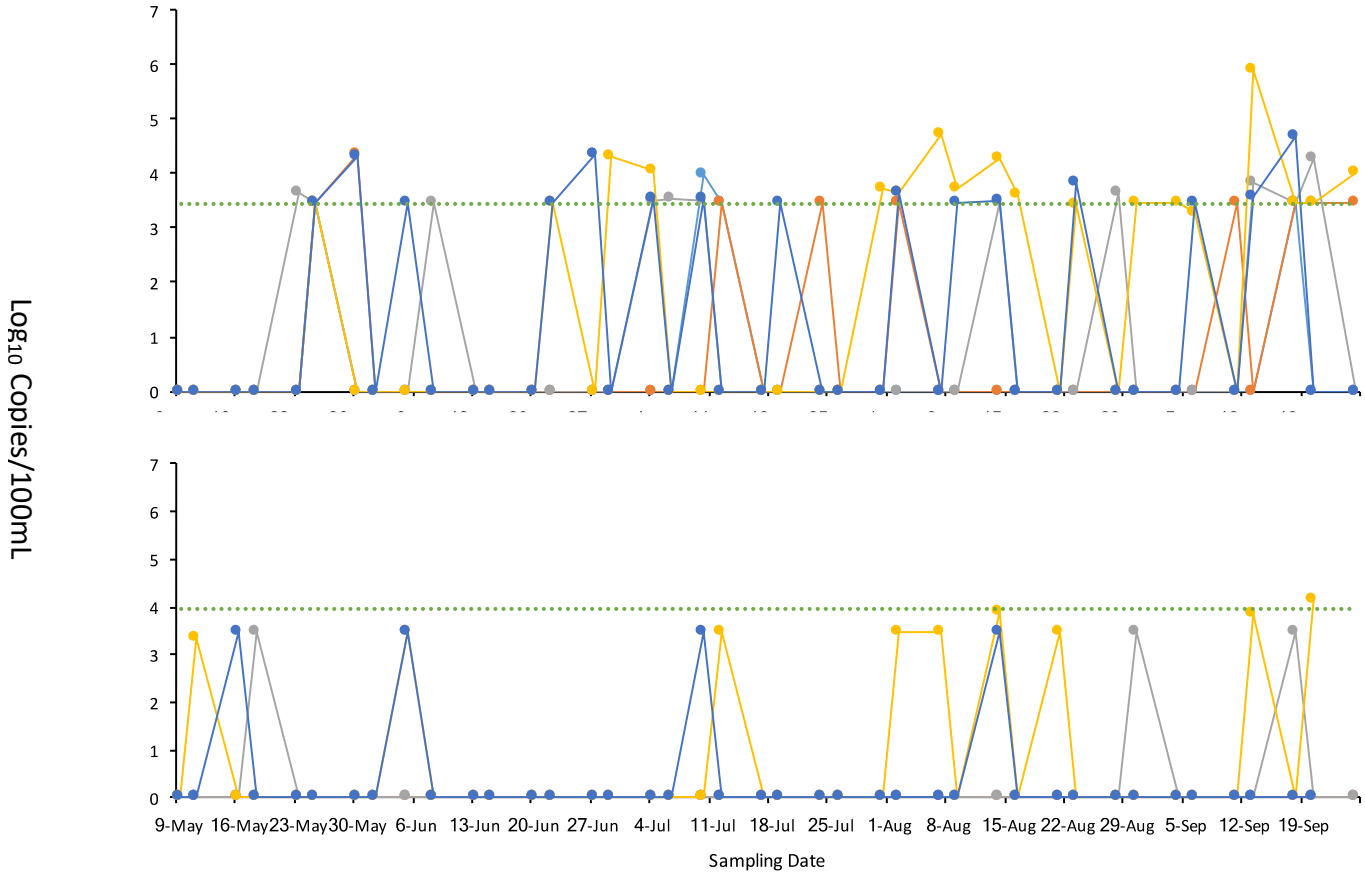


Figure 0-16: Temporal pattern of occurrence HF183 log_{10} (top) and HumM2 (bottom) concentrations at all sampling sites in Country Hills over the 21-week sampling season. Sampling site inlet WP31A is in blue, inlet WP31B in red, outfall WP31C in gray, outfall WP31D in yellow, outfall WP31E in dark blue and the limit of Quantiication$_{95}$ (LOQ) as a green dotted line.

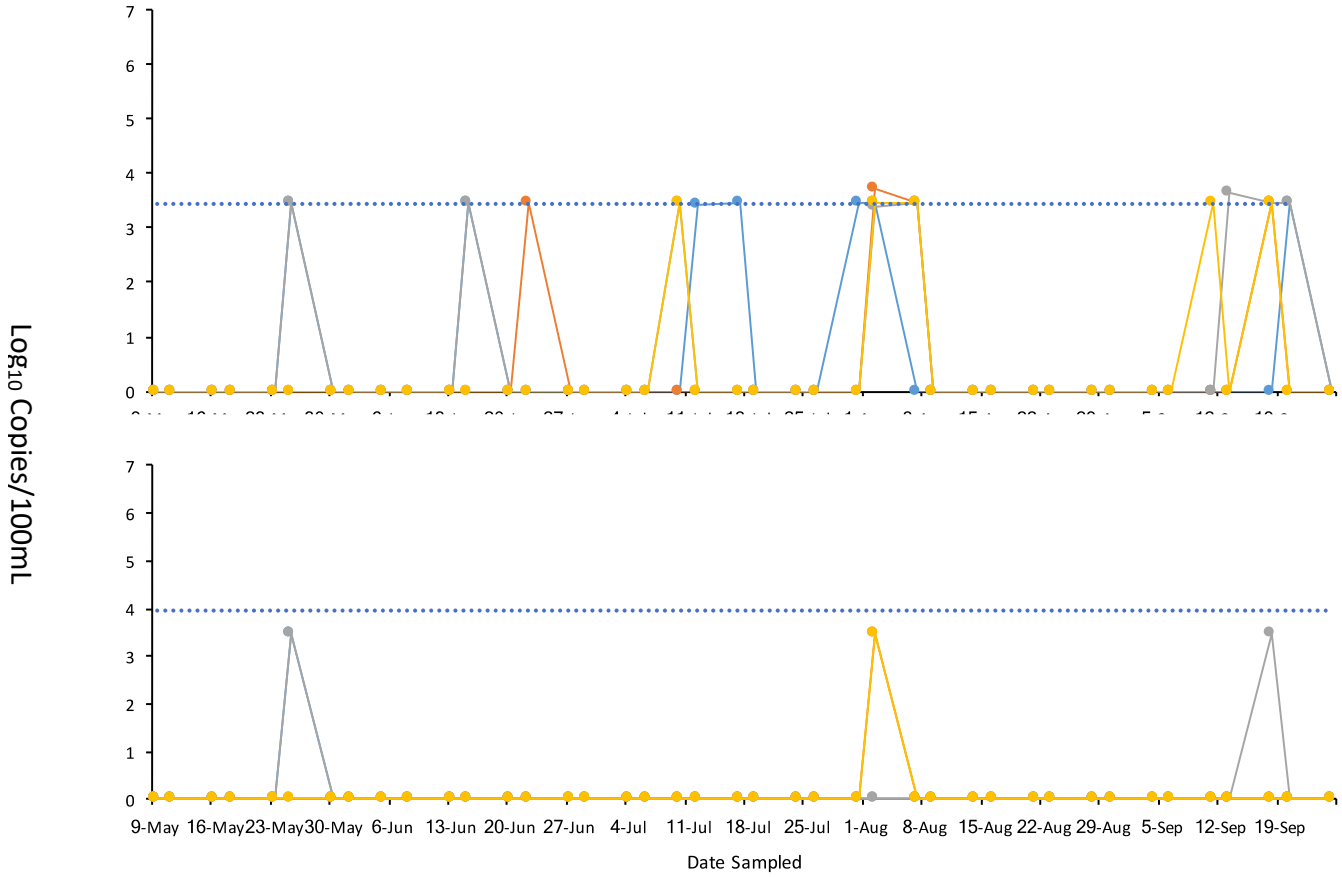


Figure 0-17: Temporal pattern of occurrence HF183 log_{10} (top) and HumM2 (bottom) concentrations at all sampling sites in Inverness over the 21-week sampling season. Sampling site outfalls/inlet is in blue, WP26B in red, WP26C in gray, outfall WP26D in yellow, and the Limit of Quantification$_{95}$ (LOQ) as a blue dotted line.

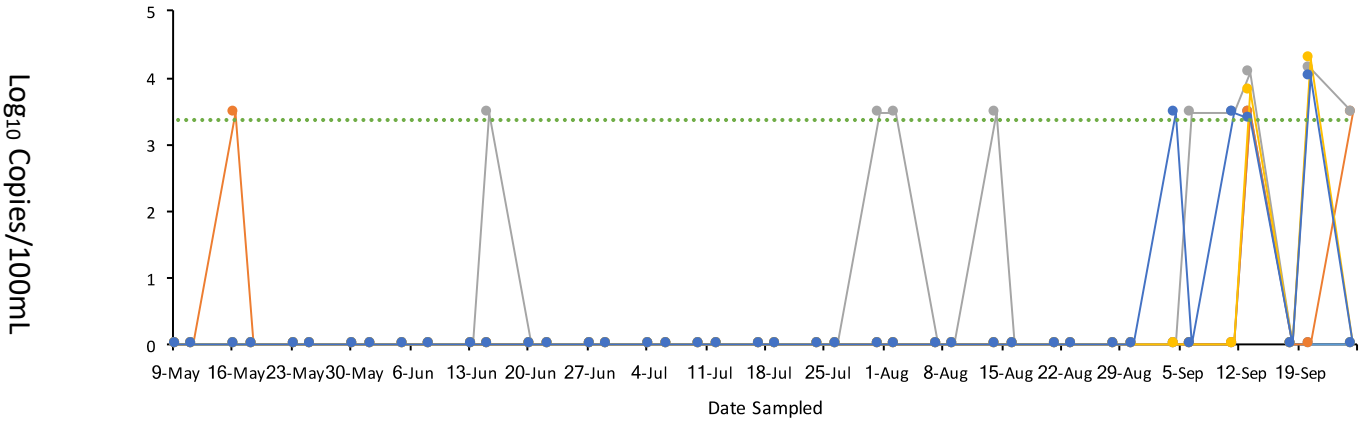


Figure 0-18: Temporal pattern of LeeSg contamination at all sampling sites in Country Hills over 21-weeks. The blue line represents WP31A, red line WP31B, gray line WP31C, yellow line WP31D, the dark blue line is WP31E and the green dotted line is the Limit of Quantification$_{95}$ (LOQ).

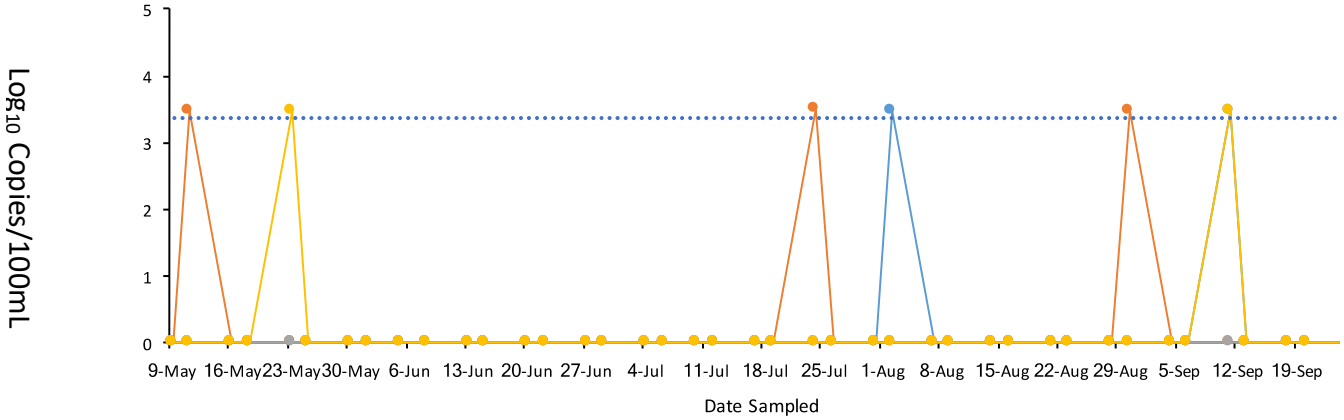


Figure 0-19: Temporal pattern of LeeSg contamination at all sampling sites in Inverness over 21-weeks. The blue line represents outfalls/inlet, red line WP26B, gray line WP26C, yellow line WP26D, the dark blue line dotted line is the Limit of Quantification$_{95}$ (LOQ).

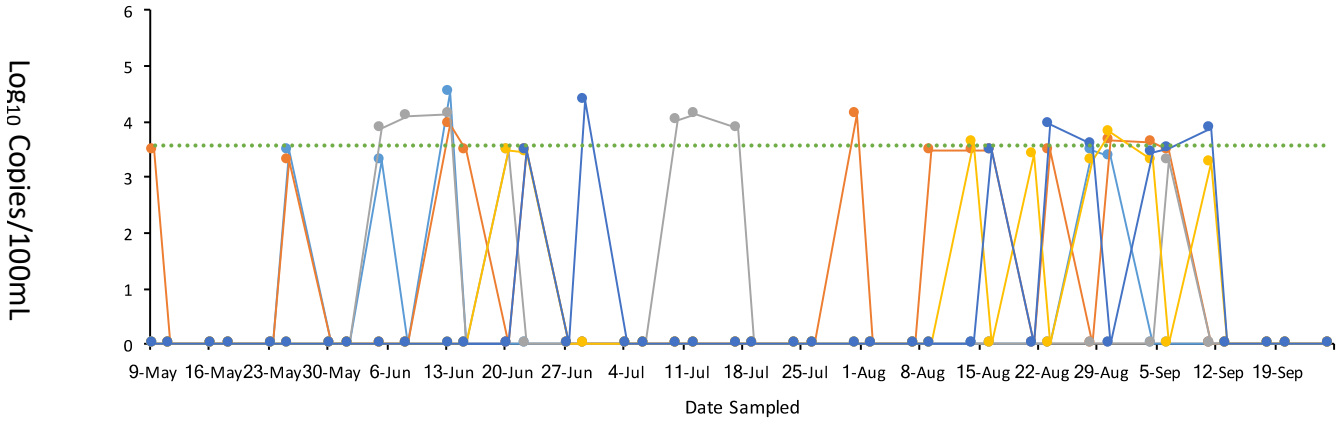


Figure 0-20: Levels of A. butzleri *in all sampling sites in Country Hills over 21 weeks. The blue line WP31A, red line is WP31B, grey line is WP31C, yellow line is WP31D, the dark blue line is WP31E and the Limit of Quantification$_{95}$ (LOQ) is a green dotted line.*

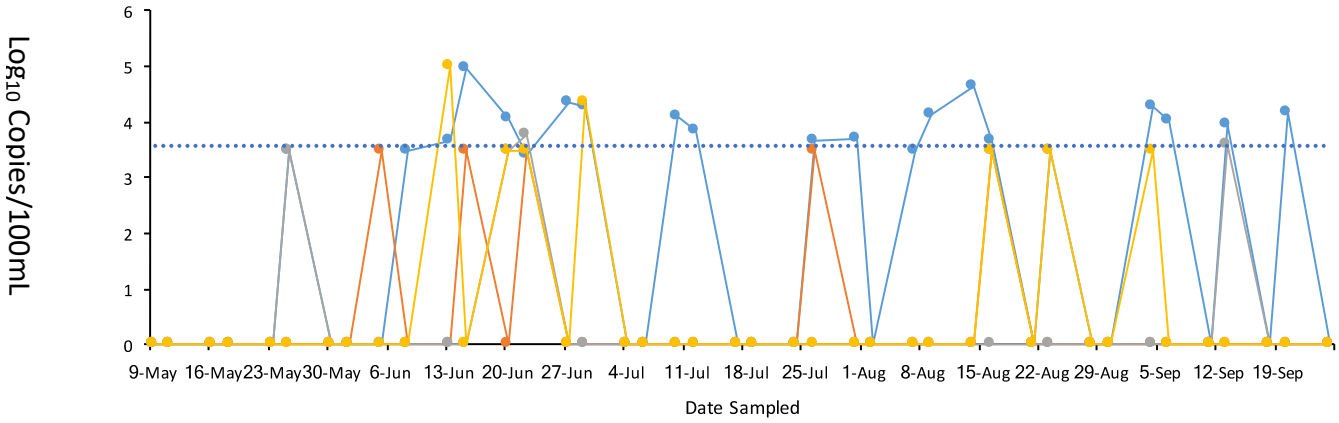


Figure 0-21: Levels of A. butzleri in all sampling sites in Inverness over 21 weeks. The blue line represents inlet outfalls/inlet, red line is WP26B, grey line is WP26C, yellow line is WP26D, and the Limit of Quantification$_{95}$ (LOQ) is a dark blue dotted line.