Assessing Public Health Risks of Stormwater Use in Alberta: Case Studies and Novel Approaches

by

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Abstract

Water scarcity is a growing concern in both the local and global spheres, with increasing urbanization, climate change, and population growth all contributing to a strain on potable and non-potable water supplies. Many jurisdictions across the world (including Alberta, Canada) are currently exploring water recycling, water reuse, and alternative source water use projects as approaches to supplement these stressed supplies. However, many alternative source waters, such as stormwater, are poorly characterized for acute (i.e., microbial) hazards, and thus require comprehensive study before they can be deemed to not be a risk to public health through their use. Consequently, this thesis focused on characterizing stormwater quality in Airdrie, Alberta (which was earmarked for non-potable use) in terms of general levels of fecal pollution, individual sources of fecal pollution, as well as for common waterborne gastroenteric and opportunistic bacterial pathogens. Key observations from this study included, firstly, that stormwater quality was typically poor and frequently exceeded recreational/ambient water quality guidelines. Secondly, fecal source signatures from a large variety of animal hosts were found to impact stormwater quality, including from humans, waterfowl, dogs, and ruminants. Human fecal signatures were detected in >25% of samples in 2021, and represented the most common tested pollution source. The concentration of human fecal markers suggested that human sewage approximated 0.1% of stormwater flows at times. Thirdly, tracing human fecal signatures in stormwater drains proved to be a useful tool for identifying cross-connections within the drainage networks in at least two cases. Fourthly, enteric and opportunistic respiratory pathogens were sporadically detected in stormwater, with *Arcobacter butzleri* found to be the most common gastroenteric pathogen, and observed in ~33% of water samples in 2021. *Legionella* spp. were found in virtually all stormwater

samples tested, albeit the respiratory pathogen *L. pneumophila* was only detected in a small number of samples and at low concentrations. Given that waterfowl (*Larus* spp. [gulls] and *Branta canadensis* [Canada geese]) were also common sources of fecal pollution in stormwater, a fecal survey was done to examine birds as a fecal source for some enteric pathogens (*Campylobacter* spp. and *Salmonella* spp.). Notably, the frequency and concentrations of *Campylobacter* spp. and *Salmonella* spp. were sparse in birds, albeit that shedding levels were high in the few birds that were infected (i.e., up to 6 $\log_{10}$ MPN/g in supershedders). The collective implication of these findings demonstrates that stormwater in Alberta is frequently contaminated by diverse fecal sources of pollution, including human sewage, and that bacterial pathogens can be common in stormwater. As a result, careful consideration of the risks from fecal contamination is necessary for stormwater to be used as an alternative water source, and the data presented in this thesis support the risk-based *Public Health Guidelines for Water Reuse and Stormwater* (2021) that were recently published by Alberta Health and Alberta Health Services.

Dedication

For my mother (Shannon), father (Richard), and sister (Kayley). I would never have been able to complete this work without your eternal love and support, and I thank you all for reminding me every day that I had it in me.

I also dedicate this thesis to my late grandfather, Raymond Carson, who taught me the value in education, and the endless search for the scientific truth.

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1. Introduction

1.1 Drivers of Alternative Water Use and Water Reuse

Water is vital for sustaining life on earth, and supplies of it are currently diminishing (He et al., 2021). By 2050, it is expected that nearly half of the urban global population will be affected by water scarcity due to climate change, exponential population growth, and increased urbanization (He et al., 2021). As a result of dwindling freshwater supplies, the use of alternative water sources, water reuse, and water recycling solutions have increasingly been developed to combat this burgeoning crisis (Murphy et al., 2017; Petterson et al., 2016; Sharvelle et al., 2017; Zaneti et al., 2013).

Alternative water sources are often water sources of variable water quality and have traditionally not been considered as sources for potable or non-potable purposes (e.g., wastewater, stormwater) in North America. Historically, most of these water sources have been managed as nuisances and not as resources. Stormwater is defined as the excess water runoff resulting from precipitation coming into contact with impervious urban surfaces, while roof-top collected rainwater is considered runoff from buildings that does not come into contact with ground surfaces, and where public access is limited or often restricted (Sharvelle et al., 2017). Water reuse, on the other hand, can be defined as secondary and subsequent use of water after it has already been used for a primary purpose. This is usually after an intermediary treatment step, with the primary and subsequent purposes not necessarily needing to be the same. Examples of water reuse include the use of wastewaters such as blackwater, greywater, and municipal wastewater (Alberta Health Services [AHS], 2021; Sharvelle et al., 2017), and in some cases

wastewater is also considered an alternative water source. Blackwater usually consists of wastewater from toilets and kitchen sinks, whereas greywater is generally defined as household wastewater that is not derived from toilets or kitchen sinks, such as from bathroom sinks, laundry machines, and showers (AHS, 2021; Sharvelle et al., 2017). Municipal wastewater is derived from municipal-level blended sewage (including greywater, blackwater, and other wastes such as from industry) (Sharvelle et al., 2017). Lastly, water recycling is defined as the reuse of water within a closed system, such as in a carwash that uses the *same* water cyclically after each round of use and with subsequent treatment (Zaneti et al., 2013).

Prospective possible uses for these source waters are extensive, including both potable (drinking water and cooking) and non-potable uses. Non-potable uses are currently being explored and can include both arable and non-arable land irrigation, aesthetic features such as water fountains, recreational purposes, dust control, clothes and vehicle washing, and toilet flushing (AHS, 2021; Sharvelle et al., 2017). Due to the potential of human exposure to microbial or chemical contaminants during potable or non-potable water use, however, an understanding of the risks of using these alternative source waters is required.

1.2 Water Quality Guidelines

1.2.1 General Water Quality Guidelines

Water quality guidelines exist to ensure the safety of the water from public health threats, including from both chemical and microbial hazards. While chemical hazards are important in the primary context of chronic health threats, for the purpose of this thesis the discussion will be mainly focused on microbial hazards, which present a more acute risk to human health. Microbial water quality guidelines or regulations exist locally, nationally, and internationally for many water sources and their intended uses, and can include guidelines/regulations for water

used for irrigation, recreation, drinking, industrial activities, or even reuse (AHS, 2021; Health Canada, 2010, 2012, 2020; Canadian Council of Ministers of the Environment [CCME], 1987; National Academies of Science, Engineering, and Medicine [NASEM], 2016; Sanz & Gawlik, 2014). In the United States, the *Clean Water Act* (see Copeland et al., 2016), and its Canadian counterpart the *Canada Water Act,* are the primary federal laws in each respective country for the regulation of general surface water quality. Organizations within each country such as the United States Environmental Protection Agency (US EPA) in the United States, as well as organizations in Canada including Health Canada and the Canadian Council of Ministers of the Environment (CCME), provide guidelines/regulations for water quality at a federal level, while also partnering with jurisdictions at the state or provincial level (US EPA, 2012a; CCME, 1987). Internationally, the World Health Organization (WHO) also produces its own guidelines for drinking water and recreational water, highlighting the need for consolidation of water quality criteria worldwide (WHO, 2017, 2021).

Most current microbial water quality measures are based on the testing of fecal indicator bacteria (FIB), with *E. coli,* for example, being used globally for over 100 years as a predominant indicator of microbial water quality, particularly when it comes to drinking water (Edberg et al., 2000; Government of Alberta, 2018; NASEM, 2016; US EPA, 2012a). The principle behind the use of these FIB as indicators of fecal pollution is that they are found in high concentrations in the human/animal intestinal tract, and can therefore be indicative of both fecal pollution and the potential consequent risk of this contamination when found in high concentrations within environmental waters (Ahmed et al., 2019a; Byappanahalli et al., 2012; Edberg et al., 2000; Field & Samadpour, 2007; Sauer et al., 2011; Steele et al., 2018). This has been corroborated in research studies carried out by the US EPA, such as the National Epidemiological and

Environmental Assessment of Recreational Water (NEEAR) studies, where a strong correlation between gastrointestinal illness risk and *Enterococcus* concentrations was observed in recreational waters affected by point-sources of human fecal contamination (Wade et al., 2003, 2006, 2010). These criteria generally call for the enumeration of FIB concentrations over a specified period of sampling (e.g., geometric mean, median, or average), as well as statistical measures to determine the acceptable limit of FIB in a single sample (e.g., statistical threshold value [STV], or single sample maximum [SSM]) (see Table 1-1).

Common FIB used both in the past and contemporarily in water quality guidelines include total coliforms, fecal coliforms, *Enterococcus,* and *E. coli* (Table 1-1). *Enterococcus* and *E. coli* are particularly common when it comes to recreational water quality guidelines and water reuse standards (Gov't of Alberta, 2018; Health Canada 2012; NASEM, 2016; US EPA, 2012a). It is important to note that while *E. coli* and *Enterococcus* are the primary FIB used in Canada and the province of Alberta for recreational water (Gov't of Alberta, 2018; Health Canada, 2012), fecal coliforms (also known as thermotolerant coliforms) and total coliforms have been utilized in previous editions of these guidelines (Alberta Environmental Protection, 1999; Health and Welfare Canada, 1992; Tobin & Ward, 1984), but are still widely used in many other international jurisdictions. Additionally, fecal coliforms are still in use in Canada for the purposes of wastewater and gray water reuse for toilet flushing (Health Canada, 2010), while total coliforms are used both for Canada's drinking water (Health Canada, 2020), and for irrigation water (CCME, 1987). In general, water quality guidelines such as those created by the US EPA have moved away from the use of fecal coliforms and total coliforms because current evidence suggests that *Enterococcus* and *E. coli* may be more robust and sensitive indicators of gastrointestinal illness (Noble et al., 2003; Wade et al., 2003).

Despite the widespread use of FIB, there is little agreement across countries and individual jurisdictions on what FIB are most appropriate to use, what concentrations of each are permissible within differing water bodies, and what end uses are permissible. For example, recreational water quality criteria from the WHO recommends the use of *Enterococcus* as a FIB exclusively, while guidelines in Canada and the United States use both *Enterococcus* and *E. coli*, though they differ in allowable concentrations of each [i.e., US EPA's geometric mean of 100 CFU/100 mL for *E. coli* in recreational water vs. Health Canada's geometric mean of 200 CFU/100 mL – see Table 1-1] (Health Canada, 2012; US EPA, 2012a; WHO, 2021).

Although recreational water quality criteria are grounded in epidemiological disease association studies, these guidelines are often used as a default for other water quality guidelines. For example, recreational water quality criteria are often used as justification for water quality standards for irrigation, water reuse, wastewater, ambient water quality (e.g., rivers, lakes streams) and aesthetic features. The argument is simple - if one can swim in it, then one should be able irrigate food crops with it or discharge their treated waste effluents at these levels. For example, FIB criteria in the Government of Alberta's *Alberta Surface Water Quality Guidelines* are based on the US EPA's *Recreational Water Quality Criteria*.

Table 1-1. Water quality guidelines for FIB commonly used in the United States and Canada, with a particular focus on recreational water quality and water reuse guidelines.

Regulation Type	FIB	Central Tendency (Type)*	Single Standard (Type)**	Reference
Recreational water quality (Alberta)	*Enterococcus*	N/A	1280 CCE/100 mL (STV)	Alberta Health, 2021
		N/A	>1280 CCE/100 mL but < 6400 CCE/100 mL†	
Recreational water quality (36/1000 Illness Risk) (USA)	*Enterococcus*	470 CCE/100 mL (GM)	2000 CCE/100 mL (STV)	US EPA, 2012a
	E. coli	126 CFU/100 mL (GM)	410 CFU/100 mL (STV)	
Recreational water quality (32/1000 Illness Risk) (USA)	*Enterococcus*	300 CCE/100 mL (GM)	1280 CCE/100 mL (STV)	US EPA, 2012a
	E. coli	100 CFU/100 mL (GM)	320 CFU/100 mL (STV)	
Recreational water quality (Canada)	*Enterococcus* (marine)	35 CFU/100 mL (GM)	70 CFU/100 mL (SSM)	Health Canada, 2012
	E. coli (freshwater)	200 CFU/100 mL (GM)	400 CFU/100 mL (SSM)	
Wastewater and Gray water reuse for the purpose of toilet and urinal flushing (Canada)	Thermotolerant (fecal) coliforms	N/A	200 CFU/100 mL (SSM)	Health Canada, 2010
	E. coli	N/A	200 CFU/100 mL (SSM)	
Drinking water (Canada)	Total coliforms	N/A	0 CFU/100 mL (SSM)	Health Canada, 2020
	E. coli	N/A	0 CFU/100 mL (SSM)	
Non-potable water reuse for indoor purposes (Texas)	Total coliforms	N/A	500 CFU/100 mL (SSM)	TWDB, 2006
	Thermotolerant (Fecal) coliforms	N/A	100 CFU/100 mL (SSM)	
Wastewater reuse (Disinfected secondary-2.2 recycled water) (California)	Total coliforms	2.2 MPN/100 mL (Median)	23 MPN/100 mL (SSM)	See Title 22
Wastewater reuse (Disinfected secondary-23 recycled water) (California)	Total coliforms	23 MPN/100 mL (Median)	240 MPN/100 mL (SSM)	See Title 22

Gray water reuse (Multiple-family Residential or commercial - Class C)	*E. coli*	2.2 MPN/100 mL (Average)	200 MPN/100 mL (SSM)	NSF 350
Gray water reuse (Single-family Residential - Class R)	*E. coli*	14 MPN/100 mL (Average)	240 MPN/100 mL (SSM)	NSF 350

* GM - Geometric mean

** These standards can exist as either a STV - Standard threshold value
or an SSM - single-standard maximum

†This higher standard of >1280 CCE/100 mL but <6400 CCE/100 mL is only reccomended if there is no evidence of human or ruminant *Bacteroides* spp. detected

CFU – Colony forming unit
MPN – most-probable-number
CCE – Calibrator cell equivalent

1.2.2 *Enterococcus* and *E. coli*

As noted above, two of the most common indicators used (particularly for recreational water quality guidelines) are *Enterococcus* and *E. coli*. Both of these organisms have previously been thought to be well-suited to this role, particularly due to the high concentrations they are found at in the human gastrointestinal tract, and their survivability and culturability when sampled from contaminated water (Ahmed et al., 2019a; Byappanahalli et al., 2012; Devane et al., 2020; Jang et al., 2017; Nshimyimana et al., 2014; Steele et al., 2018).

Enterococcus is a genus of gram-positive, non-spore forming bacteria that are obligate fermentative chemoorganotrophs that can be motile, though it depends on the species (Byappanahalli et al., 2012). Enterococci are found as flora in a large variety of animals, ranging from humans, birds, livestock animals, and pets (Ahmed et al., 2019a; Fogarty et al., 2003; Layton et al., 2010) to insects (Cox & Gilmore, 2007) and even on plants (Müller et al., 2001). While most enterococci found in the guts of animals are commensal in their hosts, a few species (such as *E. faecalis* and *E. faecium*) have been shown to cause nosocomial infections, often in part due to a penchant for antibiotic resistance (particularly vancomycin) (Arias & Murray, 2012; Byappanahalli et al., 2012; García-Solache & Rice, 2019). Importantly, this organism is also

frequently found in the environment, including in environmental and human sewage-impacted waters (Ahmed et al., 2019b; Byappanahalli et al., 2012; Devane et al., 2020).

E. coli is one of, if not the most, studied micro-organism to date, particularly due to its near ubiquitous presence across a diverse range of environments. This organism is a Gram negative, rod-shaped, facultative anaerobe, and is incredibly genetically diverse, with only ~2000 genes conserved across most strains in comparison to the ~18 000 genes across the entire pan-genome [i.e., all genes that have been identified in the species]) (Jang et al., 2017; Yu et al., 2021). Most strains are not considered pathogenic by default, unless specific virulence factors are present (e.g., the *stx1 and stx2* genes that indicate the presence of the enterohemorrhagic Shiga-toxin producing *E. coli* [STEC]) (Bryan et al., 2015; Jang et al., 2017; Yu et al., 2021). Like *Enterococcus*, *E. coli* can be found in a large range of environments, including in many different animals ranging from humans to reptiles, birds, and other mammals (Ahmed et al., 2019a; Gordon & Cowling, 2003; Fogarty et al., 2003), as well as in environmental waters, with naturalized strains having also been described in municipal sewage (Jang et al., 2017; Yu et al., 2021; Zhi et al., 2019).

As noted above, FIB including *Enterococcus* and *E. coli* can be found in a large variety of animal hosts, though it is important to note that FIB differences exist between hosts in terms of differing carriage rates, and concentrations (Ahmed et al., 2019a; Ervin et al., 2013; Fogarty et al., 2003; Gordon & Cowling, 2003; Layton et al., 2009, 2010; Lu et al., 2011a; Middleton & Ambrose, 2005; Vogt et al., 2018). For example, *E. coli* and *Enterococcus* are not always found in gull feces (36 – 80% prevalence), and only 75-85% of human fecal samples are positive for *Enterococcus* (Ahmed et al., 2019a; Ervin et al., 2013; Fogarty et al., 2003; Layton et al., 2009, 2010; Lu et al., 2011a; Middleton & Ambrose, 2005; Vogt et al., 2018). The concentrations of

both FIB can vary widely in animals, ranging from 4 – 9 $\log_{10}$ CFU/g (or 5 – 10 $\log_{10}$ copies/g by qPCR), though these concentrations can also vary heavily between different host organisms, with human fecal *Enterococcus* estimates being up to 3 $\log_{10}$ MPN or CFU/g lower in concentration than for birds (e.g., gulls), while *E. coli* concentrations appear similar between these two hosts (Ahmed et al., 2019a; Ervin et al., 2013; Fogarty et al., 2003; Layton et al., 2009, 2010; Lu et al., 2011a; Middleton & Ambrose, 2005; Vogt et al., 2018).

In addition to the above, predominance of one FIB over another in different hosts, and even host-specific differences in FIB species or genotype can exist (Ahmed et al., 2019a; Ervin et al., 2013; Fogarty et al., 2003; Gordon & Cowling, 2003; Layton et al., 2010; Middleton & Ambrose, 2005). For example, *E. coli* concentrations can be higher than *Enterococcus* concentrations by as much as 3 $\log_{10}$ in many animals (including chickens, deer, humans, and pigs), while some evidence suggests there can be nearly equivalent concentrations of *Enterococcus* and *E. coli* when found in other animals such as in dogs, gulls, geese, and cattle (Ahmed et al., 2019a; Ervin et al., 2013; Fogarty et al., 2003; Middleton & Ambrose, 2005). Lastly, it is also notable that different host species may harbor strains of host-associated *E. coli* that are more prominent over others, as well as different species of *Enterococcus* that appear more frequently than others (Gordon & Cowling, 2003; Layton et al., 2010).

Importantly, the above studies demonstrate that there are many animals with either higher FIB prevalence and/or much higher FIB concentrations in their feces than humans (dogs, gulls, some farm animals), which can potentially lead to an overassessment of the impact of human fecal sources in environmental waters where FIB is the only indicator of fecal pollution used (Ahmed et al., 2019a; Ervin et al., 2013; Fogarty et al., 2003). This can be particularly so for aquatic birds such as gulls and geese, which have been found to carry up to 8-9 $\log_{10}$ CFU/g of

Enterococcus and *E. coli* (Fogarty et al., 2003; Middleton & Ambrose, 2005). This presents a problem for risk assessment of waters when it is considered that zoonotic transmission of pathogens generally carries a lower risk to people than human-to-human transmission via human fecal input (Brown et al., 2017a; Schoen & Ashbolt, 2010; Soller et al., 2010). Predominance of one FIB over another (i.e., *Enterococcus* over *E. coli*) and/or specific species of enterococci or genotypes of *E. coli* found within environmental waters can possibly be indicative of fecal hosts, though identification of individual species and genotypes can be a time-consuming process, and the same species or genotype of FIB can often still be found in other related hosts (Gordon & Cowling, 2003; Layton et al., 2010).

In addition to the above evidence of the lack of host-specificity of FIB, there are several other outstanding issues when using FIB enumeration in isolation to indicate water quality, such as mounting evidence that *Enterococcus*, *E. coli*, and thermotolerant coliforms concentrations do not always correlate well with gastrointestinal disease risk in environmental waters where there is no known point source of human fecal contamination (Arnold et al., 2013; Colford et al., 2007; Fleisher et al., 2010; Griffith et al., 2016). Similarly, current evidence also suggests that FIB in general are not well correlated with some bacterial pathogens that may be present in environmental waters (Bradshaw et al., 2016; de Man et al., 2014; Fremaux et al., 2009; Jokinen et al., 2010; Lee et al., 2012; Savichtcheva et al., 2007; Schriewer et al., 2010; van Dyke et al., 2010), though this is with the notable exception of *Arcobacter* spp. when point-sources of wastewater are involved (Collado et al., 2008; Webb et al., 2017). Further evidence also points to FIB being a poor surrogate for pathogens (viruses and parasites) due to their assumed inability to thrive outside of a host's gastrointestinal tract in environmental waters and sediments (Ahmed et al., 2021; Lemarchand & Lebaron, 2003; Nasser et al., 2003; Wait & Sobsey, 2001). As

mentioned previously, however, environmental strains of both *Enterococcus* and *E. coli* have been found that are conversely shown to survive and replicate within environmental waters (Byappanahalli et al., 2003, 2012; Devane et al., 2020; Jang et al., 2017; Zhi et al., 2019). Based on the above, FIB concentrations in environmental waters become difficult to interpret in terms of risk without additional methods to determine fecal sources, though they still allow for a generalized estimate in environmental waters of total non-specific fecal pollution.

1.2.3 Microbial Source Tracking (MST)

Microbial source tracking (MST) methods – tools useful for assessing the host sources of fecal pollution in a water body – have been developed to counterbalance one of the principal weaknesses of FIB used to assess fecal contamination in environmental waters – namely, that FIB are non-specific to particular sources of contamination, and are found in the GI tract of many different animals (Ahmed et al., 2019a; Ervin et al., 2013; Fogarty et al., 2003; Gordon & Cowling, 2003). Given that it is generally agreed upon that human sources of fecal pollution (such as from damaged sanitary sewer infrastructure, or illicit cross-connections) cause a higher risk of illness when compared to animal contamination (though risk is still not zero), the distinction of different fecal contamination sources can be of particular importance for public health (Brown et al., 2017a; Schoen & Ashbolt, 2010; Soller et al., 2010).

While a variety of methods have been developed for MST which can range from library-dependent methods such as antibiotic resistance profiles (Field & Samadpour, 2007), to chemical markers of human waste (e.g., caffeine, aspartame – see Hachad et al., 2022; Sidhu et al., 2013), and even to sewage-sniffing canines (Van de Werfhorst et al., 2014), one of the most popular methods have been the use of molecular qPCR-based methods (Harwood et al., 2014). Most MST methods allow for the detection of host-specific microbial markers of fecal pollution and a

quantitative estimate of the number of copies of a given gene or genetic fragment within a given volume of water (Field & Samadpour, 2007; Girones et al., 2010; Harwood et al., 2014; Holcomb & Stewart, 2020). A large variety of qPCR MST markers have been developed (summarized in Table 1-2), with a particular focus on markers of human sewage contamination, as well as common culprits of fecal contamination into environmental waters including household pets, livestock, and waterfowl. By being able to track fecal pollution to specific human or animal sources, risks may be better understood, and effective corrective action more easily taken.

Table 1-2. List of PCR and qPCR markers commonly used for microbial source tracking (MST) in environmental waters for human and non-human fecal sources, including tested sensitivity and specificity of each marker.

Host	Organism (target)	Assay Name*	Target	Sensitivity	Specificity	Reference(s)
Human	*Bacteroides* spp.	**HF183**	*16S* rRNA	100%[A, B, D, F, G]	46-100%[A, B, D, F, G†]	Bernhard & Field, 2000; Haugland et al., 2010; Seurinck et al., 2005
	Bacteroidales	**HumM2**	?	93-100%[A]	75-99%[A]	Shanks et al., 2009
	Bacteroidales	BacHum	*16S* rRNA	96-100%A	59-97%[A]	Kildare et al., 2007
	Bacteroidetes	BacH	*16S* rRNA	100%A	77-100%[A]	Reischer et al., 2007
	Bacteroides spp.	HuBac	*16S* rRNA	100%D	61-68%[D]	Layton et al., 2006
	Homo sapiens	Humito	*NADH dehydrogenase subunit 5*	Not assessed	Not assessed	Martellini et al., 2005
Ruminant	*Bacteroidetes*	BacR	*16S* rRNA	100%A	79-100%[A]	Reischer et al., 2006
	Bacteroidales	**Rum2Bac**	*16S* rRNA	97%-100%A	97%-100%[A]	Mieszkin et al., 2010
	E. coli	CF193	*16S* rRNA	68-100%A, E	97-100%[A, E]	Bernhard & Field, 2000
Cattle	*Bos* spp. (bovines)	Bomito	*Nadlt5*	Not assessed	Not assessed	Martellini et al., 2005
	Bacteroidales	CowM3	Sialic acid-specific 9-O-acetylesterase secretory protein homolog	100%A	100%[A]	Shanks et al., 2010a
	Bacteroidales	BacCow	*16S* rRNA	100%A	50-95%[A]	Kildare et al., 2007
Dogs	*Bacteroidales*	**Dog3**	fatty acid coA ligase	77%	100%	Green et al., 2014
	Bacteroidales	BacCan	*16S* rRNA	63%-100%A	82-90%[A]	Kildare et al., 2007
	Bacteroides spp.	DogBact	*16S* rRNA	100%A	55%[A]	Shibata et al., 2010
	Canis spp. (canines)	DogMito	Mitochondrial DNA	100%	100%	Monteiro et al., 2021
Gull	*C. marimmamalium*	Gull4	*16S* rRNA	87%	91%	Ryu et al., 2012
	C. marimmamalium	**LeeSG**	*16S* rRNA	81%-100%A, C	86-94%[A, C‡]	Lee et al., 2013
	C. marimmamalium	GFC	*16S* rRNA	64%	94%	Green et al., 2012
Avian	*Helicobacter* spp.	GFD	*16S* rRNA	57%	100%	Green et al., 2012
Goose	*Bacteroidales*	**CGO1**	*16S* rRNA	57%	100%	Fremaux et al., 2010
Muskrat	*Bacteroidales*	Mubac	*16S* rDNA	66%	100%	Marti et al., 2011
Sheep	*Ovis* spp. (ovines)	Ovmito	*Nadlt5*	Not assessed	100%[A]	Martellini et al., 2005
Horse	*Bacteroidales*	HoF597	*16S* rRNA	50-100%A	92-100%[A]	Dick et al., 2005
Pig	*Sus* spp. (swine)	Pomito	*cytochrome c oxidase subunit II*	Not assessed	Not assessed	Martellini et al., 2005
	Bacteroidales	PF163	*16S* rRNA	87-100%A	90-100%[A]	Dick et al., 2005
	Bacteroidales	Pig2Bac	*16S* rRNA	100%A	73-100%[A]	Mieszkin et al., 2009
Cat	*Felis* spp. (felines)	CatMito	Mitochondrial DNA	100%	99.1%	Monteiro et al., 2021

Deer	*Odocoileus virginianus* (white-tailed deer)	Un-named	*MtCytB*	100%	100%	Schill & Mathes, 2008
Chicken	*Brevibacterium* spp.	LA35	*16S* rRNA	76%	100%	Weidhaas et al., 2010

* Assays in bold were used in the following studies executed for this thesis.

† The low specificity of 46% in Boehm et al., 2013 was found to be much higher when considering concentrations within the level of quantification (such as seen in human sewage), and specificity was as high as 91% when disqualifying DNQ results.

‡ The results presented by Sinigalliano et al. (2013) for LeeSG specificity did not count pigeon feces as cross-reactive, though there was a high rate of cross-reaction (e.g. 100% (n=6) of pigeon samples tested positive) in pigeon feces specifically and exclusively.

A Sensitivity/specificity was also assessed by Boehm et al., 2013.

B Sensitivity/specificity was also assessed by Shanks et al., 2010b.

C Sensitivity/specificity was also assessed by Sinigalliano et al., 2013.

D Sensitivity/specificity was also assessed by Kildare et al., 2007.

E Sensitivity/specificity was also assessed by Shanks et al., 2010a.

F Sensitivity/specificity was also assessed by Mayer et al., 2018.

G Sensitivity/specificity was also assessed by Fremaux et al., 2010.

Despite significant advantages of their use, MST methods using qPCR also have drawbacks. For example, although MST markers are highly sensitive (see Table 1-2) and can be more specific than culture methods, both cross-reactivity and inhibition can affect assay specificity and sensitivity, respectively (Boehm et al., 2013; Schrader et al., 2012; Shanks et al., 2010a,b; Sinigalliano et al., 2013). While issues with cross-reactivity may occur for commonly used MST markers, specificity appears to be preserved when these markers are detected at higher concentrations (Boehm et al., 2013; Mayer et al., 2018; Shanks et al., 2010b). Inhibition, on the other hand, occurs during qPCR when complex substances interfere with either the polymerase or DNA template, but can be detected and overcome via internal DNA controls and sample dilution (Cao et al., 2012; Haugland et al., 2010; Opel et al., 2010; Schrader et al., 2012).

To address the issue of determining human-derived fecal pollution sources, several MST markers have been designed for human sewage, particularly markers developed for the detection of human-specific *Bacteroides* spp. (see Table 1-2) (Bernhard & Field, 2000; Converse et al., 2009; Haugland et al., 2010; Kildare et al., 2007; Layton et al., 2006). The HF183 marker, which was originally developed by Bernhard & Field (2000) and improved upon by Seurinck et al. (2005) and Haugland et al. (2010), has been particularly popular due to its high sensitivity (generally ~100%) and specificity (generally 80-100%) for human sewage (Ahmed et al., 2019c; Boehm et al., 2013; Edge et al., 2010; Fremaux et al., 2009; Mayer et al., 2018). It's important to point out that despite a high sensitivity in human sewage, this marker is not perfectly sensitive (58% - 94%) in individual fecal samples, suggesting importantly that not all humans carry certain *Bacteroides* spp. (Ahmed et al., 2019c; Fremaux et al., 2009; Mayer et al., 2018). This marker in particular is also known to cross-react (though relatively infrequently) with feces from dogs and chickens, albeit the concentration in these animals is typically far lower than that observed in humans (Boehm et al., 2013; Haugland et al., 2010; Mayer et al., 2018).

Due to the potential for cross-reaction (especially with dog feces), and the importance of determining whether fecal pollution is primarily from human sewage or not, additional human fecal markers (such as HumM2) have been used alongside HF183 to more accurately determine fecal inputs (Gonzalez et al., 2020; Napier et al., 2017). Like HF183, HumM2 is not perfectly sensitive nor specific (See Table 1-2), though sensitivity and/or specificity has been found to be >90% (Boehm et al., 2013; Shanks et al., 2010b). Importantly, HumM2 was found to cross-react with elk and sheep fecal samples, though once again at a low concentration and which represented <1% of total animal fecal samples assayed (Shanks et al., 2010b).

Similar to markers for human-derived fecal pollution, the most utilized markers designed for animal sources of pollution are generally also both sensitive and specific, and are designed around the bacterial organisms commonly found in host guts (see Table 1-2). For example, markers utilized for the identification of gull fecal contamination (such as LeeSG, Gull4, and GFC) are often based on the *16S* rRNA of *Catellicoccus marimammalium* (Green et al., 2012; Lee et al., 2013; Lu et al., 2008; Ryu et al., 2012). This organism is related to *Enterococcus*, but appears to be specific to the intestines of gulls, and is also found in very high concentrations in the intestinal tract and feces of gulls based on qPCR (Brown et al., 2017b; Green et al., 2012; Koskey et al., 2014; Lee et al., 2013; Lu et al., 2008; Ryu et al., 2012). In contrast, several markers such as those designed for dogs (e.g., Dog3), Canada geese (e.g., CGO1), and ruminants (e.g., Rum2Bac) are designed around animal gut-specific *Bacteroides* spp., often using a fragment of the *16S* rRNA gene of this organism (though Dog3 has been designed around a gene fragment for the long chain fatty acid-CoA ligase enzyme) (Fremaux et al., 2010; Green et al., 2014; Mieszkin et al., 2010).

The sensitivity, specificity, and individual marker concentrations found in host feces can vary in these animals (Boehm et al., 2013; Brown et al., 2017b; Fremaux et al., 2010; Green et al., 2014; Lee et al., 2013; Sinigalliano et al., 2013; Mieszkin et al., 2010). However, both sensitivity and specificity for the MST markers used in the studies of this thesis (LeeSG, CGO1, Dog3, Rum2Bac) have generally both been found to be >75% (Boehm et al., 2013; Fremaux et al., 2010; Green et al., 2014; Lee et al., 2013; Sinigalliano et al., 2013; Mieszkin et al., 2010). There are a few exceptions, however, such as lower sensitivity reported for the CGO1 assay (57% - see Fremaux et al., 2010), while specificity was low (49.4%) for LeeSG in one study only when considering pigeon cross-reaction (Sinigalliano et al., 2013).

In terms of the practical usage of MST methods, it has been previously suggested that human sewage markers could potentially be used as an indicator of pathogens and subsequent illness risk that may be better-suited than FIB, though evaluation has shown mixed results (Ahmed et al., 2018; Boehm et al., 2018; Brown et al., 2017a; Kauppinen et al., 2019; Schoen et al., 2020; Staley et al., 2012). Studies have suggested that HF183 may have a more similar decay rate in water compared to enteric pathogens (Boehm et al., 2018), and one that differs from the decay rates of FIB such as *E. coli* and *Enterococcus* (Dick et al., 2010; Walters & Field, 2009). However, other studies show that HF183 has a differing decay rate than enteric pathogens such as *Campylobacter* spp. (Ahmed et al., 2021), suggesting that decay rates can be variable. The relationship between human sewage marker and detected enteric pathogen prevalence and concentrations in water is also variable (see Bradshaw et al., 2016; Cui et al., 2019; Fremaux et al., 2009; Sales-Ortells & Medema, 2015; Schriewer et al., 2010; Steele et al., 2018; Walters et al., 2007), while some epidemiological evidence (Napier et al., 2017) shows that relationships between HF183 and enteric illness can be inconsistent in some circumstances. The above makes sense considering that focusing completely on human sewage neglects non-human sources of fecal contamination (see Ahmed et al., 2019a, Jokinen et al., 2011), and thus the chance for indirect zoonotic transmission of common pathogens such as *Campylobacter* spp., *Salmonella*, and pathogenic *E. coli* (European Food Safety Authority [EFSA] & European Centre for Disease Prevention and Control [ECDC], 2021).

Several studies have also very recently been published that have shown the practical usage of MST to not only quantify and identify sources of pollution, but to also track these sources upstream to specific point sources of pollution before verifying when mitigation efforts have been successful (Gonzalez et al., 2020; Hachad et al., 2022; Sauer et al., 2011). For example,

Gonzalez et al. (2020) used the human sewage marker HF183 (alongside the marker humM2 and the FIB *Enterococcus*) to investigate upstream of three different creeks and rivers in Virginia fed by fecally- contaminated stormwater. Gonzalez' group were able to not only isolate hot spots where contamination was consistently detected in the Wayne Creek, Broad Creek, and Nansemond River, but also were able to delineate where human sewage was infiltrating into these waters (including infrastructure failures such as broken sewer pipes, sewer blockages, and failing septic systems) as well as successfully verify remediation efforts in 2 of 3 rivers by confirming a reduction or absence of human sewage detection after repairs. Similarly, Hachad et al. (2022) recently used increasing concentrations of HF183 to identify illicit cross-connections into the stormwater sewer from the sanitary sewer contaminating a stream north-west of Montreal, while Sauer et al. (2011) were able to track HF183 concentrations further upstream and into the stormwater drainage network flowing into the Menomonee River, though in the latter case this group was unable to locate the exact source of pollution.

It has also been suggested that MST methods can be useful for forensic purposes, both in regards to outbreaks of gastrointestinal illness, as well as for legal purposes such as litigation of parties contaminating water bodies with agricultural waste (Kauppinen et al., 2019; Teaf et al., 2018). In the former case, Kauppinen's group (2019) were able to utilize HF183 to confirm areas of human fecal contamination in the case of a large waterborne outbreak of gastreoenteritis in Finland (>900 affected), utilizing this marker also to confirm the success of remediation efforts where an infrastructure failure had previously contaminated the drinking water. While seldom employed up to this point for true forensic usage (i.e., to investigate and litigate in the case of crime), Teaf et al. (2018) speculated on and provided several case studies of how MST can and

has been used (albeit incompletely) to investigate negligence in agricultural fecal contamination into waters.

To summarize, most modern water quality guidelines use microbial water quality measures that are based solely on FIB enumeration, and this includes a wide range of water uses such as irrigation, recreation, and water reuse. MST technology can heavily supplement FIB data by specifying and quantifying the sources of fecal pollution within environmental waters. This becomes especially vital for an understanding of the risks of using alternative source waters such as stormwater, and for understanding what mitigation efforts may be required to avoid fecal contamination from certain sources (i.e., human sewage in stormwater).

1.2.4 Alternative Water Use and Water Reuse Guidelines

Few countries have health-based guidelines for water reuse and alternative source water use, and the guidelines that do currently exist are inconsistent across both countries and local jurisdictions. At least 6 countries in Europe, 27 states in the US, as well as 10 additional countries including Australia and Canada each have water reuse and/or alternative source water use guidelines of some description (NASEM, 2016; Sanz & Gawlik, 2014). The vast majority of these guidelines are quite limited in scope, and vary widely between each other in terms of allowed end uses, safety benchmarks for microbial quality, as well as what alternative source waters may be utilized (NASEM, 2016; Sanz & Gawlik, 2014). For example, guidelines from France, Israel, Mexico, and Portugal are based solely on the reuse of urban and industrial wastewater, specifically in the context of irrigation (generally agricultural) (Sanz & Gawlik, 2014). Before the publication of *Public Health Guidelines for Water Reuse and Stormwater Use* from Alberta Health Services (AHS) in 2021, criteria in Canada has only existed strictly for

wastewater reuse and greywater use in the form of toilet and urinal flushing (Health Canada, 2010).

Stormwater use guidelines exist within Canada, Australia, and in several U.S. states, though they also vary widely in their individual scope, criteria, and guiding principles (AHS, 2021; Los Angeles County Department of Public Health [LACDPH], 2016; NASEM, 2016; Natural Resource Management Ministerial Council [NRMMC] et al., 2009; Sharvelle et al., 2017; Texas Water Development Board [TWDB], 2006). While the vast majority of these guidelines use some measure of FIB enumeration, they again vary on both the concentration set as criteria, as well as what FIB are used (LACDPH, 2016; NASEM, 2016). For example, varying guidelines in California suggest using total coliforms, fecal coliforms, and/or *E. coli* (LACDPH, 2016; NASEM, 2016). Recommended FIB concentrations are also dependent on the municipality, as well as competing guidelines within municipalities such as those set out by Title 22 legislation and/or modified NSF350 requirements in California (LACDPH, 2016; NASEM, 2016). Many stormwater use guidelines also do not include many allowable end uses, or the criteria are extrapolated to be used across multiple end-uses which may have potentially different risks (LACDPH, 2016; NASEM, 2016; Schoen et al., 2017; TWDB, 2006). Additional to the above, most stormwater use guidelines currently rely solely on end-point testing of treatment processes, which can be problematic for several reasons including potential post-treatment problems from opportunistic pathogens such as *Legionella pneumophila* (Ashbolt, 2015; NASEM, 2020; Sharvelle et al., 2017).

In summary, the majority of alternative source water quality guidelines are not based on the most current science, and are rather inconsistent in their criteria across both countries and municipalities. The principles used (namely FIB enumeration alone as a proxy for pathogen

presence) tend to oversimplify and underassess the complexity of these systems, and therefore the current risks in alternative source water use and water reuse. However, different approaches to criteria for risk when using these waters are possible, and are currently being pursued.

1.3 Alberta's Approach to Water Reuse and Alternative Source Water Use

Alberta recently released (2021) the document entitled *Public Health Guidelines for Water Reuse and Stormwater Use*, and which follows an alternative approach to understanding risk in water reuse and alternative source waters similar to that proposed in Australia and the City of San Francisco (AHS, 2021; NRMMC et al., 2009; San Francisco Public Utilities Commission [SFPUC], 2020). As opposed to end-point measurements of FIB such as *E. coli*, *Enterococcus*, or fecal coliforms being the single benchmark for safety, each of these guidelines are based in the principle of quantitative microbial risk assessment (QMRA) (AHS, 2021; Haas et al., 2014; NRMMC et al., 2009; SFPUC, 2020; Sharvelle et al., 2017; WHO, 2016). QMRA is a method of calculating likelihood of acute risk of illness from microbial hazards, and does so via following sequential steps common to all environmental risk assessments: hazard identification, exposure assessment, dose-response assessment, and risk-characterization (Haas et al., 2014; WHO, 2016). Unlike traditional methods of microbial water quality based on FIB enumeration and inconsistent epidemiological associations of these FIB with illness, QMRA accounts for a far larger number of factors (Haas et al., 2014; WHO, 2016). Using QMRA also allows for standardization of acceptable risk, with recent models often using the WHO's 10^{-6} disability-adjusted life years (DALYs) per person per year (pppy) as a generally acceptable threshold for risk (Schoen et al., 2017; WHO, 2016).

Through the above framework, water quality criteria for alternative source waters are designed around the concept of 'fit-for-purpose', reflecting an acceptable level of risk to avoid excessive costs via unnecessary levels of treatment, while still maintaining public safety for water intended for certain purposes (AHS, 2021; Sharvelle et al., 2017). Using this approach, $\log_{10}$-reduction targets (LRTs) are set against an acceptable level of infection risk for a specified use of the water, thereby ensuring pathogen levels are reduced between the source (i.e., input) and the designated end use (i.e., output) (AHS, 2021; Haas et al., 2014; Schoen et al., 2017; SFPUC, 2020; Sharvelle et al., 2017; WHO, 2016). Through the use of frameworks that consider the high level of complexity and multitude of variables involved in water reuse projects, a better understanding of public health risk can be attained and acted upon.

Hazard identification, one of the first steps of QMRA, requires an understanding of various risks that can be found in an alternative source water use or water reuse system, such as fecal pollution sources (i.e., human sewage contribution), pathogen presence and concentrations, as well as general estimates of fecal pollution via FIB (AHS, 2021; Haas et al., 2014; Schoen et al., 2017; Sharvelle et al., 2017; WHO, 2016). Note, however, that while chemical hazards that pose chronic risks to users of alternative source waters are important to assess for and cannot be neglected, QMRA focusses on the acute risk demonstrated from microbial threats (Haas et al., 2014; WHO, 2016).

Hazard identification is poorly understood in some alternative source waters and some recycled water systems– even when considering the recent increase in QMRA studies on water use/reuse (Murphy et al., 2017; Petterson et al., 2016; Sales-Ortells & Medema, 2015; Schoen et al., 2017). For example, although there is considerable data on pathogen occurrence and microbial water quality in stormwater, to the author's knowledge, there is no published data

specifically on microbial hazards found within stormwater in Alberta, with the exception of Beaudry (2019). For the purposes of the contents of this thesis, stormwater use will be the main focus in the rest of this section.

Stormwater has been increasingly seen as a viable alternative source water for non-potable water use, though this was not always the case (Sharvelle et al., 2017). Alberta has historically viewed stormwater through the lens of it being a nuisance water (see *Stormwater Management Guidelines for the Province of Alberta* (1999)), and therefore management has been focused on pollution and flooding control of receiving waters, particularly through the mitigation of total suspended solids (TSS). With the recent introduction of new guidelines based partly on non-potable stormwater use, a shift in perspective is required to see stormwater as not simply a nuisance water but an untapped resource for water uses traditionally sourced from potable supplies.

Stormwater management is primarily split into two types of systems: combined sewer systems, and segregated drainage systems. Historic combined sewer systems are designed with the stormwater sewer and sanitary sewer connected so that in the event of a major rain event, both sewers can be flushed at once in a combined sewer overflow (CSO) into the nearest major water body. As a result, human fecal waste and pathogens have been demonstrated previously in these systems (McGinnis et al., 2018). Segregated drainage systems have separate stormwater and sanitary sewers, and thus should not in theory contain human fecal contamination, though this is not necessarily the case as outlined below.

1.3.1 Stormwater Quality by Traditional Standards

Even with the understanding that stormwater is increasingly seen as a viable alternative source water for non-potable uses, little is currently understood about the risks involved in its

use. From the perspective of traditional water quality, the presence of FIB such as *Enterococcus* and *E. coli* have been found in high concentrations in stormwater across multiple continents, suggesting that stormwater quality is poor from this standpoint (Ahmed et al., 2019b, 2020; Converse et al., 2011; Hachad et al., 2022; Hart et al., 2020; Kinzelman & McLellan, 2009; Lee et al., 2020; Monteiro et al., 2021; Nshimyimana et al., 2014; Olds et al., 2018; Parker et al., 2010; Sauer et al., 2011; Sidhu et al., 2012; Staley et al., 2018; Steele et al., 2018; Williams et al., 2022). Mean *Enterococcus* and *E. coli* concentrations typically range from 2-4 $\log_{10}$ CFU/100 mL, depending on factors such as individual site differences and weather conditions (Ahmed et al., 2019b, 2020; Converse et al., 2011; Hachad et al., 2022; Hart el a., 2020; Kinzelman & McLellan, 2009; Lee et al., 2020; Monteiro et al., 2021; Parker et al., 2010; Sauer et al., 2011; Sidhu et al., 2012; Staley et al., 2018; Williams et al., 2022). It is important to note, however, that concentrations can also range as high as 5-7 $\log_{10}$ CFU/100 mL for both organisms in stormwater in extreme cases (Lee et al., 2020; Parker et al., 2010; Sauer et al., 2011; Staley et al., 2018), suggesting that stormwater systems represent water of generally poor microbial quality. Most importantly, the above suggests that mean values of FIB not only frequently exceed common recreational water quality criteria (e.g., US EPA's [2012a] GM of 100 *E. coli*/100 mL), the average means themselves are very frequently higher than the single sample threshold limits (e.g., US EPA's [2012a] STV of 320 *E. coli*/100 mL), suggesting near ubiquitous criteria exceedance in studied samples.

It is important to note, however, that evidence for significant correlation in studies between enteric pathogens and FIB is often lacking in stormwater specifically (Ahmed et al., 2019b; de Man et al., 2014; Schriewer et al., 2010; Steele et al., 2018). One potential explanation for this is that notable differences have been observed in the measured decay rates between FIB

(such as *E. coli* and *Enterococcus*) and enteric pathogens, particularly in the context of viruses or protozoan parasites (Ahmed et al., 2021; Bae & Wuertz, 2012; Jones et al., 2018; Nasser et al., 2003). Additionally, while FIB are nearly ubiquitously detected in stormwater, enteric pathogens are often only sporadically detected and often at lower concentrations, making correlational analyses difficult to interpret (Ahmed et al., 2019b; McGinnis et al., 2018; Sidhu et al., 2012).

Associations between FIB and other FIB (i.e., *Enterococcus* and *E. coli*) have also been found to be inconsistent as well in stormwater, though there are often moderate-to-highly significant associations between FIB described in environmental waters (Ahmed et al., 2020; Hart et al., 2020; Lee et al., 2012; Parker et al., 2010; Schriewer et al., 2010; Sidhu et al., 2012). This makes sense considering different decay rates between these organisms in water, as well as the high environmental complexity and sources of fecal contamination in stormwater that may not make for simple relationships between FIB (Ahmed et al., 2019a, 2021; Korajkic et al., 2019; Walters & Field, 2009). In fact, even FIB that are the same species, but differ in origin (i.e., intenstinal vs. environmental strains) or are from different hosts also appear to have different decay rates (Korajkic et al., 2019). Additionally, differences may exist based on the method of detecting FIB (e.g., qPCR vs. filter-membrane culture vs. defined-substrate culture methods), though there has generally been relatively satisfactory (but not perfect) agreement between these methods (Noble et al., 2010; Raith et al., 2014; Steele et al., 2018).

The relationships between FIB and indicators of human sewage (such as HF183) can be erratic in stormwater as well, and these relationships appear especially dependent on where stormwater is surveyed and the consequent potential sources of pollution (Hachad et al., 2022; Hart et al., 2020; McGinnis et al., 2018; Nshimyimana et al., 2014; Olds et al., 2018; Sauer et al.,

2011; Staley et al., 2018; Steele et al., 2018; Williams et al., 2022). For example, some stormwater-impacted waters appear to have moderate to high correlation between human sewage markers and FIB (see Hachad et al., 2022; Hart et al., 2020; McGinnis et al., 2018; Staley et al., 2018; Steele et al., 2018), while others see low to non-existent correlation between these two (see Nshimyimana et al., 2014; Olds et al., 2018; Sauer et al., 2011; Williams et al., 2022). This makes sense considering the many fecal sources of FIB (see Ahmed et al., 2019a; Fogarty et al., 2003; Gordon & Cowling, 2003; Layton et al., 2010), as well as the decay rates that can sometimes differ between markers of human sewage and FIB such as *Enterococcus* and *E. coli* (Dick et al., 2010; Walters & Field, 2009). As an exemplar, Williams et al. (2022) found significant correlation between dog fecal marker (Dog3) and *Enterococcus*, but did not find significant correlation between FIB and evidence of gull or human fecal contamination, whereas Staley et al. (2018) found significant correlation between *E. coli* and both human and gull markers.

FIB concentration and correlation in stormwater can also differ with a multitude of other factors not directly related to human and animal sources, including weather as a temporal factor. Previous studies have found lower FIB loads and concentrations in baseflow than under conditions of high precipitation (Ahmed et al., 2020; Converse et al., 2011; Hart et al., 2020; Jokinen et al., 2010; Kinzelman & McLellan, 2009; Lee et al., 2020; Sidhu et al., 2012; McGinnis et al., 2018; Parker et al., 2010; Steele et al., 2018; Williams et al., 2022). For example, Williams et al. (2022) found that *Enterococcus* concentrations were nearly 2 orders of magnitude higher at stormwater drains (5 $\log_{10}$ CFU/100 mL) during heavy storms than light storms, with concentrations under the latter condition nearly 1 $\log_{10}$ higher than at baseflow conditions. This phenomenon is particularly prevalent during the first part of storms, often known as the “first flush” effect where FIB concentrations are understood to be highest in the first part of storms

before peak flow is reached (Converse et al., 2011; Chow et al., 2013). It is important to point out that first-flush flows are not always correlated to higher concentrations of FIB (see He et al., 2010; McCarthy, 2009; Parker et al., 2010), and first flush phenomena may be a site dependent and complex process with multiple factors involved.

Differences in *Enterococcus* and *E. coli* concentrations can also be found that are geospatial in nature and site-dependent (Hachad et al., 2022; Hart et al., 2020; Monteiro et al., 2021; Nshimyimana et al., 2014; Parker et al., 2010; Staley et al., 2018; Steele et al., 2018), though FIB concentrations can also be consistent across sites in some cases (Lee et al., 2020; Sidhu et al., 2012). Not surprisingly, some of these differences have been found to relate to how close in proximity sampling is to stormwater flow (Hart et al., 2020; Staley et al., 2018; Steele et al., 2018), though it is important to note that even in outfalls that are relatively close together geographically, large variations can still exist in FIB concentrations (Hachad et al., 2022; Hart et al., 2020; Nshimyimana et al., 2014; Staley et al., 2018; Steele et al., 2018). Whether either *Enterococcus* or *E. coli* appear most predominant can also change depending on the site, with *Enterococcus* medians occasionally 0.5-1.5 $\log_{10}$ higher than *E. coli* and vice versa in some individual sites (Monteiro et al., 2021; Sauer et al., 2011; Sidhu et al., 2012). Ahmed et al. (2020) found *E. coli* concentrations nearly always higher than *Enterococcus* concentrations in stormwater, though both of these FIB generally have similar concentrations across the literature (Monteiro et al., 2021; Olds et al., 2018; Parker et al., 2010; Sauer et al., 2011; Sidhu et al., 2012).

The vital takeaway from the above is that while stormwater is nearly universally of poor water quality based on FIB concentrations (Nshimyimana et al., 2014; Sauer et al., 2011), interpretation of risk by FIB enumeration is not straightforward. Patterns of FIB prevalence and concentration can vary heavily on temporal (Williams et al., 2022) and geospatial scales (Hachad

et al., 2022), is not always correlated to sources of human fecal pollution (Olds et al., 2018), and may not even be directly correlated to detection of enteric and opportunistic pathogens (Steele et al., 2018). The above therefore has implications not only on when and where sampling should occur for hazard identification in stormwater-impacted waterbodies, but also when and where subsequent drawing of stormwater should occur for potable or non-potable use in order to mitigate the most risk. As mentioned previously, however, differences in FIB predominance, concentrations, and prevalence must also be interpreted cautiously when considering environmentally-adapted strains of these organisms which can replicate within environmental waters such as stormwater (Byappanahalli et al., 2012; Devane et al., 2020; Jang et al., 2017). Lastly, a lack of correlation between FIB and gastrointestinal illness (Arnold et al., 2013; Colford et al., 2007) in general again means that FIB may not definitively represent the risks found in stormwater even when found in higher concentrations.

1.3.2 Sources of Fecal Pollution in Stormwater

Genetic markers for human-specific *Bacteroides* (such as HF183) have been frequently observed in stormwater worldwide, indicating the ubiquitous presence of human sewage contamination in these waters – including in stormwater and sanitary sewers that have been built separately (Ahmed et al., 2019b; Chong et al., 2013; Kinzelman & McLellan, 2009; McGinnis et al., 2018; Nshimyimana et al., 2014; Parker et al., 2010; Sales-Ortells & Medema, 2015; Sauer et al., 2011; Sidhu et al., 2013; Staley et al., 2018; Steele et al., 2018). Besides human influence, fecal contributions in stormwater have also been demonstrated from several non-human sources, including gulls, ruminants, cattle, and dogs (Bambic et al., 2015; Green et al., 2014, 2019; Sales-Ortells & Medema, 2015; Staley et al., 2018; Steele et al., 2018). Considering the above, this

suggests that fecal pollution sources can be highly diverse in stormwater, therefore presenting a challenge to hazard identification where the primary sources of fecal pollution are unknown.

Human sewage markers such as HF183 have been found in stormwater in concentrations that can range from below quantifiable limits to over 5 $\log_{10}$ copies/100 mL, though when detected in stormwater this marker has generally found to vary between 2-4 $\log_{10}$ copies/100 mL, depending on factors such as precipitation levels, location, and infrastructure (Hachad et al., 2022; Kinzelman & McLellan, 2009; Nshimyimana et al., 2014; Sauer et al., 2011; Staley et al., 2018; Steele et al., 2018). Prevalence of this marker has been noted to be very high in stormwater as well, frequently detected in half to nearly all stormwater samples (43% - 97.7%) (Hachad et al., 2022; Kinzelman & McLellan, 2009; Nshimyimana et al., 2014; Sauer et al., 2011; Sidhu et al., 2012; Steele et al., 2018). Given that *Bacteroides* is a strict anaerobe, and that the HF183 marker is generally suggested to decay to undetectable levels within approximately 5-10 days, it is believed that the presence of HF183 represents relatively fresh fecal inputs into these systems (Boehm et al., 2018; Dick et al., 2010; Walters & Field, 2009). It is understood that in storm drain systems the most likely contributions of human waste contamination include illicit cross-connections, homeless persons, leaking septic systems, CSOs, and aging infrastructure in need of repair (Kinzelman & McLellan, 2009; McGinnis et al., 2018; Nshimyimana et al., 2014; Sauer et al., 2011; Staley et al., 2018; Steele et al., 2018).

In comparison to stormwater, HF183 has been found in human sewage frequently at concentrations of 7-8 $\log_{10}$ copies/100 mL (see Ahmed et al., 2021; Mayer et al., 2018; Nshimyimana et al., 2014; Sauer et al., 2011), suggesting that concentrations of this marker in stormwater (i.e., 2-6 $\log_{10}$ copies/100 mL) very frequently represent raw human sewage flows of between <0.1% and as high as 10% in stormwater. Based on this understanding, guidelines such

as Alberta's *Public Health Guidelines for Water Reuse and Stormwater Use* (2021) suggest that stormwater should be assumed to be contaminated by up to 10% raw human sewage for the purposes of treatment and risk mitigation, unless it is verified that human fecal contamination lays at the ≤0.1% level.

Despite the potential for zoonotic pathogens such as animal-borne *Campylobacter* and *Salmonella*, non-human sources of fecal contamination have not been thoroughly investigated in stormwater in comparison to markers for human sewage, leading to a large gap in the current understanding of stormwater quality and microbial hazards (Ahmed et al., 2019b; EFSA & ECDC, 2021). Consequently, the majority of studies involved in investigating sources of fecal pollution into stormwater either primarily test for human pollution sources alone (Gonzalez et al., 2020; Hachad et al., 2022; Kinzelman & McLellan, 2009; McGinnis et al., 2018; Nshimyimana et al., 2014; Parker et al., 2010; Sauer et al., 2011; Sidhu et al., 2012), or only test for 1-2 additional animal source markers when these are tested for at all (Bambic et al., 2015; Green et al., 2019; Sales-Ortells & Medema, 2015; Staley et al., 2018; Steele et al., 2018).

The most popular animal markers tested for in stormwater are generally for birds (Ahmed et al., 2020; Lee et al., 2020; Lu et al., 2011b; Sales-Ortells & Medema, 2015; Staley et al., 2018; Steele et al., 2018; Williams et al., 2022), and dogs (Ahmed et al., 2020; Bambic et al., 2015; Green et al., 2014; Lee et al., 2020; Monteiro et al., 2021; Sales-Ortells & Medema, 2015; Steele et al., 2018; Williams et al., 2022), though ruminant-associated markers have also been found in stormwater (Bambic et al., 2015; Green et al., 2019; Lee et al., 2020; McLellan et al., 2018; Olds et al., 2018). For example, the Dog3 marker has been found at a highly variable prevalence (3-70%) in stormwater, with concentrations frequently between 1 – 3 $\log_{10}$ copies/100 mL (Green et al., 2014; 2019; Steele et al., 2018; Williams et al., 2022). In contrast, ruminant marker

Rum2Bac has been found in stormwater more frequently (37%-91%), ranging in concentration from 2 – 4 $\log_{10}$ copies/100 mL (Green et al., 2019; Lee et al., 2020). Gull markers based on *C. marimmamalium* have been detected in stormwater from 29.5% to 100% of samples tested, with a wide range of concentrations from 0.8 to 3.5 $\log_{10}$ copies/100 mL (Lu et al., 2011b; Staley et al., 2018; Steele et al., 2018).

Although birds such as gulls have previously been shown to contaminate stormwater with their feces, the level of risk to public health that avian contamination presents in environmental waters is not currently well understood (Lu et al., 2011a; Smith et al., 2020; Staley et al., 2018; Steele et al., 2018). Gull fecal contamination in particular may be hazardous due to the contemporary ecological niche they occupy as human refuse scavengers, even potentially transporting pathogens incurred from ingesting garbage across vast distances (Alm et al., 2018; Navarro et al., 2019). A recent publication from Alm et al. (2018) suggests that human sewage (HF183) can be found in the intestinal tract of gulls scavenging human waste. While evidence of human fecal contamination in stormwater is still particularly concerning, the diversity of animal sources of fecal contamination into stormwater cannot be ignored – especially when considering the zoonotic nature of enteric pathogens that can be found from these sources (Banting & Figueras Salvat, 2017; Bryan et al., 2015; EFSA & ECDC, 2021; Fitzgerald, 2015).

When it comes to the detection of MST markers within stormwater, associations in prevalence and concentrations appear to exist based on a number of environmental factors. As an example, weather appears to have a mixed relationship with MST markers in stormwater, particularly for markers of human *Bacteroides* spp. (Hart et al., 2020; McGinnis et al., 2018; McLellan et al., 2018; Sauer et al., 2011; Sidhu et al., 2012; Staley et al., 2018; Williams et al., 2022), though this can also be the case for animal markers such as for gulls (Staley et al., 2018;

Steele et al., 2018). While evidence does exist that shows that wet weather or rainfall amounts do not appear to affect HF183 prevalence and concentrations in stormwater (see Sauer et al., 2011), the vast majority of studies show distinct increases in this marker with higher precipitation levels found during wet weather events (Ahmed et al., 2020; Hart et al., 2020; McGinnis et al., 2018; McLellan et al., 2018; Sidhu et al., 2012; Staley et al., 2018; Williams et al., 2022). Animal marker differences associated with weather changes have not been frequently studied, though Staley et al. (2018) found that gull marker concentrations were significantly higher in stormwater outfalls during wet weather events than in baseline conditions, while Ahmed et al. (2020) and Williams et al. (2022) found no weather-dependent differences in gull marker concentrations in stormwater. Despite dog MST markers being sporadically detected in stormwater, both Williams et al. (2022) and Monteiro et al. (2021) observed differences in detection of dog marker (Dog3 and DogMito respectively) based on levels of precipitation.

Geospatial patterns in MST markers found in stormwater have also been noted, particularly showing differences geographically where pollution is predominantly human (Ahmed et al., 2020; Hart et al., 2020; Lee et al., 2020; Nshimyimana et al., 2014; Parker et al., 2010; Sauer et al., 2011; Sidhu et al., 2012; Staley et al., 2018; Steele et al., 2018; Williams et al., 2022) though other sites have been shown to have predominant fecal pollution by other animals (such as birds, for example) (Lee et al., 2020; Staley et al., 2018; Steele et al., 2018). Of the studies examining human sewage contamination into stormwater, differences in marker concentrations and detection have been found between different but adjacent waterbodies impacted by stormwater (see Ahmed et al., 2020; Parker et al., 2010; Sidhu et al., 2012; Staley et al., 2018; Steele et al., 2018). Differences can also exist as well as between or upstream of outfalls impacting the same water body that are relatively geographically close to each other (see

Hart et al., 2020; Lee et al., 2020; Nshimyimana et al., 2014; Sauer et al., 2011; Williams et al., 2022), with concentrations varying sometimes by several orders of magnitude.

There are many factors that may contribute to these spatial differences of human fecal contamination, including differences in land use, site infrastructure design, and proximity to high versus low stormwater flows (Hart et al., 2020; Nshimyimana et al., 2014; Parker et al., 2010; Staley et al., 2018; Steele et al., 2018; Williams et al., 2022). Spatial differences in animal contamination such as birds have found geographical differences where gull marker was found, such as where gull contamination appeared dominant in a stormwater-impacted river where a bird sanctuary existed upstream (Steele et al., 2018), or in samples taken closer to beaches than stormwater outfalls upstream (Lu et al., 2011b; Staley et al., 2018).

Spatial and temporal patterns can also intersect and influence detection and concentrations of MST markers in stormwater, suggesting a high level of complexity to the factors that can affect different fecal sources infiltrating stormwater (Hart et al., 2020; Parker et al., 2010; Sidhu et al., 2012; Staley et al., 2018; Williams et al., 2022). This is particularly so for sites where infrastructural damage or illicit cross-connections are more likely to be affected by higher precipitation levels during storms (Hart et al., 2020; Sidhu et al., 2012; Staley et al., 2018). However, this can also be seen for sites with higher concentrations of dog or gull markers, where pets or shorebirds potentially congregate and stormwater flow can wash the feces into the drainage network (Staley et al., 2018; Steele et al., 2018; Williams et al., 2022).

Converse to markers for human sewage and gulls, spatial and temporal patterns have not been frequently described for other animals (such as dogs and ruminants) in stormwater (Ahmed et al., 2020; Bambic et al., 2015; Green et al., 2014, 2019; Lee et al., 2020; Olds et al., 2018; Sales-Ortells & Medema, 2015; Steele et al., 2018). This may be due to a number of factors such

as the lower frequency of detection of animal MST markers, lower concentrations of marker when detected, and also the fact that fewer studies include these markers in general in comparison to the study of human markers (Ahmed et al., 2020; Bambic et al., 2015; Green et al., 2014, 2019; Lee et al., 2020; Olds et al., 2018; Sales-Ortells & Medema, 2015; Steele et al., 2018). Few studies show predominant ruminant marker (such as Rum2Bac) for example (see Lee et al., 2020), while dog markers are often found at low prevalence and concentrations in stormwater (Ahmed et al., 2020; Green et al., 2014, 2019; Williams et al., 2022).

To summarize, evidence of widespread human sewage contamination in stormwater (up to 10% of stormwater flows) is becoming increasingly common (Sauer et al., 2011; Steele et al., 2018), though important geospatial (Nshimyimana et al., 2014) and temporal (Hart et al., 2020) factors may affect this pollution, while animal fecal contamination is underassessed (Staley et al., 2018). This lack of investigation of animal sources is particularly concerning when considering the currently limited evidence of waterfowl fecal contamination in stormwater (Staley et al., 2018; Steele et al., 2018), and the consequent potential for zoonotic pathogens deposition (EFSA & ECDC, 2021). Evidence of whether human or animal fecal pollution is predominant in a specific stormwater pond marked for use, and the attached temporal and geospatial patterns of this pollution may be important for risk assessment, particularly when considering the higher risk of illness from human feces (Brown et al., 2017a; Schoen & Ashbolt, 2010; Soller et al., 2010).

1.3.3 Microbial Hazards in Stormwater

Regardless of whether they originate from human or non-human sources, pathogenic bacteria, protozoa, and viruses are sources of public health concern in alternative source waters such as stormwater. While the presence of protozoans (e.g., *Giardia* and *Cryptosporidium* – see

Cizek et al., 2008; de Man et al., 2014; Schreiber et al., 2019) and enteric viruses (e.g., human adenovirus and norovirus – see Sidhu et al., 2013; Steele et al., 2018) have been demonstrated in stormwater and are important to assess for acute health risk, the content of this thesis will be focusing on bacterial pathogens specifically.

Enteric and opportunistic bacterial pathogens that cause a considerable health burden can be found in environmental waters such as stormwater, where there is a high potential of exposure to these pathogens when this water is utilized for potable or non-potable purposes (Collier et al., 2021; EFSA & ECDC, 2021; Schoen et al., 2017). Enteric pathogens including *Arcobacter* spp. (Beaudry, 2019; Carney et al., 2020), *Campylobacter* spp. (de Man et al., 2014; Murphy et al., 2017; Steele et al., 2018), *Salmonella* spp. (Chong et al., 2013; Schreiber et al., 2019; Johnson et al., 2003), and pathogenic *E. coli* (McGinnis et al., 2018) have been previously detected in stormwater, as well as opportunistic pathogens such as *Legionella pneumophila* (Sales-Ortells & Medema, 2015). It is important to note that very few publications exist on the study of *Arcobacter* spp. in stormwater (See Beaudry et al., 2019; Carney et al., 2020), despite the risk that this organism may present as one of the most abundant genera in raw sewage and wastewater (Collado & Figueras, 2011; Cui et al., 2019; Fisher et al., 2014; Hsu & Lee, 2015; Lu et al., 2015; Zhang et al., 2020).

Campylobacter spp., *Salmonella* spp., and STEC are considered the respective top three bacterial causes of zoonotic gastrointestinal illness in the EU, and are estimated to cost the U.S. healthcare system approximately $57.7 million annually from waterborne transmission alone (Collier et al., 2021; EFSA & ECDC, 2021). These three organisms (as well as *A. butzleri*) are commonly found in sewage and human wastewater-impacted waters, and are known to cause moderate-to-severe gastrointestinal illness, as well as long-term sequelae and death in the case

of *Campylobacter* spp., *Salmonella* spp. and STEC (Ahmed et al., 2021; Chui et al., 2013; Cui et al., 2019; Kaakoush et al., 2015; Lisboa et al., 2019; Rana et al., 2021; Savichtcheva et al., 2007; Webb et al., 2017). *Campylobacter* spp. alone have been found responsible for several large-scale waterborne outbreaks within the last two decades, including outbreaks in Canada, New Zealand, and Norway – each occurrence causing cases of gastroenteritis in the thousands (Gilpin et al., 2020; Hrudey et al., 2003; Hyllestad et al., 2020). In line with the burden of the above pathogens, *A. butzleri* is understood to be the *Arcobacter* species with the highest prevalence found in human populations, cited in two separate studies as the 4th most frequently isolated *Campylobacter*-like species found in diarrheal stool samples (Prouzet-Mauleon et al., 2006; Vandenberg et al., 2004).

Unlike the other pathogens of concern, *L. pneumophila* primarily causes disease of the respiratory tract, and is considered one of the top 5 pathogens for highest disease burden as measured by DALYs (Cassini et al., 2018). Since at least 2007, *L. pneumophila* has also been recognized as the leading cause of waterborne illness outbreaks in drinking water in the United States (Beer et al., 2015; Benedict et al., 2017; Brunkard et al., 2011; Centers for Disease Control and Prevention [CDC], 2013).

The above pathogens pose risk to those that use stormwater for potable or non-potable uses through the fecal-oral route for the enteric pathogens, as well as via accidental inhalation for the opportunistic pathogens (Murphy et al., 2017; Petterson et al., 2016; Schoen et al., 2017). Bacterial pathogens found in stormwater can be particularly relevant when considering the large number of non-potable uses for this source water and the inherent exposure risk, such as with irrigation of community garden vegetables, street cleaning, aesthetic fountains, or recreational features (AHS, 2021; Schoen et al., 2017).

It is important to note that there are a few important differences in morphology and metabolism that may affect behaviour of the above pathogens in their pathogenesis, survivability within environmental waters, and transmission origins. *Arcobacter* spp., which are closely related to *Campylobacter* spp., are gram negative, aerotolerant, curved or helically shaped, motile via a polar flagellum, and have been found to be pathogenic in humans (Banting and Figueras Salvat, 2017; Vandamme et al., 1992). *Campylobacter* spp. are similarly gram negative, curved or helically shaped, motile, and frequently found to be pathogenic in humans (particularly *C. jejuni*, *C. coli*, and *C. lari*), though they are instead microaerophilic and therefore grow best in a low oxygen environment (Fitzgerald, 2015). Closely related to *E. coli*, *Salmonella* spp. are gram negative, motile via peritrichous flagella, rod-shaped, facultative anaerobes, though unlike most *E. coli, Salmonella* spp. are intracellular pathogens of the human intestinal tract (*S. enterica* and its various serotypes specifically, though mainly the Typhi and Typhimurium serovars) (Chen et al., 2013; Jajere, 2019). Similarly, STEC are *E. coli* that are primarily defined by being capable of producing this toxin that is encoded on the *stx*1 and *stx*2 genes, and which is highly similar to the toxin of *Shigella dysenteriae*, allowing for infection and enterohemorrhagic disease in the human intestinal tract (Bryan et al., 2015).

Differing from these pathogens, which are primarily found in the gastrointestinal tracts of warm-blooded animals as enteric pathogens, *L. pneumophila* is an opportunistic pathogen that is primarily adapted to live in environmental waters, but is also capable of colonizing engineered water systems (i.e., potable water distributions systems, premise plumbing, cooling towers, etc.) (Ashbolt, 2015; NASEM, 2020). In terms of morphology and metabolism, *L. pneumophila* is gram negative, fastidious, aerobic, and rod-shaped (Lu et al., 2015; NASEM, 2020; Sales-Ortells & Medema, 2015).

While *Arcobacter* spp., *Campylobacter* spp., *Salmonella* spp., and STEC are all known to cause gastrointestinal illness, pathogenesis can differ among these pathogens, as well as the severity of disease and potential symptomology (Bolton, 2015; Bryan et al., 2015; Ferreira et al., 2015; Jajere, 2019). For example, *Campylobacter* spp. pathogenesis is primarily through flagellar motility, adhesion (via adhesins like CadF), invasion, as well as the production of toxins such as cytolethal distending toxin (CDT) (Bolton, 2015). *Salmonella* can instead cause typhoidal or non-typhoidal (i.e., gastroenteritis) symptoms of disease in humans, with severity of disease dependent on the particular serovar and the method employed by the organism for pathogenesis (Chen et al., 2013; Jajere, 2019). *Salmonella* spp. also utilize *Salmonella* pathogenicity islands (SPI) that encode virulence factors used by this organism to invade intestinal cells, such as the type 3 secretion system utilized heavily by this organism (Jajere, 2019; Pradhan & Negi, 2019). STEC and other pathogenic *E. coli* can be of particular concern due to the enterohemorrhagic effect of Shiga toxin (Bryan et al., 2015). The principal concern with this organism is the symptoms caused during infection due to the Shiga toxin, which include intestinal hemorrhaging, and can also include hemolytic uremic syndrome in severe cases (Chui et al., 2013; Lisboa et al., 2019). In contrast to the potential severity of STEC and typhoidal *Salmonella*, gastrointestinal disease caused by *Arcobacter* spp. and *Campylobacter* spp. usually consists of self-limiting gastroenteritis, though this can still be a problem in those from vulnerable populations, such as the immunocompromised (Arguello et al., 2015; Collado & Figueras, 2011; Kaakoush et al., 2015).

Given that *L. pneumophila* is an opportunistic respiratory pathogen, this organism has a different method of pathogenicity than the enteric organisms (Newton et al., 2010). In general, *L. pneumophila* infects alveolar macrophages within the lungs, allowing itself to be phagocytosed

but defending itself with a number of mechanisms to take advantage of its host and form a protective *Legionella*-containing vacuole (LCV) (Newton et al., 2010).

It is a common misconception that enteric bacteria do not survive well outside of a host, and many bacterial pathogens have evolved a multitude of mechanisms for survival in the environment, further elucidating the risk these pathogens may bring from non-potable reuse and alternative source water use activities. For example, *Arcobacter* spp., *Campylobacter* spp., *Salmonella* spp., and *E. coli* are known to survive stressors by entering into viable but nonculturable (VBNC) states (Bronowski et al., 2017; Chaisowwong et al., 2012; Fera et al., 2008; Lisle et al., 1998; Oliver et al., 2005) and/or promote biofilm formation (Bronowski et al., 2014; da Cruz Nizer et al., 2020; Ferreira et al., 2013; Shagieva et al., 2020) that can potentially allow these organisms to retain infectivity in extreme conditions. Although an opportunistic pathogen, *L. pneumophila* can also form and sustain itself within biofilms, as well as in a VBNC form resistant to the environment (Ashbolt, 2015).

Evidence suggests that the adaptations pathogens undergo can allow for these organisms to be aerotolerant, resistant to temperature extremes, salinity, nutrient starvation, as well as (most concerningly) chemical and oxidative treatments commonly used in disinfection (Bronowski et al., 2014, 2017; Chaisowwong et al., 2012; da Cruz Nizer et al., 2020; Ferreira et al., 2015; Lisle et al., 1998; Oliver et al., 2005; Pradhan & Negi, 2019; Van Driessche & Houf, 2008; Zhi et al., 2019). For example, recent studies by Zhi et al., (2019) have demonstrated that extra-intestinal pathogenic *E. coli*, readily survive wastewater treatment and therefore raise potential concerns about water reuse and alternative source water use associated with certain water sources or the use of certain applications.

The adaptability of both enteric and opportunistic pathogens is particularly concerning when considering that alternative water source use and water reuse activities can cause exposure to contact with water containing these enteric pathogens (i.e., via recreational swimming, contact with irrigated surfaces or crops), as well as the potential for contact with aerosols (i.e., aesthetic fountains, spray features, or toilet flushing) (Schoen et al., 2017).

Several QMRA studies have been conducted on non-potable uses of stormwater (see Murphy et al., 2017; Petterson et al., 2016; Sales-Ortells & Medema, 2015; Schoen et al., 2017), suggesting considerable risks of illness from bacterial pathogens from uses including irrigation, recreation, toilet flushing, and clothes washing when water treatment is not carefully considered. Recreation may be of particular concern, when considering epidemiological evidence for stormwater associated illness in users (such as swimmers and surfers) of recreational beaches impacted by stormwater runoff (Colford et al., 2007, 2012; Haile et al., 1999; Soller et al., 2017). There are also often statistically significantly higher frequencies of waterborne outbreaks of illness in both the United States and Canada during extreme rain events and the subsequent increased stormwater runoff (Curriero et al., 2001; Thomas et al., 2006).

The enteric pathogens so far discussed are found in a large variety of aquatic and terrestrial environments, where they pose risk of infection in those that come into contact with this material via the fecal-oral route (Banting et al., 2016; Jokinen et al., 2010, 2011; Khan et al., 2013; Steele et al., 2018). For example, *Arcobacter* spp., *Campylobacter* spp., STEC, and *Salmonella* spp. have all been commonly found in human sewage and/or wastewater (Banting et al., 2016; Jokinen et al., 2010, 2011; Khan et al., 2013). Notably, *Arcobacter* spp. specifically has been found at such high abundances in wastewater that it is one of the most dominant organisms in this matrix (See Fisher et al., 2014; Lu et al., 2015; Zhang et al., 2020), and has

subsequently been detected often in environmental waters contaminated with human sewage (Collado et al., 2008; Cui et al., 2019; Webb et al., 2017). All of the above pathogens have also been found in the feces and/or meat of livestock and poultry, the feces of domestic pets (cats/dogs), and in the feces of wild birds including geese, gulls, and ducks (Ahmed et al., 2019a; Banting & Figueras Salvat, 2017; Bryan et al., 2015; Fitzgerald, 2015; Jokinen et al., 2010, 2011). STEC in particular has been associated with contaminated beef and cattle fecal discharge, as well as the environmental waters heavily affected by cattle fecal discharge (Bradshaw et al., 2016; Bryan et al., 2015; Jokinen et al., 2011). In contrast to the enteric pathogens but no less concerning, *L. pneumophila* is found ubiquitously in environmental waters, though particularly so in engineered and man-made piping systems where water has been allowed to become stagnant (Lu et al., 2015; NASEM, 2020; Sales-Ortells & Medema, 2015).

Despite the demonstrable evidence of zoonotic pathogens such as *Campylobacter* spp. and *Salmonella* spp. in gulls (Antilles et al., 2021; Kinzelman et al., 2008; Lévesque et al., 2000; Lu et al., 2011a; Migura-Garcia et al., 2017; Moré et al., 2017; Quessy & Messier, 1992; Ramos et al., 2010; Russo et al., 2021; Van Dyke et al., 2010) and geese (Feare et al., 1999; Gorham & Lee, 2016; Jokinen et al., 2010, 2011; Keller & Schriver, 2014; Rutledge et al., 2013; Vogt et al., 2018), as well as the aquatic nature of these waterfowl, the risk these birds present in environmental waters such as stormwater is underassessed. This is particularly concerning when noting that serotypes of *Campylobacter* spp. and *Salmonella* spp. found in environmental waters are frequently highly related to serotypes found in waterfowl (Jokinen et al., 2011; Mulder et al., 2020; Sheppard et al., 2011; Shrestha et al., 2019). Additionally, evidence also exists linking pathogens in water causing illness in humans that have been fecally-sourced from birds (Cody et al., 2015; Gruszynski et al., 2014). This is also consistent with recent publications suggesting

that generalist strains of *Campylobacter* spp. that have been associated with clinical infection and environmental survival have been isolated from birds (Dearlove et al., 2016), including from gulls (Broman et al., 2002; Palmgren et al., 2006) and Canada geese (Keller & Shriver, 2014). Given the potential for risk from these birds, gull and goose fecal contamination and possible pathogen deposition has therefore become a particularly important gap in understanding the microbial water quality of stormwater and other alternative source waters.

Alongside difficulties in interpreting risk of pathogens contaminating stormwater from different pollution sources, there are also current challenges in determining the risk of bacterial pathogens in stormwater when considering pathogen host specificity. Although the potential for zoonotic transmission of enteric bacterial pathogens exists, in some instances the pathogens detected in environmental waters cannot necessarily be described as host-adapted to, and therefore disease-causing in humans or animals without further validation (EFSA & ECDC, 2021). For example, while several studies suggest that gulls and/or geese commonly carry *Campylobacter* and *Salmonella* serotypes that may be pathogenic to humans (Broman et al., 2002; Keller & Shriver, 2014; Palmgren et al., 2006), evidence also suggests the majority of gull-derived *Campylobacter* spp. and *Salmonella* spp. are different genotypes than those commonly seen as pathogenic to humans (Dearlove et al., 2016; Fu et al., 2022; Griekspoor et al., 2013; Sheppard et al., 2011). A few studies show that these genotypes may not even be pathogenic to the gulls themselves (Keller & Shriver, 2014; Palmgren et al., 2006).

In contrast to bird-adapted isolates of pathogens (such as *C. jejuni* and *C. coli*) are those isolates predominantly detected in environmental waters (Mulder et al., 2020; Shrestha et al., 2019). For example, host generalist strains of *C. jejuni* and *C. coli* associated with livestock are frequently detected in environmental waters, and are thought to be highly adaptable to a large

variety of hosts including humans (Dearlove et al., 2016; Griekspoor et al., 2013; Sheppard et al., 2011). To complicate matters further, in pathogenic genera such as *Campylobacter*, not all strains and species are pathogenic in humans even if they are transmissible, leaving some uncertainty in the interpretation of risk (Ahmed et al., 2019a; Fitzgerald, 2015). In conclusion, some care must be taken in assessment of risk when bacterial pathogens are detected in alternative source waters, especially when taking into consideration that pathogen presence does not necessarily have a one-to-one relationship with risk without further validation.

In addition to the challenges posed above with risk interpretation when bacterial pathogens are detected in stormwater and other alternative source waters, issues also exist vis-à-vis the methodological constraints of pathogen detection itself (Deshmukh et al., 2016; Girones et al., 2010; Ramírez-Castillo et al., 2015). Direct testing for pathogens in environmental water has often been deemed impractical for multiple reasons, including: limitations in culturing pathogens from water due to VBNC forms, sporadic detection of these pathogens due to unevenly distributed pathogen densities, and low concentrations when pathogens are detected at all (Ahmed et al., 2019b, 2020; Deshmukh et al., 2016; Field & Samadpour, 2007; Girones et al., 2010; Oliver, 2005; Ramírez-Castillo et al., 2015; Xu et al., 1982).

Molecular techniques such as qPCR allow for more sensitive pathogen detection in environmental water samples, as well as more specific identification of pathogen contributing hosts - though they do come with their own limitations (Deshmukh et al., 2016; Girones et al., 2010; Ramírez-Castillo et al., 2015). One of the greatest limitations of qPCR technology is an inability to determine whether the targeted DNA is from a viable or non-viable organism, and thus qPCR data estimates should be interpreted with caution and are not directly equivalent to viable organism estimates (Deshmukh et al., 2016; Girones et al., 2010; Ramírez-Castillo et al.,

2015). Conversely, one of the greatest advantages that qPCR estimates have over traditional culture techniques is the ability to detect VBNC organisms, which are undetectable using traditional culture methods (Deshmukh et al., 2016; Girones et al., 2010; Oliver, 2005; Ramírez-Castillo et al., 2015; Xu et al., 1982).

Pathogen markers are also not perfectly specific, nor are they perfectly sensitive (Banting et al., 2016; Deshmukh et al., 2016; Girones et al., 2010; Ramírez-Castillo et al., 2015). For example, Banting et al. (2016) found that a large proportion of qPCR markers designed to detect *Campylobacter* were non-specific, and were likely detecting related genus *Arcobacter* in irrigation and wastewater samples. It is also worth reiterating that inhibition can cause difficulties in environmental samples being tested via qPCR, and even minor changes in sensitivity can be problematic when pathogens are only present at low concentrations to begin with (Ahmed et al., 2019c; Schrader et al., 2012).

Methodological difficulties aside, and in spite of the risk posed by these pathogens (particularly due to their adaptability to environmental waters), there are still significant gaps in the current understanding of bacterial pathogens in stormwater and other environmental waters. For example, noting that pathogenicity and host-specificity can be highly dependent on which hosts are contaminating waters with enteric pathogens (Griekspoor et al., 2013; Sheppard et al., 2011), associations between enteric pathogens and host MST markers in stormwater have also previously been examined. First and foremost, human sewage markers (such as HF183) have been correlated in environmental waters to multiple enteric pathogens including *Arcobacter* spp. (Carney et al., 2020; Cui et al., 2019; Lee et al., 2012) and *Campylobacter* spp. (Sales-Ortells & Medema, 2015; Viau et al., 2011; Walters et al., 2007). This has not been found to be universal, however, with an absence of significant associations found in a number of studies between human

sewage markers and *Campylobacter* spp. (Cui et al., 2019; Schriewer et al., 2010; Steele et al., 2018), *Salmonella* spp. (Bradshaw et al., 2016; Cui et al., 2019; Schriewer et al., 2010; Steele et al., 2018), or pathogenic *E. coli* (Bradshaw et al., 2016; Fremaux et al., 2009). Other non-human markers have been associated with particular pathogens, such as associations between ruminant markers and STEC (Bradshaw et al., 2016; Walters et al., 2007), and between gull markers and *Campylobacter* spp. (Steele et al., 2018). These results suggest a high complexity in the possible hosts that expel these pathogens into environmental waters, and further study is warranted to more fully understand the relationships between host MST markers and pathogens.

Geo-spatial and site-specific differences have also been found in regards to pathogen detection and concentration in stormwater, particularly when it comes to enteric pathogens (de Man et al., 2014; Johnson et al., 2003; Sales-Ortells & Medema, 2015; Schreiber et al., 2019; Sidhu et al., 2012; Steele et al., 2018). Several publications have shown large differences in *Salmonella* spp. detection depending on different storm drains sampled, with a prevalence of detection ranging widely from ≤25% to ≥80% (Johnson et al., 2003; Schreiber et al., 2019; Sidhu et al., 2012). In contrast, *Campylobacter* spp. detection has been found to be stably frequent across all sites in some studies, including when *Salmonella* detection frequency seems to diverge (Murphy et al., 2017; Schreiber et al., 2019; Sidhu et al., 2012). This is not always the case, however, and *Campylobacter* spp. can be found to differ across sites largely in both frequency (differences in detection frequency among sites of up to 55%) as well as concentration (differences of up to 2.7 $\log_{10}$) (de Man et al., 2014; Steele et al., 2018). Differences also exist in the densities and predominance of individual *Campylobacter* spp., such as *C. jejuni*, and *C. coli* (Sidhu et al., 2012; Steele et al., 2018). Other pathogens such as STEC are either not as frequently tested for in stormwater, or are not frequently detected in high enough frequencies to make any

meaningful comparisons (Johnson et al., 2003; McGinnis et al., 2018). Curiously, even the non-enteric pathogen *L. pneumophila* can be found to differ on a site-by-site basis, indicating that the factors causing these site-specific differences may be more complex than simply different host sources of pathogens (Sales-Ortells & Medema, 2015).

A number of different factors may explain the above geo-spatial patterns, including that sites within a study could often drain off of or into separate watersheds than others sites within the same study (Sidhu et al., 2012; Steele et al., 2018). Differing watersheds draining into stormwater sites could have a large effect on pathogen detection and concentrations due to potentially different sources of fecal contamination, such as the bird sanctuary upstream of the San Diego River in Steele et al. (2018), or the varying levels of industrial, urbanization, and/or rural land use found upstream of the sites in Sidhu et al. (2012). Infrastructural differences between sites may also play a role in dissimilarities of pathogen prevalence and concentration, such as proximity to stormwater flow (Sales-Ortells & Medema, 2015), or proximity to infrastructural damage or sanitary sewers (de Man et al., 2014). Still, differences can be found even when sites drain from the same catchment or into the same water body, highlighting the complex nature of pathogen detection and presence even when geo-spatial differences appear relatively small (Johnson et al., 2003; Schreiber et al., 2019).

Other factors have also been investigated, such as temporal differences and the effects of wet weather on pathogen detection in environmental waters. For example, Jokinen et al. (2010) found correlation between both O157:H7 and *Salmonella* to total seasonal precipitation, while Carney et al. (2020) demonstrated a relationship between *Arcobacter* spp. abundance and wet-weather sewer overflow events. The “first-flush” principle has also been studied in terms of pathogen differences, with pathogen concentrations found to be much higher in the initial onset

of a storm than when it has begun to wane (Sales-Ortells & Medema, 2015). Again, different pathogens appear to have different relationships with precipitation levels and storm events, though pathogens are generally frequently found at higher concentrations during wetter weather than during dry weather (Carney et al., 2020; Converse et al., 2011; Jokinen et al., 2010; Sales-Ortells & Medema, 2015). In terms of intrasite temporal differences, concentrations of enteric pathogens (such as *Campylobacter* spp.) have been found to differ by >1 order of magnitude, while pathogens detected at a site during one sampling date may not be found in following or preceding dates at the same site (de Man et al., 2014; Sidhu et al., 2012).

All in all, evidence suggests that enteric and opportunistic bacterial pathogens that cause significant health burdens (Cassini et al., 2018; Collier et al., 2021; EFSA & ECDC, 2021) have been found sporadically in stormwater (Sales-Ortells & Medema, 2015; Schreiber et al., 2019; Steele et al., 2018), and this posits the question of what level of risk is posed by the potable or non-potable use of this source water. These pathogens are adaptable to the environmental stressors imposed on them by stormwater (Bronowski et al., 2014; Ferreira et al., 2015), as well as the man-made strains of water treatment methods in some cases (Zhi et al., 2019). Current challenges in the estimation of pathogen numbers in stormwater and consequent risk interpretation include methodological constraints (Girones et al., 2010), and host specificity that differs by pathogen genotype indicating different consequent risk for human vs. non-human hosts depositing these pathogens (Sheppard et al., 2011). Additionally, incomplete knowledge of the temporal (de Man et al., 2014) and geo-spatial (Schreiber et al., 2019) factors that determine pathogen densities and detection make risk interpretation difficult. In combination with an incomplete understanding of the patterns of human (Sauer et al., 2011) and animal (Staley et al., 2018) sources of fecal pollution into environmental waters and their relationship to pathogens

(Bradshaw et al., 2016; Schriewer et al., 2010), as well as difficulties in interpreting the inconsistent relationship between traditional indicators to pathogens (Savichtcheva et al., 2007; Schriewer et al., 2010) and illness (Wade et al., 2003, 2006, 2010) this leaves many unknowns that must be explored to more fully understand risk in stormwater use and water reuse.

1.4 Thesis Research and Overall Objectives

There are two major knowledge gaps, and an application barrier, related to use of stormwater and recycled waters in Alberta. This thesis aims to address all three issues. Firstly, there is a paucity of data on microbial water quality in stormwater in Alberta, and this study aims to fill this knowledge gap by characterizing water quality in this matrix. In addition, the work will also focus on providing data on the prevalence and concentration of bacterial pathogens in these systems (enteric and respiratory), so as to provide credence to $\log_{10}$ reduction targets laid out in the QMRA policy framework for water reuse in Alberta (AHS, 2021). Secondly, the research will examine the sources of pollution within these systems, so as to better understand the sources of fecal loading, and therefore the risk that pathogens my pose to human health from water made fit-for-purpose. Special attention in particular is warranted for human fecal sources of pollution, as well as under-assessed avian sources (such as from gulls and Canada geese), where risk to non-potable water stormwater use is relatively uncharacterized. This element is important in supporting assumptions made in the water reuse guidance documents recently released in the province of Alberta (AHS, 2021). Thirdly, the goal of this research is to lay the foundational basis for helping municipalities navigate the newly released water reuse guidelines in Alberta, by characterizing hazards in these systems (i.e., noted above) and then using this information to navigate the regulatory approvals process to recognize these as legitimate water reuse systems in the province (AHS, 2021). Stormwater systems in Airdrie, Alberta will be used

as case-study examples of navigating regulatory compliance, while surveying for FIB, MST markers, and pathogens in urban stormwater as well as characterizing pathogen loads in some of these host sources (gull and Canada geese feces).

It is understood that due to the strengths and limitations of FIB enumeration, MST technologies, and direct pathogen testing, a combination of all of the above is required for the most accurate assessment of microbial hazards and water quality in complex water matrices (Harwood et al., 2014). By taking a multi-pronged 'toolbox' approach to the study of the quality of stormwater, the risks of using this source waters can be much better characterized within the QMRA framework, giving a greater understanding of the safety of its use. This not only applies to individual water reuse and alternative source water use projects, but can also influence how we manage risk by encouraging the development of consolidated health risk-based guidelines for water reuse and alternative source water use based on the most current science (AHS, 2021).

The following scientific objectives will be addressed in stormwater ponds in Airdrie;

1) Characterize microbial water quality in urban stormwater ponds (*E. coli* and *Enterococcus*) in relation to current water quality guidelines for acceptable uses (i.e., recreational water, irrigation water).
2) Identify major animal host sources contributing fecal pollution to these stormwater ponds, and consequently assess what risks these fecal sources might pose to public health if the water is used for specific purposes.
3) Identify the prevalence of various bacterial pathogens present in these waters, at what concentrations they are found, and where possible assess the contributions to loading associated with animals of concern (i.e., birds).

4) Characterize and quantify MST markers, enteric pathogens, and FIB in the feces of gulls and Canada geese in an effort to understand the contribution these organisms may potentially make as common animal sources of fecal contamination of stormwater and other environmental waters.

In order to accomplish these objectives, several stormwater bodies were selected for study from Airdrie, Alberta for the summer sampling seasons of 2020 and 2021. These water bodies are all directly or indirectly connected to the municipal stormwater sewer system, and thus stormwater is a major contributing source water to each of these water bodies. These stormwater ponds and creeks have also been earmarked for various non-potable reuse activities including recreational activities, non-arable land irrigation, and aesthetic fountains.

2. Materials & Methods

2.1 Airdrie Stormwater Sample Collection

Stormwater samples for this project were collected based on two approaches: a) routine sampling; and b) investigative sampling. Routine sampling entailed the (bi)weekly collection of water samples from stormwater ponds at specific sites over two field seasons (summer of 2020 and 2021). Investigative samples were taken within the drainage networks (and occasionally at an outlet/inlet into a stormpond) in order to identify the point sources of pollution within the drainage network thar were responsible for fecal pollution of stormwater. These samples were only taken in 2021.

2020 Routine Sample Collection. In the first year of sampling (summer of 2020), routine samples were taken from seven inlets (EL#1-2, EL#4-8) and one outlet (EL#3) from the East Lake stormwater pond, as well as from three inlets (WS#2-4) and one outlet (WS#1) from the Windsong stormwater pond (See Figures 2-1 and 2-2). Sampling occurred on a weekly basis starting from Aug. 17th, 2020 to Sept. 29th, 2020, with each site from East Lake and Windsong being sampled once per week, for a total of seven samples per site (n = 84 total stormwater samples for 2020). Samples were collected by hand using a sterile 1 L sampling jar as close to the respective outlet and inlet as possible, before being shipped from Airdrie, Alberta overnight on ice (~4^0 C) to the University of Alberta where samples were immediately processed (as described below).

2021 Routine Sample Collection. In the second year of sampling (summer of 2021), stormwater-impacted water bodies including the Hillcrest, King's Heights (North and South stormponds), Nose Creek Pond, and Windsong stormwater ponds, as well as the Nose Creek and

Canals/Bayside Creeks. Sites sampled included three from Hillcrest (HC#1-3), two from King's Heights North (KHN#1-2), three from King's Heights South (KHS#1-3), three inlets (WS#2-4) and one outlet (WS#4) from Windsong, one site from Nose Creek Pond (NP#1), eight outfalls from Nose Creek (N#1-8), as well as 10 outfalls from the Canals & Bayside Creek (CS#1-10) (see Fig. 2-2 to 2-6). These sites were routinely sampled on an approximately bi-weekly basis from July 19th to Sept. 8th, 2021, with each site being sampled once per date, at a total of four samples for each site. The exceptions to this were Nose Creek site N#1, as well as the Nose Creek Pond site NP#1, which were each sampled on one additional date for a total of five routine samples at these sites. The total number of routine samples amounted to 126 stormwater samples across seven stormwater-impacted water bodies, which themselves encompassed 31 separate sites. Samples were collected as described above for the 2020 sampling year, though a sterile 200 mL sampling bottle was used as opposed to the 1 L jar.

2021 Investigative Sample Collection. In the second year of stormwater sampling in Airdrie, Alberta, it was decided that sampling would also be performed at manholes upstream of any stormwater outlet/inlet sites found to have relatively consistent detection of human sewage (based on HF183 or HumM2 markers). This was done in an effort to identify specific point sources of human fecal pollution within the stormwater drainage distribution network contributing to pollution in these stormwater ponds. Investigative sampling therefore began after the first week of routine sampling (July 26th, 2021), and occurred on an approximately bi-weekly basis from (July 26th to Sept. 27th, 2021). Sites upstream of Nose Creek (N#1), East Lake (EL#4, EL#8), Hillcrest (HC#3) and Windsong (WS#2, 3 and 4) were ultimately investigated. Sampling procedures occurred as explained above for routine sampling, though the sites picked and sampling volumes varied and were dependent on the level of flow at the particular sites that were

eventually sampled. Overall, there were 55 investigative samples, with 30 samples from manholes upstream of N#1 in Nose Creek, 10 samples in the upstream drainage network of East Lake, 5 samples upstream of Hillcrest site HC#3, and 10 samples taken in the upstream drainage network of the Windsong stormpond.

2.2 Airdrie Stormwater Site Descriptions

Below are maps of the stormwater ponds, stormwater-impacted creeks, and routine sites sampled during the entirety of the Airdrie stormwater study (2020 to 2021; Figures 2-1 to 2-6) as well as an overview map of all of the water bodies studied in Airdrie, Alberta, during this period (Fig. 2-6). Also included are tables detailing both the GPS co-ordinates of each routine site studied, as well as a brief description of each site for the stormwater ponds (Table 2-1) and stormwater-impacted creeks (Table 2-2) studied.

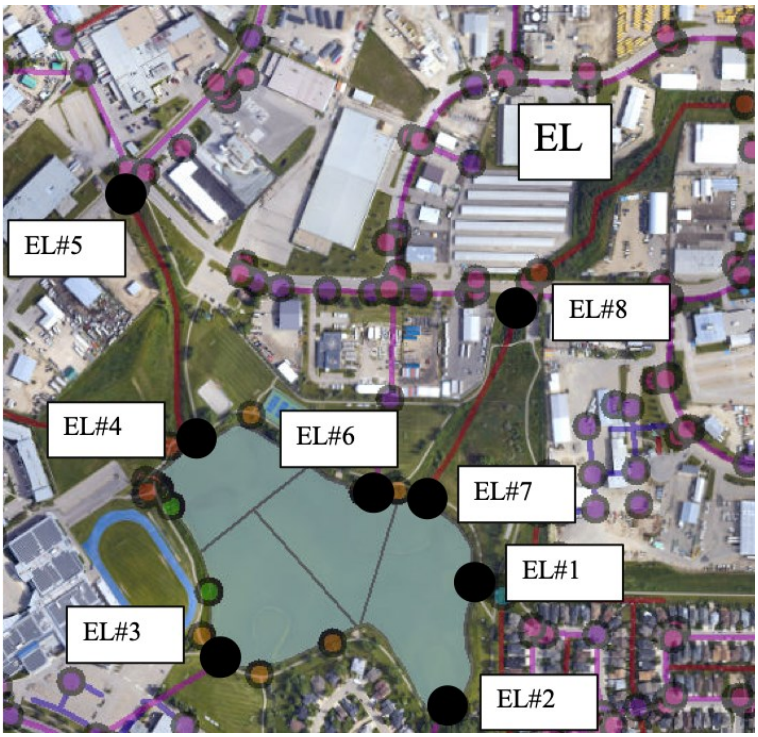

Figure 2-1. Map of the East Lake (EL) stormwater pond sampled in Airdrie, Alberta. Note the black dots representing sampling sites for this stormwater pond, including EL#1 to EL#8.

Figure 2-2. Map of Windsong (WS) and Hillcrest (HC) stormwater ponds sampled in Airdrie, Alberta. Note the black dots representing sampling sites for each stormwater pond, including WS#1-4 for Windsong, and HC#1-3 for Hillcrest.

Figure 2-3. Map of King's Heights North (KHN) and King's Heights South (KHS) stormwater ponds sampled in Airdrie, Alberta. Note the black dots representing sampling sites for each stormwater pond, including KHN#1-2 for King's Heights North, and KHS#1-3 for King's Heights South.

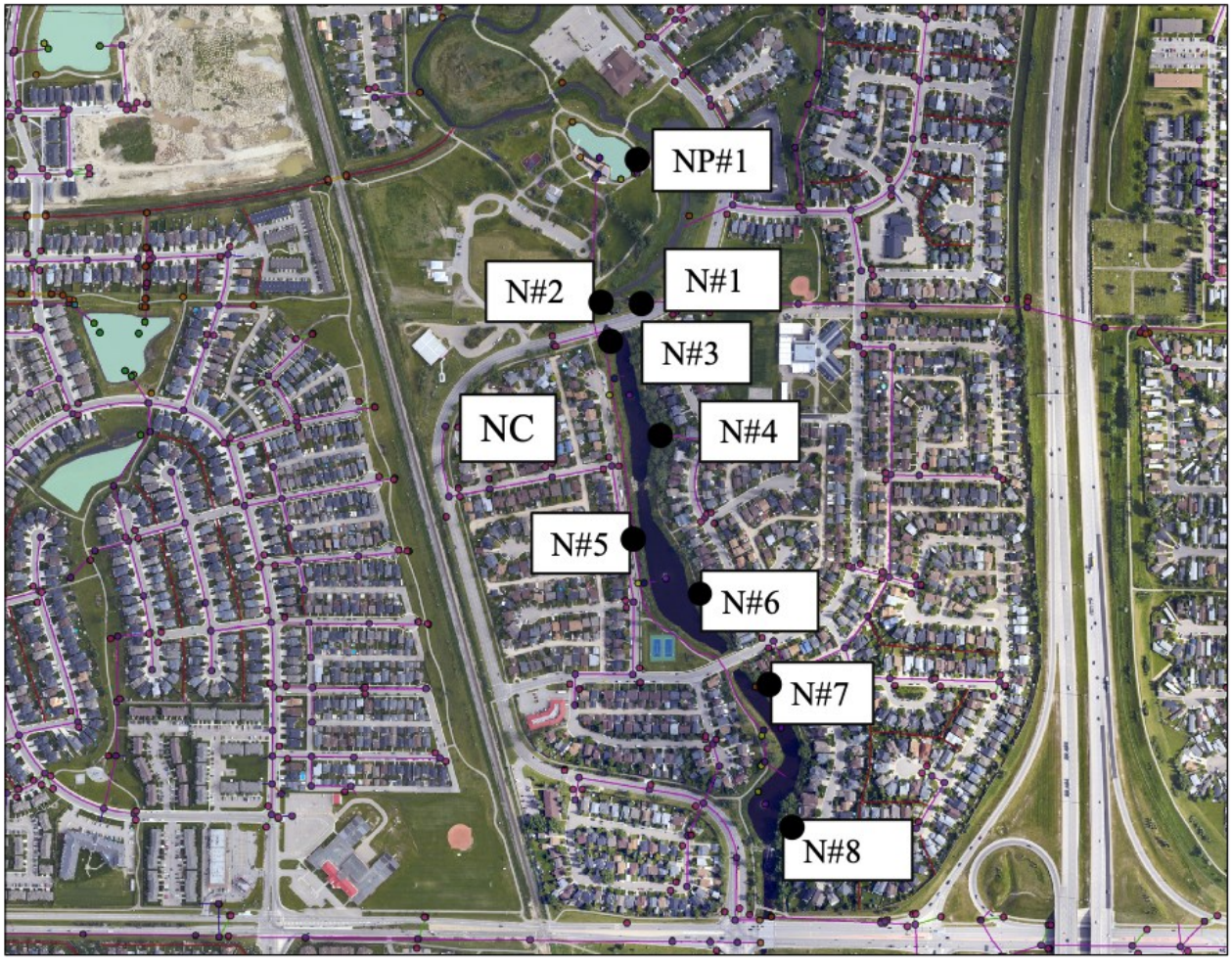

Figure 2-4. Map of the stormwater-impacted Nose Creek (NC) sampled in Airdrie, Alberta. Note the black dots representing sampling sites for each for this creek, including NP#1 from Nose Creek Pond, and N#1-N#8 for Nose Creek.

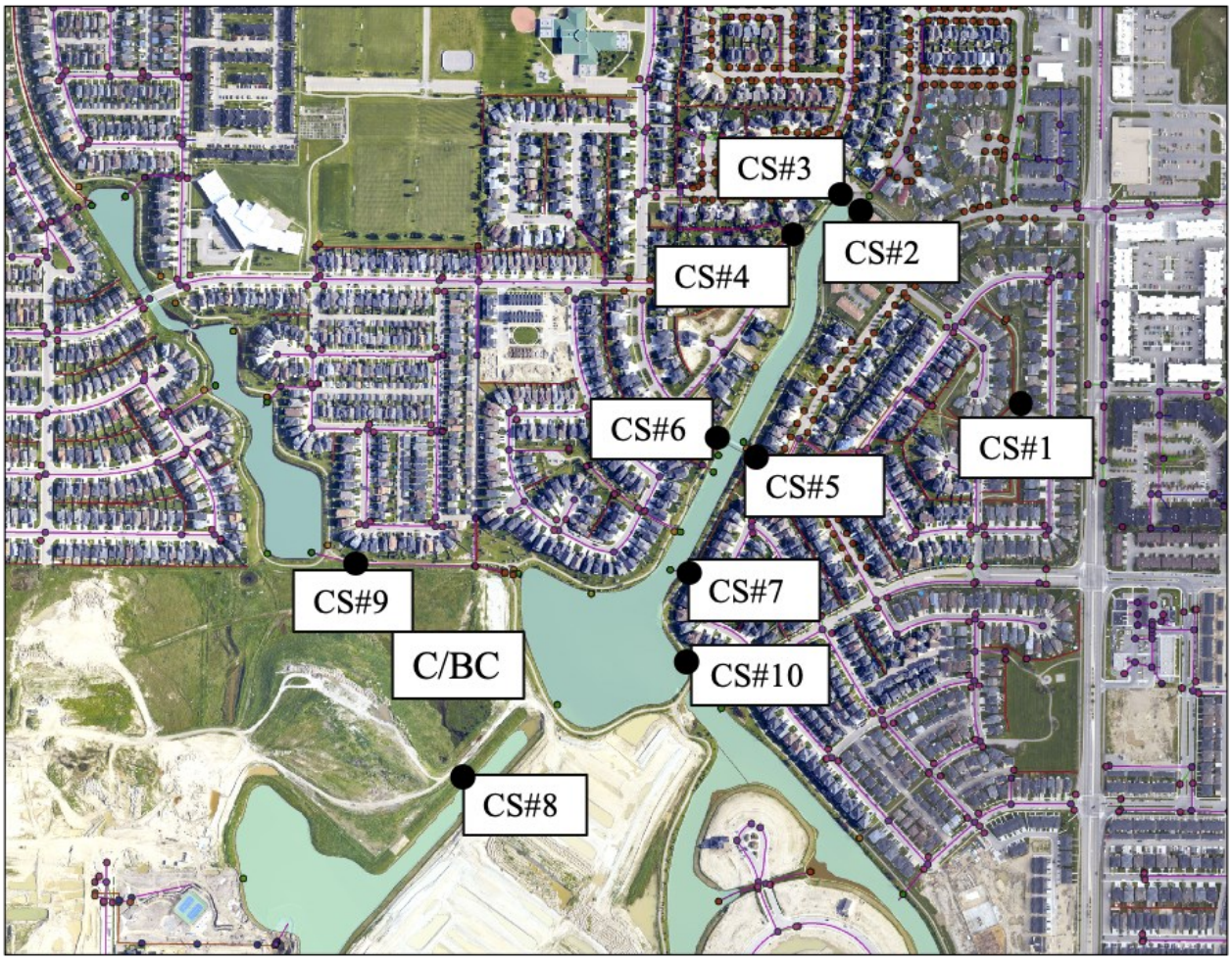

Figure 2-5. Map of the stormwater-impacted Canals & Bayside Creek (C/BC) sampled in Airdrie, Alberta. Note the black dots representing sampling sites for this creek, including CS#1-10, with CS#1 representing a sampling site just before a spray park feature utilizing water from this creek.

Figure 2-6. Map of all stormwater ponds and stormwater-impacted creeks sampled in Airdrie, Alberta in both 2020 and 2021. Starting from the bottom and appearing counter clock-wise, these included Windsong (WS), Hillcrest (HC), King's Heights North & South (KHN & KHS), East Lake (EL), Nose Creek (NC), and the Canals and Bayside Creek (C/BC). Individual water bodies are circled in red for easier viewing.

Table 2-1. GPS co-ordinates and site abbreviations of stormwater pond sites routinely sampled in 2020 and/or 2021.

Stormwater pond	Site	Co-ordinates
East Lake	EL#1	51.285454, -113.985990
	EL#2	51.283973, -113.986515
	EL#3	51.284473, -113.990754
	EL#4	51.287036, -113.991406
	EL#5	51.289850, -113.992430
	EL#6	51.286496, -113.987836
	EL#7	51.286478, -113.9877038
	EL#8	51.288621, -113.985261
Windsong	WS#1	51.255631, -114.026206
	WS#2	51.256113, -114.025876
	WS#3	51.253366, -114.027648
	WS#4	51.254323, -114.027161
Hillcrest	HC#1	51.256003, -114.014242
	HC#2	51.254197, -114.015007
	HC#3	51.255656, -114.016405
King's Heights North	KHN#1	51.263973, -113.987369
	KHN#2	51.259708, -113.987295
King's Heights South	KHS#1	51.259393, -113.984780
	KHS#2	51.256792, -113.983786
	KHS#3	51.257914, -113.987784
Nose Creek Pond	NP#1	51.280038, -114.010182

Table 2-2. GPS co-ordinates and site abbreviations of stormwater pond sites routinely sampled in 2020 and/or 2021.

Stormwater-impacted Creek	Site	Co-ordinates
Nose Creek	N#1	51.278354, -114.010189
	N#2	51.278354, -114.010776
	N#3	51.277910, -114.010542
	N#4	51.276779, -114.009813
	N#5	51.275518, -114.009992
	N#6	51.274818, -114.009018
	N#7	51.273767, -114.00776
	N#8	51.272063, -114.007332
Canals & Bayside Creek	CS#1	51.287515, -114.026755
	CS#2	51.289820, -114.029872
	CS#3	51.289934, -114.030033
	CS#4	51.289485, -114.030969
	CS#5	51.287126, -114.032436
	CS#6	51.286921, -114.031649
	CS#7	51.285562, -114.032771
	CS#8	51.283069, -114.036835
	CS#9	51.285581, -114.039151
	CS#10	51.284417, -114.032941

2.3 Airdrie Weather Data

Weather data consisting of daily antecedent total precipitation was collected during the routine Airdrie stormwater sampling dates in both 2020 (Table 2-3) and 2021 (Table 2-4) from a weather station at the nearby locality of Nier, Alberta. 'Daily antecedent total precipitation' was defined as the total precipitation (in mm) collected the day before the date of sampling. Note that investigative sampling weeks from 2021 are not displayed here, as there was a recorded zero total precipitation throughout these weeks. Notice that for 2020, the night before the Sept. 8th sampling date had the highest daily (24-hr) total antecedent precipitation of the sampling season (Table 2-

3), while for the 2021 sampling season, the weeks of the 17th and 23rd of August had the highest daily total antecedent precipitation (Table 2-4).

Table 2-3. Daily (24-hr) total antecedent precipitation (mm) on routine sampling dates for stormwater samples taken from Airdrie stormwater ponds and stormwater-impacted creeks in 2020, as reported from the nearby (approx. 23 km North-west) Nier weather station Environment Canada, 2020).

Date	24-hr Total Antecedent Precipitation (mm)
17/8/20	0
24/8/20	0
31/8/20	1.2
8/9/20	8.6
15/9/20	0
22/9/20	0
29/9/20	0

Table 2-4. Daily (24-hr) total antecedent precipitation (mm) on routine sampling dates for stormwater samples taken from Airdrie stormwater ponds and stormwater-impacted creeks in 2021, as reported from the nearby (approx. 23 km North-west) Nier weather station Environment Canada, 2021).

Date	24-hr Total Antecedent Precipitation (mm)
19/7/21	0
20/7/21	0
3/8/21	1.7
17/8/21	10.5
23/8/21	6.7
24/8/21	7.6
30/8/21	0
31/8/21	0
7/9/21	0
8/9/21	0

2.4 Culture-based Methods for Microbial Detection and Quantification in Airdrie Stormwater

2.4.1 Bacterial Control Strains Used for Culture Methods

Positive Control Strains for Assaying Culturable FIB in Airdrie Stormwater. Positive controls used for the culture methods (Colilert$^{(R)}$ and Legiolert$^{(R)}$ detection methods– see below) included; 1) *E. coli* ATCC 25922 (Colilert$^{(R)}$), and; 2) *L. pneumophila* ATCC 33152. *Escherichia coli* ATCC 25922 was aliquoted from a frozen 15% glycerol stock solution onto trypticase soy agar (TSA) (BD; ThermoFisher Scientific, Ottawa, Ontario, Canada), and incubated at 37^0 C for 24 hours. *L. pneumophila* ATCC 33152 was grown from a stock solution in a similar fashion,

though was instead incubated at 37^0 C on buffered charcoal yeast extract (BCYE) 1.5% agar (BD; ThermoFisher Scientific, Ottawa, Ontario, Canada) for 5 days.

Positive Control Strains for Assessing Campylobacter and Salmonella spp. in Avian Feces. Bacterial strains utilized for positive controls in both the gull and Canada goose fecal prevalence studies included; 1) *Campylobacter jejuni* ATCC 29428, and; 2) *Salmonella enterica* ser. Meleagridis. Growth conditions for *C. jejuni* ATCC 29428 consisted of incubation on Bolton broth agar (BBA) (Oxoid CM0983; ThermoFisher, Nepean, Ontario, Canada) for 72 hours at 37^0 C in a microaerobic environment created with MicroAero paks (Mitsubishi) within a Mitsubishi AnaeroPak Jar (ThermoFisher Scientific, Ottawa, Ontario, Canada). For *S. enterica* ser. Meleagridis positive controls, trypticase soy agar (TSA) (BD; ThermoFisher Scientific, Ottawa, Ontario, Canada) plates were used, and cultures were incubated in aerobic conditions at 37^0C for 24 hours.

2.4.2 Quantitative Detection of *E. coli* and Total Coliforms Using the Colilert$^{(R)}$ Defined-Substrate Assay (for Stormwater)

Colilert$^{(R)}$ (IDEXX Laboratories, Inc.; Westbrook, Maine, USA) was used to assay stormwater for culturable levels of *E. coli* and total coliforms in both years of sampling (2020 and 2021). This method was used as instructed by the manufacturer. Briefly; one packet of Colilert$^{(R)}$ reagent was added to a vessel containing 100 mL of stormwater sample, the bottle was shaken to mix the contents, before this solution was then poured and sealed into the Quanti-tray/2000 system and incubated at 35^0 C for 24 hours. Positive and negative controls were used for Colilert$^{(R)}$ on each day that water samples were processed. Positive controls consisted of one vessel of 100 mL DI water (HyClone^{TM} Laboratories Inc.; ThermoFisher Scientific, Ottawa, Ontario, Canada) which was spiked with 1 colony of *E. coli* ATCC 25922, while the negative

control consisted of one vessel with 100 mL of DI water only. Both positive and negative controls for Colilert(R) were otherwise processed and incubated the same way as described above. To read results, wells were recorded as either fluorescent and with yellow-coloration, or with yellow-coloration only using Colilert(R) to indicate the presence respectively of *E. coli* (the metabolism of MUG by β-glucuronidase) or total coliforms (the metabolism of ONPG by β-galactosidase), respectively. Colilert(R) results were quantified with standard MPN tables (based on the traditional 15-tube serial dilution method) using the Quanti-tray/2000 system (IDEXX Laboratories, Inc.; Westbrook, Maine, USA).

2.4.3 Quantitative Detection of *L. pneumophila* Using Legiolert(R) Defined-Substrate Assay (for Stormwater)

Legiolert(R) (IDEXX Laboratories, Inc.; Westbrook, Maine, USA) was used to assay for culturable *L. pneumophila* in Airdrie stormwater but only in the first year of sampling (2020). The Legiolert(R) method was used as instructed by the manufacturer, and is based on defined substrate approaches for the specific enrichment and selection of *L. pneumophila*. This method is similar in approach to Colilert(R) for detection of *E. coli* and total coliforms, though with a few important exceptions. Firstly, only 2 mL of water sample was used and mixed with 2 mL of Legiolert(R) pre-treatment, and incubated at room temperature for 60 seconds. Secondly, 2 mL of this solution (equivalent to 1 mL of the original sample) was added to a vessel of 100 mL of sterile water as well as Legiolert(R) reagent, before being incubated in the similar Quanti-tray/Legiolert(R) system for 7 days at 37^0 C. Positive controls used during each day samples were processed, and included a positive control (100 mL DI water) spiked with 1-4 colonies of *L. pneumophila* ATCC 33152, and a negative control of 100 mL DI water. Note that Legiolert(R) control vessels did not undergo pre-treatment, and were instead incubated at 39^0 C For 7 days, as

specified in the protocol and due to the use of potable DI water. In terms of enumeration for the Legiolert(R) assay, wells were considered positive for *L. pneumophila* if there was a brown/black coloration due to the metabolism of Legiolert(R) reagent. Results were quantified with standard MPN tables using the Quanti-tray/Legiolert(R) system (IDEXX Laboratories, Inc.; Westbrook, Maine, USA).

2.5 Molecular-based Methods for Microbial Detection

Quantitative polymerase chain reaction (qPCR) methods were used to determine MST markers, pathogens, and *Enterococcus* prevalence and concentrations in studied stormwater or avian fecal samples. Absolute quantification (via the standard curve method) was used to quantify all targeted qPCR markers with the exception of Entero1 (*Enterococcus*), which instead used relative quantification. Enumeration of *Enterococcus* was therefore performed via the ΔΔ cycle threshold (Ct) method as described in US EPA's Method 1611 (US EPA, 2012b). In brief, this method entails comparing the Ct of tested samples against the Ct of 'calibrator' controls which contain a known quantity of culturable *Enterococcus,* enumerated beforehand as CFU. The resulting ratio (normalized by the ratio of control *Onchorhyncus keta* DNA between sample and calibrator) is therefore known as a 'cell calibrator equivalent' (CCE).

2.5.1 Processing of Stormwater Samples for Molecular DNA Detection of FIB and Bacterial Pathogens

In preparation for quantitative polymerase chain reaction (qPCR) testing of Airdrie stormwater, a slightly modified version of Method 1611 DNA extraction (see US EPA, 2012b) was utilized. Briefly, 20 mL (instead of the standard 100 mL) of stormwater sample was filtered through disposable 0.4 µm-pore polycarbonate MicroFunnel™ filters (Pall Corporation, New

York, USA), with this volume being used because previous in-lab data showed an increased yield of DNA when using this smaller volume. In addition to the filtered stormwater samples, three calibrators (each containing 10 µL of the same calibrator tube) as well as one filtering blank were filtered on each date that samples were received, using 20 mL of PBS as a matrix.

After filtration, each filter was placed aseptically within a bead tube (Generite, North Brunswick, NJ, USA) and 600 µl of a lysis buffer was added containing AE buffer (10 mM Tris-Cl, 0.5 mM EDTA, pH of 9.0) (QIAGEN; Hilden, Germany) and 0.2 µg/mL *O. keta* (Salmon) sperm. At this point, tubes were spun on the Bead Mill 24 Homogenizer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at a velocity of 3.10 rotations/min. for 1 minute. After this, 350 µL of supernatant was carefully drawn from each tube, placed in a separate tube, and centrifuged for 5 minutes at 12,000 X *g*, before 250 µL of supernatant was again carefully removed from each tube and stored at -80^0 C as the final DNA extract. These homogenates were used as a source of DNA templates for qPCR tests targeting FIB (*Enterococcus*) and a variety of bacterial pathogens (*Campylobacter* spp., *Salmonella* spp., *Legionella* spp., *Arcobacter butzleri*, *L. pneumophila,* and STEC) in stormwater samples.

In 2020, testing of stormwater for STEC was done by qPCR, targeting the *stx1* and *stx2* genes from *E. coli* positive Colilert$^{(R)}$ water samples. Briefly, 100 ml stormwater samples were incubated in Colilert$^{(R)}$ in presence/absence vessels at 35^0 C for 24 hours. One mL of *E. coli*-positive cultures was centrifuged at 12,000 X *g* for 5 minutes to pellet bacteria. The supernatant was then removed, and the resulting pellet resuspended in 1 mL of PBS before the centrifugation step was repeated. The supernatant was again removed, and the bacterial pellet re-suspended in 100 µL of PBS. DNA from this volume of sample was then extracted by boiling at 95^0 C for 10 minutes, and used as a template for qPCR detection for STEC.

2.5.2 qPCR Cycling Conditions and Reagents

All qPCR assays performed on either Airdrie stormwater samples or gull and Canada goose fecal samples were run on the Applied Biosystems™ 7500 Real-Time PCR system (Applied Biosystems™; ThermoFisher Scientific, Ottawa, Ontario, Canada) using the fast cycling option. All qPCR runs were performed using 1x PrimeTime® GeneExpression Master Mix (Integrated DNA Technologies, Coralville, Iowa, USA), 200 µg/mL bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, Mississippi, USA), as well as the appropriate primers/probes (See Table 2-5) to total reaction volumes of 5 µL of sample and 15 µL of the above reagents. All gull qPCR runs were 2-step reactions set at the following cycling conditions: 3 minutes of initial denaturation at 95^0 C, before 40 cycles of denaturation and subsequent annealing/extension at 95^0 C for 5 seconds and 60^0 C for 30 seconds respectively. Fluorescence thresholds were set to 0.1 for all assays with the exception of the following assays when Airdrie stormwater was tested at a threshold of 0.05; LeeSG (gull marker), CGO1 (goose marker), VD16S (*Campylobacter* spp. marker), and Lu16S (*Legionella* spp. marker). All qPCR reactions were run in MicroAmp Fast Optical 96-well plates (Applied Biosystems, Foster City, California, USA), with positive controls (calibrators or plasmid standards where appropriate) run in triplicate wells, while negative controls (no template controls [NTC], filtering blanks) and samples were run in duplicate wells (with the exception of pathogen markers assayed in Airdrie stormwater in 2020, which were run in singlicate).

2.5.3 qPCR Controls

Each qPCR run used either the appropriate calibrators (if measuring *Enterococcus* concentrations or inhibition via the Sketa assay), or a standard curve of 1:10 plasmid dilutions of the appropriate marker (50K copies/reaction diluted to 5 copies/reaction), as well as a no template

control (NTC) and filtering blanks. The Sketa assay was used as a sampling process control (SPC) for all US EPA Method 1611 extracted samples tested, as well as to determine whether inhibition occurred in individual samples relative to the filtered calibrators (US EPA, 2012b). Following this protocol, if Sketa Cts in individual samples were found to differ largely (≥3 Cts) from calibrator Cts, the sample was considered inhibited and was re-tested at 1:5 and 1:25 dilutions.

2.5.4 Airdrie Stormwater qPCR

Airdrie stormwater samples from both years (2020 and 2021) were tested by qPCR for the FIB *Enterococcus*, five different fecal-contributing hosts (human sewage [HF183 and HumM2], dogs [Dog3], ruminants [Rum2Bac], gulls [LeeSG], and Canada geese [CGO1]), as well as six different enteric or opportunistic bacterial pathogens (*A. butzleri* [*hsp60*], *Campylobacter* spp. [VD16S], *Salmonella* spp. [*invA*], STEC [*stx*1 and *stx*2], *Legionella* spp. [Lu16S] and *L. pneumophila* [*mip*]) (see Table 2-3).

Table 2-5. Conditions for all qPCR assays using the ABI 7500, including target, primer, and probe names, sequences, and concentrations where applicable.

Target Name	Target	Target Organism (locus)	Primer/Probe	Primer/Probe Target Sequnce (5'-3')	Primer/Probe Conc.	Reference
Fecal Indicator Bacteria (FIB)						
Entero1	*Enterococcus* spp.	*23S* rRNA	Entero1-F	GAGAAATTCCAAACGAACTTG	1 uM	Ludwig & Schleifer (2000)
			Entero1-R	CAGTGCTCTACCTCCATCATT	1 uM	
			Entero1-P	6FAM-TGGTTCTCTCCGAAATAGCTTTAGGGCTA-TAMRA	80 nM	US EPA Method 1611
Microbial Source Tracking (MST)						
HF183	Humans	*Bacterioides* spp. (*16S* rRNA)	HF183-F	ATCATGAGTTCACATGTCCG	1 uM	Haugland et al. (2010)
			BFDrev	CGTAGGAGTTTGGACCGTGT	1 uM	
			BFD-FAM	FAM-CTGAGAGGAAGGTCCCCCACATTGGA-TAMRA	80 nM	
HumM2	Humans	Unknown (alpha-1,2-mannosidase analog)	HumM2-F	CGTCAGGTTTGTTTCGGTATTG	200 nM	Shanks et al. (2009)
			HumM2-R	TCATCACGTAACTTATTTATATGCATTAGC	200 nM	
			HumM2-P	FAM-TATCGAAAATCTCACGGATTAACTCTTGTGTA CGC-TAMRA	125 nM	
Dog3	Dogs	fatty acid coA ligase	Dog3 - F	TTTTCAGCCCCGTTGTTTCG	1 µM	Green et al. (2014)
			Dog3 - R	TGAGCGGGCATGGTCATATT	1 µM	
			Dog3 - P	6FAM-AGTCTACGCGGGCGTACT-MGBNFQ	100 nM	
Rum2Bac	Ruminants	*Bacteroidales* (*16S* rRNA)	BacB2-590F	ACAGCCCGCGATTGATACTGGTAA	200 nM	Mieszkin et al. (2010)
			Bac708Rm	CAATCGGAGTTCTTCGTGAT	200 nM	
			BacB2-626P	6FAM-ATGAGGTGGATGGAATTCGTGGTGT-TAMRA	200 nM	
CGO1	Canada geese	*Bacteroides* spp. (*16S* rRNA)	CGO1-F	GTAGGCCGTGTTTTAAGTCAGC	300 nM	Fremaux et al. (2010)
			CGO1-R	AGTTCCGCCTGCCTTGTCTA	300 nM	
			CGO1-P	6FAM-CCGTGCCGTTATACTGAGACACTTGAG-TAMRA	100 nM	
LeeSG	Gulls	*Catellicoccus marimammalium* (*16S* rRNA)	LeeSG-F	AGGTGCTAATACCGCATAATACAGAG	250 nM	Lee et al. (2013)
			LeeSG-R	GCCGTTACCTCACCGTCTA	250 nM	
			LeeSG-P	6FAM-TTCTCTGTTGAAAGGCGCTT-NFQMGB	125 nM	
Pathogen Markers						
VD16S	*Campylobacter* spp.	*16S* rRNA	VD16S-F	CTGCTTAACACAAGTTGAGTAGG	300 nM	Van Dyke et al. (2010)
			VD16S-R	TTCCTTAGGTACCGTCAGAA	300 nM	
			VD16S-P	6FAM-CGCTCCGAAAAGTGTCATCCTCC-BHQ1	100 nM	
invA	*Salmonella* spp.	*invA*	invA-F	GCGTTCTGAACCTTTGGTAATAA	450 nM	Daum et al. (2002)
			invA-R	CGTTCGGGCAATTCGTTA	450 nM	
			invA-P	6FAM-TGGCGGTGGGTTTTGTTGTCTTCT-MGBNFQ	125 nM	
hsp60	*A. butzleri*	*hsp60*	Abutz-F	CTCTTCATTAAAAGAGATGTTACCAATTTT	300 nM	de Boer et al. (2013)
			Abutz-R	CACCATCTACATCTTC**W**GCAATAATTACT	300 nM	
			Abutz-P	6FAM-CTTCCTGATTGATTTACTGATT-NFQMGB	100 nM	
Stx1	STEC	*Stx1*	Stx1-F	CATCGCGAGTTGCCAGAAT	450 nM	Chui et al. (2013)
			Stx1-R	GCGTAATCCCACGGACTCTTC	450 nM	
			Stx1-P	6FAM-CTGCCGGACACATAGAAGGAAACTCATCA-TAMRA	125 nM	
Stx2	STEC	*Stx2*	Stx2-F	CCGGAATGCAAATCAGTC	450 nM	Chui et al. (2013)
			Stx2-R	CAGTGACAAAACGCAGAACT	450 nM	
			Stx2-P	6FAM-ACTGAACTCCATTAACGCCAGATATGA-TAMRA	125 nM	
Lu16S	*Legionella* spp.	*16S rRNA*	Leg-F1C	GATTAGCCTGCGTCCGATTAG	1 µM	Lu et al. (2015)
			Leg-R1C	GAAATTCCACTACCCTCTCCCA	1 µM	
			Leg-P	6FAM-AGTGTCAGTATTAGGCCAGGTAGC-TAMRA	100 nM	

mip	*L. pneumophila*	*mip*	mip-F mip-R mip-P	AGGATAAGTTGTCTTATAGCA TTAAGAACGTCTTTCATTTG 6FAM-TAATCCGGAAGCAATGGCTAA-TAMRA	300 nM 300 nM 100 nM	Mentasti et al. (2015)
Sample Processing and Inhibition Controls						
Internal Amplification Control (IAC)	synthetic gene	randomized sequence	IAC-F IAC-R IAC-P	CTAACCTTCGTGATGAGCAATCG GATCAGCTACGTGAGGTCCTAC VIC-AGCTAGTCGATGCACTCCAGTCCTCCT-NFQMGB	400 nM 400 nM 100 nM	Deer et al. (2010)
Sketa	*Onchorhynchus keta*	rRNA ITS	Sketa-F Sketa-R Sketa-P	GGTTTCCGCAGCTGGG CCGAGCCGTCCTGGTC VIC-AGTCGCAGGCGGCCACCGT-TAMRA	1 µM 1 µM 80 nM	Haugland et al. (2005) US EPA Method 1611

2.6 Airdrie Stormwater Data Reporting and Analysis

Before data was analyzed, qPCR marker data (both MST and pathogen markers), *Enterococcus* data, and culture data (including *E. coli* and total coliforms) were normalized per 100 mL of stormwater and respectively reported as copies/100 mL, cell calibrator equivalents (CCE)/100 mL, and most-probable-number (MPN)/100 mL. Data analysis of all quantifiable FIB, MST marker, and pathogen marker estimates first began by $\log_{10}$-normalizing all estimates, though non-detects for *E. coli* (<1 MPN/100 mL) were substituted for the lowest limit of detection (1 MPN/100 mL) to accommodate this normalization. Estimates of MST and pathogen markers were defined as non-detect (ND) when the Ct was ≥40 and amplification was not found, detectable but not quantifiable (DNQ) when amplification was detected but was under the 95% limit of quantification (LOQ_{95}), and quantifiable when detection exceeded this LOQ_{95}.

Statistics. Estimates of *Enterococcus* via qPCR and *E. coli* via the defined-substrate Colilert$^{(R)}$ method were tested for normality via the Shapiro-Wilk test, and were found to have skewed distributions (including after $\log_{10}$ normalization). Other statistical tests performed on FIB results included the non-parametric Spearman-rank, Fisher's exact, Kruskal-Wallis, and Mann-Whitney U-tests. The Spearman-rank test wesas used to assess the correlation between FIB (*E. coli*, total coliforms, and *Enterococcus*) for both years of sampling, while the Kruskal-

Wallis and Mann-Whitney U-tests were only used in 2020 to test for site-specific statistical differences because of the requirements for group sample size (n=7 routine samples per site in 2020 versus n=4 in 2021). The Kruskal-wallis test was used for each stormwater pond in 2020 (East Lake and Windsong) to determine whether concentrations of FIB were significantly different across sites (at p = 0.05, though made more stringent via the Bonferroni correction), while the Mann-Whitney U-test was then used to determine which pairs of sites within each stormwater pond were specifically significantly different (again using a p-value of 0.05 that was again further refined by the Bonferroni correction). The Kruskal-Wallis test was used as well to assess whether the same sites sampled in Windsong in 2020 were statistically different than those same sites in 2021. Fisher's exact test was used when comparing frequencies of criteria exceedance between *Enterococcus* and *E. coli* across both geo-spatial and temporal lines. Where more than two categories (I.e., ponds or sampling weeks) were compared and results were significant, pairwise Fisher's exact tests were used (with the Bonferroni correction) to determine which comparisons were significant.

Estimates of MST and pathogen detection were also evaluated (where appropriate) with the non-parametric Fisher's exact test, and in the same way when >2 categories were tested as described for FIB (i.e., pairwise testing with the Bonferroni correction). This testing included for various comparisons in detection between markers, such as along geospatial (e.g., between stormwater ponds) and temporal (e.g., between sampling weeks) lines. McNemar's test was instead used to evaluate whether human sewage (i.e., HF183) detection was more likely to be detected than other individual animal MST markers in samples positive for MST host sources, while this test was also used to evaluate which MST markers were more likely to co-occur with *A. butzleri* detection.

2.7 Microbial Characterization of Aquatic Avian Feces

2.7.1 Avian Fecal Sample Collection

Gull fecal sample collection. Given the predominance of aquatic bird fecal contamination commonly seen in stormponds, a fecal survey of urban gulls and Canada geese was done to assesses FIB occurrence, MST markers and pathogen carriage in these animals. Gull fecal samples (n=39) were collected during the summer (May-July) and fall (October) of 2021 in urban areas of south-central Edmonton, Alberta (particularly parking lots of supermarkets, malls, and fast-food establishments) frequented by gulls. First, gull species was visually identified for each subject, before individual gulls were observed to defecate to ensure specimen freshness and to confirm the identification of individual samples. Fresh fecal samples were swabbed from the ground (generally non-porous concrete or asphalt) using the ESwab^{TM} system (COPAN Diagnostics, Murrieta, CA) before being stored at 4^0 C until samples could be processed ≤12 hours from being sampled. Once at the laboratory, individual fecal samples were first weighed (within their pre-weighed individual tubes) before being transferred to a separate conical tube where phosphate buffered saline (PBS) (HyClone^{TM} Laboratories Inc.; ThermoFisher Scientific, Ottawa, Ontario, Canada) was added until a total volume of 20 mL was reached. Individual samples were then homogenized into a slurry through intensive vortexing. Individual fecal slurries were then used for both the miniaturized MPN-qPCR assay (as described below), as well as for use by qPCR testing (also described below).

Canada goose fecal sample collection. Canada goose fecal samples (n=49) were collected from Hawrelak park exclusively, located in central Edmonton, Alberta during the summer of 2019. Similar to the gull sampling, individual goose scats were confirmed visually by watching

birds defecate, before sterile plastic spoons were used to collect samples into individual 15 mL conical tubes from park grass or asphalt surfaces. Samples were then stored at 4^0 C until processing at the laboratory (which began within an hour of collection). Individual fecal samples were aliquoted into 1 g of feces each per sterile tube, before PBS (HyClone™ Laboratories Inc.; ThermoFisher Scientific, Ottawa, Ontario, Canada) was added to a final volume of 10 mL (in the case of Canada geese 1-41) or 100 mL (in the case of Canada geese 42-49). The majority of individual fecal slurries (27 of 49) were used for the miniaturized MPN-qPCR assay (described below), though qPCR testing was done on fecal composite samples, which were created as described below and made up of 25 of 49 of the individual Canada goose fecal samples.

After sub-sampling from individual fecal slurries for the MPN-qPCR assay, five goose fecal composites were constructed by pooling homogenized slurries of between 3 and 9 individual fecal goose samples into a single sample (the five composites being made up of 25 of 49 of the original individual Canada goose fecal samples total) before samples were again homogenized by vortexing. This was originally done for a separate study, which aimed to test *E. coli* survivability in pooled Canada goose feces over varying periods of time (unpublished). Testing by qPCR on Canada goose feces was therefore performed on the fecal composite samples (n=5 total), as opposed to individual fecal slurries.

2.7.2 Quantitative Detection of *Campylobacter* and *Salmonella* spp. in Avian Feces Using a Miniaturized Most-Probable-Number (MPN) Assay

A 3-tube miniaturized most-probable-number (MPN) assay adapted from Banting et al. (2016), was used to assay for culturable *Campylobacter* spp. and *Salmonella* spp. in individual gull and Canada goose fecal samples.

Miniaturized MPN Assay for Gull Feces. Aliquots of each individual gull fecal slurry (1 mL each in triplicate wells) were added to deep-well microplates (2 mL, 96-well) (Greiner Bio-One, Frickenhausen, Germany), where gull fecal samples were serially diluted (1:10 at each step) until they were 10^{-7} of their original fecal concentration. This was done in either BB media (Oxoid CM0983; ThermoFisher, Nepean, Ontario, Canada) with *Campylobacter* supplement (Dalynn Biologicals Inc., Calgary, Alberta, Canada) for *Campylobacter* spp. testing, or in trypticase soy broth (TSB) (BD; ThermoFisher Scientific, Ottawa, Ontario, Canada) for *Salmonella* spp. assessment. For positive controls, 10 colonies of *C. jejuni* ATCC 29428 or 4 colonies of *S. enterica* ser. Meleagridis were picked and spiked into 1 mL of BB or TSB respectively, and this concentration was diluted to 10^{-4} before 1 mL aliquots were added to the appropriate microplate and further diluted within the plate as a positive control. Plates were covered with hard-shell lids, and incubated under microaerobic conditions using the Mitsubishi AnaeroPak Jar system with Anaero-MicroAero Paks for 42-44 hours at 42^0 C for *Campylobacter* spp. testing, or were incubated in an aerobic environment for 24 hours at 37^0 C for *Salmonella* spp. testing. After the incubation period, 100 µl of TSB (BD; ThermoFisher Scientific, Ottawa, Ontario, Canada) in the microplates used for *Salmonella* spp. testing were subsampled into another deep-well microplate filled with 900 µl of Rappaport Vassiliadis R10 (RV) broth (BD; ThermoFisher Scientific, Ottawa, Ontario, Canada) with Novobiocin supplement (Dalynn Biologicals Inc., Calgary, Alberta, Canada), before this plate was incubated aerobically for 16-18 hours at 42^0 C. Following incubations, 50 µl aliquots of each well of the *Campylobacter* spp. (BB-filled) or *Salmonella* spp. (RV-filled) microplates were boiled at 95^0 C for 10 mins to extract DNA, before being diluted either 1:20 or 1:10 (for the *Salmonella* spp. and *Campylobacter* spp. assay, respectively) in water in preparation for qPCR confirmation of growth in the individual wells.

Miniaturized MPN assay for Canada Goose Feces. The Canada goose miniaturized MPN-qPCR assay protocol was the same as for gulls, though there were a few alterations. For example, 100 µl aliquots were initially used in the first sets of wells, and individual Canada goose fecal samples were diluted down to 10^{-7} in most geese fecal preparation (except for pooled goose samples 30-36, which were diluted down to 10^{-3} of the original concentration). In terms of positive controls, only 1 colony of the appropriate organism (*C. jejuni* ATCC 29428 or *S. enterica* ser. Meleagridis) was used for positive controls, spiked and homogenized into 1 mL of PBS, before 100 µl was seeded into triplicate wells. One well on each plate was additionally left uninoculated (media only) on each plate to serve as a negative control.

qPCR Confirmation and Enumeration of Miniaturized MPN Assay. Using qPCR to confirm whether wells of the MPN assay were positive for either *Campylobacter* spp. (VD16S qPCR assay; Van Dyke et al. [2010]) or *Salmonella* spp. (*invA* assay; Daum et al. [2002]), MPN enumeration was executed using standard 3-tube MPN tables, with wells considered positive when cycle thresholds (Cts) were <35 and there was no detected inhibition (Table 2-5). A duplex assay was run using 100 copies of internal amplification control (IAC) per reaction when the *Salmonella* spp. MPN wells were tested by qPCR, due to concerns of inhibition from the (diluted) RV broth (see Table 2-5). Inhibition was considered to occur if a shift of ≥3 Cts was observed for the IAC assay in any of the wells.

2.7.3 Avian Fecal Sample Processing and Preparation for qPCR

Gull Fecal sample processing and preparation for qPCR. Gull fecal slurries were filtered in 1 mL and 0.1 mL aliquots in 10 mL of PBS through disposable 0.4 µm-pore polycarbonate MicroFunnel™ filters (Pall Corporation, New York, USA) for a modified US

EPA Method 1611 DNA extraction (US EPA, 2012b). DNA extraction otherwise occurred in a manner as described above for Airdrie stormwater samples.

Canada Goose Fecal sample processing and preparation for qPCR. As mentioned above, five fecal slurry samples were constructed from 3 – 9 individual goose fecal samples, and these were the samples that would undergo later qPCR testing. These samples were originally intended to be used as part of different study (unpublished). Since much of this methodology was irrelevant to this particular study, only the methods necessary for the results presented will be described. Briefly, fecal composite slurries made as described above were spiked into 250 mL transportation bottles of deionized (DI water (HyClone^{TM} Laboratories Inc.; ThermoFisher Scientific, Ottawa, Ontario, Canada) at a 1:200 or 1:2000 dilution (i.e., equivalent to 1 g of feces in 200 or 2000 mL of water, depending on the week they were sampled). For each composite fecal slurry tested, duplicate bottles at each were then filtered based on a slightly modified US EPA Method 1611 protocol (as described above for Airdrie stormwater sampling), where 20 mL of water was filtered instead of 100 mL (US EPA, 2012b).

2.7.4 Avian Feces qPCR

Gull feces qPCR. Gull fecal samples underwent qPCR testing for *Enterococcus* (Entero1), gull (LeeSG) and Canada goose (CGO1) MST markers, two enteric pathogens (*Campylobacter* spp. [VD16S] and *Salmonella* spp. [*invA*]), as well as the HF183 human sewage marker (see Table 2-5). Due to lab error, it was not possible to use the calibrators that were filtered alongside gull fecal samples for either Sketa Ct estimation, or for *Enterococcus* Ct estimation, though this error did not affect any of the individual gull fecal samples tested. Instead, results from calibrator extracts from 9 weeks of Airdrie stormwater sampling that occurred the same summer and fall

as the gull sampling were pooled and substituted as a replacement for the Ct values of Sketa and *Enterococcus* that were not detected in the failed original calibrators.

Canada goose feces qPCR. Goose fecal samples underwent qPCR testing as described above for gulls for markers including *Enterococcus* (Entero1), Canada goose (CGO1), *Campylobacter* spp. (VD16S), and *Salmonella* spp. (*invA*), though results were averaged from the 8 bottles filtered (20 mL each) from each fecal composite. There were no issues with calibrators for Canada Goose fecal composites, so they were utilized as described above and in US EPA Method 1611 (US EPA, 2012b).

2.7.5 Avian Fecal Data Reporting and Analysis

Gull data analysis. Data analysis was performed for qPCR results of MST, FIB, and pathogen markers by averaging results between the 1 mL and 0.1 mL aliquots, after first normalizing these results to wet weight (i.e., per 1 gram) of feces. Results for qPCR markers (MST and pathogen markers) and *Enterococcus* were thus respectively reported as copies/g and CCE/g. Quantifiable results were $\log_{10}$ normalized. Markers were considered not detected (ND) in a sample if there was no detectable marker (i.e., no Ct observed), considered detectable but not quantifiable (DNQ) if copy numbers were detected but did not exceed the 95-percentile limit of quantification (LOQ_{95}), and were considered quantifiable if they were both detected and the copy number exceeded the LOQ_{95}. Geometric means of *Enterococcus*, MST markers, and enteric pathogens (*Campylobacter* spp. and *Salmonella* spp.) were calculated by first substituting all ND and DNQ values as '1', and adding a constant of 1 to every other value before log-transformation in order to compensate for the inability of log-transformation and later geometric mean tabulation when values were missing or '0'.

Statistics. In terms of statistical analysis, the Shapiro-Wilk test was first used to confirm that distributions of LeeSG and *Enterococcus* marker were not normally distributed (including after $\log_{10}$ transformation). Next, the Spearman-Rank correlational test was used to determine whether a statistically significant correlation could be found between LeeSG concentrations and *Enterococcus* concentrations in gull feces.

Canada goose data analysis. MPN data analysis and qPCR data analysis occurred in the same manner for Canada goose fecal samples as for gull fecal samples with the following exception. As fecal samples used in qPCR (but not the miniaturized MPN assays, which used individual goose fecal samples) were made up of fecal composites of 3 – 9 fecal samples, data was normalized by wet weight (i.e., per 1 gram of feces) for each fecal composite and thus was not representative of fecal samples from individual geese.

3. Microbial Water Quality of Stormwater

3.1 Introduction

Fecal indicator bacteria (FIB) such as *Enterococcus* and *E. coli* have long been used to indicate the potential health risks of fecal contamination in water, and the relationship between the two has led to the derivation of microbial water quality guidelines for a variety of source waters based on the enumeration of FIB (Health Canada, 2010, 2012, 2020; NASEM, 2016; US EPA, 2012a). FIB share a number of traits that assist serving this purpose, including; high culturability from water, being found in high concentrations within the human/animal intestinal tract (see Ahmed et al., 2019a; Ervin et al., 2013), and correlating with gastrointestinal illness in recreational contexts when a clear point-source of human fecal contamination is present (Wade et al., 2003, 2006, 2010). Over time, use of FIB has expanded to include its utilization in monitoring the water quality of source waters used for irrigation, recreation, or potable and non-potable water reuse (CCME, 1987; Health Canada, 2010, 2012, 2020, NASEM, 2016; Sanz & Gawlik, 2014; US EPA, 2012a). Guidelines for recreational water quality are often seen as being appropriate for other applications or source waters (i.e., irrigation, non-potable stormwater use), as there is a tendency to assume that if the water is 'safe' enough to swim in, then this quality should be safe to irrigate or discharge our wastes at.

Generally, current water quality standards have been designed around the enumeration of FIB in water in terms of both a method of measuring central tendency (such as geometric mean), and as a method of determining unacceptable concentrations within a single sample (such as a standard threshold value [STV] or single sample maximum) (Gov't of Alberta, 2018; Health Canada, 2010, 2012; US EPA, 2012a). Canada (and by extension Alberta) both currently recognize the use of *E. coli* and *Enterococcus* as the primary FIB for microbial recreational water

quality assessments, with Alberta recently adopting criteria from the US EPA's *Recreational Water Quality Criteria* (2012) (Gov't of Alberta, 2018; Health Canada, 2012).

Despite FIB being traditionally used as indicators of fecal contamination in water, (and consequent illness risk), several caveats of their use exist. These include that FIB concentrations do not appear to correlate well with health outcomes where there is no apparent point source of human fecal contamination (Arnold et al., 2013; Colford et al., 2007; Fleisher et al., 2010; Griffith et al., 2016), the existence of indigenous environmental strains of FIB (Byappanahalli et al., 2012; Devane et al., 2020), the lack of host specificity of FIB (Ahmed et al., 2019a; Fogarty et al., 2003; Gordon & Cowling, 2003; Layton et al., 2010) considering differing risks from human versus non-human fecal pollution sources (Brown et al., 2017a; Schoen & Ashbolt, 2010; Soller et al., 2010), and a frequent lack of correlation between FIB and enteric pathogens (de Man et al., 2014; Schriewer et al., 2010; Steele et al., 2018). In spite of these caveats, obtaining estimates of FIB can still be useful for acquiring data on general fecal contamination in stormwater, and the majority of currently used water quality guidelines are based on end-point FIB measurement (Gov't of Alberta, 2018; NASEM, 2016; US EPA, 2012a), therefore making non-potable use of stormwater dependent on these guidelines.

FIB contamination has been found to be ubiquitous in stormwater (Ahmed et al., 2019b, 2020; Hachad et al., 2022; Hart el a., 2020; Lee et al., 2020; Monteiro et al., 2021; Nshimyimana et al., 2014; Olds et al., 2018; Sauer et al., 2011; Williams et al., 2022), though gaps still exist in understanding the patterns and concentrations of FIB in this matrix, particularly in regards to the variation in prevalence, concentrations, geo-spatial and temporal patterns in stormwater in Canada specifically (Hachad et al., 2022; Johnson et al., 2003; Staley et al., 2018).

As a result of the above, a scoping study was carried out to assess microbial water quality (*Enterococcus* and *E. coli* in particular) within Airdrie stormwater ponds and stormwater-impacted creeks. The goals of the study were to; 1) understand the prevalence, concentrations, and predominance of *Enterococcus* and *E. coli* in Airdrie stormwater; 2) establish whether concentrations of FIB frequently exceed commonly used water quality standards, such as Alberta's *Alberta Surface Water Quality Guidelines*, and; 3) explore the potential for factors that may affect FIB prevalence and concentrations such as spatial (e.g. site differences) and temporal (e.g., weather-dependent) factors.

3.2 Results

Three FIB were principally studied in Airdrie stormwater, including *E. coli*, *Enterococcus*, and total coliforms. Traditional standards used in the Government of Alberta's *Alberta Surface Water Quality Guidelines* (though based on the US EPA's criteria) currently include STV and GM values for allowable *E. coli* or *Enterococcus* concentrations for safe recreational use of water and as guidelines for ambient water quality. As total coliforms are not included in these criteria (albeit present in previous criteria), the focus on this section is primarily on *E. coli* and *Enterococcus* presence in the stormwater ponds studied, though total coliforms will be addressed where appropriate as an indicator of total non-specific fecal pollution.

FIB distributions detected in East Lake and Windsong stormwater ponds sampled in 2020, as well as in the Hillcrest, Nose Creek, Canals, King's Heights, and Windsong stormwater impacted water bodies sampled in 2021, varied widely between each other, as well as between and within individual sites in a single stormwater pond. Consequently, traditional water quality standards based on *E. coli* and *Enterococcus* concentrations were exceeded with differing

frequencies and magnitudes in different stormwater sites and ponds. It is important to note that total coliform concentrations were frequently above the upper Limit of Quantification (LOQ) of the assay used to test for these bacteria, particularly in samples collected in 2021 (96 of 126 samples, 76.2%), providing an obstacle in meaningful analysis of the distribution of total coliforms in both years of sampling.

As can be seen in Figures 3-1 and 3-2, FIB were widely distributed in routine samples of Airdrie stormwater in both years of sampling (2020 and 2021). *Enterococcus* concentrations were overall relatively high in samples taken from 2020 and 2021, with a median of 2.5 $\log_{10}$ CCE/100 mL in the former year, and 2.9 $\log_{10}$ CCE/100 mL in the latter year (Figures 3-1, 3-2). Concentrations of *Enterococcus* were highly variable in stormwater, however, ranging from 1.6 $\log_{10}$ CCE/100 mL to 5.1 $\log_{10}$ CCE/100 mL in 2020, and from 1.4 $\log_{10}$ CCE/100 mL to 6.5 $\log_{10}$ CCE/100 mL in 2021. *E. coli* concentrations instead ranged from ND to $\geq$3.4 $\log_{10}$ MPN/100 mL (the upper detectable range of the assay) in both years, while median values were found to be just shy of 1 $\log_{10}$ MPN/100 mL in 2020, and 1.8 $\log_{10}$ MPN/100 mL in 2021. It is important to note, however, that different stormwater ponds and creeks were sampled each year (except Windsong, which was consistent), meaning that the two years of data are not necessarily directly comparable on a year-to-year basis. Overall, however, the data demonstrates that stormwater is a relatively poor-quality source water based on current guidelines.

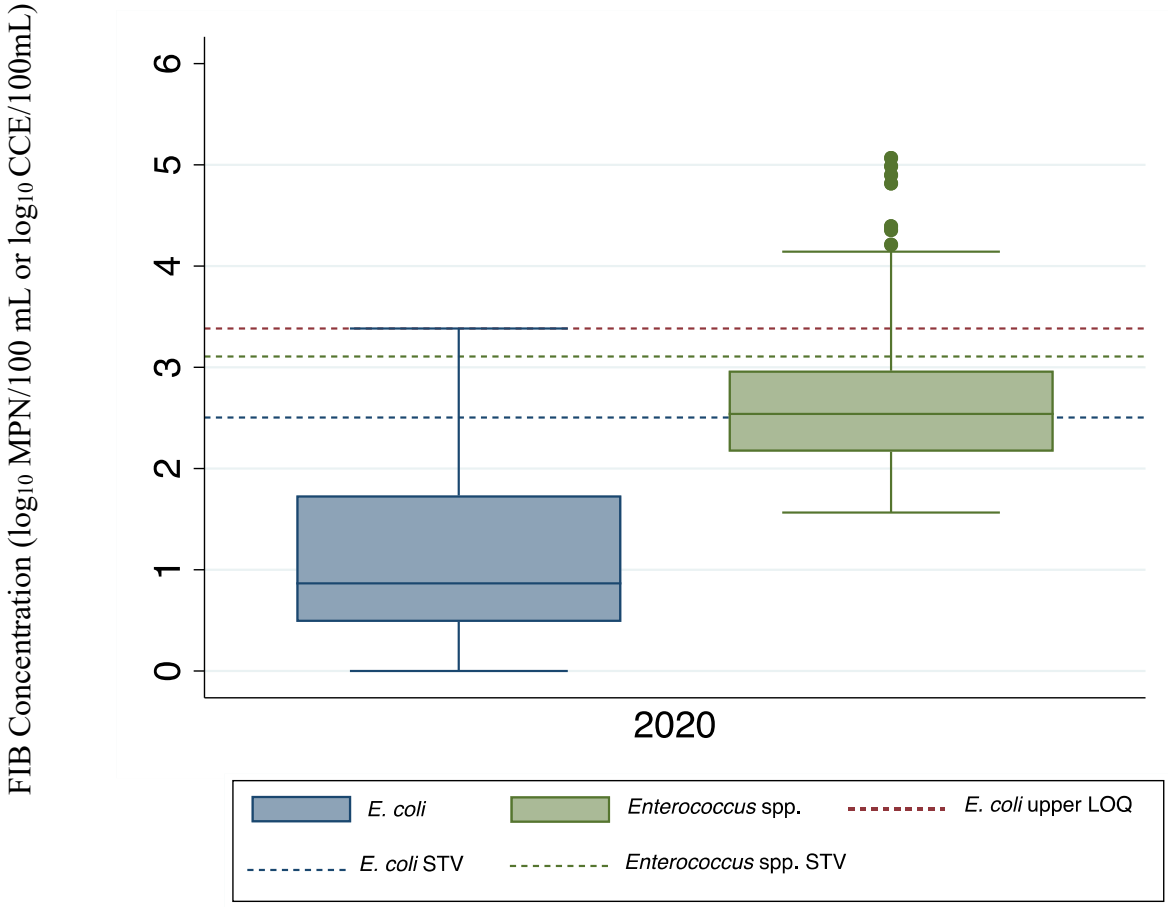

Figure 3-1. Box and whisker plots of total fecal indicator bacteria (FIB) distributions in all routine sites combined from both East Lake and Windsong stormwater ponds in Airdrie, Alberta, Canada (sampled in 2020). The solid line within each box is representative of the median FIB concentration, outer horizontal edges of each box represent the 25th and 75th percentile values of concentration, while whiskers represent ±1.5*interquartile range (IQR). Outliers are represented by colored dots outside the range of the upper whisker. Note the dotted lines representing acceptable STVs for *E. coli* (320 MPN/100 mL) and *Enterococcus* (1280 CCE/100 mL), as well as the upper limit of quantification (LOQ) of the Colilert$^{(R)}$ assay (2419.60 MPN/100 mL).

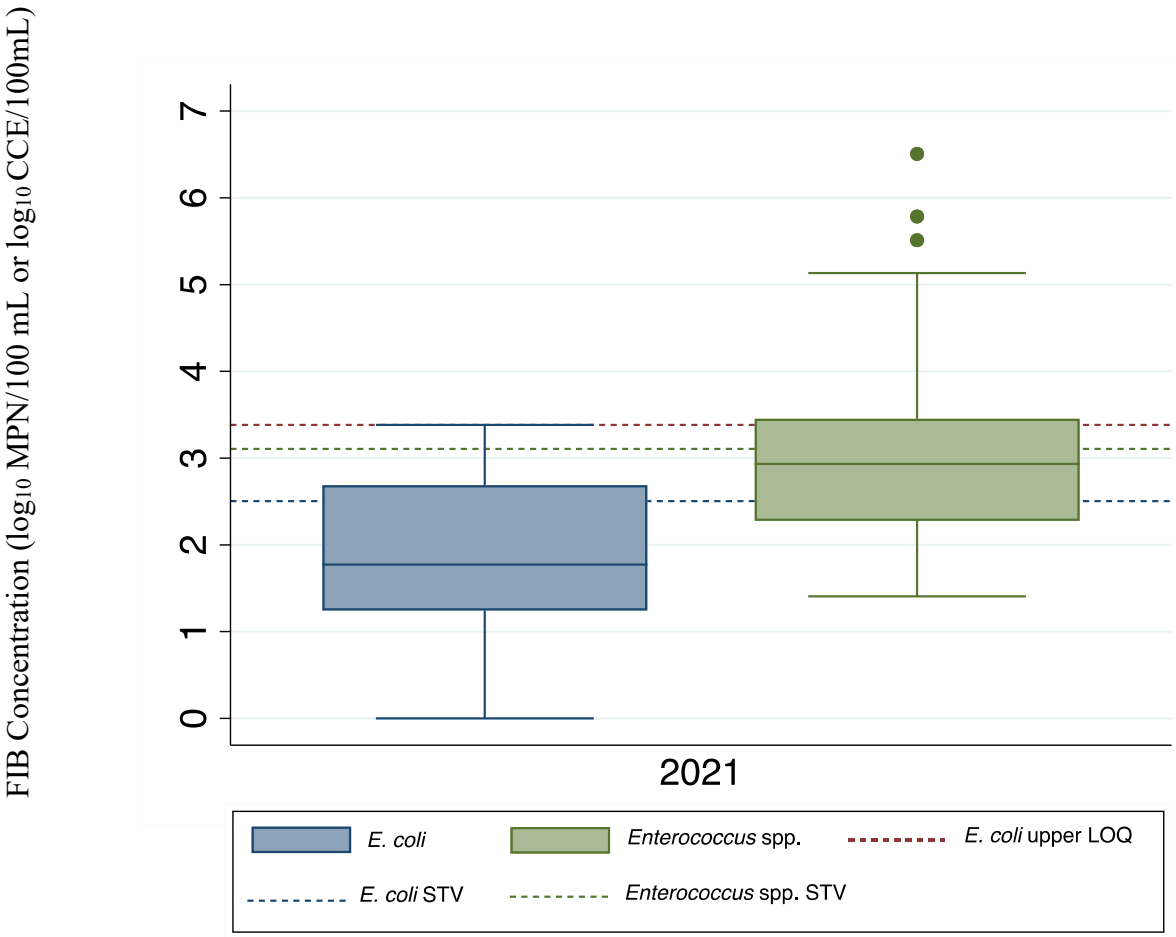

Figure 3-2. Box and whisker plots of total fecal indicator bacteria (FIB) distributions in all sampled routine stormwater sites combined from Windsong, Hillcrest, King's Heights, Nose Creek, and Canals in Airdrie, Alberta, Canada (sampled in 2021). The solid line within each box is representative of the median FIB concentration, outer horizontal edges of each box represent the 25th and 75th percentile values of concentration, while whiskers represent ±1.5*interquartile range (IQR). Outliers are represented by colored dots outside the range of the upper whisker. Note the dotted lines representing acceptable STVs for *E. coli* (320 MPN/100 mL) and *Enterococcus* (1280 CCE/100 mL), as well as the upper limit of quantification (LOQ) of the Colilert$^{(R)}$ assay (2419.60 MPN/100 mL).

3.2.1 Microbial Water Quality Between Stormwater Ponds and Sites

3.2.1.1 Microbial Water Quality in 2020

Enterococcus and *E. coli* concentrations varied widely between and within stormwater ponds during 2020 sampling (see Fig. 3-3), while total coliform distributions were either relatively stable or skewed by samples detected frequently above the upper LOQ. Using the Kruskal-Wallis test, *E. coli* concentrations were found to differ significantly between East Lake and Windsong stormwater ponds ($p= 0.0029$), with the latter pond having higher concentrations, though it was notable that *Enterococcus* concentrations were not significantly different between the ponds. *Enterococcus* concentrations varied largely across East Lake sites, ranging from a minimum of 1.6 $\log_{10}$ CCE / 100 mL to 4.9 $\log_{10}$ CCE/100 mL, while ranging comparably from 1.7 $\log_{10}$ CCE/100 mL to 5.1 $\log_{10}$ CCE/100 mL across Windsong sites. *E. coli* concentrations also ranged widely between both the lower and upper LOQ (0 $\log_{10}$ MPN/100 mL to ≥3.4 $\log_{10}$ MPN/100 mL) in samples from both East Lake and Windsong. Statistically significant differences in *Enterococcus* concentrations were found via the Kruskal-Wallis test between East Lake sites ($p=0.0001$), as well as between Windsong sites ($p=0.024$). Via the same test, *E. coli* concentrations were also found to be statistically different across East Lake sites ($p=0.005$), though they were not found to be statistically significantly different across Windsong sites. Note, however, that 5 of 28 Windsong samples (17.9%) were found to be above the upper LOQ for the *E. coli* assay used, including two samples from WS#2, one from WS#3, and two from WS#4, in comparison to only one sample from East Lake that exceeded this LOQ value (from EL#8).

In light of significant differences between both *Enterococcus* and *E. coli* in East Lake sites, as well as *Enterococcus* in Windsong sites, each stormwater pond was tested for specific between-site differences via the Mann-Whitney U-test. The EL#8 site was found to be

statistically significantly different in *Enterococcus* concentration (using the Bonferroni correction), from sites EL#1, EL#3, EL#4, EL#6, and EL#7 (exact p=0.0006 for each comparison). In addition, EL#5 itself also had a marginally significant difference in *Enterococcus* concentration from EL#1 when considering the Bonferroni correction (p=0.0012). EL#8 was also found to have significantly higher *E. coli* concentrations compared to EL#1, EL#3, EL#6, and EL#7 (respective exact p=0.0012, 0.0006, 0.0006, 0.0006), though the difference between EL#8 and EL#1 was only marginal based on the Bonferroni correction. Using the same statistical test as above (with a Bonferroni-corrected p-value of 0.0083), WS#4 was found to only be significantly different from WS#1 in *Enterococcus* spp. concentration (exact p=0.0012).

Based on these results, EL#8 and WS#4 appeared to be the two sites most heavily affected by fecal pollution in 2020, having the highest medians of both *Enterococcus* (3.1 $\log_{10}$ CCE/100 mL and 3.9 $\log_{10}$ CCE/100 mL, respectively) and *E. coli* (2.3 $\log_{10}$ MPN/100 mL and 3.2 MPN/100 mL respectively) (Fig. 3-3). These two sites aside, *Enterococcus* and *E. coli* median values did not vary between the other sites in East Lake and Windsong by >1 log in concentration, staying generally within 2-3 $\log_{10}$ CCE/100 mL for *Enteroccocus*, and <1 to <2 $\log_{10}$ MPN/100 mL for *E. coli* (Fig. 3-3). As a result, both FIB had distributions that appeared to mirror each other, particularly at the most affected sites of WS#4 and EL#8.

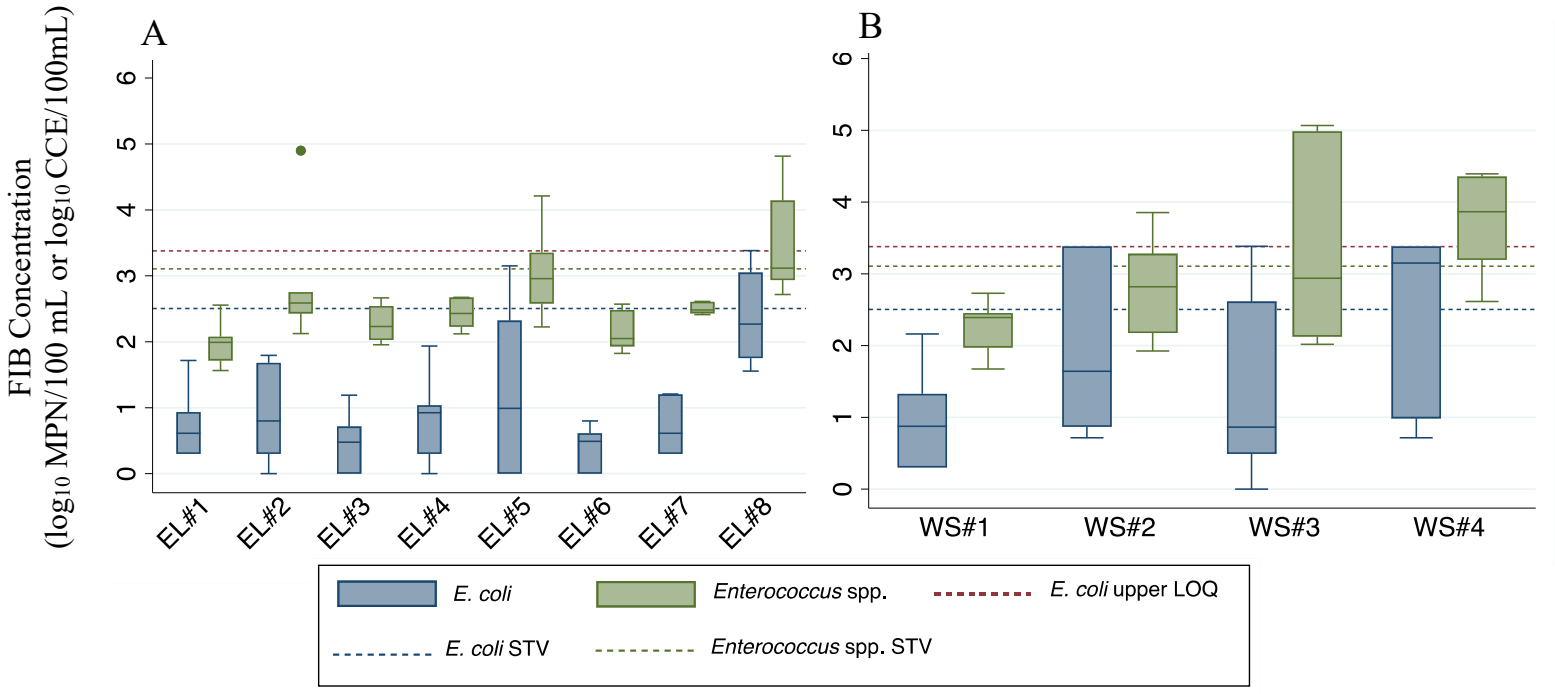

Figure 3-3. Box and whisker plots of fecal indicator bacteria (FIB) distributions for sampling sites from East Lake (A), and Windsong (B) stormwater ponds in Airdrie, Alberta, Canada (sampled in 2020). The solid line within each box is representative of the median FIB concentration, outer horizontal edges of each box represent the 25th and 75th percentile values of concentration, while whiskers represent ±1.5*interquartile range (IQR). Outliers are represented by colored dots outside the range of the upper whisker. Note the dotted lines representing acceptable STVs for *E. coli* (320 MPN/100 mL) and *Enterococcus* (1280 CCE/100 mL), as well as the upper limit of quantification (LOQ) of the Colilert$^{(R)}$ assay (2419.60 MPN/100 mL).

3.2.1.2 Microbial Water Quality in 2021

During the 2021 sampling season, there was again high variation for *Enterococcus* and *E. coli* across stormwater ponds and sites (Fig. 3-4), while total coliforms concentrations were almost universally above the upper detectable limit. Statistically significant differences in *Enterococcus* (but not *E. coli*) concentrations between the four studied stormwater ponds and two stormwater-impacted creeks were observed by the Kruskal-Wallis statistical test (p=0.0002).

Subsequently, it was observed that Nose Creek sites contained statistically significantly higher concentrations of *Enterococcus* than both the Canals ($p<0.0001$) and the King's Heights South stormwater pond ($p=0.0005$) by pairwise Mann-Whitney U-tests using the Bonferroni correction. Amongst the four stormwater ponds (Windsong, Hillcrest, King's Heights North, and King's Heights South) and two stormwater-impacted creeks (Nose Creek and the Canals) tested, *Enterococcus* ranged most dramatically in Windsong from a minimum of 1.6 $\log_{10}$ CCE/100 mL in a sample from WS#2 to a maximum of 6.5 $\log_{10}$ CCE/100 mL from a sample in WS#3 (Fig. 3-4C). In contrast, the Hillcrest stormwater pond had the least dramatic range of *Enterococcus* concentrations from a minimum of 1.6 $\log_{10}$ CCE/100 mL in a HC#2 sample to 3.5 $\log_{10}$ CCE/100 mL at the same site (Fig. 3-4A). In terms of *E. coli*, concentrations ranged from <1 $\log_{10}$ MPN/100 mL to the upper LOQ of 3.4 $\log_{10}$ MPN/100 mL in the above stormwater ponds. It is important to note that nearly 33% of samples from Windsong (5 of 16 sampes, 31.3%) were detected at greater than the upper LOQ for *E. coli*, while ≤25% of samples from each other stormwater pond and creek were above this upper LOQ.

In a direct comparison between 2020 and 2021, *E. coli* concentrations were statistically significantly higher in the latter year in Windsong site studied ($p=0.0171$) by the Kruskal-Wallis test, though differences in *Enterococcus* between both years at this stormwater pond were insignificant. More specifically, 3 of 4 of the Windsong sites sampled in both years had higher median *E. coli* concentrations in the more recent year of sampling (2021), while 2 of 4 of these sites also had higher *Enterococcus* median values in the second year of sampling, though all differences were <1 $\log_{10}$ higher in concentration. The converse was seen at Windsong site WS#4, which was the site with the highest median *Enterococcus* and *E. coli* concentration in

2020, but had smaller median estimates for both FIB in 2021 (though differences were again <1 $\log_{10}$ in concentration).

3.2.3 Associations Between *Enterococcus*, *E. coli*, and Total Coliforms

Individual FIB were found to be significantly correlated to each other via the Spearman rank-test in Airdrie stormwater during both 2020 (Table 3-1) and 2021 (Table 3-2). In the 2020 sampling year, correlation was moderate between the three studied FIB (i.e., $\rho > 0.5$ but < 0.75), and quite similar regardless of which FIB were paired together (Table 3-1.).

Table 3-1. Spearman correlation coefficients for FIB concentrations from all collected stormwater samples from Airdrie, Alberta in 2020 (n=84).

	Correlation (ρ)	
FIB	Total coliforms	*E. coli*
Enterococcus spp.	0.61*	0.56*
E. coli	0.55*	-

* $p < 0.0001$

In 2021, however, correlation was relatively weak ($\rho<0.5$) between *Enterococcus* and total coliforms, as well as between *E. coli* and total coliforms, while *E. coli* was correlated with *Enterococcus* to a higher degree in 2021 than in 2020 (Tables 3-1, 3-2). Note, however, that total coliforms concentrations in 2021 were frequently at or above the upper LOQ in comparison to results in 2021, and it is probable that this had an effect on the correlational results of 2021.

Table 3-2. Spearman correlation coefficients for FIB concentrations from all collected routine stormwater samples from Airdrie, Alberta in 2021 (n=126).

	Correlation (ρ)	
FIB	Total coliforms	*E. coli*
Enterococcus spp.	0.34†	0.70*
E. coli	0.31†	-

* $p < 0.0001$

† $p < 0.001$

On a site by site basis (Table 3-3), as well as on a sample by sample basis (Table 3-4) *Enterococcus* and *E. coli* criteria exceedance were generally in agreement where if criteria exceedance occurred for one FIB (either STV or GM violations), it frequently occurred for the other, though this could also often be discordant. For example, 28 of 43 sites (65.1%) tested in 2020 and 2021 exhibited concordance in whether GM criteria for either FIB were exceeded or not (Table 3-3), while 83.3% of individual routine samples (175 of 210) from both years were themselves concordant on whether STV criteria for either FIB were exceeded or not. On a per site basis, *Enterococcus* GM criteria exceedance was significantly discordant from *E. coli* GM criteria exceedance (McNemar's test; $p = 0.0001$), and the same could be said on a per sample basis for STV criteria (McNemar's test; $p = 0.0019$). Notably, when discordant results occurred, samples and sites were generally more likely to exceed *Enterococcus* criteria (either STV or GM) without exceeding *E. coli* criteria than the other way around, suggesting overall that *Enterococcus* could be potentially slightly more sensitive as a fecal pollution marker than *E. coli*.

Table 3-3. Two-by-two table of the number of Airdrie stormwater sites in both years that exceeded either the *Enterococcus* GM (>300 CCE/100 mL), the *E. coli* GM (>100 MPN/100 mL), both, or neither (n=43).

	Enterococcus GM exceeded	*Enterococcus* GM not exceeded
E. coli GM exceeded	17	0
E. coli GM not exceeded	15	11

Table 3-4. Two-by-two table of the number of individual Airdrie stormwater samples collected both years that exceeded either the *Enterococcus* STV (>1280 CCE/100 mL), the *E. coli* STV (>320 MPN/100 mL), both, or neither (n=210).

	Enterococcus STV exceeded	*Enterococcus* STV not exceeded
E. coli STV exceeded	39	8
E. coli STV not exceeded	27	136

3.2.4 Traditional Water Quality Standard Exceedances

Due to the variable and frequently high concentrations of *E. coli* and *Enterococcus* that were observed in Airdrie stormwater in both 2020 and 2021, FIB data was analyzed based on percent of samples exceeding the recreational standards set out in the Government of Alberta's *Environmental Quality Guidelines for Alberta Surface Waters* (2018) as noted above (see introduction for greater details). To reiterate, these standards consist of a single sample threshold value (STV) for acceptable concentrations of *E. coli* or *Enterococcus* in a sample (with no more than 10% of sample as a site allowed to exceed this value in a given month), as well as a method of calculating central tendency via the geometric mean (GM) of FIB concentrations within the same site over a set period of time.

3.2.4.1 Traditional Water Quality Standard Exceedances in 2020

In both the East Lake and Windsong stormwater ponds, recreational standards for *E. coli* and *Enterococcus* were frequently violated at multiple sites and in both stormwater ponds sampled in 2020 (Table 3-5). For example, Windsong sites had a particularly frequent level of exceedance, with 3 of 4 sites sampled violating the STV standard (i.e., having at least 10% of samples greater than the recommended STV) of both *E. coli* and *Enterococcus*, as well as violating the allowable GM of 300 CCE/100 mL in these three sites (Table 3-5). The magnitude of recreational standard exceedance was also high, with these three Windsong sites having between 28.6% and 85.7% of samples above the recommended STV for both FIB, as well as *Enterococcus* GMs within these sites that ranged between approximately double and >1 order of magnitude higher than the recommended GM. This observation was especially pronounced for WS#4, which not only violated all STV and GM standards, but also exceeded these criteria with the majority of samples (≥57.1% STV exceedance) and to a higher extent (i.e., an *Enterococcus* GM 1.3 $\log_{10}$ CCE/100 mL higher than the recommended GM) than at other sites (Table 3-5).

In comparison to Windsong, East Lake had both fewer sites violating traditional water quality criteria and had fewer sites exceeding these standards with a high magnitude, with the exception of at the site EL#8 (Table 3-5). Alongside Windsong site WS#4, EL#8 also violated all STV and GM standards for both FIB, as well as having a similarly high magnitude of exceedance for these standards. Of note, WS#4 and EL#8 had the highest GMs of both *Enterococcus* and *E. coli* in comparison to any of the other sites sampled in 2020, and were therefore the only sites in that year that exceeded the recommended *E. coli* GM of 100 MPN/100 mL.

Table 3-5. Frequencies of FIB concentrations over acceptable standard threshold values (STVs) and $\log_{10}$ geometric means (GMs) according to Alberta recreational standards in Airdrie stormwater sites sampled during 2020 from the East Lake and Windsong stormwater ponds.*

Stormwater Pond	Site	n	Frequency of samples > 1280 CCE/100 mL for *Enterococcus* (%)	Frequency of samples > 320 MPN/100 mL for *E. coli* (%)	$\log_{10}$ site GM for *Enterococcus* (>300 CCE/100mL in bold)	$\log_{10}$ site GM for *E. coli* (>100 MPN/100 mL in bold)
East Lake	EL#1	7	0	0	2.00	0.83
	EL#2	7	**1/7 (14.3)**	0	**2.86**	0.93
	EL#3	7	0	0	2.29	0.63
	EL#4	7	0	0	2.43	0.94
	EL#5	7	**2/7 (28.6)**	**1/7 (14.3)**	**3.00**	1.43
	EL#6	7	0	0	2.18	0.53
	EL#7	7	0	0	**2.50**	0.78
	EL#8	7	**4/7 (57.1)**	**3/7 (42.9)**	**3.50**	**2.42**
	Total East Lake	56	**7/56 (12.5)**	4/56 (7.1)	N/A	N/A
Windsong (2020)	WS#1	7	0	0	2.26	1.07
	WS#2	7	**2/7 (28.6)**	**2/7 (28.6)**	**2.78**	1.88
	WS#3	7	**3/7 (42.9)**	**2/7 (28.6)**	**3.23**	1.47
	WS#4	7	**6/7 (85.7)**	**4/7 (57.1)**	**3.73**	**2.41**
	Total Windsong	28	**11/28 (39.3)**	**8/28 (28.6)**	N/A	N/A
Total	Total	84	**18/84 (21.4)**	**12/84 (14.3)**	N/A	N/A

*Note that values in bold represent sites where >10% of samples are above the recommended STV, or the GM calculated for the site is greater than the recommended GM.

The frequency of exceedance of STVs appeared to follow temporal patterns in 2020, peaking during the weeks of Aug. 17th and Sept. 8th for both *E. coli* and *Enterococcus* in ≥33.3% of samples each week and for each of the two organisms tested (Table 3-6). This is in sharp contrast to any of the other sampling dates in 2020, particularly for *E. coli*, where 0 to 1 samples exceeded the relevant STV per week with the exception of these two peak weeks just described (Table 3-6). It was also during these two weeks that 24-hr total antecedent precipitation was observed at its highest during that time of sampling in 2020, with the other weeks having little to no estimated 24-hr total antecedent precipitation. These two weeks aside, STV exceedance was consistent (but low) in frequency across all weeks for both of the aforementioned FIB (Table 3-6). However, an important caveat to note is that there was no statistically significant differences in *Enterococcus* and *E. coli* STV criteria exceedances in 2020 between weeks when tested by Fisher's exact test.

Table 3-6. Frequencies of FIB concentrations over acceptable standard threshold values (STVs) stratified by date of sampling in 2020 for all routine Airdrie stormwater samples combined (n=84).

Dates	n	Frequency of Samples > 1280 CCE/100 mL for Enterococcus (%)	Frequency of samples > 320 MPN/100 mL for E. coli (%)
17/8/20	12	5/12 (41.7)	4/12 (33.3)
24/8/20	12	2/12 (16.7)	1/12 (8.3)
31/8/20	12	1/12 (8.3)	1/12 (8.3)
8/9/20*	12	4/12 (33.3)	4/12 (33.3)
15/9/20	12	2/12 (16.7)	1/12 (8.3)
22/9/20	12	2/12 (16.7)	1/12 (8.3)
29/9/20	12	2/12 (16.7)	0
Total	84	18/84 (21.4)	12/84 (14.3)

* Note that this date was found with 24 hour total antecedent precipitation >5 mm.

In summary, FIB criteria exceedance in both East Lake and Windsong was relatively high in 2020, though this appeared to dependent on the stormwater pond, individual sites, as well as 24-hr total antecedent precipitation and potentially other temporal factors. Recreational water quality criteria exceedance was therefore particularly high in Windsong in comparison to East Lake, and STV criteria exceedance for both FIB was the highest on Aug. 17th and Sept. 8th in 2020, which were the weeks sampled with the highest 24-hr total antecedent precipitation. While *Enterococcus* and *E. coli* behaved concordantly in the majority of sites and samples, *Enterococcus* criteria exceedance (particularly STV by sample and GM by site) was slightly more frequently detected than *E. coli* criteria exceedance. Consequently, sites with *E. coli* criteria

exceedance were more likely to exceed the *E. coli* STV in >10% of samples than they were to exceed either the *E. coli* GM alone, or both criteria combined. These results suggest that FIB criteria exceedance in Airdrie stormwater in 2020 were dependent on a large host of factors, and the complexity within this matrix was high in that year.

3.2.4.2 Traditional Water Quality Standard Exceedances in 2021

In the second year of sampling, there was again considerable variation of recreational water quality criteria exceedance for both of the studied FIB in Airdrie stormwater, though exceedance frequency and magnitude were both relatively high at most sites. Nose Creek had an especially high frequency of recreational water quality criteria violations, and every single site sampled from this creek exceeded the 10% STV and GM criteria for *Enterococcus*, while the majority of sites also exceeded both criteria for *E. coli* (Table 3-7). Compared to other stormwater ponds and stormwater-impacted creeks, Nose Creek also had the one of highest magnitudes of FIB criteria exceedance, with over half of samples (21 of 38 samples, 55.3%) and just under one third of samples (12 of 38 samples, 31.6%) violating the STV criteria for *Enterococcus* and *E. coli* respectively (Table 3-7). This is in addition to site GM values that were greater than the *Enterococcus* GM criteria by ≥1 log in 5 of 9 Nose Creek sites. The sites of particular concern in Nose Creek were N#4 and N#6, with N#4 having the highest GM of *Enterococcus* of any stormwater site in both years of sampling (4.2 $\log_{10}$ CCE/100 mL), while 100% of samples (n=4) from N#6 exceeded the *Enterococcus* STV (Table 3-7). Notably, N#4 and N#6 are spatially close to each other, and both drain from the same neighborhood to the east of Nose Creek (see site maps in Chapter 2).

In contrast to Nose Creek, the Canals & Bayside Creek was the least impacted stormpond by fecal pollution according to the selected criteria (though exceedance was still high), with 3 of 10 sites from the Canals not exceeding any of the criteria (Table 3-7). These also happened to be the only three stormwater sites from sampling done in 2021 that did not to at least violate one STV or the GM criteria for either *Enterococcus* or *E. coli*. In addition, only *Enterococcus* criteria and not *E. coli* criteria were violated in 3 of 10 Canal sites. Canal sites CS#4 and CS#6 were particularly incongruent with this pattern, violating all of the criteria for both FIB, including with the majority of samples (75%) violating the *Enterococcus* STV for both sites, as well as the *E. coli* STV in the case of CS#4. Similar to problem sites N#4 and N#6 in Nose Creek, CS#4 and CS#6 both drained from the same neighborhood, though this time draining a neighborhood to the north-west of the Canals & Bayside Creek.

Table 3-7. Frequencies of FIB concentrations over acceptable standard threshold values (STVs) and log10 geometric means (GMs) according to Alberta recreational standards in Airdrie stormwater sites sampled in 2021 from stormwater-impacted creeks including Nose Creek and the Canals & Bayside Creek.*

Stormwater-impacted Creek	Site	n	Frequency of samples > 1280 CCE/100 mL for *Enterococcus* (%)	Frequency of samples > 320 MPN/100 mL for *E. coli* (%)	Log_{10} site GM for *Enterococcus* (>300 CCE/100mL in bold)	Log_{10} site GM for *E. coli* (>100 MPN/100 mL in bold)
Nose Creek	N#1	5	**2/5 (40.0)**	**2/5 (40.0)**	**3.29**	**2.03**
	N#2	4	**3/4 (75.0)**	**1/4 (25.0)**	**3.59**	**2.18**
	N#3	4	**2/4 (50.0)**	**1/4 (25.0)**	**3.44**	**2.18**
	N#4	4	**3/4 (75.0)**	**2/4 (50.0)**	**4.19**	**2.81**
	N#5	4	**2/4 (50.0)**	**2/4 (50.0)**	**3.73**	**2.48**
	N#6	4	**4/4 (100.0)**	**2/4 (50.0)**	**3.85**	**2.54**
	N#7	4	**1/4 (25.0)**	**1/4 (25.0)**	**2.87**	1.53
	N#8	4	**2/4 (50.0)**	**1/4 (25.0)**	**3.49**	1.83
	NP#1	5	**2/5 (40.0)**	0	**3.08**	1.07
	Total Nose Creek	38	**21/38 (55.3)**	**12/38 (31.6)**	N/A	N/A
Canals/Bayside Creek	CS#1	4	0	0	2.22	1.19
	CS#2	4	0	0	**2.79**	1.39
	CS#3	4	**1/4 (25.0)**	0	**2.93**	1.53
	CS#4	4	**3/4 (75.0)**	**3/4 (75.0)**	**3.52**	**2.52**
	CS#5	4	**1/4 (25.0)**	**1/4 (25.0)**	**2.85**	**2.09**
	CS#6	4	**3/4 (75.0)**	**1/4 (25.0)**	**3.50**	**2.02**
	CS#7	4	0	0	2.16	1.31
	CS#8	4	**1/4 (25.0)**	**2/4 (50.0)**	**2.63**	**2.05**
	CS#9	4	0	0	2.00	1.30
	CS#10	4	**1/4 (25.0)**	0	2.28	1.41
	Total Canals/Bayside	40	**10/40 (25.0)**	**7/40 (17.5)**	N/A	N/A
Total	Total	78	**31/78 (39.7)**	**19/78 (24.4)**	N/A	N/A

*Note that values in bold represent sites where >10% of samples are above the recommended STV, or the GM calculated for the site is greater than the recommended GM.

As was the case in the sampling from 2020, evidence of criteria exceedance suggested that Windsong sites were particularly influenced by fecal pollution (Table 3-8). All Windsong sites violated both *Enterococcus* STV and GM criteria, while 3 of 4 sites violated the STV and GM criteria for both FIB. Over half of Windsong samples from this year (9 of 16 samples, 56.3%) had *Enterococcus* concentrations higher than the recommended STV, while just under half (7 of 16 samples, 43.8%) exceeded *E. coli* STV values. The *E. coli* GM for WS#4 was also the highest of any stormwater site sampled in either 2020 or 2021 at a concentration of 2.8 $\log_{10}$ MPN/100 mL, once again suggesting this site to be the most contaminated.

The three other stormwater ponds studied in 2021 appeared to have a high amount of fecal pollution, though this was dependent on site and the tested FIB (Table 3-8). In HC#1 for example, the only criterion surpassed was the *E. coli* STV in one sample, whereas the other two sites in Hillcrest observed frequent criteria exceedance for both FIB. Similarly, KHN#2 in King's Heights North did not exceed any *E. coli* criteria despite exceeding *Enterococcus* criteria. King's Heights South sites appeared to show a curious pattern of incongruence between STV and GM criteria for the same organism, suggesting the interpretation of fecal influence into these sites could change heavily depending on the tested organism and particular criteria used.

Table 3-8. Frequencies of FIB concentrations over acceptable standard threshold values (STVs) and log10 geometric means (GMs) according to Alberta recreational standards in Airdrie stormwater sites sampled in 2021 from stormwater ponds including Hillcrest, King's Heights (North & South), and Windsong.*

Stormwater-impacted Creek	Site	n	Frequency of Samples > 1280 CCE/100 mL for *Enterococcus* (%)	Frequency of samples > 320 MPN/100 mL for *E. coli* (%)	Log_{10} site GM for *Enterococcus* (>300 CCE/100mL in bold)	Log_{10} site GM for *E. coli* (>100 MPN/100 mL in bold)
Hillcrest	HC#1	4	0	**1/4 (25.0)**	2.22	1.76
	HC#2	4	**1/4 (25.0)**	**1/4 (25.0)**	**2.71**	**2.04**
	HC#3	4	**2/4 (50.0)**	**1/4 (25.0)**	**2.69**	1.96
	Total Hillcrest	12	**3/12 (25.0)**	**3/12 (25.0)**	N/A	N/A
King's Heights North	KHN#1	4	**2/4 (50.0)**	**2/4 (50.0)**	**3.13**	**2.73**
	KHN#2	4	**2/4 (50.0)**	0	**3.46**	1.81
	Total King's Height North	8	**4/8 (50.0)**	**2/8 (25.0)**	N/A	N/A
King's Heights South	KHS#1	4	**1/4 (25.0)**	**1/4 (25.0)**	2.47	1.88
	KHS#2	4	0	**2/4 (50.0)**	**2.58**	2.00
	KHS#3	4	0	**1/4 (25.0)**	**2.53**	1.81
	Total King's Heights South	12	1/12 (8.3)	**4/12 (33.3)**	N/A	N/A
Windsong (2021)	WS#1	4	**3/4 (75.0)**	0	**3.04**	1.66
	WS#2	4	**2/4 (50.0)**	**2/4 (50.0)**	**2.69**	**2.31**
	WS#3	4	**2/4 (50.0)**	**2/4 (50.0)**	**3.89**	**2.75**
	WS#4	4	**2/4 (50.0)**	**3/4 (75.0)**	**3.60**	**2.84**
	Total Windsong	16	**9/16 (56.3)**	**7/16 (43.8)**	N/A	N/A
Total	Total	48	**17/48 (35.4)**	**16/48 (33.3)**	N/A	N/A

* Note that values in bold represent sites where >10% of samples are above the recommended STV, or the GM calculated for the site is greater than the recommended GM.

On a temporal scale of exceedance, there was a consistent observed pattern based on the dates of sampling and FIB criteria violations across all stormwater ponds sampled in Airdrie in 2021 (see Table 3-9). *Enterococcus* STV exceedance, for example, ranged from <10% of samples collected on July 19th/20th during routine sampling to a peak of nearly 75% of the samples collected the weeks of Aug. 17th/23rd before tapering off in following sampling weeks. *E. coli* STV exceedance followed a highly similar tendency as above, with <5% exceedance in samples from July 19th/20th to exceedance in nearly 75% of samples taken the weeks of the Aug. 17th/23rd. Corresponding to these peaks in FIB criteria violations, 24-hr total antecedent precipitation was highest during the week of the 3rd of August, and especially Aug. 17th and Aug. 23rd. Differences were dramatic between different sampling weeks with differing weather, such as a 41.9% increase in *Enterococcus* STV exceedance between sampling periods of Aug. 3rd and Aug.17th/23rd, and a 54.8% decrease in *Enterococcus* STV exceedance between the sampling period of Aug.17th/23rd and Aug. 30th/31st in all routine samples collected from all sites in 2021 during those times (Table 3-9). *E. coli* had a similar tendency, increasing by 29.1% between July 19th/20th and Aug. 3rd, and decreasing by 64.5% of exceedance in all samples from Aug. 3rd to the next consecutive weeks of Aug. 17th/23rd.

Statistically significant differences were observed for both *Enterococcus* and *E. coli* STV criteria exceedances by sampling week according to Fisher's exact test ($p<0.001$ in both cases). Specifically, Aug. 3rd had significantly more frequent *Enterococcus* STV criteria exceedances than July 19th ($p=0.0007$), while Aug. 17th/23rd had significantly more *Enterococcus* STV criteria exceedances than both July 19th and Aug. 30th ($p<0.0001$ in both cases) when tested pairwise by Fisher's exact test with the Bonferroni correction. Similarly, Aug. 17th/23rd had statistically significantly more *E. coli* STV criteria exceedances than July 19th ($p<0.0001$), Aug. 3rd

(p=0.0048), and Aug. 30th (p<0.0001) by the same test. Taken together, observed weather patterns and FIB criteria exceedance appeared to have the potential of being connected, though further studies would be required to get a solid and quantitative relationship between these two in Airdrie stormwater.

Table 3-9. Frequencies of Standard Threshold Value (STV) exceedance for both *Enterococcus* and *E. coli* concentrations in all routine 2021 Airdrie stormwater samples as stratified temporally by sampling date.

Dates	n	Frequency of Samples > 1280 CCE/100 mL for Enterococcus (%)	Frequency of samples > 320 MPN/100 mL for E. coli (%)
19/7/21, 20/7/21	31	3/31 (9.7)	1/31 (3.2)
3/8/21	31	16/31 (51.6)	10/31 (32.3)
17/8/21*, 23/8/21*, 24/8/21*	31	23/31 (74.2)	22/31 (71.0)
30/8/21, 31/8/21	31	6/31 (19.4)	2/31 (6.5)
7/9/21	2	0	0
Total	126	48/126 (38.1)	35/126 (27.8)

* Note that these dates were found with 24 hour total antecedent precipitation >5 mm.

To summarize, both *Enterococcus* and *E. coli* were frequently detected in Airdrie stormwater during both the 2021 and 2020 sampling seasons at high concentrations. Certain ponds and creeks, such as Windsong in both years and Nose Creek in 2021 had sites with much

higher and more frequent fecal pollution than others. This was also the case for individual sites within a stormwater pond, with some sites being affected with higher frequencies and magnitudes of FIB criteria violations than others. Weather may have also been important in determining FIB criteria exceedance in both years of the study, with the highest frequency of STV violations occurring in samples collected during the weeks with the highest levels of 24-hr total antecedent precipitation. While fecal pollution was overall higher in stormwater sites sampled in 2021 in comparison to those sampled in 2020, and *Enterococcus* more frequently detected above criteria limits than *E. coli* in both years, agreement was often lower in 2021 between types of criteria (GM vs STV) and the FIB studied (*Enterococcus* vs *E. coli*). STV and GM criteria were less in agreement for *E. coli* than for *Enterococcus* on a per site basis, and *E. coli* STV violations in >10% of samples at a site was sometimes more frequently seen alone than violating the *E. coli* GM at the same site. Overall, FIB criteria exceedance appeared to be dependent on a large number of factors, and this was similar for sampling that occurred in both 2021 and 2020.

3.3 Discussion

In studying traditional marker of water quality in Airdrie stormwater, several key findings were made that were not only vital for understanding the particular stormwater system and reuse projects in the City of Airdrie, but could also have implications in other similar systems across Alberta and Canada in general. To begin with, FIB prevalence was nearly universally high in routine stormwater samples across both years of sampling, and routinely violated recreational/ambient water quality criteria at most sampling sites (Government of Alberta, 2018; US EPA, 2012a). In spite of this, distinctive patterns of FIB concentrations were also observed. For example, *Enterococcus* criteria violations were nearly always slightly more frequent than *E. coli* criteria violations as a whole for each year, suggesting that *Enterococcus* qPCR may be a

more sensitive measure of fecal pollution in stormwater. Another difference included the fact that certain stormwater ponds and stormwater-impacted creeks (i.e., Windsong and Nose Creek) were found to be much more polluted with FIB than others, where specific sites within the same water body appeared to exceed water quality criteria at a far higher frequency than other sites. Consequently, FIB are subject to considerable spatial variability and likely dependent upon fecal influences occurring in each of the individual drainage networks feeding into a single pond. Additionally, temporal patterns of high FIB criteria exceedance appeared to occur on a few sampling weeks each year (the week of Sept. 8th in 2020, and Aug. 17th/23rd in 2021) and which had higher 24-hr total antecedent precipitation (>5 mm), though there were also distinctive weeks each year with high FIB exceedance without excessive rainfall (Aug. 17th in 2020 and Aug. 3rd in 2021). Lastly, correlation was only moderate ($0.5>\rho<0.8$) between *Enterococcus* and *E. coli* in both years of study, further suggesting the relationships that may exist between these two FIB as likely complex and driven by multiple factors (e.g., host sources of contamination, decay rates, environmental strains, etc.).

As mentioned above, the universality of high concentrations of FIB in stormwater (including *E. coli* and *Enterococcus*) has become increasingly well documented in recent years (Ahmed et al., 2020; Converse et al., 2011; de Man et al., 2014; Hachad et al., 2022; Hart et al., 2020; Kinzelman & McLellan, 2009; Lee et al., 2020; Monteiro et al., 2021; Nshimyimana et al., 2014; Olds et al., 2018; Parker et al., 2010; Sauer et al., 2011; Schreiber et al., 2019; Sidhu et al., 2012; Staley et al., 2018; Steele et al., 2018; Williams et al., 2022). In the current study, *E. coli* and *Enterococcus* were found in 100% of routine stormwater samples studied, with the former organism found at site-specific median concentrations of between 1 and 2 $\log_{10}$ MPN/100 mL, and the latter at site-specific median concentrations of between 2 and 3 $\log_{10}$ CCE/100 mL,

depending on the year sampled. Median concentrations of *E. coli* in Airdrie stormwater ponds are consistent with concentrations reported in a few studies (Hart et al., 2020; Olds et al., 2018; Sidhu et al., 2012), though other studies suggest higher medians and averages of this FIB, often by 1-2 orders of magnitude (Ahmed et al., 2020; de Man et al., 2014; Hachad et al., 2022; Kinzelman & McLellan, 2009; Lee et al., 2020; Monteiro et al., 2021; Sauer et al., 2011; Schreiber et al., 2019). Many studies found high concentrations of *Enterococcus* (Hart et al., 2020; Olds et al., 2018; Sidhu et al., 2012; Steele et al., 2018; Williams et al., 2022), though a few studies showed slightly higher (Monteiro et al., 2021; Sauer et al., 2011) or lower concentrations (Ahmed et al., 2020; Parker et al., 2010), suggesting that variation also exists for *Enterococcus* concentrations across the literature. It must be noted with regards to *Enterococcus*, however, that these studies generally measured viable organisms (i.e., CFU/100 mL or MPN/100 mL), which is not necessarily equivalent or comparable to the qPCR-based estimates (using CCE/100 mL) utilized in the current study (see Noble et al., 2010; Raith et al., 2014).

In comparison to our study, a diverse range of occurrences of both *E. coli* and *Enterococcus* have also been observed in several other studies of stormwater (Ahmed et al., 2020; de Man et al., 2014; Hachad et al., 2022; Hart et al., 2020; Nshimyimana et al., 2014; Parker et al., 2010; Sauer et al., 2011; Sidhu et al., 2012; Staley et al., 2018; Steele et al., 2018). The range of *E. coli* in our study (i.e., <1 $\log_{10}$ MPN/100 mL to >3.4 $\log_{10}$ MPN/100 mL) was similar to that observed within other stormwater studies (Hachad et al., 2022; Hart et al., 2020; Nshimyimana et al., 2014; Parker et al., 2010; Sidhu et al., 2012; Staley et al., 2018), but others have reported much higher minimum values of *E. coli* than those observed in our study (2 to 3 $\log_{10}$ MPN/100 mL) (Converse et al., 2011; de Man et al., 2014; Lee et al., 2020; Monteiro et al., 2021; Sauer et al., 2011; Schreiber et al., 2019). Ranges of *Enterococcus* concentrations found

in other studies often had a wide range of concentrations found (Ahmed et al., 2020; de Man et al., 2014; Hart et al., 2020; Sauer et al., 2011; Sidhu et al., 2012; Steele et al., 2018), while a few studies showed much tighter ranges of concentration for this organism (Converse et al., 2011; Monteiro et al., 2021; Parker et al., 2010).- Not surprisingly, the data demonstrate that stormwater is: a) a relatively poor-quality water source; and b) that stormwater is subject to episodic influxes of major fecal pollution.

Exceedance of US EPA's (2012a) microbial water quality criteria (and other similar criteria) occurred frequently in the present study, and this has been routinely observed in many other studies (Converse et al., 2011; de Man et al., 2014; Hachad et al., 2022; Kinzelman & McLellan, 2009; Lee et al., 2020; Monteiro et al., 2021; Nshimyimana et al., 2014; Olds et al., 2018; Parker et al., 2010; Sauer et al., 2011; Sidhu et al., 2012; Staley et al., 2018). For example, Converse et al. (2011) found single sample limits of 400 MPN/100 mL were exceeded in 4 of 5 studied sites, while both this study and Parker et al. (2010) found >85% of samples exceeded a single sample limit of 104 MPN/100 mL for *Enterococcus*. In contrast, our 2021 study found that just greater than 33.3% of samples exceeded *Enterococcus* STV criteria, while only 25.0% of samples violated *E. coli* STV criteria, suggesting that the high criteria exceedance found in the present study in Airdrie was actually less severe than that seen in other locations. Importantly, however, approximately 75.0% of sites in 2021 and half of the sites studies in Airdrie in 2020 still exceeded water quality criteria, suggesting that FIB contamination could appear more prevalent when compared site-by-site as opposed to on a sample-by-sample basis.

FIB concentrations have been generally found to be similar between each other in stormwater (Converse et al., 2011; de Man et al., 2014; Hart et al., 2020; Lee et al., 2020; Monteiro et al., 2021; Olds et al., 2018; Parker et al., 2010; Sauer et al., 2011; Sidhu et al., 2012).

In opposition to the above, however, Ahmed et al. (2020), found *E. coli* concentrations to be consistently higher than *Enterococcus* (often by ~1 $\log_{10}$) as opposed to the converse found in Airdrie stormwater. This suggests overall that while these observations are rare, it is possible to have a bias in a certain area for one FIB over another. While the present study measured *Enterococcus* via the qPCR-based calibrator-cell equivalent (CCE) as opposed to viable estimates of most-probable-number (MPN) for *E. coli*, making it not possible to compare the two, this comparison may be valuable in future studies.

As in the present study, variation in FIB concentrations is commonly found among stormwater-impacted sites, and can be explained (at least in part) by site-specific geo-spatial differences (Hachad et al., 2022; Hart et al., 2020; Nshimyimana et al., 2014; Parker et al., 2010; Sidhu et al., 2012; Staley et al., 2018; Steele et al., 2018). These can include differing upstream land uses (Nshimyimana et al., 2014; Sidhu et al., 2012; Staley et al., 2018; Steele et al., 2018), proximity to high flows or differing sources of contamination (Hachad et al., 2022; Hart et al., 2020; Parker et al., 2010; Staley et al., 2018), as well as infrastructural differences between sites (e.g., whether sites are above or below grade (Hart et al., 2020; Parker et al., 2010). Similar to in Airdrie stormwater ponds, temporal patterns of *E. coli* and *Enterococcus* have also commonly been observed in various other studies (Ahmed et al., 2020; Converse et al., 2011; de Man et al., 2014; Hart et al., 2020; Kinzelman & McLellan, 2009; Lee et al., 2020; Parker et al., 2010; Sidhu et al., 2012; Steele et al., 2018). For example, many studies show correlation between higher FIB concentrations and wet weather conditions in comparison to dry baseline conditions (Ahmed et al., 2020; Converse et al., 2011; Hart et al., 2020; Kinzelman & McLellan, 2009; Lee et al., 2020; Parker et al., 2010; Sidhu et al., 2012; Steele et al., 2018), though this is not always the case

(Monteiro et al., 2021), and can even itself by site-specific (Hart et al., 2020; Parker et al., 2010; Sidhu et al., 2012).

The moderately strong correlation between *Enterococcus* and *E. coli* concentrations in Airdrie stormwater was highly consistent with results seen in some studies of stormwater (Ahmed et al., 2020; Lee et al., 2020; Schriewer et al., 2010), though correlation has been shown to be much higher ($\rho \geq 0.8$) elsewhere (Hart et al., 2020; Parker et al., 2010; Sidhu et al., 2012). Curiously, high correlation appeared to be conditional in a few of these publications, with Parker et al. (2010) finding high correlation in 2 of 3 studied sites, though a third site had no significant correlation between these two FIB at all. Similarly, Sidhu et al. (2012) found high correlation between *Enterococcus* and *E. coli* concentrations only under wet-weather conditions, whereas correlation was more similar to that seen in the present study under dry-weather conditions. Overall, this suggests that the relationship between these two FIB can be complex in stormwater, and it cannot be assumed that a strong relationship exists between both of them without further investigating in a specific geographical area or set of temporal conditions.

A number of factors may explain the variation in regards to the results found in our study on the correlation between FIB (i.e., *Enterococcus* vs. *E. coli*), distributions and concentrations of FIB, the predominance of one FIB over another, and geo-spatial and temporal differences in FIB concentrations. For example, it is important to note that our study used a qPCR method of determining *Enterococcus* marker concentrations (US EPA Method 1611 – see US EPA, 2012b), whereas many studies utilized direct culture methods such as membrane filtration (e.g., US EPA Method 1600, or ISO 7899-2) (Ahmed et al., 2020; de Man et al., 2014; Olds et al., 2018; Sauer et al., 2011; Schreiber et al., 2019; Sidhu et al., 2012; Steele et al., 2018; Williams et al., 2022), or Enterolert$^{(R)}$ (Converse et al., 2011; Hart et al., 2020; Monteiro et al., 2021; Parker et al.,

2010). Notably, studies have shown very high correlation between these methods, finding consistency in criteria exceedance in >85% of samples measured by qPCR vs. culture methods, though qPCR-based estimates (i.e., calibrator cell equivalents [CCE]) were found consistently <1 $\log_{10}$ higher than traditional culture estimates (i.e., most-probable-number [MPN] or colony forming unit [CFU] estimates) (Boehm et al., 2013; Gonzalez & Noble, 2014; Noble et al., 2010; Raith et al., 2014). In spite of this, criteria for qPCR-based measurement of *Enterococcus* are often set at ≥0.5 $\log_{10}$ higher than criteria for viable *E. coli* (See US EPA, 2012a), which should in theory compensate for the consistently higher qPCR-based estimates of *Enterococcus* in comparison to culture-based estimates of *E. coli* (Gonzalez & Noble, 2014; Raith et al., 2014).

A few additional important distinctions that may explain differences in FIB distribution and criteria exceedance (particularly for *E. coli*) between the present study and others are differences in climate, geographical location, and site infrastructure (Ahmed et al., 2020; de Man et al., 2014; Hachad et al., 2022; Kinzelman & McLellan, 2009; Lee et al., 2020; Monteiro et al., 2021; Sauer et al., 2011; Schreiber et al., 2019). For example, many studies examining FIB in stormwater are executed in metropolitan areas of high population density and/or take place in coastal areas with frequent rainfall and generally wet climates in comparison to Airdrie (see Ahmed et al., 2020; Converse et al., 2011; de Man et al., 2014; Hachad et al., 2022; Kinzelman & McLellan, 2009; Lee et al., 2020; Monteiro et al., 2021; Sauer et al., 2011), while site infrastructure differences between studies (such as networks built with combined sewer outfalls [CSOs] versus those built without) de Man et al., 2014; Monteiro et al., 2021; Parker et al., 2010; Sauer et al., 2011) can also contribute to these results by affecting flow and dilution of FIB.

In addition to the above, it is important to re-emphasize that FIB contamination can come from multiple host sources (i.e., human vs animal – see Ahmed et al., 2019a; Ervin et al., 2013),

as well as be naturalized to the environment (Byappanahalli et al., 2012; Devane et al., 2020). This can affect FIB concentrations in Airdrie stormwater and elsewhere for several reasons, including that animal sources may be transported differently (i.e., washed over nearby surfaces into stormwater ponds due to excessive precipitation) than FIB from human feces such as from cross-connections, combined sewer outfalls, or leaky/broken sanitary sewer infrastructure (Ahmed et al., 2020; Converse et al., 2011; Monteiro et al., 2021; Nshimyimana et al., 2014; Olds et al., 2018; Staley et al., 2018; Steele et al., 2018; Williams et al., 2022). Additionally, both prevalence and concentrations of FIB can differ greatly among different human and animal hosts, including whether *Enterococcus* or *E. coli* is more dominant, potentially affecting the proportion of these organisms seen in environmental waters in relation to which hosts are the primary contributors of fecal pollution (Ahmed et al., 2019a; Ervin et al., 2013; Layton et al., 2009, 2010). Lastly, decay rates can also be significantly different between different FIB (i.e., *Enterococcus* and *E. coli*) as well as between the same FIB coming from different hosts, or even between fecally-sourced and environmental strains of FIB adapted to live naturally in the environment (Korajkic et al., 2019; Walters & Field, 2009).

In conclusion, both *Enterococcus* and *E. coli* were found at high concentrations almost universally in Airdrie stormwater sites, resulting in a high frequency of water quality criteria exceedances. Importantly, both geo-spatial and temporal patterns of FIB concentrations and consequent criteria exceedance were apparent, demonstrating that episodic and significant influxes of fecal pollution occurred in most stormwater ponds, and often at multiple sites within a pond. From a water reuse viewpoint, it is important to appreciate that water in most stormwater ponds does not consistently meet microbial water quality standards for recreation, irrigation, treated wastewater, or even ambient water.

4. Tracking Sources of Fecal Pollution in Stormwater to Assess the Health Risks of Water Reuse

4.1 Introduction

In terms of recreational/ambient water quality, it is commonly accepted that exposure to human fecally-contaminated water causes much higher risks than animal feces when it comes to gastrointestinal illness, making the elucidation of fecal sources contaminating a water system via microbial source tracking (MST) of paramount importance (Brown et al., 2017a; Schoen & Ashbolt, 2010; Soller et al., 2010). While many MST methods have been developed to for this purpose, library-independent quantitative polymerase chain reaction (qPCR) based assays have gained popularity in recent years (Field & Samadpour, 2007; Hachad et al., 2022; Sidhu et al., 2013; Van de Werfhorst et al., 2014). These methods entail the quantification of gene targets that are specific to microorganisms found in a particular host, such as the commonly used HF183 marker adapted from the *16S* rRNA gene of *Bacteroides* spp. found in human feces (Field & Samadpour, 2007; Haugland et al., 2010). As such, qPCR markers for fecal sources have been developed for a large variety of animal hosts, including humans (Haugland et al., 2010; Shanks et al., 2009), waterfowl (Fremaux et al., 2010; Lee et al., 2013; Lu et al., 2008; Ryu et al., 2012), domestic pets (Green et al., 2014; Kildare et al., 2007), and livestock (Mieszkin et al., 2009, 2010) to name a few.

Similar to other MST methods, qPCR-based methods have their advantages and disadvantages as a methodology. Advantages include relatively high sensitivity and specificity specificity (>80% for many markers – see Boehm et al., 2013; Shanks et al., 2010a,b; Sinigalliano et al., 2013), and the ability to detect viable but non-culturable (VBNC) organisms (Field & Samadpour, 2007). Disadvantages include that markers may not be representative of viable

organisms and therefore fresh fecal pollution, and that sensitivity and specificity can be affected negatively by inhibitory substances found in the environment (Opel et al., 2010; Schrader et al., 2012) and cross-reactions with other animal feces (Boehm et al., 2013; Shanks et al., 2010a,b; Sinigalliano et al., 2013).

MST markers can have diverse uses as forensic tools for investigation of fecal contamination events (Gonzalez et al., 2020; Hachad et al., 2022; Teaf et al., 2018; Kauppinen et al., 2019). Recent studies have used MST markers to effectively investigate infrastructure failures or illicit cross-connections in drainage systems via an up-the-pipe investigative method – e.g., following increasing concentrations of an MST marker, such as HF183 from human *Bacteroides* spp., to a physical contamination source in the drainage network, where dye-testing and re-testing of said markers can be used to confirm successful repairs and mitigation efforts (Gonzalez et al., 2020; Hachad et al., 2022; Sauer et al., 2011).

The use of MST markers has its advantages over traditional FIB enumeration, though the use of MST markers alone in environmental waters also come with complications. For example, human *Bacteroides* marker HF183 is not always correlated to gastrointestinal illness (Napier et al., 2017), FIB (Hachad et al., 2022; Hart et al., 2020; McGinnis et al., 2018; Nshimyimana et al., 2014; Sauer et al., 2011; Staley et al., 2018; Steele et al., 2018; Williams et al., 2022), or enteric pathogens (Bradshaw et al., 2016; Cui et al., 2019; Fremaux et al., 2009; Sales-Ortells & Medema, 2015; Sidhu et al., 2013; Steele et al., 2018; Walters et al., 2007), suggesting that fecal indicator estimates can still be informative of general water quality where fecal sources cannot be fully characterized.

The most current concern in stormwater is the growing evidence that human sewage contamination is common in stormwater, even in systems where sanitary and stormwater sewers

have been built as separate infrastructure (Gonzalez et al., 2020; Hachad et al., 2022; Kinzelman & McLellan, 2009; Nshimyimana et al., 2014; Parker et al., 2010; Sales-Ortells & Medema, 2015; Sauer et al., 2011; Sidhu et al., 2012; Staley et al., 2018; Steele et al., 2018). While detection of human sewage in stormwater is often quite sporadic, it is estimated that as high as 10% of total flows are made up of human sewage in some cases (Ahmed et al., 2021; Mayer et al., 2018; Nshimyimana et al., 2014; Sauer et al., 2011; Sharvelle et al., 2017).

Animal sources of contamination in stormwater have been much less frequently investigated in comparison to human sewage (Bambic et al., 2015; Green et al., 2019; Sales-Ortells & Medema, 2015; Staley et al., 2018; Steele et al., 2018). Of particular interest in stormwater is fecal contamination from waterfowl (Lu et al., 2011b; Staley et al., 2018; Steele et al., 2018), a host source which is currently underassessed in terms of the risk they contribute to environmental waters, and yet have been increasingly shown to be carriers of zoonotic pathogens such as *Campylobacter* spp. and *Salmonella* spp. (Antilles et al., 2021; Broman et al., 2002; Palmgren et al., 2006; Russo et al., 2021). Regardless of the animal in question, both geo-spatial and temporal (i.e., weather-related) factors have not been thoroughly investigated when it comes to MST marker contamination in stormwater despite evidence of the detection of these markers (Ahmed et al., 2020; Bambic et al., 2015; Green et al., 2014, 2019; Hart et al., 2020; Lee et al., 2020; Nshimyimana et al., 2014; Olds et al., 2018; Sales-Ortells & Medema, 2015; Steele et al., 2018; Williams et al., 2022), producing a large gap in the current understanding of the multiple sources of fecal contamination in this matrix.

In light of these gaps in knowledge, the primary research objectives for this chapter of the thesis were to; 1) characterize sources of fecal pollution in stormwater ponds using MST targets (humans, gulls, Canada geese, dogs, and ruminants) across a number of Aidrie stormwater

ponds and stormwater-impacted creeks; 2) in the case of sites potentially impacted by human sewage markers, confirm and demonstrate the effectiveness of up-the-pipe (i.e., forensic) investigative techniques to locate point-sources of human contamination in drainage networks, and; 3) evaluate potential factors contributing to marker prevalence and concentrations that may require further study, including geo-spatial and temporal (e.g. wet weather) factors.

4.2 Results

MST marker results presented below for both 2020 and 2021 Airdrie stormwater samples were analyzed in a multitude of ways. To begin with, general MST marker occurrence and concentrations in routine water samples were analyzed in each stormwater pond to determine the most predominant fecal sources of pollution, as well as general diversity of the polluting hosts. Next, MST marker results for routine samples were aggregated by the date of sampling, in order to determine whether temporal factors such as 24-hr total antecedent precipitation played a role in marker occurrence and concentration. Analysis of routine samples primarily focused on finding evidence of human sewage contamination via MST markers (particularly HF183), and more specifically on the potential associations between human sewage detection and FIB exceedance (Chapter 3) as well any temporal factors that may mediate this relationship.

In cases where evidence for human sewage contamination was found (chronic or sporadic), specific investigations were carried out to trace the cause of this pollution in the drainage network (i.e., potential cross connections, infrastructure failures, etc.). These 'case-study' investigations occurred specifically in storm drainage networks feeding into Nose Creek (chronic contamination) as well as the stormpond facilities in Hillcrest that were found to be sporadically contaminated with human feces.

4.2.1 Overall Occurrence of MST marker in Stormwater Ponds and Effluents

Overall, human and several animal marker signatures were detected in Airdrie stormwater samples during both 2020 as well as 2021. Animal fecal signatures included gull (LeeSG), Canada goose (CGO1), dog (Dog3), and ruminants (Rum2Bac), although the ruminant signature was only detected in 2021. Frequencies as well as concentrations of individual markers varied widely, and appeared to be dependent on the stormwater pond, individual sites in a stormpond, 24-hr total antecedent precipitation, and other temporal factors. Human sewage contamination was sporadically detected in all stormwater ponds as well as stormwater-impacted creeks studied in both years, the exception being at King's Heights South stormpond. All in all, evidence suggested that humans were the most frequently detected fecal pollution source in stormponds, though there was a wide diversity of fecal hosts detected in both years within Airdrie stormwater.

4.2.1.1 Occurrence of MST Markers in 2020

In 2020, all of the assayed MST markers were detected in at least one sample and at varying frequencies and concentrations, with the exception of the ruminant marker (Rum2Bac), which was not detected (Table 4-1). During this year of sampling, gulls and humans (based on HF183 marker) were respectively the highest and 2nd highest detected fecal contributors of all stormwater samples combined at 9.5% and 6.0% frequency of detection, respectively (Table 4-1). The Canada goose marker was found in 2.4% of samples, though the dog marker was only detected in one sample that year. HumM2, a second but less sensitive human marker than HF183, was only detected in a single sample in that year as well, specifically in Windsong. It is important to note that not one particular MST marker tested (i.e., HF183, LeeSG, CGO1, Dog3, Rum2Bac) comprehensively identified the source(s) of the majority of fecal contamination into East Lake

and Windsong, with the highest frequency of marker detection being only 12.5% of samples in East Lake positive for the gull marker (LeeSG) (Table 4-1).

Table 4-1. Frequency (%) of microbial source tracking (MST) marker detection within Airdrie stormwater ponds routinely sampled in 2020.

Stormwater Pond	n	Human		Gull	Canada Goose	Dog	Ruminant
		HF183	HumM2	LeeSG	CGO1	Dog3	Rum2Bac
East Lake	56	3/56 (5.4)	ND	7/56 (12.5)	1/56 (1.8)	1/56 (1.8)	ND
Windsong (2020)	28	2/28 (7.1)	1/28 (3.6)	1/28 (3.6)	1/28 (3.6)	ND	ND
Total	84	5/84 (6.0)	1/84 (1.2)	8/84 (9.5)	2/84 (2.4)	1/84 (1.2)	ND

Spatial differences in HF183 detection were observed in both stormwater ponds tested in 2020, as well as between these stormwater ponds. In terms of spatial differences in East Lake, human sewage was only detected in three sites at EL#3, EL#4, and EL#8 (Fig. 4-1). The EL#3 site is the outlet for this stormwater pond, and it was specifically located to the south of EL#4 as pictured in Fig. 4-1, though both of these sites were much further away from EL#8, which was the source of a drainage swale upstream of East Lake. Curiously, HF183 was not detected at EL#5, despite this site supplying stormwater via a drainage swale to EL#4. Converse to East Lake, the only two sites where HF183 was detected in Windsong (WS#3 and WS#4) appeared to be much closer to each other than the sites in East Lake, draining from neighborhoods to the south and west of this stormwater pond (Fig. 4-2). Another potential difference between these stormwater ponds is the appearance of more commercial properties draining into East Lake where HF183 was detected, whereas Windsong appeared to be surrounded primarily by residential neighborhoods (Figures 4-1, 4-2).

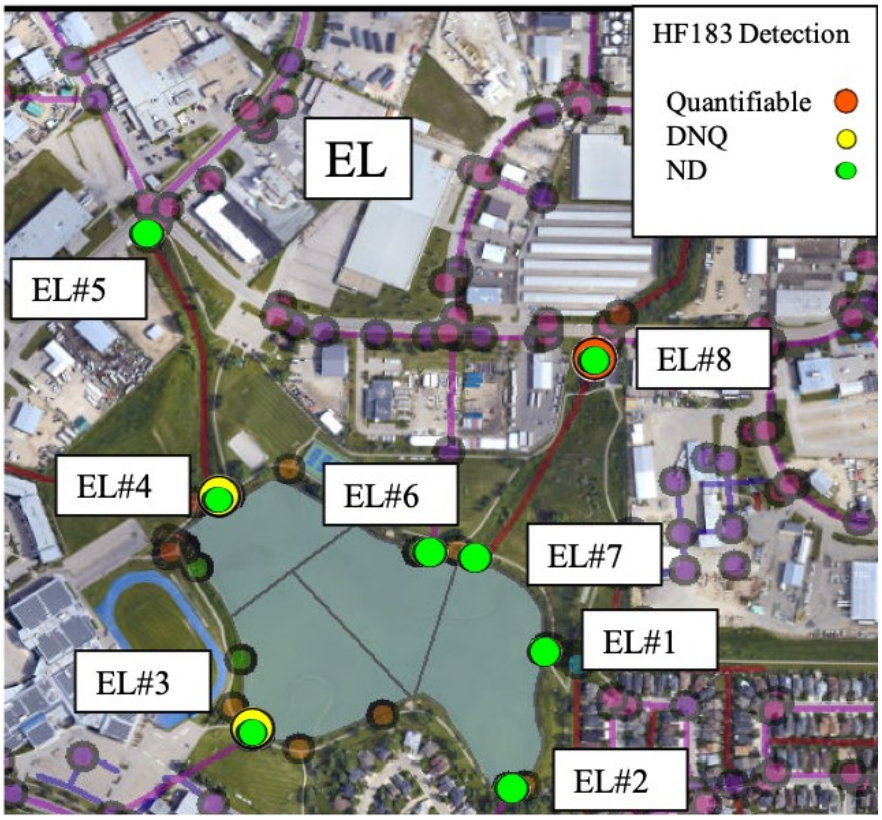

Figure 4-1. Map of human sewage marker (HF183) detection within sites from the East Lake (EL) stormwater pond in Airdrie, Alberta, 2020. Note the colored dots representative of HF183 not being detected (green) in all East Lake sites (EL#1-8).

Figure 4-2. Map of human sewage marker (HF183) detection within sites from the Windsong (WS) stormwater pond in Airdrie, Alberta, 2020. Note the colored dots representative of HF183 detection that is quantifiable (red), detectable but not quantifiable (yellow), and not detected (green) in Windsong sites (WS#1-4).

Marker concentrations in 2020 were generally low and were mostly found to be detectable but not quantifiable (DNQ), particularly for the gull marker (LeeSG), and which did not have a single sample above this limit. Only two samples were quantifiable for HF183, and were found at concentrations of 4.5 $\log_{10}$ copies/100 mL and 4.8 $\log_{10}$ copies/100 mL in samples respectively taken from WS#4 on Sept. 8th and EL#8 on Sept. 29th. The only detectable goose marker was found at a concentration of 3.5 $\log_{10}$ copies/100 mL in a sample from EL#6 on Sept. 29th, whereas the only quantifiable dog marker detected was found in EL#8 on Sept. 8 at 3.8 $\log_{10}$ copies/100 mL.

4.2.1.2 Occurrence of MST Markers in 2021

In 2021, all of the MST markers in the panel (human, dog, runminant, goose, gull) were detected in stormwater samples collected from Airdrie, though human sewage (HF183), dogs, and ruminants were the top 3 most frequently detected sources in routine samples at occurrences of 26.2%, 11.9%, and 9.5% respectively (Table 4-2). In total, 47 of 126 total routine samples (37.3%) were positive for one or more MST marker. In samples where any MST marker used in this study were detected (47 of 126 samples), HF183 was statistically significantly more likely by McNemar's test to be detected than gull ($p = 0.0018$; Table 4-3), dog ($p = 0.0014$; Table 4-4), ruminant ($p = 0.0001$; Table 4-5), and goose ($p < 0.0001$; Table 4-6) markers. Note that as in 2020, the majority of samples were not necessarily characterized by any one particular MST marker of the panel used in this study. Human sewage influence into these stormwater ponds is further corroborated by the second human marker, HumM2, which was found in 7.1% of all routine samples tested that year, with 7 of 9 (77.8%) routine samples positive for HumM2 also being positive for HF183. Converse to human sewage marker HF183, the Canada goose marker (CGO1) was only detected in the Canals & Bayside Creek site in 2021, suggesting geese were not a predominant contamination source in Airdrie stormwater ponds that year (Table 4-2). Gulls were also only detected at Nose Creek, Hillcrest, and Windsong in routine samples, and were detected in <5% of total routine samples in 2021. In contrast, the dog and ruminant marker were detected in every water body sampled in 2021 at least once, with the exception of the ruminant marker being absent from the Canals & Bayside Creek (Table 4-2).

With the sole exception of the King's Heights South stormwater pond, human sewage contamination was observed in every stormwater pond and stormwater-impacted creek in 2021 (Table 4-2). Of particular note was Nose Creek, a stormwater-impacted creek where the majority

of routine samples (22 of 38 samples, 57.9%) were found positive for HF183, as well as a large number of samples positive for HumM2 (8 of 38 samples, 21.1%), suggesting a high frequency of human fecal contamination in this creek. Both Windsong and Hillcrest stormponds had the second highest occurrence of HF183, with positivity in 25% of samples, though this was proportionally less than half the frequency this marker was found at in Nose Creek (Table 4-2). Differences in HF183 detection were geo-spatially significant between stormponds and creeks ($p<0.001$) by Fisher's exact test, while pairwise iterations of this test found that HF183 detection was significantly higher in Nose Creek samples than both Canals ($p = 0.0001$) and King's Heights South ($p = 0.0004$) samples when considering the Bonferroni correction for multiple comparisons.

Table 4-2. Frequency (%) of microbial source tracking (MST) marker detection within stormwater-impacted ponds and creeks from the routine sampling sites in 2021.

Stormwater Pond/Creek	n	Human		Gull	Canada Goose	Dog	Ruminant
		HF183	HumM2	LeeSG	CGO1	Dog3	Rum2Bac
Nose Creek	38	22/38 (57.9)	8/38 (21.1)	3/38 (7.9)	ND	6/38 (15.8)	6/38 (15.8)
Canals/Bayside Creek	40	3/40 (7.5)	ND	ND	3/40 (7.5)	3/40 (7.5)	ND
Hillcrest	12	3/12 (25.0)	1/12 (8.3)	1/12 (8.3)	ND	2/12 (16.7)	2/12 (16.7)
King's Heights North	8	1/8 (12.5)	ND	ND	ND	1/8 (12.5)	1/8 (12.5)
King's Heights South	12	ND	ND	ND	ND	1/12 (8.3)	2/12 (16.7)
Windsong (2021)	16	4/16 (25.0)	ND	2/16 (12.5)	ND	2/16 (12.5)	1/16 (6.3)
Total	126	33/126 (26.2)	9/126 (7.1)	6/126 (4.8)	3/124 (2.4)	15/126 (11.9)	12/126 (9.5)

Table 4-3. Two-by-Two table of MST marker positive Airdrie stormwater samples collected in 2021, as stratified by HF183 versus gull MST marker detection (n=47).

	HF183 Detected	HF183 Not Detected
Gull (LeeSG) Marker Detected	5	1
Gull (LeeSG) Marker Not Detected	28	13

Table 4-4. Two-by-Two table of MST marker positive Airdrie stormwater samples collected in 2021, as stratified by HF183 versus dog MST marker detection (n=47).

	HF183 Detected	HF183 Not Detected
Dog (Dog3) Marker Detected	9	6
Dog (Dog3) Marker Not Detected	24	8

Table 4-5. Two-by-Two table of MST marker positive Airdrie stormwater samples collected in 2021, as stratified by HF183 versus ruminant MST marker detection (n=47).

	HF183 Detected	HF183 Not Detected
Ruminant (Rum2Bac) Marker Detected	8	4
Ruminant (Rum2Bac) Marker Not Detected	25	10

Table 4-6. Two-by-Two table of MST marker positive Airdrie stormwater samples collected in 2021, as stratified by HF183 versus goose MST marker detection (n=47).

	HF183 Detected	HF183 Not Detected
Goose (CGO1) Marker Detected	0	3
Goose (CGO1) Marker Not Detected	33	11

From a spatial scale encompassing all of the stormwater-impacted creeks and ponds in the City of Airdrie, the majority of human sewage contamination in stormwater in 2021 was detected from the drainage originating from the center and the south of the City, where Nose creek and the Windsong and Hillcrest stormwater ponds lay, respectively (Fig. 4-3). In comparison, the Canals & Bayside Creek in the north-west, East Lake to the north-east and both King's Heights stormwater ponds in the south-east were observed to be minimally impacted by human sewage contamination in 2021 (Fig. 4-3).

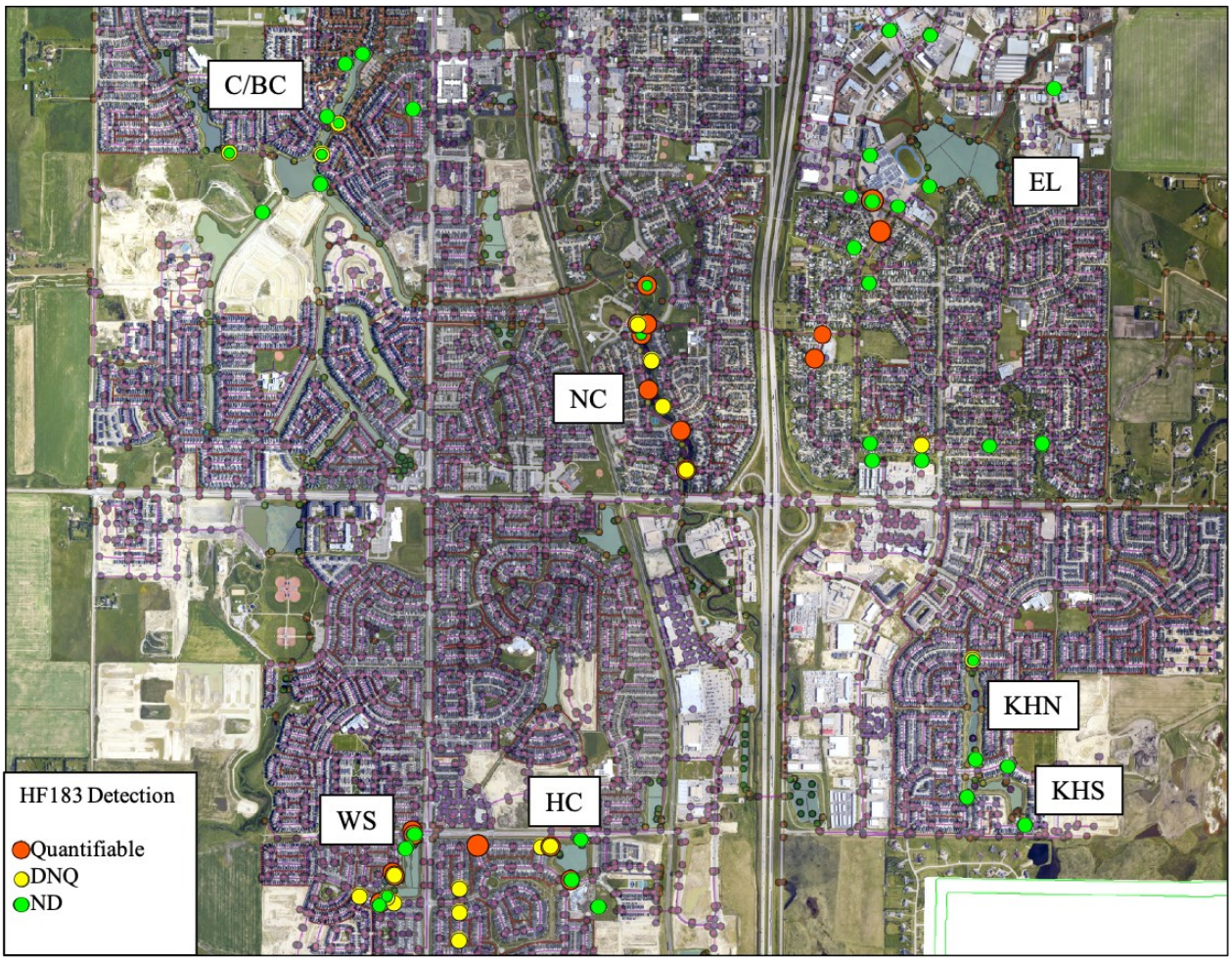

Figure 4-3. Map of human sewage marker (HF183) detection within sites from all studied stormwater ponds and stormwater-impacted creeks in Airdrie, Alberta, 2021. Starting from the bottom left and appearing counter clock-wise, these include Windsong (WS), Hillcrest (HC), King's Heights North & South (KHN & KHS), East Lake (EL), Nose Creek (NC), and the Canals and Bayside Creek (C/BC). Note the colored dots representative of HF183 detection that is quantifiable (red), detectable but not quantifiable (yellow), and not detected (green).

Windsong and Hillcrest were the stormwater ponds with the highest frequency of HF183 detection, though this could be dependent on site. For example, HF183 was sporadically detected

at the WS#2, WS#3 and WS#4 sites in Windsong, while the outlet (WS#1) did not test positive during the entire sampling season (Fig. 4-4). Likewise, while HF183 was occasionally detected in the HC#2 and HC#3 sites of Hillcrest, it was not detected in HC#1.

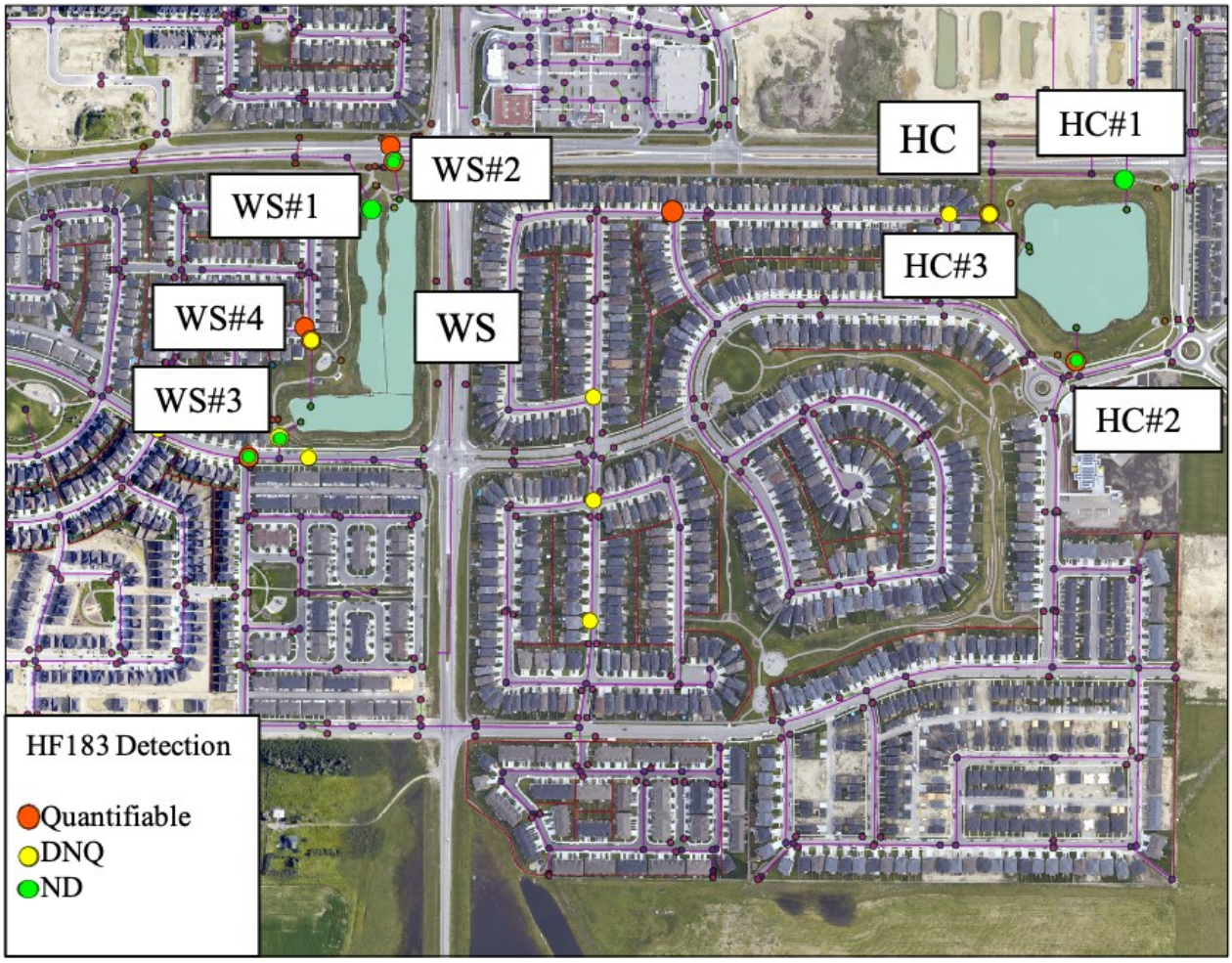

Figure 4-4. Map of human sewage marker (HF183) detection within sites from Windsong (WS) and Hillcrest (HC) stormwater ponds in Airdrie, Alberta, 2021. Note the colored dots representative of HF183 detection that is quantifiable (red), detectable but not quantifiable (yellow), and not detected (green) in Windsong (WS#1-4) and King's Heights South (HC#1-3) sites.

Nose Creek was also impacted heavily by human sewage, with HF183 detected in at least one sample at each site, though the most frequent pollution came from N#1 where all 5 samples

in 2021 tested positive (Fig. 4-5). These sites collected stormwater from the neighborhoods to the east and west of the creek, with N#1 in particular collecting stormwater from much further east in addition to the surrounding area.

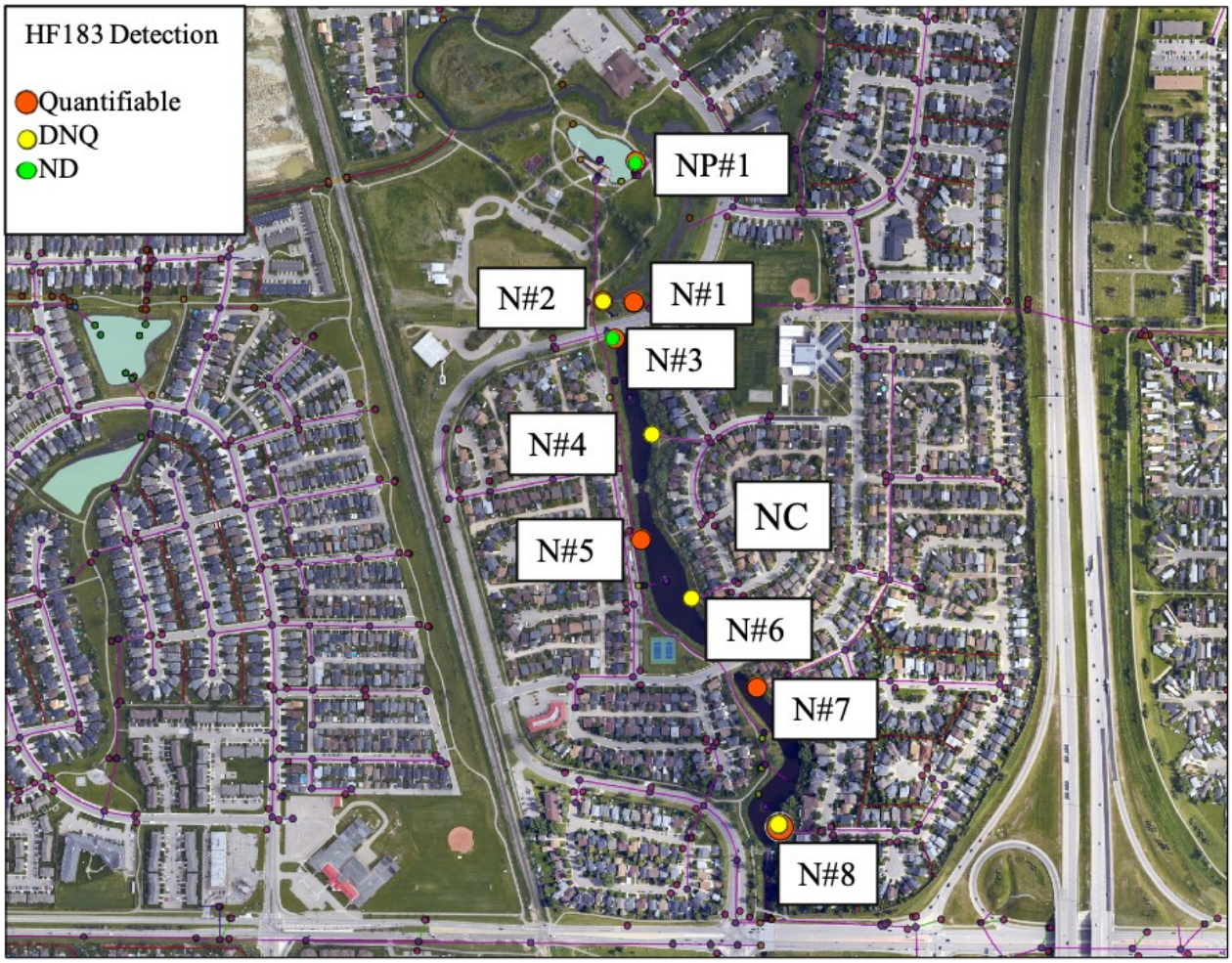

Figure 4-5. Map of human sewage marker (HF183) detection within sites from the Nose Creek (NS) stormwater-impacted creek in Airdrie, Alberta, 2021. Note the colored dots representative of HF183 detection that is quantifiable (red), detectable but not quantifiable (yellow), and not detected (green) in Nose Creek Pond (NP#1) and Nose Creek sites (NC#1-8).

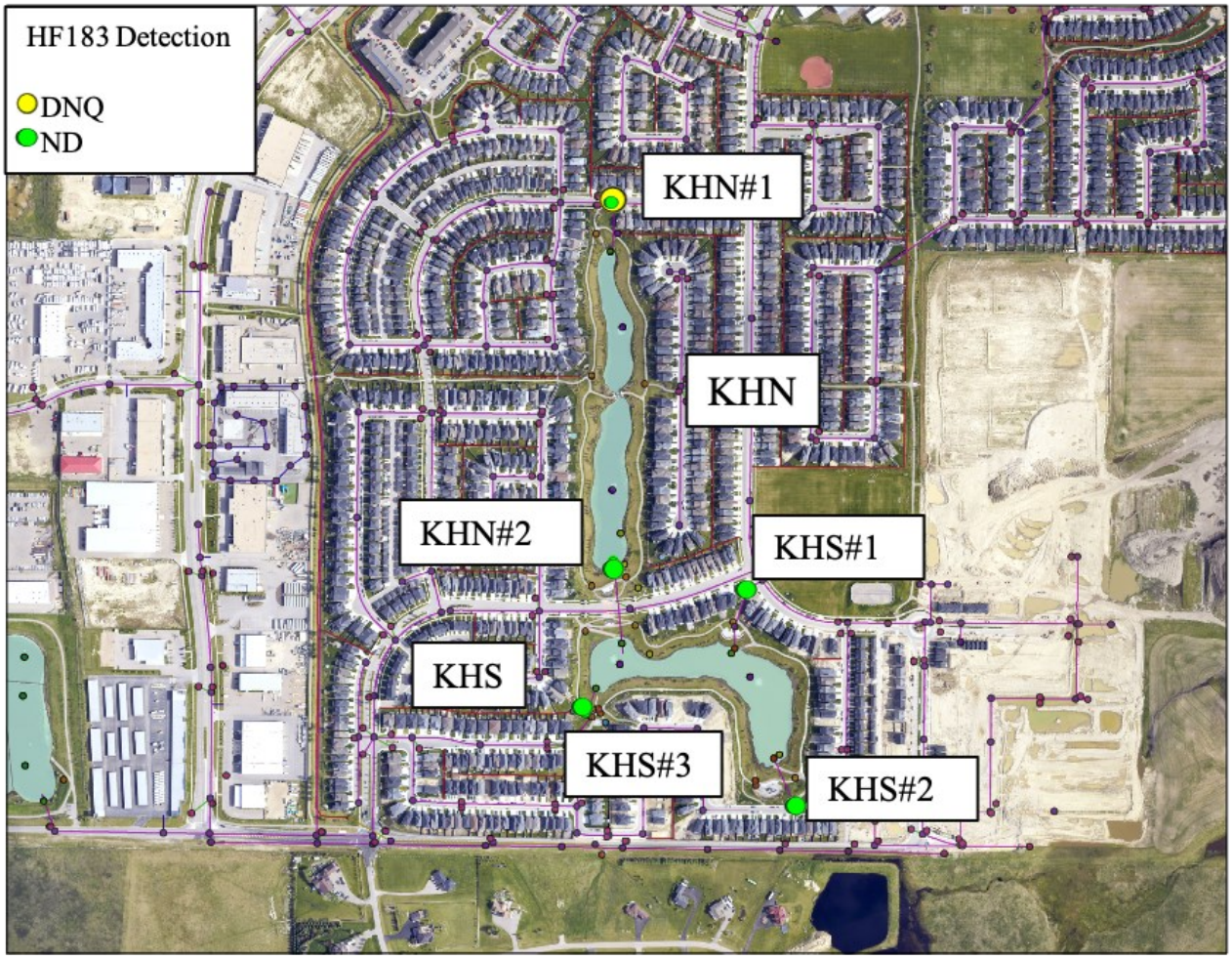

Figure 4-6. Map of human sewage marker (HF183) detection within sites from King's Heights North (KHN) and King's Heights South (KHS) stormwater ponds in Airdrie, Alberta, 2021. Note the colored dots representative of HF183 detection that is quantifiable (red), detectable but not quantifiable (yellow), and not detected (green) in King's Heights North (KHN#1-2) and King's Heights South (KHS#1-3) sites.

Contrasting the above was the King's Heights North and King's Heights South stormwater ponds (Fig. 4-6) as well as the Canals & Bayside Creek (Fig. 4-7), where HF183 was very infrequently detected, and only in a minority of sites. For example, HF183 was only detected in one site in the King's Heights North stormwater pond (KHN#1), three sites in the Canals

(CS#6, CS#7, and CS#9), and was not at all detected in the King's Heights South stormwater pond (Figures 4-6, 4-7). When detected in these sites, HF183 was also only ever detected at a DNQ concentration.

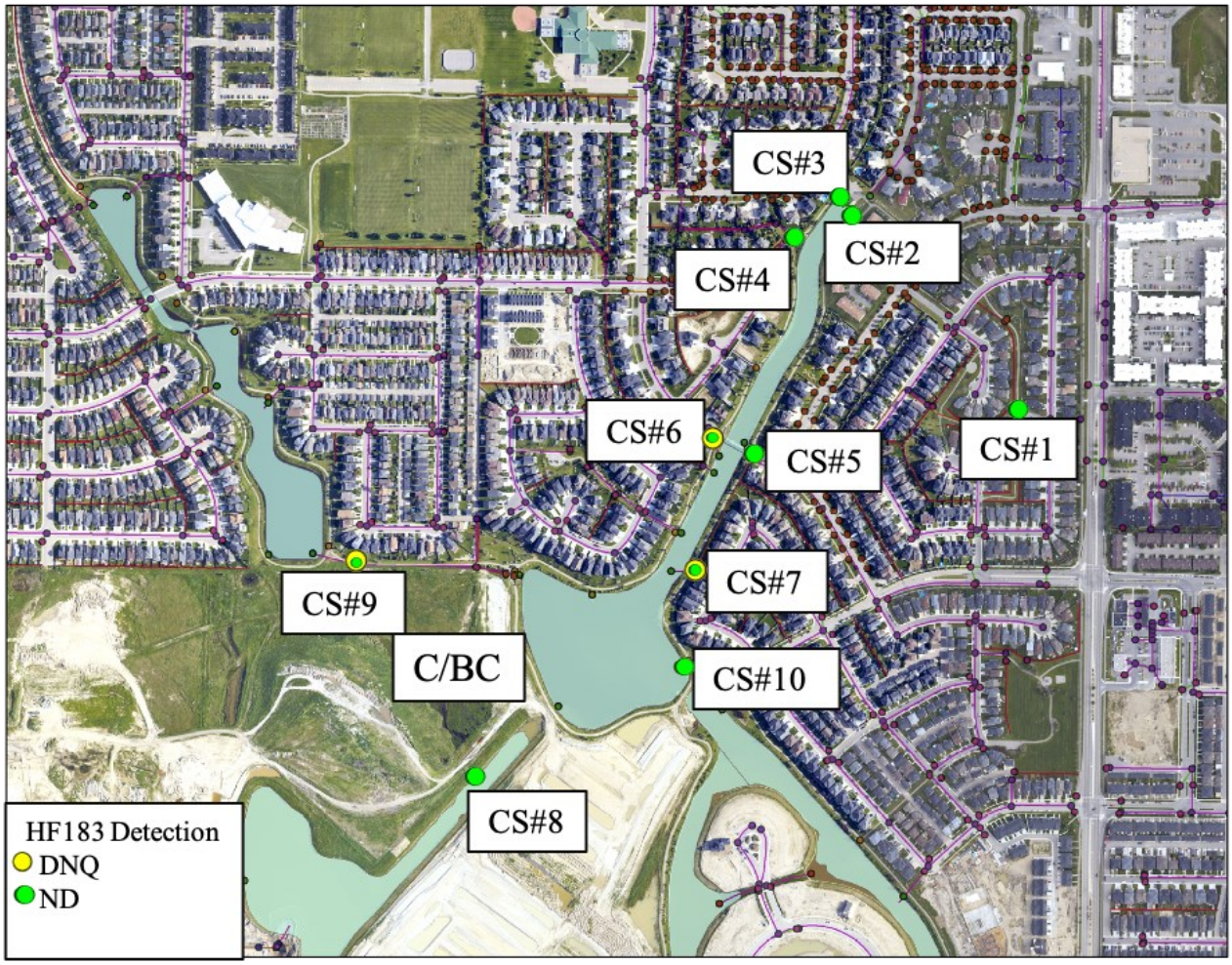

Figure 4-7. Map of human sewage marker (HF183) detection within sites from the Canals & Bayside Creek (C/BC) stormwater-impacted creek in Airdrie, Alberta, 2021. Note the colored dots representative of HF183 detection that is quantifiable (red), detectable but not quantifiable (yellow), and not detected (green) in Canals sites (CS#1-10).

While the majority of samples positive for MST markers were found at a DNQ concentration, this was not always the case for a few markers in particular. In routine samples positive for the dog marker, 13 of 15 (86.7%) of these samples were quantifiable, and ranged in concentration from 3.7 $\log_{10}$ copies/100 mL to 4.9 $\log_{10}$ copies/100 mL. Only three samples from

the 2021 dataset were positive for the goose marker, with two of them being quantifiable at 4.3 $\log_{10}$ copies/100 mL from a sample collected from CS#8 the week of the 30th of August, 2021, and 3.8 $\log_{10}$ copies/100 mL from a sample collected the same week from the CS#10 site. Of routine samples positive for HF183, 14 of 33 (42.4%) were at a quantifiable concentration for this marker, and had a range of concentration from 3.6 $\log_{10}$ copies/100 mL to 4.3 $\log_{10}$ copies/100 mL. This range was only representative of routine sampling of stormwater inlets and outlets, however, and HF183 was found in much higher concentrations at times in the special investigative sampling upstream of some of these sites as discussed in later sections.

In spite of being sampled fewer times in 2021 than in 2020, Windsong had a higher frequency of HF183 detection in 2021 (4 of 16 samples [25.0%] in 2021 versus 2 of 28 samples [7.1%] in 2020), as well as markers detected in 2021 that were not detected in 2020 (Dog3, Rum2Bac) and vice versa (HumM2, CGO1) (Tables 4-1, 4-2). The gull marker was detected in both years of sampling, though ≤2 samples were positive for this marker per year in this stormwater pond. Frequency of detection was generally low for each MST marker found in Windsong for both years of sampling (the previously mentioned exception of HF183 in 2021 notwithstanding), with each marker being positive in ≤2 samples per year. Quantifiable MST marker results were similar in both years, with 2 of 4 HF183 positive samples quantifiable in Windsong in 2021 at 3.9 $\log_{10}$ copies/100 mL and 4.2 $\log_{10}$ copies/100 mL, similar to the single quantifiable sample collected in 2020 at 4.5 $\log_{10}$ copies/100 mL. Dog3 marker was also quantifiable in the 2 positive samples in 2021 at 3.8 $\log_{10}$ copies/100 mL and 4.1 $\log_{10}$ copies/100 mL, though this marker was not detected in Windsong in 2020. When detected, all other markers (LeeSG, Rum2Bac, HumM2, CGO1) were DNQ in the Windsong stormwater pond for both years suggesting low level comtamination from these sources. At least in the Windsong

stormwater pond, multiple fecal sources (but especially human) were therefore detected in stormwater across two years of sampling, albeit sporadically.

4.2.2 Temporal Occurrence of MST Marker Detection

4.2.2.1 Temporal Occurrence of MST Markers in 2020

MST marker occurrence but not concentration appeared to follow a temporal pattern in 2020, with the highest diversity in markers detected during the weeks of Sept. 8th and Sept. 29th, which were also the only weeks with detected human sewage contamination in the form of HF183 and HumM2 (though HF183 detection was not significantly different from these weeks than others by Fisher's exact test) (Table 4-7). With the exception of gull marker (LeeSG), MST markers detected in 2020 samples were not consistently detected on a weekly basis. The gull marker, however, was detected in at least 1 sample each week for most weeks, and spiked in frequency to 25.0% of the samples collected during the Sept. 15th sampling week (Table 4-7). As a possible temporal factor, 24-hr daily total antecedent precipitation was found to be highest in the 2020 sampling season for the Sept. 8th sampling date (8.6 mm of precipitation), and this sampling date happened to have a higher diversity and frequency of MST marker detection than most other sampling dates that year. This is with the exception of Sept. 29th, which had no recorded rainfall, but also had a higher diversity and occurrence of MST markers (Table 4-7). Overall, this suggested the possibility that MST marker detection in 2020 could be mediated by temporal factors, which could include antecedent precipitation level and other unknown temporal factors, though this would require further study to confirm any particular association.

Table 4-7. Frequency (%) of microbial source tracking (MST) marker detection within Airdrie stormwater ponds routinely sampled in 2020, as stratified temporally by date of sampling.*

Dates	n	Human		Gull	Canada Goose	Dog	Ruminant
		HF183	HumM2	LeeSG	CGO1	Dog3	Rum2Bac
17/8/20	12	ND	ND	1/12 (8.3)	ND	ND	ND
24/8/20	12	ND	ND	1/12 (8.3)	ND	ND	ND
31/8/20	12	ND	ND	ND	ND	ND	ND
8/9/20†	12	3/12 (25.0)	ND	1/12 (8.3)	ND	1/12 (8.3)	ND
15/9/20	12	ND	ND	3/12 (25.0)	ND	ND	ND
22/9/20	12	ND	ND	1/12 (8.3)	1/12 (8.3)	ND	ND
29/9/20	12	2/12 (16.7)	1/12 (8.3)	1/12 (8.3)	1/12 (8.3)	ND	ND
Total	84	5/84 (6.0)	1/84 (1.2)	8/84 (9.5)	2/84 (2.4)	1/84 (1.2)	ND

* Note that routine samples in taken in 2020 were collected on a weekly basis for 7 weeks total.

† Note that this date was found with 24 hour total antecedent precipitation >5 mm.

4.2.2.2 Temporal Occurrence of MST Markers in 2021

When stratified temporally by weeks of sampling in 2021, some markers differed in frequency depending on the weeks sampled, while others were either consistently detected at a low frequency or were too infrequently detected to form any observable temporal patterns (Table 4-8). The majority of routine samples positive for non-HF183 MST markers in 2021 were from the Aug. 17th and Aug. 23rd sampling weeks, the sampling dates of these weeks showing the highest amounts of 24-hr total antecedent precipitation from the 2021 sampling season (>5 mm).

This was particularly so for the dog marker, with 14 of 15 (93.3%) routine samples positive for this marker being collected from these two weeks, while 7 of 12 (58.3%) and 5 of 6 (83.3%) routine samples positive for ruminant and gull marker respectively were sampled during the single week of Aug. 17th. Human sewage marker HF183 followed the above pattern to a degree as well, with a higher frequency of detection the weeks of Aug.17th/23rd. Notably, the frequency of detection for this marker in routine Airdrie stormwater samples was still >25% in all sampling rounds except for Aug. 3rd, and detection of HF183 was not significantly different across weeks according to Fisher's exact test (Table 4-8). Only 3 of 31 (9.7%) samples were positive for HF183 from samples collected on Aug. 3rd, despite the level (albeit small; <5 mm) of 24-hr total antecedent precipitation for that date (Table 4-8). The Canada goose marker and the second human sewage marker (HumM2) were the exceptions to the above, as the former was very infrequently detected, and the latter was distributed equally at a low frequency across most sampling weeks (Table 4-8).

It is also important to note that whether MST markers were at a high enough concentration to be quantifiable or not also appeared to be temporally dependent in 2021. For example, 7 of 14 (50.0%) samples quantifiable for HF183 were all detected on Aug. 17th, while 13 of 14 (92.9%) samples quantifiable for Dog3 were found during sampling the weeks of Aug.17th/23rd. Taken together, the above suggests that both the frequency of detection and concentration of MST markers had the potential for a relationship with temporal factors that could include antecedent precipitation, though it would again require further study to determine whether a statistically significant relationship between these factors actually exists.

Table 4-8. Frequency (%) of microbial source tracking (MST) marker detection within Airdrie stormwater ponds routinely sampled in 2021, as stratified temporally by date of sampling.*

Dates	n	Human		Gull	Canada Goose	Dog	Ruminant
		HF183	HumM2	LeeSG	CGO1	Dog3	Rum2Bac
19/7/21, 20/7/21	31	9/31 (29.0)	2/31 (6.5)	ND	ND	1/31 (3.2)	1/31 (3.2)
3/8/21	31	3/31 (9.7)	2/31 (6.5)	1/31 (3.2)	1/31 (3.2)	ND	2/31 (6.5)
17/8/21†, 23/8/21†, 24/8/21†	31	12/31 (38.7)	2/31 (6.5)	5/31 (16.1)	ND	14/31 (45.2)	7/31 (22.6)
30/8/21, 31/8/21	31	8/31 (25.8)	3/31 (9.7)	ND	2/31 (6.5)	ND	1/31 (3.2)
7/9/21	2	1/2 (50.0)	ND	ND	ND	ND	1/2 (50.0)
Total	126	33/126 (26.2)	9/126 (7.1)	6/126 (4.8)	3/126 (2.4)	15/126 (11.9)	12/126 (9.5)

* Note that routine samples taken in 2021 were collected on an approximately biweekly basis for 4 weeks total (5 weeks for N#1 and NP#1 sites).

† Note that this date was found with 24 hour total antecedent precipitation >5 mm.

MST marker diversity and prevalence in Windsong sites were observed to follow temporal patterns in 2021 but not in 2020, though very few samples in 2020 (4 of 28, 14.3%) were positive for MST markers in comparison to in 2021 (5 of 16 samples, 31.3%). This tendency of higher MST marker occurrence in 2021 appeared especially pronounced when daily (24-hr) total antecedent precipitation was highest, such as for the sampling date of Aug. 17th. It was during this sampling date alone that 3 of 4 of the HF183 positive samples found in Windsong were detected, while Rum2Bac and Dog3 were also only detected on this date. The gull marker (LeeSG) was detected in Windsong in both years, though it did not appear to follow any temporal

pattern in either year at this stormwater pond. Notably, sampling in 2020 occurred both more frequently (weekly in 2020 vs. roughly bi-weekly in 2021) and later in the year (August-September in 2020 vs. July-August in 2021) than in 2021, though it is unclear if these differences had any direct effect on MST occurrence and concentrations. Importantly, differences in HF183 detection in 2021 across temporal (i.e., biweekly) lines were not significantly different by Fisher's exact test.

4.2.3 Patterns of HF183 Detection Alongside FIB in Stormwater in 2020 and 2021

While some variation did occur, HF183 detection was not found to be significantly influenced in 2020 or 2021 whether FIB STV criteria exceedance occurred or not according to Fisher's exact test, suggesting that human sewage contamination (via HF183 detection) was not a good predictor of FIB STV criteria exceedance on an individual sample basis. Although HF183 was only detected infrequently in Airdrie stormwater in 2020 (5 of 84 samples, 6.0%), this appeared true for 2021 as well, when 26.1% of samples (33 of 126) were positive. For example, of the total 33 routine samples collected in 2021 that were positive for HF183, just over half (18 of 33, 54.5%) were detected in samples where STVs were exceeded by either *Enterococcus*, *E. coli*, or both, suggesting that human sewage marker was nearly just as likely detected under high or low FIB concentrations. This also, not surprisingly, suggests that multiple fecal sources contribute collectively to FIB exceedances.

4.2.4 Investigative Studies - Identifying Point Source of Human Fecal Sewage in Stormwater Drainage Networks

As a result of the evidence that human sewage contamination was potentially polluting Airdrie stormwater ponds and receiving bodies (i.e., Nose Creek), follow-up investigations occurred upstream of select sites in Nose Creek, Windsong, and Hillcrest, as well as East Lake based on data from 2020 and 2021. The main objective of these investigations was therefore to trace point sources of human sewage contamination (through the use of the HF183 and HumM2 MST markers) into the stormwater drainage system of Airdrie in an effort to identify exactly where contamination was coming from (i.e., cross connection, leaky sewerage systems, homeless populations, etc.) so as to remediate these problems if possible.

4.2.4.1 Case Study #1 – Nose Creek

Due to the fact that Nose Creek sites had the highest and most consistent concentrations of the human marker (HF183), especially at the N#1 site (see Fig. 4-8), a special investigation was conducted over several weeks aimed at sampling the stormwater drainage network upstream of this inlet and in an effort to isolate a point source of human contamination. Investigative sampling therefore began on manholes upstream of N#1 starting the week of July 27th, when both HF183 and HumM2 were detected in relatively high concentrations in the north trunks draining into manholes N1-15C2 and N1-15C62, though this marker was not found in the East trunk of the latter manhole (Fig 4-9, Table 4-9). While N1-15C2 (south east drainage) was upstream of N1-15C62, it was in the latter manhole where HF183 and HumM2 concentrations were greater (by >1 order of magnitude for HF183). Considering the differences in marker concentration, and the lack of human sewage marker detection in the East trunk of N1-15C62, it was surmised that the primary source of human sewage contamination to outfall N#1 in Nose Creek was likely coming from north and to the upstream of manhole N1-15C62, while what appeared to be a smaller portion of contamination appeared to also have come from the south drainage trunk at

manhole N1-15C2 (Fig. 4-9, Table 4-9). No other animal MST marker was detected in these investigative samples except for the human markers.

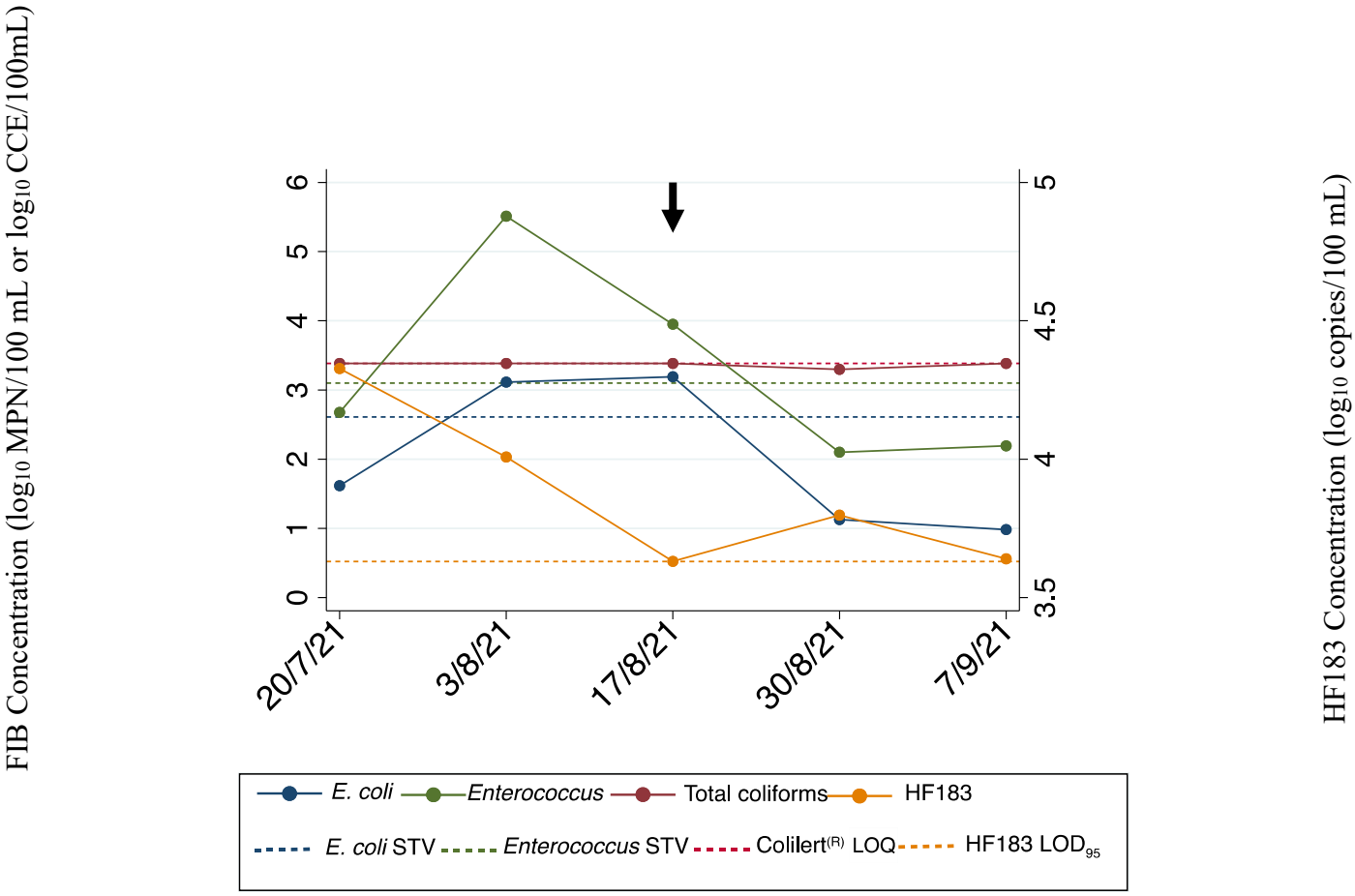

Figure 4-8. Line graph of FIB (E. coli, Enterococcus, and total coliforms) and HF183 concentrations and detection over time in Nose Creek stormwater site N#1. Note that the left y-axis is representative of FIB concentrations in MPN/100 mL for *E. coli* and total coliforms, and CCE/100 mL for *Enterococcus* concentrations. The right y-axis represents HF183 marker concentrations in marker copy numbers/100 mL. Note also the dotted lines representing acceptable STVs for *E. coli* (320 MPN/100 mL) and *Enterococcus* (1280 CCE/100 mL), as well as the upper quantifiable limit of the Colilert$^{(R)}$ assay (2419.60 MPN/100 mL) and the LOD_{95} of the HF183 assay (4272 copies/100 mL). Black arrows represent sampling dates where rainfall was >5 mm the day of sampling, the day before, or both.

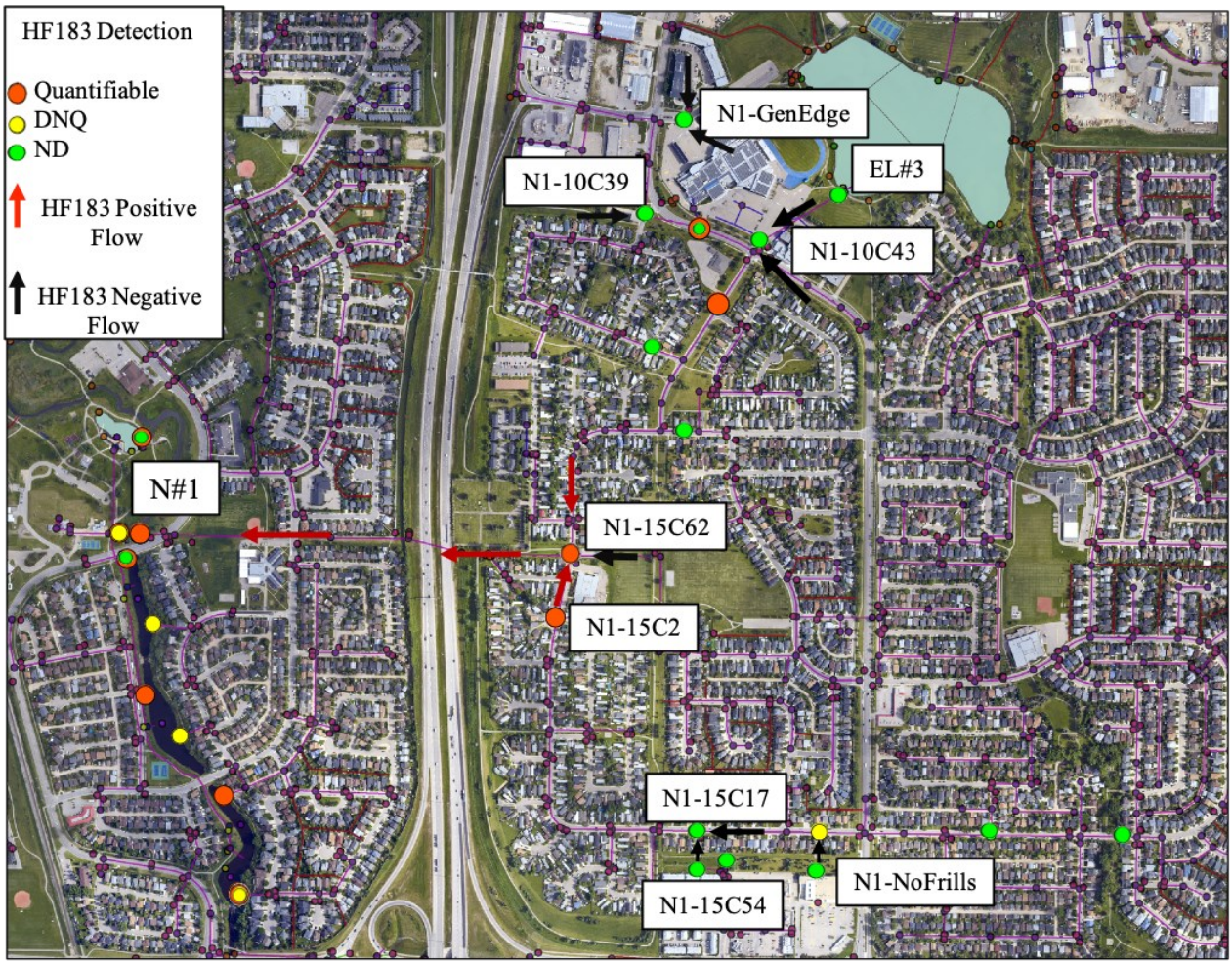

Figure 4-9. Map of Nose Creek stormwater drainage network and the manholes tested for human sewage marker HF183 upstream of HF183 positive outfall site N#1 from the first three weeks of the investigation. Note the manholes upstream of N#1 where HF183 was positive and quantifiable (red dots), detectable but not quantifiable (yellow dots), or not detected (green dots). Note also the flow of stormwater through the system, represented by presumed HF183 positive flow (red arrows) based on upstream site HF183 detection and concentrations, as well as flow with no demonstrable HF183 detection (black arrows).

In the subsequent investigative sampling weeks of Aug. 9th and Aug. 24th, the team sought to explore the human fecal signature of contamination in the south/southeast and the

north/northeast drainage trunks converging and draining into the highly contaminated manhole N1-15C62. As depicted by the black arrows in Fig. 4-9, as well as Table 4-9, none of these manholes tested positive for human sewage markers, with the exception of the east trunk of N1-15C17 in the south-east, though only HumM2 was detected at a DNQ concentration.

Table 4-9. Evidence of human sewage contamination and FIB results from investigative samples taken upstream of the N#1 site in Nose Creek during the first half of the investigation (weeks 1-3).

Sampling Date	Site	HF183	HumM2
		log10 copies/100 mL	
26/7/21	N1-15C2-N	4.06	DNQ
	N1-15C62-N	5.25	4.32
	N1-15C62-E	ND	ND
9/8/21	N1-10C39-N	ND	ND
	N1-10C43-E	ND	ND
	N1-10C43-W	ND	ND
	N1-15C54-N	ND	ND
	N1-15C17-E	ND	DNQ
24/8/21	N1-10C39-W	ND	ND
	N1-GenEdge-S	ND	ND
	N1-GenEdge-N	ND	ND
	N1-NoFrills-N	ND	ND

Collectively, the data suggested that two sources of human fecal pollution may be contributing contamination to the stormwater drain flowing in Nose Creek at site N#1, including: a) a dominant source of human fecal pollution coming from the north drainage trunk flowing into manhole N1-15C62, but downstream of N1-10C39; and b) a less dominant (and possibly

intermittent) source of human fecal pollution flowing into N1-15C62 from the south (and flowing through N1-15C2). As such, the team focused their sampling efforts during Sept. 7th and 8th on the north drainage trunk between manholes N1-15C62 (i.e., highly contaminated with human sewage) and N1-10C39 (i.e., not contaminated with human sewage), on manholes draining in nearby neighborhoods, and manholes even further upstream to the far south-east (Fig. 4-10, Table 4-10). The pattern of human sewage contamination in N1-15C62 was again confirmed (i.e., contamination in the north trunk but not the east trunk), though human sewage was not detected in any great concentration with the single exception of HF183 and HumM2 found at the south trunk of N1-10C49, suggesting that the primary point source of human sewage contamination was in the local vicinity of this manhole (Fig. 4-10, Table 4-10).

In the final rounds of investigative sampling, the highest HF183 concentrations for the entire investigative study were observed at N1-10C41-N and again at N1-10C49-S on Sept. 27th (Fig 4-10, Table 4-10), suggesting that the human fecal source of pollution was in close proximity to these manholes. Just upstream of N1-10C41 was a multiuser community center, and subsequent tracer dye studies using rhodamine (and carried out by city employees) confirmed that several of the toilets within the multiuser center were cross-connected to the stormwater system. It was further confirmed that human sewage contamination was not likely coming from other upstream sites to the west and north, with an absence of HF183 detection in N1-10C39, N1-10C41-W and N1-10C43-N (draining East Lake) (Fig. 4-10, Table 4-10). The study clearly demonstrated that a cross-connection in this multi-user community facility was the dominant source of human feces contributing to pollution of stormwater draining into Nose Creek at the N#1 site.

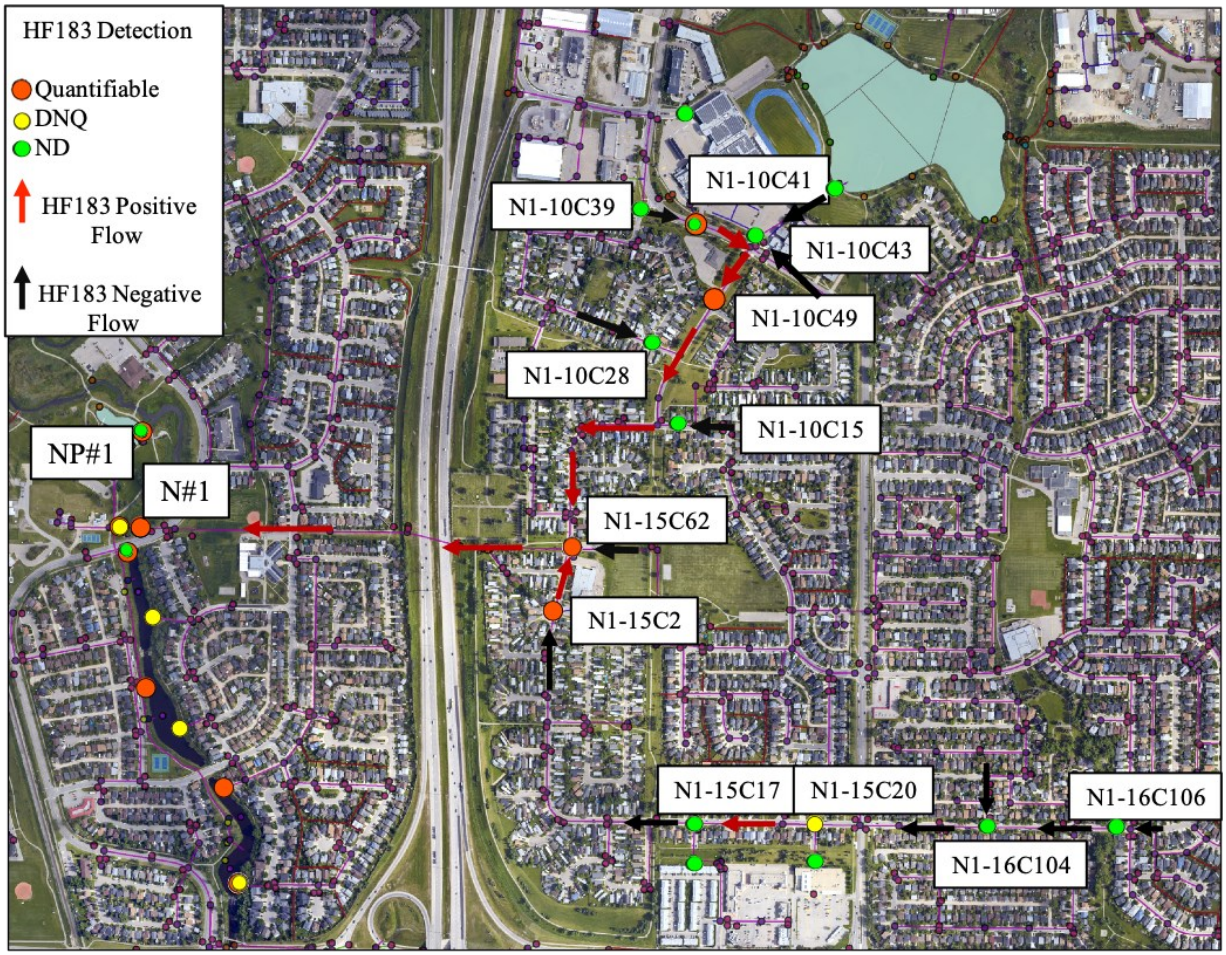

Figure 4-10. Map of Nose Creek stormwater drainage network and the manholes tested for human sewage marker HF183 upstream of HF183 positive outfall site N#1 from the final two weeks of the investigation. Note the manholes upstream of N#1 where HF183 was positive and quantifiable (red dots), detectable but not quantifiable (yellow dots), or not detected (green dots). Note also the flow of stormwater through the system, represented by presumed HF183 positive flow (red arrows) based on upstream site HF183 detection and concentrations, as well as flow with no demonstrable HF183 detection (black arrows).

Table 4-10. Human sewage MST results from investigative samples taken upstream of the N#1 site in Nose Creek during the second half of the investigation (weeks 4-5).

Sampling Date	Site	HF183	HumM2
		log10 copies/100 mL	
7/9/21	N#1	3.64	ND
	N1-10C39-E	ND	ND
	N1-10C28-E	ND	ND
	N1-10C49-S	4.75	DNQ
	N1-10C15-E	ND	ND
8/9/21	N1-15C62-E	ND	ND
	N1-15C2-E	ND	ND
	N1-15C2-S	ND	ND
	N1-15C17-W	ND	ND
	N1-15C20-W	DNQ	ND
	N1-16C104-E	ND	ND
	N1-16C104-N	ND	ND
	N1-16C106-E	ND	ND
	NP1	ND	ND
27/9/21	N1-10C49-S	5.36	4.31
	N1-10C41-N	6.08	6.05
	N1-10C41-W	ND	ND
	N1-10C43-N	ND	ND
	N1-10C39-E	ND	ND

The end result of the above investigation is best summarized in Fig. 4-11 and Table 4-11. As can be seen in these two figures below, HF183 concentrations were tracked upstream of N#1 ultimately to the N1-10C41 manhole, where the human HF183 marker was 2.4 $\log_{10}$ higher in concentration at this site than that observed in the effluent at Nose Creek (N#1), demonstrating

a steady increase by ~1 $\log_{10}$ of this marker between each individual site tested in the direct pathway from N#1 (the outfall) to this terminal distal point of contamination (Fig. 4-11, Table 4-11). This high human sewage contamination was also confirmed by a second marker, HumM2, which was found at nearly the same high level of concentration as the HF183 marker at the terminal manhole, and followed a similar pattern of increasing between N#1 and N1-10C41 (Table 4-11). This case study exemplifies the utility of using MST (i.e., HF183) to trace human fecal contamination sources in stormwater drainage networks, and potentially allows municipalities to successfully identify infrastructural issues that contribute pollution to receptor water bodies (i.e., stormwater ponds, rivers, and creeks).

Table 4-11. Human sewage MST results from select investigative samples highlighting the most direct proposed path of human fecal contamination from a local community center in Airdrie, Alberta to the N#1 outfall feeding Nose Creek.

Sampling Date	Site	HF183	HumM2
		$\log_{10}$ copies/100 mL	$\log_{10}$ copies/100 mL
7/9/21	N#1	3.64	ND
8/9/21	N1-15C62-N	4.40	ND
27/9/21	N1-10C49-S	5.36	4.31
	N1-10C41-N	6.08	6.05

Figure 4-11. Map of Nose Creek stormwater drainage network summarizing the most relevant manholes tested for human sewage marker HF183 upstream of HF183 positive outfall site N#1. Note the manholes upstream of N#1 where HF183 was positive and quantifiable (red dots), detectable but not quantifiable (yellow dots), or not detected (green dots). Note also the flow of stormwater through the system, represented by presumed HF183 positive flow (red arrows) based on upstream site HF183 detection and concentrations, as well as flow with no demonstrable HF183 detection (black arrows).

4.2.4.2 Case Study #2 – Hillcrest Stormpond

Throughout the summer of 2021, an additional investigation was carried out to try and identify fecal sources of pollution impacting the Hillcrest stormpond. Within the Hillcrest stormpond, both HC#2 and HC#3 sites were occasionally positive for the HF183 human marker

throughout the routine sampling campaign in 2021. Since HF183 detections were more frequently observed in HC#3, investigative sampling was performed in the storm drainage network upstream of HC#3 (Fig. 4-12).

On Aug. 24th, HF183 was detected (at a DNQ concentration) just upstream and to the west of the HC#3 site, and samples were taken upstream to the west and south on Sept. 7th and 27th. This marker was not at a quantifiable concentration with the single exception of the western trunk of HC3-52C6, which was found to have a relatively high HF183 concentration of 4.02 $\log_{10}$ copies/100 mL (Fig. 4-12).

Using robots, the City of Airdrie carried follow up studies in the storm drain during December 2021 that found an illicit cross-connection upstream of HC3-52C16, and likely represented a primary source of human sewage entering into the Hillcrest Stormpond via the HC#3 inlet (Fig. 4-12). DNQ detection of HF183 (but not for HumM2), in a manhole upstream of the cross-connected house (HC3-52C16) suggested the possibility of additional sources contributing human sewage to the Hillcrest stormwater pond at HC#3. Notably, identification of a physical cross-connection upstream of the Hillcrest stormwater pond, and where HF183 was found persistently, albeit at low levels, demonstrates that these MST tools are fairly sensitive in pinpointing the actual causes of pollution.

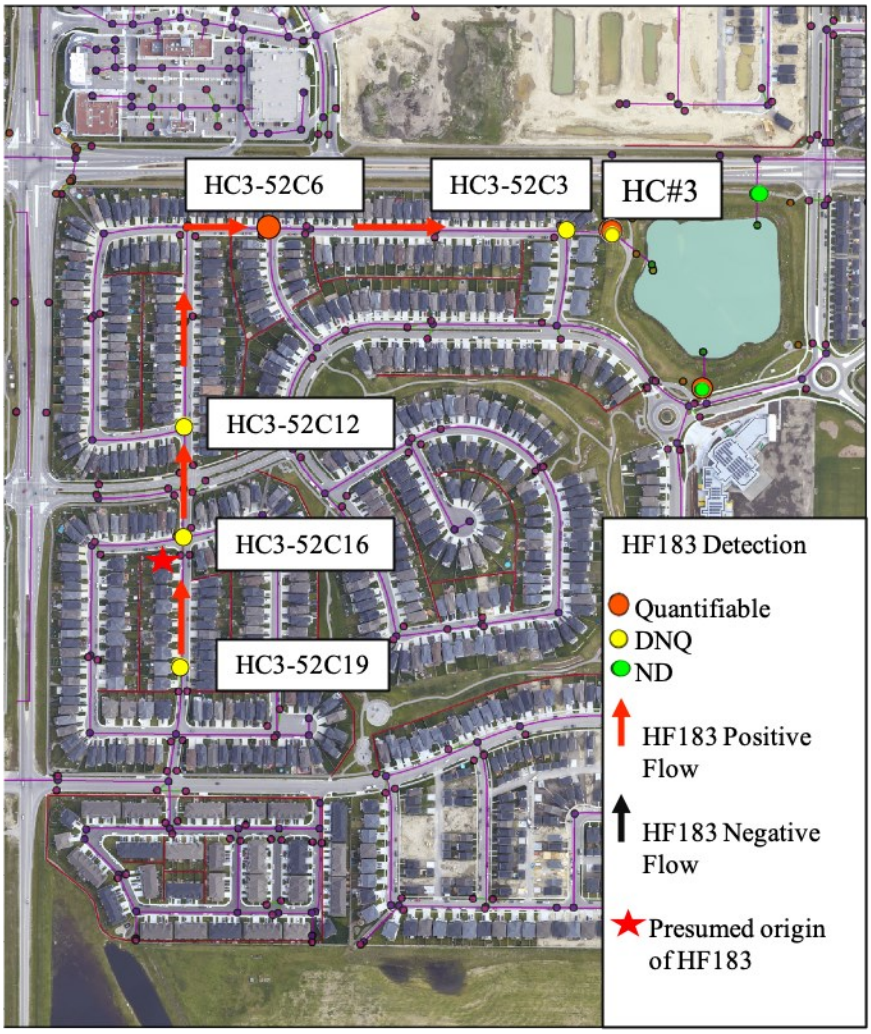

Figure 4-12. Map of Hillcrest stormwater drainage network and manholes tested for human sewage marker HF183 upstream of HF183 positive outfall site HC#3. Note the manholes upstream of HC#3 where HF183 was positive and quantifiable (red dots), or detectable but not quantifiable (yellow dots). Note also the flow of stormwater through the system, represented by presumed HF183 positive flow (red arrows) based on upstream site HF183 detection and concentrations. A red star represents the presumed site of origin of HF183 contamination in the form of a cross-connection as revealed by personal correspondence with the City of Airdrie.

4.3 Discussion

Several important observations, with potentially significant implications were made regarding the sources of fecal pollution impacting stormwater in Airdrie. First and foremost, human fecal contamination (via the HF183 marker, and often confirmed by the HumM2 marker) was generally found to be the most frequently detected fecal pollution source in stormwater and often at high concentrations, while all other markers for animal sources were very sporadically detected and often at relatively low concentrations. Furthermore, MST methods were shown to be extremely valuable for investigating and identifying point sources of human fecal pollution in drainage system by tracing fecal signatures from stormwater outfalls back into the upstream drainage networks in the city. In the case of the Nose Creek, a multiuser center in the northeast quadrant of the City of Airdrie was identified as a source of human fecal contamination and tracer dye testing confirmed that several of the toilets in the facility were cross-connected to the storm drains. In the case of the Hillcrest Stormpond, a persistent but low-level signature of human pollution coming into the stormpond was traced back to at least one house in the community, and for which robotic drone surveillance confirmed a domestic cross-connection of sewer lines to storm drains. In both of these cases, persistent occurrence of human markers in stormwater samples even at very low levels (i.e., DNQ), proved useful for pin-pointing the actual source of pollution (i.e., cross connections).

An increasing body of evidence suggests that human sewage markers (i.e., HF183) are frequently (albeit sporadically) detected in stormwater across the world (Ahmed et al., 2020; Gonzalez et al., 2020; Hachad et al., 2022; Hart et al., 2020; Kinzelman & McLellan, 2009; Lee et al., 2020; Nshimyimana et al., 2014; Sales-Ortells & Medema, 2015; Sauer et al., 2011; Sidhu

et al., 2012; Staley et al., 2018; Steele et al., 2018; Williams et al., 2022), and stormwater from Airdrie, Alberta was no exception. HF183 was sporadically detected in Airdrie stormwater, with just greater than 25% of routine samples testing positive in 2021 for example, and concentrations generally ranging from DNQ to >4 $\log_{10}$ copies/100 mL. Other studies have demonstrated even higher concentrations (generally 4 – 7 $\log_{10}$ copies/100 mL) and prevalence (usually between 50-75% of samples) in stormwater of other jurisdictions, though a wide range of variability in both these parameters is still quite common (Ahmed et al., 2020; Gonzalez et al., 2020; Hachad et al., 2022; Hart et al., 2020; Kinzelman & McLellan, 2009; Lee et al., 2020; Nshimyimana et al., 2014; Sales-Ortells & Medema, 2015; Sauer et al., 2011; Sidhu et al., 2012; Staley et al., 2018; Steele et al., 2018; Williams et al., 2022). Noting that the above concentrations of HF183 in stormwater are estimated to be 1-3 orders of magnitude lower than the concentration of HF183 in raw human sewage (generally 7-8 $\log_{10}$ copies/100 mL – see Ahmed et al., 2021; Mayer et al., 2018; Nshimyimana et al., 2014; Sauer et al., 2011), storm flows can therefore typically be composed of 0.1% to 10% of raw human sewage. The data from Airdrie suggests that the lower end of this range applies to most of the sites monitored in this study. The methods and observations described in this study therefore support the approach used in the Government of Alberta's *Public Health Guidelines for Water Reuse and Stormwater Use*, in which different $\log_{10}$ reduction credits for stormwater use are based on the levels of human sewage contamination as determined by HF183.

Recent studies have also begun utilizing HF183 and other similar markers of human sewage to pinpoint sanitary sewer infrastructural failures and illicit cross-connections (Ahmed et al., 2020; Gonzalez et al., 2020; Hachad et al., 2022; Sauer et al., 2011; Sercu et al., 2011). For example, Gonzalez et al. (2020) conducted three separate case studies wherein infrastructure

failures were isolated in each case through the the use of targeted HF183 sampling, before remediation efforts and confirmatory re-sampling were executed successfully in multiple cases. Similarly, Hachad et al. (2022) used HF183 (though in conjunction with a number of other indicators in a "combined index" approach) in a similar manner, and were able to isolate three individual domestic cross-connections tying into the stormwater sewer upstream of contaminated outfalls. Lastly, the dilution effect of HF183 from human sewage source to outfall through the stormwater network found in the present study was also demonstrated by Ahmed et al. (2020), where HF183 concentrations were higher in upstream manholes by 2-3 $\log_{10}$ copies/100 in comparison to concentrations at studied outfalls.

While detection of animal markers (i.e., for gulls, Canada geese, dogs, and ruminants) was found to be even more sporadic than for human sewage, the positive detection of all the tested animal markers suggested a large variety of animal sources contributing feces to this system, and this has been increasingly found in more recent studies (Ahmed et al., 2020; Bambic et al., 2015; Green et al., 2014, 2019; Lee et al., 2020; Lu et al., 2011b; McLellan et al., 2018; Monteiro et al., 2021; Olds et al., 2018; Sales-Ortells & Medema, 2015; Staley et al., 2018; Steele et al., 2018; Williams et al., 2022). The most prevalent animal markers in Airdrie stormwater were dogs (in 2021) and gulls (in 2020), though both the concentrations and prevalence of animal markers were all relatively low (each marker characterizing <12% of samples, for example), this is consistent with the literature, which has found patterns animal fecal markers in stormwater to be quite sporadic (Ahmed et al., 2020; Bambic et al., 2015; Green et al., 2014, 2019; Lee et al., 2020; Lu et al., 2011b; McLellan et al., 2018; Monteiro et al., 2021; Olds et al., 2018; Sales-Ortells & Medema, 2015; Staley et al., 2018; Steele et al., 2018; Williams et al., 2022). The above is particularly concerning when it comes to stormwater fecal contamination from waterfowl (Lu

et al., 2011b; Staley et al., 2018; Steele et al., 2018), which are currently underassessed in terms of the risk they contribute to environmental waters, and have been increasingly shown to be carriers of zoonotic pathogens such as *Campylobacter* spp. and *Salmonella* spp. (Antilles et al., 2021; Broman et al., 2002; Palmgren et al., 2006; Russo et al., 2021). Infrequent evidence of ruminant contamination suggested that this came possibly from deer as seen in Lee et al. (2020) as opposed to fecal contamination from upstream agricultural activities and livestock (Bambic et al., 2015; Green et al., 2019). Importantly, the data demonstrates that animal sources cannot be discounted, including waterfowl, domestic pets, and wild animals.

Temporal differences in MST markers from both human and animal fecal sources appeared to exist in Airdrie stormwater, with antecedent rainfall found as a factor (among others) with the potential to affect the detection and concentrations of these markers. For example, human sewage marker HF183 was detected slightly (though not statistically significantly) more frequently during the sampling periods where 24-hour antecedent daily precipitation was >5 mm (Sept. 8th, 2020, and Aug. 17th/23rd in 2021), while the gull, ruminant, and dog markers also saw an uptick in prevalence during this same level of precipitation (though only in 2021). Similarly, many studies have found that higher levels of precipitation and the presence of wet weather events increased the prevalence and/or concentration of HF183 in stormwater (see Ahmed et al., 2020; Gonzalez et al., 2020; Hart et al., 2020; McGinnis et al., 2018; Olds et al., 2018; Sidhu et al., 2012; Staley et al., 2018; Williams et al., 2022) with few exceptions (see Sauer et al., 2011). Importantly, human sewage leaks into stormwater could be missed when only baseline weather conditions have been analyzed, with several studies finding human sewage at some sites only during wet weather (Ahmed et al., 2020; Sidhu et al., 2012; Staley et al., 2018), with Gonalez et al. (2020) only able to isolate some infrastructural problems when sampling was done under wet

weather conditions and HF183 could be identified.-Still other studies suggest a lack of significant relationships between these parameters, especially between wet weather and avian fecal contamination (Ahmed et al., 2020; Steele et al., 2018; Williams et al., 2022). These results suggest that there may not necessarily be a cut-and-dry relationship between precipitation levels and non-human MST marker detection.

Geo-spatial differences in HF183 frequencies of detection were found in Airdrie stormwater-impacted ponds and creeks, with both individual sites within stormwater ponds as well as the Nose Creek and Windsong stormwater pond being particularly contaminated in comparison to the other water bodies studied (though only Nose Creek was significantly different from the Canals and King's Heights South stormwater ponds). This is consistent with the current literature, with multiple studies suggesting HF183 occurrence and concentration variation between differing outfalls within the same general geo-spatial area, as well as between sites collected from differing but nearby stormwater-impacted water bodies (Ahmed et al., 2020; Nshimyimana et al., 2014; Parker et al., 2010; Sidhu et al., 2012; Staley et al., 2018; Steele et al., 2018). Conversely, a few studies have shown that human sewage contamination is so prevalent that similar concentrations and detection are commonly found across most studied sites, offering little in the way of variation (Hachad et al., 2022; Hart et al., 2020; Lee et al., 2020; Sauer et al., 2011).

Causes of geo-spatial variation found in HF183 detection frequency and concentrations can appear for several diverse reasons (Ahmed et al., 2020; Nshimyimana et al., 2014; Parker et al., 2010; Sidhu et al., 2012; Staley et al., 2018; Steele et al., 2018). For example, several studies suggest that land use differences in areas draining into stormwater sites can heavily affect HF183 marker variability (Nshimyimana et al., 2014; Sidhu et al., 2012; Steele et al., 2018). Instead,

others have found that differing site infrastructure (such as culvert and ditch systems vs. pipe outfalls) as well as where infrastructure has been sampled (such as further upstream in manholes versus further downstream at outlets themselves) (Parker et al., 2010; Staley et al., 2018; Steele et al., 2018; Williams et al., 2022). In contrast to the above, little geo-spatial variation has been observed for animal markers (such as those for domestic pets and waterfowl) in stormwater, though this can often likely be attributed to the fact that these markers are often found to be less prevalent and in lower density than human sewage markers (Ahmed et al., 2020; Lee et al., 2020; Monteiro et al., 2021). Exceptions include that Lee et al. (2020) found statistically significant differences in ruminant marker (Rum2Bac) between outfalls, while Steele et al. (2018) found that stormwater draining into the San Diego River (which had a bird sanctuary upstream) had significantly higher influence of gull fecal marker than the nearby Tourmaline Creek.

In our study, high FIB concentrations and HF183 detection and concentrations tended to diverge along both temporal and geo-spatial lines, and this is consistent with the poor or absent correlational relationship between FIB and HF183 found in several studies (Hart et al., 2020; Nshimyimana et al., 2014; Olds et al., 2018; Sauer et al., 2011; Williams et al., 2022). Moderate correlation between these two has also been found infrequently (see Hachad et al., 2022; Staley et al., 2018; Steele et al., 2018). Geo-spatial differences have been observed to effect HF183 to diverge from FIB in behavior in some studies (Hachad et al., 2022; Hart et al., 2020; Lee et al., 2020), as well as act in tandem in other studies (Nshimyimana et al., 2014; Parker et al., 2010). Interestingly, Steele et al. (2018) also noticed that *Enterococcus* appeared to form a temporal pattern and peaked with higher storm sizes before tapering off in days following a storm, whereas HF183 appeared to continue to be detected at the tail end of the studied storms, independent of storm size. Divergence between FIB and human sewage MST markers can likely be due to

variable behavior of fecal pollution from the many other animal hosts of FIB (Ahmed et al., 2019a; Ervin et al., 2013; Layton et al., 2009, 2010).

The unclear relationships between individual FIB and human sewage marker HF183 noted above was also the case between high FIB concentrations and animal MST markers studied, with again very variable relationships when considering both temporal and geo-spatial differences (Ahmed et al., 2020; Lee et al., 2020; Staley et al., 2018; Steele et al., 2018). Importantly, a lack of strong correlation between FIB and either the human or animal markers tested suggests that the relationship between the two may be complex, and that these markers may not comprehensively cover all of the potential sources of FIB in these systems. All in all, the above suggests that while FIB and HF183 and other animal MST markers can be found to have a similar relationship with wet weather (particularly when they are well correlated), this cannot be assumed, as this relationship with wet weather events appears muddied under some circumstances.

Alongside the above explanatory factors, it is important to emphasize that several key limitations could contribute to the lack of detection of some MST markers, as well as inconsistent relationships between various human and animal MST markers and FIB. The first of which would be issues with marker sensitivity and specificity, with the markers used in this current study and other similar studies not scoring 100% on either front (see Boehm et al., 2013; Shanks et al., 2010a, b; Sinigalliano et al., 2013). In terms of sensitivity, inhibitory substances have been demonstrated to cause a lack of detection where MST markers may be present (Opel et al., 2010; Schrader et al., 2012). Difficulties with sensitivity may help potentially explain why animal MST markers are often rarely detected, with the MST assays used potentially having difficulty picking up animal markers when they are present in low concentrations to begin with, particularly when

the dominant source of fecal contamination appears human in nature (such as in the current study). Additionally, this lack of detection can show misleading relationships between MST markers and FIB, temporal factors, and geo-spatial factors, possibly explaining the lack of strong relationships and/or presence of contradictory relationships between these factors seen in the current literature.

In terms of specificity, cross-reaction has been shown to be a problem in the case of several MST markers where feces from non-host animals causes false-positive "detection", incorrectly inflating the quantities of supposedly host-specific markers found (see Boehm et al., 2013; Shanks et al., 2010a,b; Sinigalliano et al., 2013). This has been found to be the case particularly for HF183 in the presence of both canine and chicken feces, though studies have also found that cross-reaction produces low concentrations of false-positive results so that higher concentrations of HF183 are still likely representative of true-positive results for human sewage (Ahmed et al., 2019c; Boehm et al., 2013; Shanks et al., 2010b). While cross-reactivity has been shown for other animal MST markers (such as canine and avian markers) (Boehm et al., 2013; Sinigalliano et al., 2013), detection and concentration of these markers was in general too low in the current Airdrie study for specificity to be a major concern in comparison to the potential lack of sensitivity.

Additional to the above limitations, it is worth re-emphasizing that many molecular methods do not differentiate viable from non-viable DNA, particularly without further methods used to differentiate these two strata (see Emerson et al., 2017; Field & Samadpour, 2007; Harwood et al., 2014). As a result, overestimation of viable indicators of fecal pollution can potentially occur, suggesting active pollution is occurring when pollution may be more sporadic. As mentioned previously though, the HF183 marker at least has been found to degrade generally

within one week of being deposited into environmental waters, suggesting when this marker is detected in large quantities and represents fecal pollution that is relatively recent (Boehm et al., 2018; Dick et al., 2010; Walters & Field, 2009).

Another clear limitation is the fact that the MST markers used in individual studies have not been standardized, with several popular markers being used for the most commonly tested hosts (reviewed by Ahmed et al., 2019b; Harwood et al., 2014; Holcomb & Stewart, 2020). For example, some of the most frequently used markers for the detection of dog feces include the BacCan, DogBact, and Dog3 markers based on host-specific bacteria, whereas markers based on canine mitochondrial DNA have also recently been used (Ahmed et al., 2019b; Harwood et al., 2014; Holcomb & Stewart, 2020; Monteiro et al., 2021). Similarly, many avian markers are currently used to investigate environmental waters for fecal pollution, including but not limited to the LeeSG, Gull4, and GFD markers (Ahmed et al., 2019b; Harwood et al., 2014; Holcomb & Stewart, 2020; Monteiro et al., 2021; Sinigalliano et al., 2013). Admittedly, variations of the HF183 marker can be considered to be informally adopted as the standard marker for the investigation of human sewage in environmental waters in comparison to markers previously used (such as HumM2, BacHum, BacH, HuBac – mostly based on human *Bacteroides* 16S rRNA with the exception of HumM2) due to how often it has been adopted (Ahmed et al., 2016, 2019b, c; Harwood et al., 2014). However, it is still notable that competing markers which are not based on human-derived *Bacteroides* spp., such as the *Lachnospiraceae*-based (see Feng et al., 2018) Lachno3 and virally-based markers such as those for crAssphage, human adenovirus (HAdV), pepper mild mottle virus (PMMoV), and human polyomavirus (HPyV) are all being increasingly used for MST purposes (Ahmed et al., 2018, 2019b; Harwood et al., 2014; Holcomb & Stewart, 2020). With such a large variety of markers for each MST source host and little consistency

between studies, interpretation of marker detection and concentrations is fraught with difficulty, particularly when making comparisons between studies to more fully understand overarching patterns of human and animal fecal pollution in environmental waters. Nevertheless, and in conclusion, the above study suggests that human sewage is a predominant source of fecal pollution in stormwater systems in Airdrie.

5. Presence of Enteric and Opportunistic Pathogens in Stormwater

5.1 Introduction

Enteric pathogens of concern in stormwater include viruses (Sidhu et al., 2013; Steele et al., 2018), protozoans (Cizek et al., 2008; de Man et al., 2014; Schreiber et al., 2019), as well as bacteria (Chong et al., 2013; de Man et al., 2014; Murphy et al., 2017; Sales-Ortells & Medema, 2015; Schreiber et al., 2019; Steele et al., 2018). For the purposes of this thesis, occurrence of the enteric pathogenic bacteria *Arcobacter* spp., *Campylobacter* spp., *Salmonella* spp., and STEC were assessed in stormwater. The latter three pathogens are often cited as the top three bacterial causes of zoonotic illness (EFSA & ECDC, 2021). Emerging enteric pathogens such as *Arcobacter* spp. (particularly *A. butzleri*) are also of growing concern (Collado & Figueras, 2011; Chieffi et al., 2020), with this genus emerging as one of the most frequent *Campylobacter*-like organisms isolated from patients presenting with gastrointestinal illness (Prouzet-Mauleon et al., 2006; Vandenberg et al., 2004). Opportunistic respiratory pathogens such as *L. pneumophila* also pose a major public health threat, causing one of the heaviest burdens of waterborne disease in the United States due to Legionairre's disease (Cassini et al., 2018).

Multiple studies have observed the presence of the above organisms in stormwater, including *Campylobacter* spp. (de Man et al., 2014; Murphy et al., 2017; Steele et al., 2018), *Salmonella* spp. (Chong et al., 2013; Schreiber et al., 2019; Johnson et al., 2003), STEC (McGinnis et al., 2018), *Arcobacter* spp. (Beaudry, 2019; Carney et al., 2020), and *L. pneumophila* (Sales-Ortells & Medema, 2015). Enteric pathogens pose risks to public health via the fecal-oral route when stormwater is used for certain non-potable uses such as irrigation of community gardens and recreation, while the respiratory pathogen *L. pneumophila* can pose a

risk through exposure to aerosols through uses such as recreational spray features, fountains, and irrigation (Ashbolt, 2015; Murphy et al., 2017; NASEM, 2019; Petterson et al., 2016; Sales-Ortells & Medema, 2015; Schoen et al., 2017). *Arcobacter* spp. occurrence is particularly underassesed in stormwater (Beaudry et al., 2019; Carney et al., 2020) especially when considering its abundance in human sewage (Cui et al., 2019; Fisher et al., 2014; Lu et al., 2015; Zhang et al., 2020), its associations with markers of human sewage (i.e. human-derived *Bacteroides* spp.) (Carney et al., 2020; Cui et al., 2019; Lee et al., 2012), and the levels of FIB (Collado et al., 2008; Webb et al., 2017) in sewage-polluted environmental waters. These pathogens are particularly concerning because many of them are well-adapted to surviving the stressful conditions (e.g., salinity, temperature, starvation) in environmental waters, using several mechanisms such as (but not limited to) viable but non-culturable (VBNC) forms, and biofilm formation as examples (Ashbolt, 2015; Bronowski et al., 2014, 2017; Chaisowwong et al., 2012; da Cruz Nizer et al., 2020; Ferreira et al., 2015; Lisle et al., 1998; Oliver et al., 2005; Pradhan & Negi, 2019; Van Driessche & Houf, 2008; Zhi et al., 2019). The most concerning evidence suggests the development of resistance to chemical and oxidative processes used in water treatment (da Cruz Nizer et al., 2020; Lisle et al., 1998; Oliver et al., 2005; Zhi et al., 2019).

Although enteric pathogens have been detected in stormwater and other environmental waters, little is currently understood regarding their concentrations, association with other parameters of water quality, or their spatiotemporal occurrence. Current evidence suggests a mixed relationship between enteric pathogens and FIB (Bradshaw et al., 2016; Collado et al., 2008; de Man et al., 2014; Fremaux et al., 2009; Jokinen et al., 2010; Lee et al., 2012; Savichtcheva et al., 2007; Schriewer et al., 2010; Van Dyke et al., 2010; Webb et al., 2017), as well as between enteric pathogens and markers of human sewage (such as HF183) (Bradshaw et

al., 2016; Carney et al., 2020; Cui et al., 2019; Fremaux et al., 2009; Lee et al., 2012; Schriewer et al., 2010; Sales-Ortells & Medema, 2015; Steele et al., 2018; Viau et al., 2011; Walters et al., 2007).

Both geospatial and temporal factors have been found to affect pathogen detection and density in stormwater, though these factors have not been intensively studied (Carney et al., 2020; de Man et al., 2014; Johnson et al., 2003; Jokinen et al., 2010; Sales-Ortells & Medema, 2015; Schreiber et al., 2019; Sidhu et al., 2012; Steele et al., 2018). Differences in upstream land use (Steele et al., 2018), proximity to infrastructural damage and sanitary sewage infiltration (de Man et al., 2014), and proximity to high stormwater flows (Sales-Ortells & Medema, 2015) can all be potential factors in explaining some of the geo-spatial differences in pathogen occurrence, though more work must be done to fully explain these effects. Temporal factors such as the effect of weather on pathogens in stormwater have also been studied, showing that pathogen concentrations are generally higher when higher precipitation and wet weather events are present, though these relationships can still be variable, and dependent on a multitude of other factors such as pathogen type and site-specific variables (Carney et al., 2020; Jokinen et al., 2010; Sales-Ortells & Medema, 2015). As with geo-spatial factors that can contribute to sporadic pathogen detection, work must be done to more fully understand this phenomenon as well.

Considering current gaps in knowledge on the spatiotemporal occurrence of enteric and opportunistic pathogens in stormwater, this thesis aimed to acquire baseline data in stormwater systems in Airdrie, Alberta. The goals of this study were to; 1) characterize the frequency of detection, concentration, and general distribution of enteric and opportunistic pathogens in Airdrie stormwater; 2) determine relationships between the occurrence and concentration of enteric pathogens in relation to FIB with special consideration for concentrations of FIB that

exceed chosen water quality standards; 3) identify potential host sources of fecal pollution contributing to the loading of enteric pathogens using microbial source tracking, and; 4) explore the potential for geo-spatial, temporal, and weather-dependent factors that may have an effect on pathogen prevalence and density.

5.2 Results

5.2.1 General Pathogen Occurrence in Stormwater

Generally, pathogen occurrence was infrequent in Airdrie stormwater in 2020 (Table 5-1) and in 2021 (Table 5-2). The exceptions were *Legionella* spp. (which was nearly universally found in both years), and *A. butzleri* in 2021 (found in 31% of routine samples in 2021). For all other enteric and opportunistic pathogens, detection was <10% of samples in both years (Tables 5-1, 5-2). It is important to note that *Salmonella* spp. were not detected in any of the samples tested in either 2020 or 2021, while STEC was only detected in a handful of samples in 2020, and *L. pneumophila* only detected by qPCR in 2021 (Tables 5-1, 5-2). Note, however, that *L. pneumophila* was detected by the Legiolert$^{(R)}$ defined substrate system in 2020 (this assay being utilized only in that year), being found in 7 of 84 (8.3%) samples.

Table 5-1. Occurrence (%) of enteric and opportunistic pathogens found in all routine samples collected from Airdrie stormwater ponds in 2020 based on qPCR, and inclusive of data from Windsong and East Lake stormwater ponds.

Sample Type	n	*A. butzleri*	*Campylobacter* spp.	STEC	*Salmonella* spp.	*Legionella* spp.	*L. pneumophila*
		*hsp*60	VD16S	*stx*1 & *stx*2	*invA*	Lu16s	*mip*
Total (Routine)	84	2/84 (2.4)	8/84 (9.5)	2/84 (2.4)	ND*	69/84 (82.1)	ND*

* this marker was not detected (ND) in any routine samples that year

Table 5-2. Occurrence (%) of enteric and opportunistic pathogens found in all routine and investigative upstream manhole samples collected from Airdrie stormwater ponds and creeks in 2021, based on qPCR and inclusive of samples from Nose Creek, Canals, Windsong, East Lake, Hillcrest, King's Heights North, and King's Heights South.†

Sample Type	n	*A. butzleri*	*Campylobacter* spp.	STEC	*Salmonella* spp.	*Legionella* spp.	*L. pneumophila*
		*hsp*60	VD16S	*stx*1 & *stx*2	*invA*	Lu16s	*mip*
Routine	126	39/126 (31.0)	6/126 (4.8)	ND	ND	112/126 (88.9)	4/112 (3.6)*
Investigative	55	16/55 (29.0)	ND	ND	ND	49/55 (89.0)	3/49 (6.1)*
Total	181	55/181 (30.4)	6/181 (3.3)	ND	ND	161/181 (89.0)	7/161(4.3)*

* Note that *mip* was only tested on samples positive for *Legionella* spp. marker (Lu16S)
† Note that only investigative samples were taken for East Lake in 2021, with other investigative sampling occurring upstream only of Nose Creek, Windsong, and Hillcrest sites.

In addition to testing for *L. pneumophila* by qPCR in both years of study, testing was also performed for culturable *L. pneumophila* in 2020, though only 8.3% (7 of 84) samples were positive, and generally at the relatively low concentration of 2.0 $\log_{10}$ MPN/100 mL. This data suggested that only a relatively small proportion of stormwater samples contained viable *L. pneumophila*, with this result potentially due to enhanced detection associated with selective growth enrichment used to culture the organism to a detectable concentration – a similar phenomenon reported for *Campylobacter* spp. by Beaudry et al. (2019).

5.2.2 Pathogen Occurrence by Stormwater Pond

5.2.2.1 Pathogen Occurrence by Stormwater Pond in 2020

During this year, *Campylobacter* spp. were the most commonly detected enteric pathogen, though this pathogen was only detected in 9.5% (8 of 84) of samples, with 7 positive samples from East Lake and only one sample taken from Windsong (Table 5-3). In contrast, *A.*

butzleri and STEC were rarely detected, with the latter only being found in one site in Windsong (WS#2) during two sampling dates. Separate from the enteric pathogens, *Legionella* spp. were detected almost universally in Airdrie stormwater in 2020, despite the lack of detection of *L. pneumophila* by qPCR specifically.

Most samples positive for pathogens via qPCR were detected at the DNQ level, suggesting low levels of pathogen concentrations in these samples. For example, all *Campylobacter* spp. detections were at DNQ levels in 2020, while only one of the two *A. butzleri* positive samples was quantifiable, at 3.9 $\log_{10}$ copies/100 mL. The glaring exception to this was for the *Legionella* genus-specific marker that was detected in the vast majority of samples in 2020, and which ranged from 3.6 $\log_{10}$ copies/100 mL to 7.0 $\log_{10}$ copies/100 mL, with every positive sample being quantifiable.

Table 5-3. Occurrence (%) of bacterial pathogen detection within stormwater ponds routinely sampled in 2020, as stratified by stormwater ponds sampled.

Stormwater Pond	n	*A. butzleri*	*Campylobacter* spp.	STEC	*Legionella* spp.	*L. pneumophila*
		hsp60	VD16S	stx1 & stx2	Lu16s	*mip*
East Lake	56	1/56 (1.8)	7/56 (12.5)	ND	50/56 (89.3)	ND
Windsong	28	1/28 (3.6)	1/28 (3.6)	2/28 (7.1)	19/28 (67.9)	ND
Total	84	2/84 (2.4)	8/84 (9.5)	2/84 (2.4)	69/84 (82.1)	ND

Culturable *L. pneumophila* was detected via Legiolert$^{(R)}$ in 4 of 28 (14.3%) samples from Windsong, and in 3 of 56 (5.4%) East Lake samples. With the exception of the sample collected

from EL#3 on Aug. 31st at 2.6 $\log_{10}$ MPN/100 mL, all other positive samples were found at a concentration of 2.0 $\log_{10}$ MPN/100 mL, suggesting relatively low levels in the water. The majority of *L. pneumophila* positive samples in Windsong (3 of 4, 75.0%) came from the WS#4 site, with the last sample being positive at WS#2. In comparison, *L. pneumophila* positive samples in East Lake were more evenly distributed between the EL#3, EL#5, and EL#8 sites.

Curiously, the genus-specific qPCR marker for *Legionella* spp. was detected in only 4 of 7 (57.1%) samples positive for viable *L. pneumophila* (Table 5-4). As mentioned previously, none of the samples positive for *L. pneumophila* through Legiolert(R) were positive via the *L. pneumophila* specific qPCR target (*mip*), suggesting different sensitivities in detection between these two methods for this organism. The Legiolert(R) assay uses 1 mL of water as the measurand. This is in contrast to molecular assays where an equivalent volume of only ~167 uL of water is tested (i.e., 20 mL of water is filtered, the DNA extracted to a volume of 600 uL and for which only 5 uL is used in the qPCR assay [equivalent to 1/120 of the original 20 mL sample volume that was filtered]), possibly explaining the decreased sensitivity of qPCR compared to culture-based methods in environmental samples.

Table 5-4. Two-by-two table of Legionella spp. detection (Lu16S) via qPCR versus viable *L. pneumophila* detection via Legiolert(R) in 2020 Airdrie stormwater (n=84).

	Lu16S Positive	Lu16S Negative
Legiolert(R) Positive	4	3*
Legiolert(R) Negative	65	12

* All Legiolert(R) positive but Lu16S negative samples were collected from WS#4

5.2.2.2 Pathogen Occurrence by Stormwater Pond in 2021

In 2021, the enteric pathogens *A. butzleri* and *Campylobacter* spp. were frequently detected in Airdrie stormwater alongside *Legionella* spp. and on occasion, the opportunistic pathogen *L. pneumophila* (Table 5-5). STEC was not detected the second year of sampling, and *Salmonella* was again not detected in any samples. The most prominently detected enteric pathogen was *A. butzleri*, which was detected in 31.0% (39 of 126) of routine stormwater samples from that year and was detected in every tested water body except the Hillcrest stormwater pond. The detection of this pathogen was found to be statistically significantly different ($p = 0.001$) between stormwater ponds and creeks by Fisher's exact test, with detection of *A. butzleri* found to be statistically significantly more frequently detected in Nose Creek than both Hillcrest ($p < 0.001$), and the Canals ($p<0.01$) under pairwise Fisher's exact tests with the application of the Bonferroni correction. In comparison, *Campylobacter* was detected in <5% (6 of 126) of routine samples in 2021, but was not detected at all in Hillcrest or Windsong stormwater ponds. Once again, the genus-specific marker for *Legionella* spp. was detected in the vast majority of routine samples (112 of 126, 88.9%), though a small minority of these positive samples were also positive for *L. pneumophila.* Notably, 3 of the 4 routine samples that were positive for *L. pneumophila* were found in Hillcrest sites specifically (Table 5-5).

When detected, most pathogens were at a DNQ concentration with the exception of *Legionella* spp. *A. butzleri* was quantifiable only in 16 of 39 (41.0%) positive routine samples, though the marker ranged from 3.7 $\log_{10}$ copies/100 mL to 6.2 $\log_{10}$ copies/100 mL in

quantifiable samples. Concentrations of *Campylobacter* spp. and *Legionella pneumophila*, however, were DNQ in all cases. Diverging from the above, *Legionella* spp. marker was quantifiable in all but 1 routine sample that tested positive, and ranged from 4.5 $\log_{10}$ copies/100 mL to 7.5 $\log_{10}$ copies/100 mL.

Table 5-5. Occurrence (%) of bacterial pathogen detection within stormwater-impacted creeks and ponds routinely sampled in 2021, as stratified by stormwater ponds sampled.

Stormwater Pond/Creek	n	*A. butzleri*	*Campylobacter* spp.	*Legionella* spp.	*L. pneumophila*
		*hsp*60	VD16S	Lu16s	*mip*
Nose Creek	38	21/38 (55.3)	3/38 (7.9)	33/38 (86.8)	1/33 (3.0)
Canals/Bayside Creek	40	8/40 (20.0)	1/40 (2.5)	39/40 (97.5)	ND
Hillcrest	12	ND	ND	11/12 (91.7)	3/12 (25.0)
King's Heights North	8	3/8 (37.5)	1/8 (12.5)	7/8 (87.5)	ND
King's Heights South	12	2/12 (16.7)	1/12 (8.3)	12/12 (100.0)	ND
Windsong (2021)	16	5/16 (31.3)	ND	10/16 (62.5)	ND
Total	126	39/126 (31.0)	6/126 (4.8)	112/126 (88.9)	4/112 (3.6)*

* Note that *mip* was only tested on samples positive for *Legionella* spp. marker (Lu16S)

Directly comparing the two years of Windsong samples, the largest difference between samples was the frequency of detection of *A. butzleri* (5 of 16 [31.3%] samples in 2021, and 1 of 28 [3.6%] samples in 2020), as well as STEC and *Campylobacter* spp. being detected only in 2020 (albeit in ≤2 samples each). In terms of STEC testing, methods were different in 2020 (culture followed by qPCR) compared to 2021 (qPCR only), which could possibly account for the difference in occurrence of this marker. In 2021, *A. butzleri* varied in concentration at Windsong sites from 3.7 $\log_{10}$ copies/100 mL to 6.2 $\log_{10}$ copies/100 mL in 5 of 16 (31.3%) positive samples, while there was only one sample in 2020 quantifiable for this pathogen, and at a concentration of 3.9 $\log_{10}$ copies/100 mL. Despite the lack of enteric pathogen diversity in the Windsong stormwater pond in 2021, the total number of samples positive for enteric pathogens was proportionally higher (5 of 16, 31.3%) in that year in comparison to in 2020 (4 of 28 samples, 14.3%). *L. pneumophila* was not detected via qPCR in the Windsong stormwater pond in either year of sampling, whereas *Legionella* spp. were detected at a similar frequency in both years (10 of 16 samples [62.5%] in 2021 vs. 19 of 28 samples [67.9%] in 2020). Concentrations of *Legionella* spp. also ranged widely in both years, from 4.9 $\log_{10}$ copies/100 mL to 7.0 $\log_{10}$ copies/100 mL in 2021, and 3.6 $\log_{10}$ copies/100 mL to 7.0 $\log_{10}$ copies/100 mL in 2020.

5.2.3 Temporal Patterns of Pathogen Occurrence

5.2.3.1 Temporal Patterns of Pathogen Occurrence in 2020

When 2020 stormwater samples were analyzed temporally, slight differences were observed in the occurrence of enteric pathogen bacteria (though they were not significant by Fisher's exact test), and *Legionella* spp. detection appeared to be relatively stable over time. The

sampling week of Sept. 8th was the only week where *A. butzleri*, *Campylobacter* spp., and STEC were all detected during the same week. It was also during this week where daily total antecedent precipitation was highest for the sampling season that year, at >5 mm the day before sampling. Unlike *Campylobacter* spp., which was detected relatively consistently nearly each week at a low frequency, STEC was only detected during the Aug. 17th and Sept. 8th sampling weeks.

5.2.3.2 Temporal Patterns of Pathogen Occurrence in 2021

Analyzed through a temporal lens, pathogen detection appeared to be consistent in frequency regardless of sampling period in 2021, with the possible exception of *Campylobacter* spp. (though enteric pathogens in general did not see significantly different detection across weeks as tested by Fisher's exact test). *A. butzleri* was detected frequently, with around 33% of samples positive in each week with the exceptions of the weeks of Aug. 30th/31st and Sept. 7th, where this pathogen was detected in 25.8% (8 of 31) of samples or not detected at all, respectively. The bulk of *Campylobacter* spp. detection, however, occurred during the weeks of Aug. 17th and Aug. 23rd, which was also the two weeks with the highest 24-hr total antecedent precipitation for the dates of sampling during the 2021 sampling season. In contrast, the *Legionella* genus marker was almost universally detected each sampling week (77.4-100% detection) in Airdrie stormwater samples.

5.2.4 Patterns of Co-detection and Association between Enteric Pathogens, MST Markers, and FIB

5.2.4.1 Patterns of Co-detection and Association between Enteric Pathogens, MST Markers, and FIB in 2020

In order to determine the potential fecal source contributing pathogens to stormwater, an assessment of the patterns of co-detection between pathogens and MST was carried out. However, both enteric pathogens and MST markers were infrequently detected in 2020, and co-detection between pathogens and MST markers as well as association between enteric pathogens and high FIB concentrations were therefore rarely observed. For example, detection of enteric pathogens was not found to be significantly different between different combinations of MST markers (human or animal) being detected in 2020. Co-detection between pathogens and MST markers was found in only three samples that year, two of which being between *Campylobacter* spp. (VD16S) and the human sewage marker (HF183) at WS#3 and EL#3. In contrast, out of STEC (2 of 84 samples) and *A. butzleri* positive samples from that year (2 of 84), only 1 of 2 STEC positive samples were co-detected with another MST marker, this being the gull marker (LeeSG). In all cases, pathogens and the corresponding MST markers were detected at a DNQ concentration.

Detection of enteric pathogens was also not found to be significantly more likely to occur in 2020 when FIB STV criteria were exceeded by Fisher's exact test, suggesting that FIB STV criteria exceedance was not predictive of pathogen detection on an individual sample basis. FIB concentrations were rarely high when enteric pathogens were detected in 2020, except at the WS#2 site on the Aug.17th and Sept. 8th sampling dates. On both dates, both *Enterococcus* and *E. coli* STVs were exceeded, with STEC being the lone enteric pathogen detected on the former date, and both STEC and *A. butzleri* being detected on the latter date. When FIB GMs were considered, it was found that the only sites with GM exceedance (*Enterococcus* only) and pathogen detection were WS#2 and WS#3. Only one sample was found in 2020, where the same

sample exceeded FIB, had observable MST marker detection (LeeSG in this case), and had observable enteric pathogen detection (STEC).

5.2.4.2 Patterns of Co-detection and Association between Enteric Pathogens, MST Markers, and FIB in 2021

While the only enteric pathogens detected in Airdrie stormwater in 2021 were *A. butzleri* and *Campylobacter* spp., these pathogens were frequently co-detected with MST markers, as well as with high concentrations of FIB. This was the case in the majority of samples positive for either of these pathogens, with the most frequent MST markers co-detected with these pathogens in 2021 being human (HF183) and dog (Dog3) markers, while many samples positive for *A. butzleri* and *Campylobacter* spp. also exceeded FIB STVs.

Based on MST, there appeared to be multiple contributing host fecal sources associated with *Campylobacter* loading. Despite *Campylobacter* spp. being detected in only 6 of 126 (4.8%) routine samples, in 5 of 6 (83.3%) of these samples, one ore more fecal MST markers were observed, and included markers from gulls, dogs, and humans. The dog marker (Dog3) was most frequently co-detected with *Campylobacter* spp., (i.e., in half of *Campylobacter* spp. positive samples), while the human marker (HF183) was found in 33% of these samples. A single sample each were also positive for the gull and goose marker. Only one of the samples positive for *Campylobacter* spp. did not have discernable MST marker detection. One *Campylobacter* spp. positive sample (found in Nose Creek) was positive for both the gull and human marker.

A. butzleri was detected in far more samples than *Campylobacter* spp. (39 of 126 [31.0%]), and MST markers were detected in 23 of these 39 samples (59.0%) from 2021 (Table 5-6). There was often a large variety of MST markers detected alongside *A. butzleri*. The most

frequently detected fecal marker associated with *A. butzleri* was from humans followed by dogs, ruminants, gulls, and then Canada geese. The vast majority of samples where co-detection between *A. butzleri* and an MST marker occurred was with the human sewage marker HF183, found in 17 of 39 *A. butzleri* positive samples (43.6%). The largest variety and frequency of MST markers co-detected alongside *A. butzleri* was found in Nose Creek samples (Table 5-6). Windsong appeared to be the stormwater pond with the second-highest variety of MST marker co-detected with *A. butzleri* (Table 5-6).

Table 5-6. Occurrence (%) of MST markers detected within routine stormwater samples positive for *A. butzleri* in 2021 Airdrie stormwater.

Stormwater Pond/Creek	n	Human		Gull	Canada Goose	Dog	Ruminant
		HF183	HumM2	LeeSG	CGO1	Dog3	Rum2Bac
Nose Creek	21	16/21 (76.2)	4/21 (19.0)	3/21 (14.3)	ND	6/21 (28.6)	5/21 (23.8)
Canals/Bayside Creek	8	0	ND	ND	1/8 (12.5)	0	ND
King's Heights North	3	0	ND	ND	ND	0	1/3 (33.3)
King's Heights South	2	ND	ND	ND	ND	1/2 (50.0)	0
Windsong (2021)	5	1/5 (20.0)	ND	2/5 (40.0)	ND	1/5 (20.0)	0
Total	39	17/39 (43.6)	4/39 (10.3)	5/39 (12.8)	1/39 (2.6)	9/39 (23.1)	6/39 (15.4)

* Also included are *A. butzleri* positive samples where MST markers were not detected, or where multiple MST markers were simultaneously detected

Although *A. butzleri* was not statistically significantly more likely to be detected under McNemar's test when human sewage marker HF183 was detected versus any of the animal

markers combined (see Table 5-7), *A. butzleri* was observed to be statistically significantly more likely to be detected by human sewage than any individual animal marker, including gulls ($p < 0.0001$; Table 5-8), dogs ($p = 0.0023$; Table 5-9), ruminants ($p = 0.0023$; Table 5-10), and geese ($p < 0.0001$; Table 5-11).

Table 5-7. Two-by-Two table of *A. butzleri* positive Airdrie stormwater samples collected in 2021, as stratified by HF183 versus other animal MST marker detection (n=39).

	HF183 Detected	HF183 Not Detected
Animal MST Marker Detected	7	5
Animal MST Marker Not Detected	10	17

Table 5-8. Two-by-Two table of *A. butzleri* positive Airdrie stormwater samples collected in 2021, as stratified by HF183 versus gull MST marker detection (n=39).

	HF183 Detected	HF183 Not Detected
Gull (LeeSG) Marker Detected	4	1
Gull (LeeSG) Marker Not Detected	13	21

Table 5-9. Two-by-Two table of *A. butzleri* positive Airdrie stormwater samples collected in 2021, as stratified by HF183 versus dog marker detection (n=39).

	HF183 Detected	HF183 Not Detected
Dog (Dog3) Marker Detected	6	2
Dog (Dog3) Marker Not Detected	11	20

Table 5-10. Two-by-Two table of *A. butzleri* positive Airdrie stormwater samples collected in 2021, as stratified by HF183 versus ruminant MST marker detection (n=39).

	HF183 Detected	HF183 Not Detected
Ruminant (Dog3) Marker Detected	6	2
Dog (Dog3) Marker Not Detected	11	20

Table 5-11. Two-by-Two table of *A. butzleri* positive Airdrie stormwater samples collected in 2021, as stratified by HF183 versus goose MST marker detection (n=39).

	HF183 Detected	HF183 Not Detected
Goose (CGO1) Marker Detected	0	1
Goose (CGO1) Marker Not Detected	17	21

Despite nearly half of all samples from Nose Creek (16 of 38, 42.1%) being positive for both *A. butzleri* and HF183, only one single sample was positive for both of these markers from all remaining outlets and inlets studied in 2021, including sites from Windsong, the Canals & Bayside Creek, Hillcrest, and both King's Heights stormwater ponds (Tables 5-12, 5-13). It was also found by Fisher's exact test that *A. butzleri* was significantly more likely to be detected when HF183 was detected and vice versa (e.g., both simultaneously not detected) in Nose Creek samples than for one of these to be detected alone (see Table 5-12, $p = 0.013$), though this was not the case (by the same test) for samples taken from the other stormwater ponds and creeks in Airdie, where there was no significantly higher likelihood of these parameters being dependent on one another (Table 5-13).

Table 5-12. Two-by-two table of Nose Creek 2021 routine Airdrie stormwater samples positive for *A. butzleri* (*hsp60*), human sewage marker (HF183), both, or neither (n=38).

	HF183 Detected	HF183 Not Detected
A. butzleri Detected	16	5
A. butzleri Not Detected	6	11

Table 5-13. Two-by-two table of 2021 routine Airdrie stormwater samples (minus Nose Creek samples) positive for *A. butzleri* (*hsp60*), human sewage marker (HF183), both, or neither (n=88).

	HF183 Detected	HF183 Not Detected
A. butzleri Detected	1	17
A. butzleri Not Detected	10	60

Enteric pathogen detection in 2021 could be found with high concentrations of FIB as well, with non-concordance being statistically insignificant by McNemar's test (Table 5-14). This association between high FIB concentrations and enteric pathogen detection could vary both between the individual pathogens detected, as well as between stormwater-impacted ponds and creeks. For example, *Campylobacter* was only ever found in samples exceeding the STV of *Enterococcus* and *E. coli*. In contrast, 53.8% (21 of 39) of the samples positive for *A. butzleri* were co-associated with concentrations of *Enterococcus*, *E. coli*, or both that exceeded their respective STVs. On the pond and creek level, the majority of samples from Nose Creek (14 of 21, 66.7%) and Windsong (4 of 5, 80.0%) positive for *A. butzleri* exceeded STV criteria for either *Enterococcus*, *E. coli*, or both. In contrast, only a single sample positive for *A. butzleri* from each of King' Heights North, King's Heights South, and Canals & Bayside Creek sites also had concentrations of FIB exceeding STVs of either FIB.

Table 5-14. Two-by-two table of 2021 routine Airdrie stormwater samples of enteric pathogen detection status versus FIB STV criteria exceedance (either *Enterococcus*, *E. coli*, or both) (n=126).

	FIB STV Criteria Exceeded	FIB STV Criteria Not Exceeded
Enteric Pathogen(s) Detected	24	18
Enteric Pathogen(s) Not Detected	31	53

Curiously, *Enterococcus* concentrations exceeding STV concentrations were more frequently observed alongside *A. butzleri* than *E. coli* STV exceedances. In this case, *Enterococcus* STV criteria were exceeded in 20 of 39 [51.3%] total *A. butzleri*-positive samples vs. 13 of 39 [33.3%] total *A. butzleri*-positive samples that exceeded *E. coli* STV criteria. This

suggested the potential for *Enterococcus* as a possibly more sensitive indicator of this pathogen than *E. coli*. Additionally, 12 of 39 (30.8%) of *A. butzleri* positive samples exceeded only the *Enterococcus* STV (and not the *E. coli* STV), whereas only one sample exceeded the *E. coli* STV without exceeding the *Enterococcus* STV.

In conclusion, a potential interplay between enteric pathogens, FIB concentrations, and HF183 co-detection was observed in Airdrie stormwater in 2021, though this appeared mostly in Nose Creek samples, and mostly for *A. butzleri*. This enteric pathogen in particular was found to be significantly associated with HF183 detection in Nose Creek in comparison to other ponds and creeks, and was also significantly more likely to be detected alongside HF183 than any other MST marker utilized in this study.

5.3 Discussion

In summary, while enteric bacterial pathogens were detected in both years of study (2020/2021), these pathogens were generally found infrequently and often at low concentrations, with the sole exception of *A. butzleri* in 2021 (which was itself found in ~33% of routine samples in 2021). Nevertheless, an important finding from the current study related to the pattern of detection between the human sewage marker HF183 and enteric pathogens in stormwater. For example, the human sewage marker HF183 was most often associated with *A. butzleri* occurrence when compared to all other individual animal MST markers, suggesting that human sewage appears to be a predominant source of this pathogen in stormwater. Indeed, several studies have demonstrated that the genus *Arcobacter* is a dominant microbial member of municipal sewage (Cui et al., 2019; Fisher et al., 2014; Lu et al., 2015; Zhang et al., 2020), and for which *A. butzleri* is a common species (González et al., 2007; Webb et al., 2017). In some cases this pathogen was

detected in the absence of the HF183 marker, and for which other animal markers of fecal contamination were observed, suggesting that animal fecal sources may also contribute *A. butzleri* to stormwater ponds/effluents. Known animal host reservoirs for *A. butzleri* include chickens (González et al., 2007), geese (Atabay et al., 2008), and dogs (Houf et al., 2008).

In comparison to the present study, other studies have also demonstrated co-detection of the human sewage marker HF183 with enteric bacterial pathogens at a high frequency and similar to what was observed in Nose Creek samples in the present study (Beaudry, 2019; Sales-Ortells & Medema, 2015; Sidhu et al., 2012; Steele et al., 2018). For example, several studies found between 54.5% and 93.8% of *Campylobacter* spp. positive samples were also positive for HF183 (Sales-Ortells & Medema, 2015; Sidhu et al., 2012; Steele et al., 2018). While being detected much less frequently in general, *Salmonella* spp. has been cited to co-occur with HF183 in up to 100% of samples positive for this pathogen (Sidhu et al., 2012; Steele et al., 2018). Curiously, overall percentage of co-detection of HF183 in *A. butzleri* positive samples found in Airdrie was very similar between stormwater studied in Calgary by Beaudry (2019), with each study both finding co-detection of ~43%. Overall, while frequency of co-occurrence was often relatively high between human sewage and enteric pathogens, it is important to re-emphasize that a high amount of variability could be noted, and that the detection of one did not necessarily indicate the detection of the other. Nevertheless, with the exception of the Sales-Ortells and Medema study (2015) noted above, human feces appeared to be the dominant source of pollution in the above cases, and collectively the data supports the idea that this is a major source of enteric pathogens in stormwater in general.

Animal markers (including those for geese, gulls, dogs, and ruminants) were each also individually co-detected rarely with *A. butzleri* positive samples (2.6% to 23.1% of samples,

depending on the animal target), and were also rarely found in *Campylobacter* spp. positive samples in 2021 (though it should be noted that <5% of total routine samples were positive for *Campylobacter* spp. to begin with). In contrast, Steele et al. (2018) and Sales-Ortells & Medema (2015) both found that the vast majority of *Campylobacter* positive samples (75.8% - 100%) were also positive for either dog or bird markers. Steele et al. (2018) also found similar results for *Salmonella* positive samples (88.9% positive for bird marker), though the dog marker was found in just less than half of *Salmonella* positive samples. Differing from both these studies and the present study, Beaudry (2019) found that individual animal markers (including for gulls, dogs, geese, and ruminants) were co-detected in ≤10% of *A. butzleri* positive samples.

Correlational analyses performed in various other studies on environmental waters are consistent with the above frequencies of co-detection, with generally mixed correlation found between markers of human *Bacteroides* (such as HF183) and enteric bacterial pathogens (Bradshaw et al., 2016; Carney et al., 2020; Cui et al., 2019; Fremaux et al., 2009; Lee et al., 2020; McGinnis et al., 2018; Sales-Ortells & Medema, 2015; Savichtcheva et al., 2007; Schriewer et al., 2010; Viau et al., 2011; Walters et al., 2007). *Arcobacter* spp. specifically appear to have strong correlation with human *Bacteroides* spp., particularly when environmental waters are known to be heavily impacted by human sewage (Carney et al., 2020; Cui et al., 2019; Lee et al., 2020). In contrast, *Campylobacter* spp. has been observed to be correlated to human *Bacteroides* spp. in some studies (see Sales-Ortells & Medema, 2015; Viau et al., 2011; Walters et al., 2007), while a weak or distinct lack of correlation has been observed in others (Cui et al., 2019; Fremaux et al., 2009; Schriewer et al., 2010; Steele et al., 2018). STEC and *Salmonella* spp., however, have generally been found to lack significant correlation with the presence of

HF183 (Bradshaw et al., 2016; Cui et al., 2019; Fremaux et al., 2009; Savichtcheva et al., 2007; Schriewer et al., 2010; Steele et al., 2018; Viau et al., 2011).

Congruent with the current literature, enteric (and opportunistic) bacterial pathogens are generally only sporadically detected in stormwater and other environmental waters, and often at concentrations around assay detection limits (Beaudry, 2019; Chong *et al.*, 2013; De Man *et al.*, 2014; Johnson *et al.*, 2003; McGinnis *et al.*, 2018; Murphy *et al.*, 2017; Sales-Ortells and Medema, 2015; Schreiber *et al.*, 2019; Sidhu *et al.*, 2012; Steele *et al.*, 2018). *Salmonella* spp. and STEC were not detected in Airdrie stormwater in 2021 (though there were two STEC positive samples found in 2020), and these organisms in particular have been scarcely detected in other studies (Beaudry, 2019; McGinnis et al., 2018). For example, occurrence for the latter organism has been reported to be between 8-14% in stormwater (see Beaudry, 2019; McGinnis et al., 2018), while other studies report a lack of detection (Johnson et al., 2003), though *Salmonella* spp. has been found in between 1-56% of samples in several studies (Beaudry, 2019; Johnson et al., 2003; McGinnis et al., 2018; Schreiber et al., 2019; Sidhu et al., 2012; Steele et al., 2018). In contrast to *Salmonella* spp. and STEC, *Campylobacter* spp. are quite frequently found in the vast majority (52.3-100%) of stormwater samples (de Man et al., 2014; Murphy et al., 2017; Sales-Ortells & Medema, 2015; Schreiber et al., 2019; Sidhu et al., 2012; Steele et al., 2018). Notably, concentrations of this organism (when it is enumerated at all) are very often found between 1-2 $\log_{10}$ copies/100 mL when detected, suggesting a ubiquitous presence but a relatively low density (de Man et al., 2014; Murphy et al., 2017; Sales-Ortells & Medema, 2015; Steele et al., 2018).

Arcobacter spp. (including *A. butzleri*) (see Beaudry, 2019; Carney et al., 2020) as well as the opportunistic pathogen *L. pneumophila* (Sales-Ortells & Medema, 2015) have rarely been assessed in stormwater, leaving less capability for comparisons to be made. Sales-Ortells &

Medema (2015) found *L. pneumophila* more frequently than in our study (25% versus <5% of samples), though concentrations were generally less than or equal to 2 $\log_{10}$ copies/100 mL. With that said, *Legionella* spp. were nearly universally detected in Alberta stormwater (as opposed to the rarely detected species *L. pneumophila*). It is important to note that although *L. pneumophila* dominates the clinical case loads associated with legionellosis, many *Legionella* species are known to cause disease in humans, including (but not limited to) *L. longbeachae*, *L. bozemanii*, *L. feeleii*, *L. dumoffii, L. wadsworthii, L. waltersii, L. micdadei, L. rubrilucens and L. anisa,* albeit these species are typically regarded as less pathogenic than *L. pneumophila* (Chambers et al., 2021; Cunha et al., 2016). At the same time, many other *Legionella* species are non-pathogenic, and consequently, further work is needed to verify whether the widespread presence of *Legionella* spp. seen in stormwater (and often at high concentrations based on qPCR [i.e., 10^7 copies of 16S gene/100 mL of water]), poses a risk to human health (i.e., due to aerosolization from aesthetic water fountains, recreational spray features, etc.). In terms of *A. butzleri*, Beaudry (2019) found this pathogen in ~25% of routine samples (compared to ~33% of samples in 2021 in the current study). High FIB concentrations themselves did not appear to be observed any more frequently when *A. butzleri* was detected (with the exception of *Enterococcus* at Nose Creek) than the converse, suggesting potentially poor predictability of this pathogen by FIB enumeration. This is similar to a study by Carney et al., (2020) which demonstrated a dominance of *Arcobacter* spp. in stormwater under wet weather conditions, and the presence of which did not correlate with FIB such as enterococci. In contrast, other studies found *Arcobacter* spp. correlated relatively well to FIB in environmental waters heavily impacted by wastewater (see Collado et al., 2008; Lee et al., 2012; Webb et al., 2017),

Geo-spatial patterns of enteric pathogen density (*A. butzleri* in particular) were observed in the current study, and this was consistent with other studies (albeit with other studies mostly focusing on *Campylobacter* spp., *Salmonella* spp., and STEC) (Beaudry, 2019; de Man et al., 2014; Sales-Ortells & Medema, 2015; Steele et al., 2018). For example, Beaudry (2019) observed that *A. butzleri* detection could be found in nearly half of samples at some sites, but as low as 10% at other sites draining into the same stormwater pond. This pattern appeared most dramatic for *Salmonella* spp. detection, with a few studies showing sites with ≤25% detection of this pathogen that could be adjacent to sites with >80% detection despite both sites draining similar areas (Johnson et al., 2003; Schreiber et al., 2019). These differences in geo-spatial patterns can be explained by differing site infrastructure (i.e., sampling of stormwater from combined sewer versus separate stormwater sewer sites – de Man et al., 2014), differing upstream land use, and the plethora of human and animal host sources contributing feces to a stormwater system (Sales-Ortells & Medema, 2015; Steele et al., 2018).

Although differences in pathogen density based on temporal factors (such as those potentially caused by weather differences) were not observed in the current study, these differences were observed elsewhere (Carney et al., 2020; de Man et al., 2014; McGinnis et al., 2018; Sales-Ortells & Medema, 2015; Sidhu et al., 2012; Steele et al., 2018). Results from correlational analysis in environmental surface waters between precipitation and enteric pathogens have found generally positive relationships between the two, suggesting that higher precipitation overall leads to higher densities of enteric pathogens in agricultural storm runoff, for example (see Jokinen et al., 2010; Wilkes et al., 2009, 2011). A number of factors may be explanatory to the lack of temporal-dependent differences seen in Airdrie stormwater pathogen densities, with the most obvious being the lack of detection of most enteric pathogens tested for,

with the exception of *A. butzleri*. Airdrie weather was also reported to be relatively dry during the periods of sampling for both years of the current study (2020 and 2021), with total daily antecedent precipitation rarely greater than 5 mm, potentially explaining in part the consistent results seen in *A. butzleri* concentrations and prevalence over time. Additionally, it is possible that consistent leakage of human sewage facilitated the occurrence of this pathogen in stormwater, or that *A. butzleri* were able to survive in this aquatic environment for longer periods of time (Chieffi et al., 2020; Fera et al., 2008; Ferreira et al., 2013, 2015).

Overall, exceedances of FIB criteria did not correlate well with occurrence of enteric pathogens in stormwater – a finding that fits within the overall picture of what other studies have demonstrated. Poor to strong correlations between bacterial enteric pathogens and FIB in environmental waters have been found (Bradshaw et al., 2016; de Man et al., 2014; Fremaux et al., 2009; Jokinen et al., 2010; Savichtcheva et al., 2007; Schriewer et al., 2010; Van Dyke et al., 2010), and this uncertainty in association is likely driven by the complexity and dynamics of pathogen occurrence, such as sources of fecal pollution impacting the environment, varying levels of FIB populations in human and animal hosts (Ahmed et al., 2019a; Ervin et al., 2013), and the heterogenous mobilization of these sources into stormwater. Importantly, the results of this study (alongside previous evidence) suggest that the use of FIB enumeration alone overall may not accurately predict the presence of enteric pathogens in stormwater when fecal pollution sources are not well understood or characterized.

One important factor that may contribute to some of the results observed in the current study, particularly when considering the lack of detection of pathogens such as *Campylobacter* spp. and *Salmonella* spp. in comparison to other studies, would be limitations in sensitivity. For example, the majority of studies of *Campylobacter* suggest this pathogen was found at between

1 – 2 $\log_{10}$ copies/100 mL or MPN/100 mL (see Murphy et al., 2017; Sales-Ortells & Medema, 2015; Steele et al., 2018), suggesting that concentrations may have been too low to be detectable in the current study. It is important to note, however, that qPCR estimates of bacteria have been observed to include nonviable DNA and to often overestimate culture estimates (though this is most well studied in FIB) as consistently high as 1 $\log_{10}$ (see Noble et al., 2010; Raith et al., 2014). In general, sensitivity can potentially be lower as well in molecular-based techniques when considering the lack of an enrichment step found in culture-based methods (Beaudry, 2019; Girones et al., 2010). Beaudry (2019) observed that as many as 75% of stormwater samples were contaminated with *A. butzleri* based on culture compared to only 25% by molecular methods, the difference of which could be attributed to the very low numbers found in the water and the ability of culture-based methods to amplify these low levels of bacteria to detectable levels. Additionally, many qPCR targets are multi-copy genes such as the *16s rRNA* gene targeted in the VD16S assay for *Campylobacter* spp. used in the current study (see Van Dyke et al., 2010), and therefore more sensitive than single-copy genes for detection for other pathogens (e.g., *invA* gene of *Salmonella* – see Daum et al., 2002).

In conclusion, enteric pathogens were detected sporadically in Airdrie stormwater, suggesting the potential for public health risks if this water is utilized without care or caution. Collectively, the general lack of correlation with FIB exceedances, coupled with the observation that human sewage often impacts stormwater (and is a dominant source of pathogen loading), supports the QMRA-based approach proposed by Alberta Health to manage stormwater use based on meeting $\log_{10}$ reduction targets against pathogens.

6. Characterization of Pathogen Carriage and Fecal Indicator Bacteria in Avian (*Larus* spp. and *Branta canadensis*) Sources of Stormwater Fecal Pollution

6.1 Introduction

While human fecal pollution is often the primary concern for environmental waters (such as stormwater) from a public health perspective, the level of risk presented by animal sources of fecal pollution (such as from waterfowl) is currently poorly understood (Brown et al., 2017a; Schoen & Ashbolt, 2010; Soller et al., 2010). Admittedly, avian sources of pollution are less risky than human sources in general (Brown et al., 2017a; Schoen & Ashbolt, 2010; Soller et al., 2010; Smith et al., 2020), though this does not mean that the risk posed by this source is zero. This is particularly the case when noting evidence of disease from enteric bacteria with links to avian feces (Cody et al., 2015; Gruszynski et al., 2014). MST data presented in Chapter 4 of this thesis (particularly for 2020) as well as data from other studies done in our laboratory (Beaudry, 2019) have suggested that aquatic birds are often the 2nd or 3rd most common fecal source of microbial pollution in Albertan stormwater, and as such, this chapter examines the prevalence and occurrence of enteric bacterial pathogens in these urban bird populations. Of particular concern is the carriage of *Campylobacter* spp. and *Salmonella* spp. in gulls (*Larus* spp.) and Canada geese (*Branta canadensis*) (Antilles et al., 2021; Broman et al., 2002; Keller & Shriver, 2014; Kinzelman et al., 2008; Lévesque et al., 2000, Lu et al., 2011a; Rutledge et al., 2013; Van Dyke et al., 2010; Feare et al., 1999; Gorham & Lee, 2016; Jokinen et al., 2011; Migura-Garcia et al., 2017; Moré et al., 2017; Palmgren et al., 2006; Quessy & Messier, 1992; Russo et al., 2021; Vogt et al., 2018).

These pathogens are not ubiquitously found in all gull species (*Larus* spp.), and there is therefore a wide variability in both the prevalence and concentrations of these pathogens shed by infected in gulls. Trends of high variability in prevalence of these two pathogens are also seen in Canada geese (See Rutledge et al., 2013; Van Dyke et al., 2010; Feare et al., 1999; Jokinen et al., 2011; Vogt et al., 2018), though data on concentrations of these pathogens is often lacking (Gorham & Lee, 2016). Variability in pathogen prevalence and concentrations can depend on many factors such as bird colony location, specimen age, sampling season, and species (in the case of gulls) (Feare et al., 1999; Keller & Shriver, 2014; Kinzelman et al., 2008; Lévesque et al., 2000; Migura-Garcia et al., 2017; Moré et al., 2017; Palmgren et al., 2006; Quessy & Messier, 1992; Russo et al., 2021; Rutledge et al., 2013; Vogt et al., 2018).

Complicating matters, current evidence suggests that not all *Campylobacter* or *Salmonella* serotypes found in birds are pathogenic to humans, and host-specificity can be restricted to bird hosts exclusively depending on the specific serovars in question (Dearlove et al., 2016; Fu et al., 2022; Griekspoor et al., 2013; Sheppard et al., 2011). In spite of the above, it should also be noted that generalist strains of these pathogens have also been found in gull feces, including but not limited to serovars associated with clinical infection (Broman et al., 2002; Palmgren et al., 2006).

Another problem faced in the risk interpretation of gull and Canada goose fecal contamination into waters such as stormwater is the fact that these birds frequently carry high concentrations of FIB (such as *E. coli* and *Enterococcus*) in their feces (Ahmed et al., 2019a; Ervin et al., 2013; Fogarty et al., 2003; Jokinen et al., 2010; Layton et al., 2009, 2010; Lu et al., 2011a; Middleton & Ambrose, 2005). Prevalence of these organisms in gulls and Canada geese appear to vary, but are generally found in the vast majority of birds sampled (Fogarty et al., 2003;

Lu et al., 2011a; Middleton & Ambrose, 2005; Vogt et al., 2018). Given that current recreational water quality guidelines (see Government of Alberta, 2018; US EPA, 2012a) primarily focus on enumerating FIB that are non-specific to a particular a host, this may make it difficult to differentiate the fraction of fecal contamination and subsequent pathogen contributions that are either human or bird derived in these waters. Consequently, this can lead to challenges to QMRA-based risk interpretation considering the higher risk provided by human sewage contamination in comparison to avian (Brown et al., 2017a; Schoen & Ashbolt, 2010; Soller et al., 2010).

To help overcome these challenges, MST markers have been developed to quantify gull (Green et al., 2012; Lee et al., 2013; Lu et al., 2008; Ryu et al., 2012) as well as Canada goose (Fremaux et al., 2010) fecal contributions into the environment. Through the use of MST markers, evidence of avian fecal pollution in stormwater has been especially strong for waterfowl, and several studies have demonstrated that gull spp. (Converse et al., 2012; Kinzelman & McLellan, 2008; Lee et al., 2013; Lu et al., 2011b; Staley et al., 2018; Steele et al., 2018) and Canada geese (Fremaux et al., 2010; Gorham & Lee, 2016) frequently pollute this alternative source water.

Understanding the relationship between pathogen occurrence, FIB and MST markers is important for understanding water quality and risks to public health. For example, Alberta has recently adopted new recreational water quality standards encompassing both *Enterococcus* qPCR and MST into their risk analysis, and is exploring the application of these guidelines to alternative water quality standards. In a three-year study on recreational water quality in Alberta (i.e., lakes, rivers, etc), MST data demonstrated that gulls and geese were the most dominant source of fecal pollution in natural recreational water venues across Alberta (Table 6-1 and Figure 6-1). Notably, human fecal pollution was largely absent at these sites (Fig 6-1). These findings

challenge the derivation and universal application of current recreational water quality standards developed by the US EPA (i.e., the NEEAR studies), largely due to the fact that US EPA study sites were chosen based on known human point sources of fecal pollution impacting water quality at these sites (wastewater, combined sewer outfalls, etc.) (US EPA 2012a; Wade et al., 2003, 2006, 2010). The Alberta data challenges the universality of simply applying the US EPA recreational water quality criteria to recreational water in Alberta, and by association to stormwater and its potential use (e.g., irrigation). As a consequence, Alberta adopted the *Enterococcus* standard of 1280 CCE/100 mL only at sites where MST data suggests that human (or cattle) sources of pollution are present (Alberta Health, 2021). Interestingly, in almost all recreational sites violating the 1280 CCE/10 mL standard for *Enterococcus* in Alberta, gulls and geese were found to be the dominant contributors of fecal pollution, but notably, neither *Campylobacter* or *Salmonella* could be detected in these samples (Fig 6-1). These findings led the province to adopt the recommendation of using 6400 CCE/100 mL as the *Enterococcus* standard for beaches devoid of human (and cattle) sources of pollution (Alberta Health, 2021). These same criteria could be considered for alternative water supplies, such as stormwater, as they encompass multiple sources of pollution into the risk assessment.

Table 6-1. Microbial source tracking marker detections in 20 mL beach samples from 2016 – 2017 (n = 804) that exceeded two *Enterococcus* CCE thresholds (>1280 CCE/100ml or >6400 CCE/100ml) [re-printed from Alberta Health report].

		Number of detections						
		Human		**Cattle**		**Bird**		
***Enterococcus* threshold – CCE/100 mL**	**# of exceedances**	**HF183**	**HumM2**	**CowM3**	**Rum2Bac**	**LeeSG**	**CGO**	**LeeSG and/or CGO1**
>1280	166	3 (1.8%)	1 (0.6%)	0 (0%)	1 (0.6%)	109 (66%)	32 (19%)	120 (72%)
>6400	53	1 (1.9%)	0 (0%)	0 (0%)	1 (1.9%)	43 (81%)	16 (30%)	44 (83%)

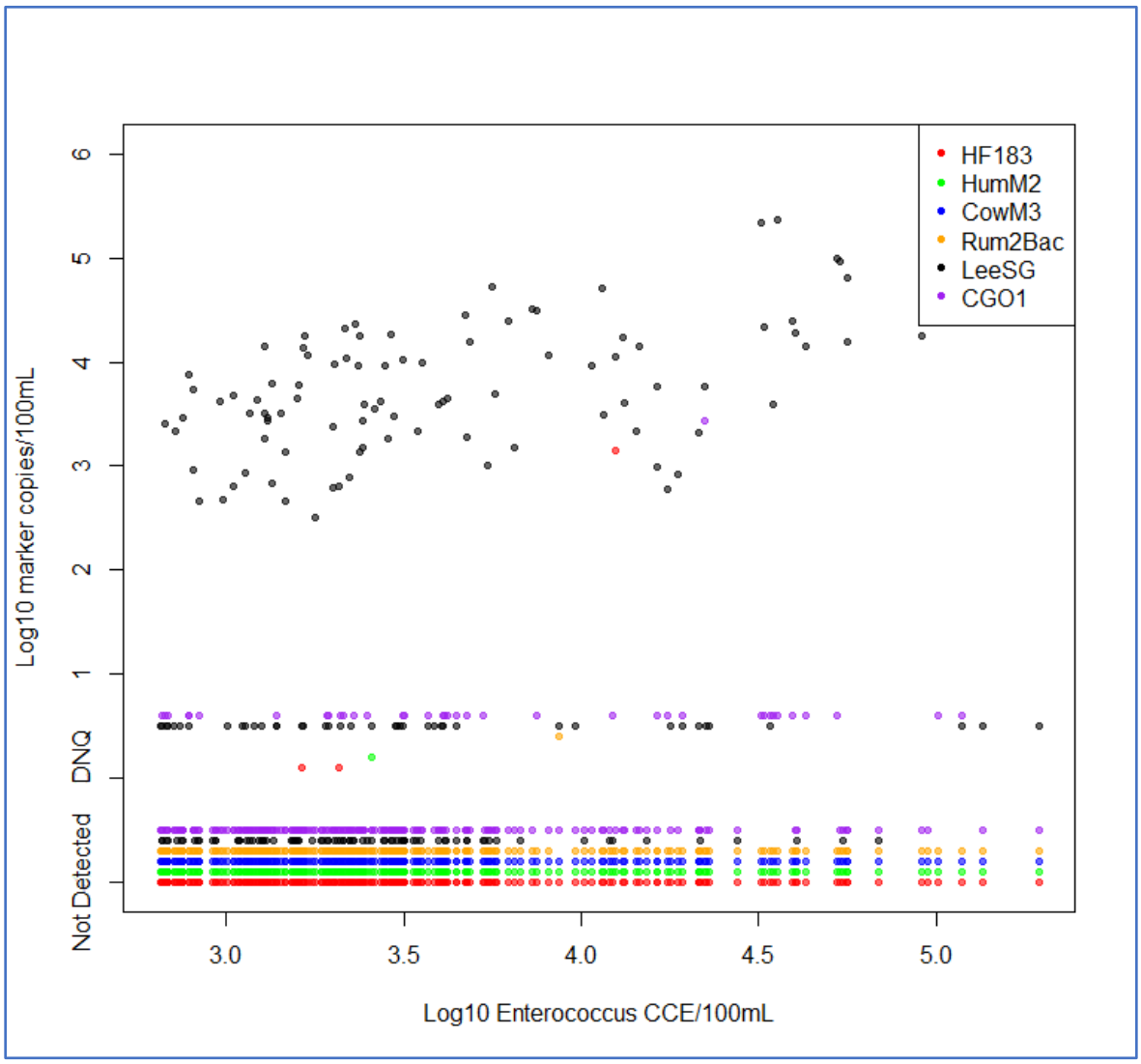

Figure 6-1. Relationship between the concentrations of 6 MST markers and Enterococcus in beach water samples in which the Enterococcus values were greater than the beach action values [BAV] (640 CCE; 2016 year) or the STV (1280 CCE; 2017 year). Note the dominance of seagull (LeeSG) related fecal pollution in these samples. In all beach samples where *Enterococcus* values exceeded 640 CCE/100 mL (n=145), and for which the vast majority were shown to be contaminated by bird feces (gull and/or geese), neither *Campylobacter* nor *Salmonella* were detected by qPCR in any of the samples. For a breakdown of these samples, see Table 6-1. Markers are as follows: HF183 – human; HumM2 – human; CowM3 – cattle; Rum2Bac – ruminants; LeeSG = gulls; CGO1 – Canada goose. [figure re-printed from Alberta Health report].

To further support this decision, a study was commissioned by Alberta Health to better understand the relationship between zoonotic bacterial pathogens, FIB and MST markers in aquatic birds (gulls and geese) and in order to provide further credence to this newly derived recreational water quality standard and its possible application to alternative water sources impacting public health (i.e., stormwater use).

With this in mind, the following scientific goals for this research were to; 1) estimate the prevalence, concentrations, and general distribution of enteric bacterial pathogens (*Campylobacter* spp. and *Salmonella* spp.) in urban gulls and Canada geese; 2) estimate the prevalence and concentrations of commonly used MST markers (LeeSG and CGO1) and FIB (*Enterococcus*) in gull and Canada goose feces; and 3) assess the ratios of enteric pathogen markers, MST markers, and FIB in waterfowl feces to determine the protective nature of these new guidelines for general water quality in Alberta.

6.2 Results

Pathogen carriage (*Campylobacter* spp. and *Salmonella* spp.) and shedding, as well as characterization of the concentrations of MST markers (gull marker LeeSG & Canada goose marker CGO1), and FIB (*Enterococcus*) in bird fecal samples were analyzed. This included 39 gulls, inclusive of *Larus californicus* (n=9), *Larus delawarensis* (n=29) and a single unidentified gull. While 49 total individual *Branta canadensis* fecal samples were also collected, 27 individual Canada goose fecal samples were tested for pathogens (*Campylobacter* spp. and *Salmonella* spp.), and 25 individual goose fecal samples were used to construct 5 composite fecal slurries which were tested for CGO1 and *Enterococcus* occurrence.

Overall prevalence, geometric means (where applicable), and ranges were analyzed for each pathogen, MST marker, and *Enterococcus* in water fowl fecal samples, while analyses were also performed to understand the ratio between these three elements. Results were analyzed both by bird spp., and were also stratified by gull versus goose results for each applicable marker/assay.

6.2.1 Enteric Bacterial Pathogen Estimates

Estimates of *Campylobacter* spp. and *Salmonella* spp. in individual gull fecal samples and individual Canada goose fecal samples showed that *Campylobacter* spp. were particularly prevalent in Canada geese but not gulls, though *Salmonella* spp. were infrequently found in birds regardless of species (Tables 6-2, 6-3). Culturable *Campylobacter* spp. were found in just over 50% of Canada geese fecal samples, but in <15% of gull fecal samples. In contrast, culturable *Salmonella* spp. were found in <12% of Canada goose fecal samples, and only in a single gull fecal sample. Fecal shedding of *Campylobacter* spp. varied considerably in both gulls (e.g., up to ~3 $\log_{10}$ MPN/g in California gulls) and geese (up to ~4 $\log_{10}$ MPN/g), while concentrations of *Salmonella* in Canada geese varied by up to ~3 $\log_{10}$ MPN/g between fecal samples (Table 6-2). The geometric mean of culturable *Campylobacter* spp. was found to be very low in all gull spp. (<1 $\log_{10}$ MPN/g), though it was found to be 2.0 $\log_{10}$ MPN/g in Canada geese. However, some gulls and geese could shed concentrations of *Campyloboacter* spp. as as high as 4.9 $\log_{10}$ MPN/g and 5.9 $\log_{10}$ MPN/g feces respectively, suggesting the potential for supershedders in these populations. Estimates by qPCR of *Campylobacter* spp. but not necessarily *Salmonella* spp. were similar to the culturable estimates in birds in terms of prevalence. Conversely, *Salmonella* spp. were not detected by qPCR in any of the gull fecal samples (n=39) or Canada

goose fecal composites (n=5), despite the detection of culturable *Salmonella* spp. in both gull and Canada goose feces (Tables 6-4, 6-5). Interestingly, only two fecal samples were positive by both qPCR and MPN methods for *Campylobacter* spp. (including the only sample quantifiable for *Campylobacter* spp. in gull feces), while another two samples were only positive for *Campylobacter* spp. by MPN, and two samples only positive by qPCR. In these cases of discordant detection, relatively low concentrations of *Campylobacter* spp. were observed (57.8 to 61.5 MPN/g in the case of the MPN assay, and DNQ in the case of the qPCR samples), suggesting variation in detection around the lower limits of sensitivity of both assays.

Table 6-2. Prevalence and range of viable *Campylobacter* spp. and *Salmonella* spp. in urban ring-billed gull (*Larus delawarensis),* California gull (*Larus californicus*) and Canada Goose (*Branta canadensis*) fecal samples.

Bird spp.	n	MPN					
		Campylobacter spp.	*Salmonella* spp.	*Campylobacter* spp. Range (log_{10} MPN/g)	*Salmonella* spp. Range (log_{10} MPN/g)	*Campylobacter* spp. Geomean (MPN/g)	*Salmonella* spp. *Geomean* (MPN/g)
L. californicus	9	2/9 (22.2)	ND	1.79 – 4.85	N/A	0.74	N/A
L. delawarensis	29	2/17† (11.8)	1/29 (3.4)	1.76*	1.75	0.11	N/A
Unidentified Gull	1	ND	ND	N/A	N/A	N/A	N/A
Total Gull	39	4/27† (14.8)	1/39 (2.6)	1.76* - 4.85	N/A	0.32	N/A
B. canadensis	27	14/27 (51.9)	3/27 (11.1)	1.71 – 5.90	2.70 – 5.90	1.98	0.43
Total Birds	66	18/54† (33.3)	4/66 (6.1)	1.71 - 5.90	2.70 – 5.90	1.26	0.20

* n = one sample less than shown because *Campylobacter* spp. was detected but could not be quantified per gram of feces in one sample

† Smaller n due to lab error the final week of gull sampling

Table 6-3. Prevalence and range of *Campylobacter* spp. (VD16S) in urban ring-billed gull (*Larus delawarensis)* and California gull (*Larus californicus*) fecal samples.

Bird spp.	n	qPCR	
		Campylobacter spp. (%)	*Campylobacter* spp. Range (log_{10} copies/g)
L. californicus	9	3/9 (33.3)	DNQ - 5.74
L. delawarensis	29	1/29 (3.4)	DNQ*
Unidentified Gull	1	ND	N/A
Total Gull	39	4/39 (10.3)	DNQ - 5.74
B. canadensis	5†	3/5 (60.0)†	5.29 - 6.25†

* DNQ - *Campylobacter* spp. marker was detected but could not be quantified per gram of feces in one sample
† Goose *Campylobacter* spp. qPCR estimates are from composite samples of 3 - 9 geese (with n=25 Canada geese samples total being used to construct composites), and do not therefore represent individual geese tested for *Campylobacter* spp., unlike estimates for gulls

6.2.2 *Enterococcus* Estimates

Enterococcus was found in almost all gull and Canada goose feces sampled except for one fecal sample collected from *L. californicus* (Table 6-4). Concentrations of this FIB ranged widely in all birds, though particularly in *L. delawarensis*, where it was observed that

Enterococcus could vary by as much as 5.6 $\log_{10}$ CCE/g between fecal samples (range of 2.82 - 8.44 $\log_{10}$ CCE/g). Despite this high variability, geometric means of *Enterococcus* were relatively high in gull feces, being >6.5 $\log_{10}$ CCE/g regardless of gull species (Table 6-4). In contrast to individual gull fecal samples, *Enterococcus* concentrations in Canada goose fecal composites did not appear to vary heavily between each other, differing by a maximum of ~2 $\log_{10}$ CCE/g between fecal composites. The geometric mean overall also appeared to be lower for Canada goose feces than in gull feces, though it differed by <1 $\log_{10}$ CCE/g from gull estimates, regardless of species.

Table 6-4. Prevalence, geometric mean, and range of *Enterococcus* in urban ring-billed gull (*Larus delawarensis),* California gull (*Larus californicus*), and Canada goose (*Branta canadensis*) fecal samples.

Bird spp.	n	*Enterococcus* Prevalence (%)	*Enterococcus* Geometric Mean ($\log_{10}$ CCE/g)	*Enterococcus* Range ($\log_{10}$ CCE/g)
L. californicus	9	8/9 (88.9)	6.57	6.00 - 8.76
L. delawarensis	29	29/29 (100.0)	6.53	2.82 - 8.44
Unidentified Gull	1	1/1 (100.0)	N/A	4.40
Total Gull	39	38/39 (97.4)	6.86	2.82 - 8.76
B. canadensis	5*	5/5 (100.0)*	5.78*	4.60 - 6.35*

* Goose *Enterococcus* spp. qPCR estimates are from composite samples of 3 - 9 geese (with n=25 Canada geese samples total being used to construct composites), and do not therefore represent individual geese tested for *Enterococcus*, unlike the estimates for gull fecal samples

6.2.3 MST Marker Estimates

While gull-specific marker LeeSG appeared to be relatively sensitive (i.e., found in 87.2% of gulls - see Table 6-5), and was often found at high concentrations in gulls (i.e., upper limit of 11.39 $\log_{10}$ copies/g), the Canada goose marker CGO1 was found to be less sensitive (found in only 1 of 5 goose fecal composites) and at much lower concentrations. Prevalence and concentrations of LeeSG appeared to be similar between gull species, with concentrations that varied widely (e.g., as much as ~3.5 $\log_{10}$ copies/g between California gull samples) and could be as high as 11.39 $\log_{10}$ copies/g as in one ring-billed gull sample. Geometric mean concentrations of gull marker exceeded 7 $\log_{10}$ copies/g in gull fecal samples regardless of species, further emphasizing the high concentration this marker was often found. In contrast, Canada goose marker was only found in one of five fecal composites at a concentration of 5.1 $\log_{10}$ copies/g, suggesting that the Canada goose marker (CGO1) was not highly sensitive in Canada goose fecal composites, and was not found in concentrations as high as the gull-specific marker seen in gull feces.

Table 6-5. Prevalence, geometric mean, and range of gull marker (LeeSG) in ring-billed gull (*Larus delawarensis)* and California gull (*Larus californicus*) fecal samples collected in Edmonton, Alberta.

Gull spp.	n	LeeSG Prevalence (%)	LeeSG Geometric Mean ($\log_{10}$ copies/g)	LeeSG Range ($\log_{10}$ copies/g)
L. californicus	9	8/9 (88.9)	8.25	7.75 - 11.21
L. delawarensis	29	25/29 (86.2)	7.10	DNQ - 11.39
Unidentified Gull	1	1/1 (100.0)	N/A	6.79
Total Gull	39	34/39 (87.2)	7.36	DNQ - 11.39

6.2.4 Relationships and Correlation Between FIB, MST, and Pathogen Marker Concentrations

While pathogen markers, MST markers, and *Enterococcus* were not universally found positive or quantifiable in all gull and Canada goose fecal samples, ratios between the geometric means of these parameters were analyzed. When comparing geometric means, *Enterococcus* spp. was found at a ratio of 3.4 x 10^6 CCE/g: 1 MPN/g of *Campylobacter* spp., while LeeSG was found at a ratio of 5.3 x 10^6 copies/g: 1 MPN/g of *Campylobacter* spp., and 1.6 copies/g: 1 CCE/g of *Enterococcus* spp. Looking at individual gull samples, ratios of *Enterococcus*: *Campylobacter* spp. could range from 1.2 x 10^2 CCE/g: 1 MPN/ g to 3.8 x 10^4 CCE/g: 1 MPN/g, while ratios of LeeSG: *Campylobacter* spp. could range from 2.5 x 10^4 copies/g: 1 MPN/g to 1.9 x 10^6 copies/g: 1 MPN/g.

The ratio of *Enterococcus* to *Campylobacter* spp. in Canada goose samples (again using geometric means) was found to be 6.3 x 10^3 CCE/g: 1 MPN/g, while the ratio of *Salmonella* spp. to *Enterococcus* was found to be 2.2 x 10^5 CCE/g: 1 MPN/g. Comparing *Enterococcus* to CGO1 in the single Canada goose composite sample where the latter was detected, these markers were found at a ratio of 4.9 CCE/g: 1 copy/g, with the ratio of *Campylobacter* to CGO1 (both found by qPCR) observed at 1.4 x 10^1 copies/g: 1 copy/g.

6.3 Discussion

Overall, the present study provided several key observations regarding carriage of zoonotic bacterial pathogens (i.e., *Campylobacter* spp. and *Salmonella* spp.), FIB (in the form of *Enterococcus*), and avian-specific MST markers (LeeSG for gulls, and CGO1 for Canada geese)

in aquatic birds, as well as the relationship between these variables in terms of impacting water quality. The data aims to improve our understanding of health risks associated with animal fecal contamination in natural waters, including stormwater.

Both *Campylobacter* spp. and *Salmonella* spp. were found in the feces of gulls and Canada geese, albeit at highly variable concentrations, with both pathogens showing mixed results in their distribution and occurrence when compared to previous studies. In terms of gull feces, occurrence of *Campylobacter* spp. has been found to be higher in some studies (see Broman et al., 2002; Kinzelman et al., 2008; Lu et al., 2011a; Russo et al., 2021) compared to the current study (14.8% by MPN assay and 10.8% by qPCR), although these results are still consistent with other studies (i.e., 11.8% to 15.9% occurrence - see Keller & Shriver, 2014; Moré et al., 2017; Quessy & Messier, 1992). *Campylobacter* carriage in gulls has been reported to be as low as 5.2% (see Antilles et al., 2021) but as high as 59% (Moriarty et al., 2011), suggesting a wide range of occurrence across multiple studies (Broman et al., 2002; Kinzelman et al., 2008; Lévesque et al., 2000; Lu et al., 2011a; Moré et al., 2017; Palmgren et al., 2006; Quessy & Messier, 1992; Russo et al., 2021).

Despite a relatively large and recent body of work on *Campylobacter* spp. occurrence in gull feces, few studies have actually attempted to quantify this pathogen (Lévesque et al., 2000; Lu et al., 2011a; Moriarty et al., 2011). Of the studies that quantified this pathogen in gull feces, concentrations of *Campylobacter* spp. were generally found to be higher than those reported in the current study, with Lévesque et al. (2000) finding this organism to range from 3.3 $\log_{10}$ CFU/g to 7.1 $\log_{10}$ CFU/g compared to our range of 1.8 $\log_{10}$ MPN/g to 4.9 $\log_{10}$ MPN/g for example. Similarly, the average found in Moriarty et al. (2011) of 2.9 $\log_{10}$ organisms/g was also much higher than the geometric mean reported herein of 0.32 $\log_{10}$ MPN/g.

Corresponding to the above, *Salmonella* spp. have been rarely detected in gull feces, with several studies suggesting 10% or less occurrence (Kinzelman et al., 2008; Moriarty et al., 2011; Palmgren et al., 2006; Quessy & Messier, 1992; Russo et al., 2021), and similar to the 2.6% occurrence found in Albertan gulls. However, it is important to note that an occurrence of between 26.3 – 51.2% has also been recorded elsewhere (Antilles et al., 2021; Moré et al., 2017; Rodríguez et al., 2012), suggesting a much higher frequency of this pathogen in some gull species. Again, this pathogen is rarely quantified in gulls, and was found in concentrations ranging from 2.4 $\log_{10}$ CFU/g to 9.4 $\log_{10}$ CFU/g by Lévesque et al. (2000), in comparison to the one positive sample found in this study at a concentration of 1.8 $\log_{10}$ MPN/g.

In evaluating *Campylobacter* spp. in Canada goose feces, the prevalence and concentrations of this pathogen in our study (i.e., 51.9% of individual samples by culture, and 60.0% of fecal composite samples by qPCR) were consistent with some studies (Fallacara et al., 2001, 2004; Jokinen et al., 2010, 2011; Moriarty et al., 2011), but discrepant with others (Feare et al., 1999; Keller & Shriver, 2014; Rutledge et al., 2013; Vogt et al., 2018). For example, several studies suggest little, or infrequent, detection of this pathogen in Canada goose feces (i.e., 0 – 11.2% - see Feare et al., 1999; Keller & Shriver, 2014; Nagamori et al., 2022; Pacha et al., 1988; Rutledge et al., 2013; Vogt et al., 2018), whereas others indicate much higher occurrence ranging from 25.9% to 51.8%, and with this higher range being more consistent with the current study (see Fallacara et al., 2001, 2004; Jokinen et al., 2010, 2011; Moriarty et al., 2011). Concentrations of *Campylobacter* shed in the feces of geese are rarely investigated, though Moriarty et al. (2011) found a mean estimate of 3.7 $\log_{10}$ MPN/g, and a range of <1 $\log_{10}$ MPN/g to >5 $\log_{10}$ MPN/g – a finding very similar results to the current study (i.e., a range of 1.7 $\log_{10}$ MPN/g to 5.9 $\log_{10}$ MPN/g).

Converse to *Campylobacter* occurrence in Canada geese, *Salmonella* carriage is typically low, with prevalence estimates of ≤10% being commonly found in the literature (Fallacara et al., 2001, 2004; Feare et al., 1999; Jokinen et al., 2011), albeit prevalence as high as 15.9% has also been reported (see Jokinen et al., 2010). It is important to note that several papers have reported the absence of this organism in goose feces (Nagamori et al., 2022; Moriarty et al., 2011; Vogt et al., 2018). As *Salmonella* was only detected by culture in 11.1% of samples in the current study (and not at all by qPCR in composite samples), this suggests that our estimates were similar to previous studies.

In comparison to each other, we observed that both the prevalence and concentrations of *Campylobacter* spp. and *Salmonella* spp. were in general higher in Canada geese than in gulls. Varying from this relationship, *Enterococcus* was instead found in slightly higher concentrations in gulls than in geese. Moriarty et al. (2011) found similar results between Canada geese and gulls for *Enterococcus*, though *Campylobacter* prevalence (but not concentration) was higher in gulls, and *Salmonella* was not detected in any of the studied birds, thus precluding any comparison. Diverging from this, Keller & Shriver (2014) found that the prevalence of *Campylobacter* spp. in Canada geese could be higher, similar, or lower depending on the species of *Larus* being compared, with prevalence being highly dependent on both gull species and specimen host age.

In the present study, *Enterococcus* was found to be quite common among both gulls (i.e., *Larus delawarensis* and *Larus californicus*) as well as among Canada geese, though concentrations and occurrence could be variable and in some cases differed from some previous studies (Ervin et al., 2013; Fogarty et al., 2003; Layton et al., 2009, 2010; Lu et al., 2011a; Middleton & Ambrose, 2005). In terms of occurrence, *Enterococcus* was found more frequently

in gulls in comparison to the current literature (i.e., >97% occurrence versus 36.4% - 55%), though the limited data on geese suggest that this FIB is found at just as high a prevalence as reported herein (Fogarty et al., 2003; Layton et al., 2009, 2010; Middleton & Ambrose, 2005; Moriarty et al., 2011). Moriarty et al. (2011), however, found a prevalence of 99% for this FIB in gull feces, suggesting that prevalence could also be high for this host. In terms of concentrations, the large ranges of *Enterococcus* concentrations found in both gulls (2.8 $\log_{10}$ CCE/100 mL to 8.8 $\log_{10}$ CCE/100 mL) and geese (4.6 $\log_{10}$ CCE/100 mL to 6.4 $\log_{10}$ CCE/100 mL) where this organism was detected in the current study are consistent with other studies (Ervin et al., 2013; Fogarty et al., 2003; Layton et al., 2009, 2010; Lu et al., 2011a; Middleton & Ambrose, 2005), while the geometric means of *Enterococcus* found in our study (6.9 and 5.8 $\log_{10}$ CCE/g for gulls and geese respectively) were similar to some studies (particularly for geese) (Lu et al., 2011a; Middleton & Ambrose, 2005, Moriarty et al., 2011) though they differed from others (Ervin et al., 2013; Fogarty et al., 2003; Layton et al., 2009). For examples, studies that diverged have found in gulls higher averages of *Enterococcus* between 7 to >9 $\log_{10}$ CFU, MPN, and copies/g (i.e., depending on the units – see Ervin et al., 2013; Fogarty et al., 2003), though Layton et al. (2009) found a low median of just >3 $\log_{10}$ CFU/g of *Enterococcus* in gull feces.

In terms of MST marker occurrence and concentration, Brown et al. (2017b) found high prevalence of the LeeSG marker in gulls, with 100% of samples (n = 37) positive for the marker (similar to the high level of occurrence [87.2%]) observed in this thesis, and concentrations ranging from 4.6 to 9.8 $\log_{10}$ copies/g of gull feces, though this was slightly lower than the upper limit observed in the present thesis (i.e., 11.44 $\log_{10}$/g). Additionally, Brown et al., (2017b) observed a median of 8.4 $\log_{10}$ copies/g, which was within an order of magnitude to our estimate of the geometric mean calculated as 7.4 $\log_{10}$ copies/g. In a few studies assessing the effectiveness

of the LeeSG marker, Boehm et al. (2013) found that this marker was 100% sensitive and >90% specific to gull feces, whereas a companion study by Sinigalliano et al. (2013) found that this marker was again 100% sensitive, though specificity was only >85% when pigeon fecal cross-reaction was included as a true positive.

In contrast to the above, CGO1 was not found to be particularly sensitive to Canada goose feces (being found in only 1 out of 5 fecal composites in the present study at 5.1 $\log_{10}$ copies/g), though this marker was also found to be relatively insensitive (57%) by those that designed the assay (Fremaux et al., 2010). Importantly, the median concentration of this marker found by Fremaux et al. (2010) was just <5 $\log_{10}$ copies/g, suggesting a similar concentration to the one positive sample found in the current study, though it is worth nothing that concentrations found by Fremaux et al. could be as high as 8.8 $\log_{10}$ copies/g. This low sensitivity of the marker for geese (i.e., few geese who carry the marker) suggests that contamination of stormwater with goose feces may be underestimated in studies that use the CGO1 marker.

The statistical relationships between FIB, MST markers (i.e., LeeSG and CGO1), and enteric pathogens (such as *Campylobacter* spp. and *Salmonella* spp.) can be potentially important, particularly when considering whether MST markers found in environmental waters can be used as indicators of enteric pathogens. In the current study, there was no statistically significant relationship found between the LeeSG marker in gulls and *Enterococcus* concentrations, while *Campylobacter* spp. and *Salmonella* spp. were found too infrequently to meaningfully assess their relationship with either of these parameters. Lu et al. (2011a), however, found moderate statistical correlation between LeeSG and *Campylobacter* spp. in gull feces, as well as between *Enterococcus* and *Campylobacter* spp. In contrast, Moriarty et al. (2011) found

no significant correlation between *Campylobacter* spp. and *Enterococcus* for the feces of either gulls or Canada geese, suggesting the results found by Lu et al. (2011a) are not universal.

Regardless of the above, the observed ratios of MST markers, FIB, and enteric pathogens can be potentially useful for the same purposes as described above. Lu et al. (2011a) found that ratios of *Enterococcus* to *Campylobacter* spp. could vary by sampling date anywhere between 0.01:1 to as high as 954.4:1, suggesting a large amount of variation between these two organisms within individuals. In contrast, when using the ratio between average values of *Enterococcus: Campylobacter* spp. found in Moriarty et al. (2011), this ratio can be observed to be as high as 11,619:1. For Canada geese, this result was a much lower ratio of 5.2:1 *Enterococcus*: *Campylobacter* spp. (Moriarty et al., 2011). In comparison, we observed an overall ratio of 3.4 x 10^6 CCE/g: 1 MPN/g (*Enterococcus*: *Campylobacter* spp.) in gulls, suggesting overall differing values for these ratios in gulls among the literature. This likely implies that both intra-population differences as well as inter-geographical differences may play a large part in differing ratios between this FIB and pathogens in gulls to an extent that these ratios may be difficult to consistently predict. This is particularly the case when considering the inconsistent statistical relationships found between LeeSG, *Campylobacter* spp., and *Enterococcus* as demonstrated in the present study and others (Lu et al., 2011a; Moriarty et al., 2011), and suggests that FIB monitoring may not be predictive of *Campylobacter* occurrence in stormwater (or *Salmonella* for that matter).

It should be noted that both large and small geographical differences have been found to make substantial impacts in carriage rates of pathogens in gulls (Antilles et al., 2021; Lévesque et al., 2000; Moré et al., 2017; Quessy & Messier, 1992), while the effects of temporal factors such as bird age, the season samples are collected, and the year of sampling have all been shown

to have varying impacts on enteric pathogen occurrence in gulls (Antilles et al., 2021; Broman et al., 2002; Keller & Shriver, 2014; Kinzelman et al., 2008; Lévesque et al., 2000; Palmgren et al., 2006). It is also important to note that many of the above factors have also been explored (albeit to a limited capacity as well) in Canada geese (see Fallacara et al., 2001, 2004; Feare et al., 1999; Keller & Shriver, 2014; Middleton & Ambrose, 2005; Rutledge et al., 2013; Vogt et al., 2018). Overall, the above suggests that there are many factors that have not yet been fully explored but may affect, and at least partially explain, patterns of enteric pathogens in gulls and the potential public health threat from avian fecal contamination of water resources.

Indeed, several researchers have argued that public health risks from bird fecal contamination into environmental waters may be relatively low, especially when considering other sources of enteric pathogens, such as human sewage contamination (Brown et al., 2017a; Schoen & Ashbolt, 2010; Soller et al., 2010; Smith et al., 2020). Smith et al. (2020) argues that avian fecal pollution is potentially overestimated, noting that very few avian taxa have been studied to any sufficient degree, while few papers have fully investigated and addressed the transmission of zoonotic bacterial pathogens from animal hosts, suggesting not enough is known to assume widespread public health risks from these hosts. Additionally, *Campylobacter* spp. and *Salmonella* spp. associated with avian hosts (including those found in environmental waters) have been increasingly found to differ in terms of lineage from more generalist strains that are known to more commonly cause disease in humans, domestic animals, livestock, and poultry (Dearlove et al., 2016; Fu et al., 2022; Griekspoor et al., 2013; Mulder et al., 2020; Sheppard et al., 2011; Shrestha et al., 2019). It is still important to point out, however, that generalist strains of *Campylobacter* spp. and *Salmonella* spp. associated with other human and animal hosts have been found in gulls (Broman et al., 2002; Palmgren et al., 2006), as well as these same strains

often found in environmental waters (Mulder et al., 2020; Shrestha et al., 2019). Additionally, enteric pathogens such as *Campylobacter* spp. can exhibit rapid host-switching, as well as broad host ranges in general, suggesting that there may still be an appreciable (though not yet well characterized) risk of zoonotic transmission from avian sources (Dearlove et al., 2016). When considering the high concentrations of *Campylobacter* spp. sometimes found in waterfowl in the current study (such as estimated maximums of 4.9 and 5.9 $\log_{10}$ MPN/g in gulls and geese) and reported by others (see Lévesque et al., 2000; Lu et al., 2011a; Moriarty et al., 2011), this fraction of potentially human-infectious *Campylobacter* may be of some concern. Moriarty et al. (2011) for example estimated that gulls and Canada geese excreted *Campylobacter* spp. at concentrations as high as 4.6 $\log_{10}$ MPN and 6.1 $\log_{10}$ MPN respectively per day on average from an individual bird's feces, suggesting avian sources can represent a large potential reservoir for this pathogen that warrants further investigation.

In conclusion, given the large variation in pathogen and FIB occurrence in aquatic bird hosts such as gulls and geese – and for which these birds are commonly found on stormwater ponds - it is not surprising that poor correlations were noted between FIB and *Campylobacter/Salmonella* occurrence in stormwater (Chapter 5). Coupled with complex and dynamic microbial transport processes (e.g., weather-related mobilization of fecal pathogens on a landscape and into the drainage network) and the challenge of having many other host sources contaminating stormwater (i.e., humans, dogs, ruminants, etc), the data presented in this chapter provides further credence for the province's adoption of a QMRA-based framework for managing risks associated with stormwater use. This contrasts the adoption of traditional water quality standards based on FIB occurrence that are typical of most jurisdictions considering water reuse. This concept is expanded further in the next chapter of this thesis.

7. General Discussion

The paucity of data on microbial stormwater quality in Alberta simply emphasizes the importance of the current study towards filling these knowledge gaps, while also revealing several other significant findings. An overview of each of these key findings is provided below.

Firstly, and perhaps not surprisingly, stormwater quality tends to very poor from a microbiological perspective. High concentrations of FIB, and the consequential exceedance of traditional water quality standards - i.e., recreational water quality criteria, and by association, irrigation and wastewater effluent water quality criteria - were widespread in nearly all stormwater-impacted ponds and creeks studied in Airdrie, Alberta, regardless of the FIB used in the assessment of water quality (i.e., total coliforms, *E. coli* or *Enterococcus*). Microbial water quality based on *Enterococcus* criteria was found to be more frequently exceeded than *E. coli* criteria. Collectively, these findings reinforce the concept that the use of stormwater as an alternative water source requires a management strategy that seeks to reduce the concentration of pathogenic microbes in the water before it can be used (for most purposes). As such the data supports the province's risk assessment strategy based on achieving $\log_{10}$ treatment reduction credits against select pathogen groups (viruses, bacteria, protozoa) to ensure that stormwater is made 'fit-for-purpose'. Secondly, human sewage appeared to be the primary source of fecal pollution in most stormwater ponds, with over a quarter of samples taken in 2021 positive for the HF183 human fecal marker, and over half of Nose Creek samples positive for this marker alone. Additionally, MST investigations aimed at tracking these human signatures of fecal pollution managed to pinpoint upstream sites in the drainage networks associated with cross-connections in at least two separate investigations (upstream of Nose Creek and Hillcrest). Thirdly, enteric pathogens were only sporadically detected in stormwater, with the exception of *A. butzleri*, which

had a relatively high rate of detection (i.e., in nearly 33% of samples in 2021), and was most often associated with human fecal pollution. Fourthly, both geo-spatial and temporal patterns were noted for the detection of FIB, MST markers, and pathogens, with Nose Creek having the highest occurrence of HF183 and the pathogen *A. butzleri*, as well as the highest occurrence of both variables when associated with *Enterococcus* STV water quality exceedances (26.3% of samples), suggesting that *Enterococcus* detection by qPCR may be the most sensitive FIB for monitoring fecal pollution and pathogen occurrence in stormwater. Lastly, data regarding FIB, MST markers, and enteric pathogens from avian fecal pollution sources (such as those also seen in Airdrie stormwater) provided important information about pathogen loading from these sources and potential risks posed to human health. For example, *Salmonella* spp. were not detected in stormwater even when bird MST markers were detected, and which coincided with a lack of occurrence of this pathogen in the feces of urban gulls or Canada geese. By contrast, *Campylobacter* spp. were detected on occasion in stormwater, and for which the prevalence of *Campylobacter* in birds was also more common, with individual birds occasionally shedding high numbers in their feces (i.e., supershedders). Each of the significant findings noted above are discussed in more detail below.

Importantly, a large body of research has demonstrated that both *E. coli* and *Enterococcus* can be frequently found in high concentrations in stormwater, often exceeding recreational water quality standards and sometimes at an even higher frequency than as seen in the current study (Converse et al., 2011; de Man et al., 2014; Hachad et al., 2022; Kinzelman & McLellan, 2009; Lee et al., 2020; Monteiro et al., 2021; Nshimyimana et al., 2014; Olds et al., 2018; Parker et al., 2010; Sauer et al., 2011; Sidhu et al., 2012; Staley et al., 2018). As noted previously, while FIB concentrations were high in the present study, very often exceeding recreational water quality

criteria, geo-spatial variation was also shown to clearly exist. For example, even in relatively "clean" stormwater-impacted water bodies, high variation could still occasionally be seen on a site-by-site basis. This was of course not unexpected, with many studies suggesting that geo-spatial site differences (site infrastructure, upstream land use, proximity to high flows, etc.) can have a considerable impact on FIB concentrations (Hachad et al., 2022; Hart et al., 2020; Nshimyimana et al., 2014; Parker et al., 2010; Sidhu et al., 2012; Staley et al., 2018; Steele et al., 2018). Like geo-spatial variation, temporal variation in FIB distributions was also noted, with peak occurrence of FIB in this study occurring during both wet weather conditions (24 hr antecedent rainfall $\geq$5 mm) as well as under baseline flows. It is therefore somewhat unclear what role wet weather played in FIB criteria exceedance during this study. While other studies have found wet weather and increases in FIB concentrations to be generally congruent (Ahmed et al., 2020; Converse et al., 2011; Hart et al., 2020; Kinzelman & McLellan, 2009; Lee et al., 2020; Parker et al., 2010; Sidhu et al., 2012; Steele et al., 2018), other evidence suggests little correlation between precipitation and FIB concentrations (Monteiro et al., 2021), or even occasional peaks of FIB during relatively dry periods (Hart et al., 2020; Parker et al., 2010).

A large caveat to the above, however, is that much of the research used to derive traditional microbial water quality guidelines that protect public health (such as standards set for recreational water by the US EPA [2012]) come from studies where recreational water was directly impacted by point-sources of human sewage pollution (Wade et al., 2003, 2006, 2010). Conversely, there are unclear associations between the concentration of FIB and illness when the contamination source is from animals [or unknown sources] (Arnold et al., 2013; Colford et al., 2007; Fleisher et al., 2010; Griffith et al., 2016). One of the most important aspects (as outlined

by AHS' *Public Health Guidelines for Stormwater Use and Water Reuse*) is therefore to closely characterize source waters for human sewage (among other sources of microbial hazards).

Along the above lines, this study provided evidence of a relatively high frequency of occurrence of the human sewage marker HF183 in stormwater ponds compared to other animal markers of fecal pollution. The ability to track HF183 in the drainage network to cross-connections leaking into storm drains provided critical evidence that human feces was indeed a primary source of fecal pollution in these stormwater systems. Overall, human sewage contamination was found in ~25% of samples from each stormwater pond, with 58% of Nose Creek stormwater effluent samples having presented evidence of human sewage contamination. Animal markers were found in much smaller proportions by comparison. Considering both how widespread human fecal pollution into Airdrie stormwater was, as well as considering the concentrations of these human fecal markers in stormwater effluents (i.e., between 3 – 4 $\log_{10}$ copies/100 mL of HF183) it is estimated that approximately 1 of every 1000 litres of stormwater flow is coming from raw human sewage. This is based on the finding that raw municipal sewage typically contains 7-8 $\log_{10}$ copies/100 mL of the HF183 fecal human marker (Ahmed et al., 2021; Mayer et al., 2018; Nshimyimana et al., 2014; Sauer et al., 2011). This is consistent with other studies, which have demonstrated that as much as 10% of stormwater effluents may be comprised of raw human sewage (Ahmed et al., 2021; Nshimyimana et al., 2014; Sauer et al., 2011). Collectively, these findings support the approaches outlined in the new public health guidance documents that set different $\log_{10}$ reduction targets for stormwater impacted by human feces [e.g, 10% and 0.1% sewage content] (AHS, 2021), and importantly, the information provided in this thesis offers a methodological approach to quantitate these levels for compliance purposes.

Sampling of bacterial pathogens (*Campylobacter, Salmonella,* STEC and *A. butzleri*) provided some important information towards improving our knowledge of microbial hazards present in stormwater. *Arcobacter butzleri* was the most frequently detected pathogen, often observed in samples exceeding microbial water quality standards and/or when human feces were detected. For example, over 75% of samples positive for *A. butzleri* were found alongside the HF183 marker within split samples, further suggesting the potential for this pathogen to be related to the human sewage source(s). *Campylobacter* and STEC were only sporadically detected, and *Salmonella* was not detected in any water samples. Geo-spatial variation was observed in terms of *A. butzleri* occurrence at both the pond level and site-specific basis within a pond. As an example, the King's Heights North site KHN#1 appeared to harbor all instances of *A. butzleri* detection of the two sites from that pond, suggesting a contamination profile specific to that site. Taking into consideration that this pathogen has frequently been associated with human sewage (Cui et al., 2019; Fisher et al., 2014; Lee et al., 2012; Lu et al., 2015; Zhang et al., 2020), as well as sometimes strongly correlated to FIB (Collado et al., 2008; Webb et al., 2017), *A. butzleri* is of significant concern for stormwater management. A previous outbreak of gastrointestinal illness was noted by Fong et al. (2007) to occur from groundwater contaminated with feces, and was associated with *Arcobacter* spp. as one of the potential causative agents. Importantly, *Arcobacter* spp. have also been found in a wide variety of other diverse environmental waters such as irrigation water, river water, lake water, spring water, and sea water, suggesting that this pathogen can be widely found in the aquatic environment (Banting et al., 2016; Collado et al., 2008; Hsu & Lee, 2015; Lee et al., 2012; Sciortino et al., 2021). In spite of a paucity of epidemiological data, this organism has been implicated in moderate to severe gastrointestinal illness and outbreaks (Chieffi et al., 2020; Collado & Figueras, 2011; Figueras et

al., 2014) as well as traveller's diarrhea (Jiang et al., 2010), and bacteremia in rarer cases (Arguello et al., 2015). Additionally, it has been suggested that *Arcobacter* spp. can also grow in the environment (Banting & Figueras-Salvat, 2016; Fisher et al., 2014). Beaudry (2019) found that *A. butzleri* isolates from stormwater carried a number of important virulence genes suggesting that many of the isolates found in stormwater were likely pathogenic. Despite the high occurrence of *A. butzleri* found in Airdrie stormwater, there is a general paucity of data on the occurrence of *Arcobacter* spp. in stormwater in general (Beaudry, 2019; Carney et al., 2020). Moreover, to the author's knowledge, there are no risk assessment studies designed around *Arcobacter* spp. found in stormwater. In contrast, risk assessment studies for environmental waters and water reuse, which instead often utilize *Campylobacter* spp. and *Salmonella* spp. occurrence (thus utilizing these organisms' dose-response models) as reference pathogens (de Man et al., 2014; Murphy et al., 2017; Sales-Ortells & Medema, 2015; Schoen et al., 2017). However, the data presented in this thesis suggests that it may be more prudent to focus attention on *A. butzleri* as a reference pathogen for microbial risk assessment associated with stormwater use.

Overall, the data suggest that the patterns of occurrence of fecal indicators, sources of pollution, and pathogens clearly relates to the demographics of the contributing drainage network at each site, making it difficult to generalize about overall stormwater quality across ponds or among sites within a single pond/receiving body. This is important, as it suggests that future monitoring programs should focus on sampling at all inlets/outfalls in order to understand the conditions of the individual drainage networks that impact water quality in a stormpond. Additionally, while this study did not (nor was it designed to) assess relationships between FIB, MST and pathogens in dry and wet weather conditions, the potential also exists that weather-

dependent variation may cause fluctuation in occurrence and concentrations of these parameters in stormwater.

Water quality monitoring is recognized as an important element of public health management, including water reuse (AHS 2021), but Alberta's new *Public Health Guidelines for Water Reuse and Stormwater Use* do not explicitly state that the water must meet certain criteria before it can be used (e.g., irrigation water quality standards). Rather, the guidelines focus on meeting $\log_{10}$ treatment reduction targets against pathogens, and it is these treatment targets that provide the measure of safety for stormwater use. Nevertheless, water quality monitoring remains important for a couple of reasons. Firstly, and as revealed in this thesis, the monitoring of FIB and MST markers provide collective information about the integrity of the storm drain network itself, and can help identify infrastructure problems/failures leading to increased risks of using stormwater as an alternative water source. A stormpond deemed to be relatively 'clean' (i.e., low FIB concentrations) and free of human sewage (i.e., HF183 not detected) may not retain this status over time. Indeed, the prevalence of human fecal contamination in stormwater has been shown to coincide with aging infrastructure (Gonzalez et al., 2020; Hachad et al., 2022; Sauer et al., 2011), and infiltration of human sewage into stormwater drainage is therefore more commonly expected in older neighborhoods. Consequently, monitoring programs help establish baseline water quality conditions, and which can subsequently reveal deteriorating conditions within the drainage network that increase risk to public health. Secondly, water quality monitoring can help identify sites within a stormpond for which water quality may be more suitable for use (low FIB and no HF183). Indeed, although stormwater microbial water quality was generally poor, some sampling sites in the current study had reasonable water quality with generally low FIB concentrations, no evidence of human sewage contamination, nor occurrence

of enteric bacterial pathogens. This was the case for both the EL#1 and WS#1 sites sampled in 2020 from the East Lake and Windsong stormwater ponds respectively. Several studies have used bacterial water quality monitoring in storm ponds to model transport, fate and hydrodynamics of micobial pollutants in storm effluents, identifying zones of good water quality even in heavily polluted systems (Allafchi et al., 2019, 2020, 2021a, 2021b). Thirdly, identifying specific host sources of fecal pollution allows for targeted interventions to be implemented to reduce fecal pollution in urban environments, such as improving detection of cross connections in municipal plumbing programs (Gonzalez et al., 2020; Hachad et al., 2022; Sauer et al., 2011).

There are also implications in terms of which FIB are more effective to use for monitoring purposes, particularly when considering differences in FIB performance and behaviour as demonstrated in the current study. Of the two primarily studied, *Enterococcus* may be the more sensitive indicator as it consistently exceeded water quality criteria at a higher rate than *E. coli*, and these exceedences were more often associated with human pollution (HF183) and bacterial pathogen occurrence. As mentioned previously, the distribution of *Enterococcus* and *E. coli* in terms of concentrations and prevalence can differ between animal and human hosts (Ahmed et al., 2019a; Ervin et al., 2013; Layton et al., 2009, 2010), but given the predominance of human sewage impacting stormponds, *Enterococcus* qPCR testing is recommended as the better indicator for stormwater monitoring. This aligns with the findings of the NEEAR studies, where *Enterococcus* detected by qPCR showed strong correlation with gastrointestinal illness in recreational water impacted by human sewage, including over a wide range of concentrations (Wade et al., 2003, 2006, 2010).

When considering specific reccomendations for stormwater monitoring in municipalities, such as the City of Airdrie, one of the most important determinants is the level of exposure of

this water to the public, and therefore the health risk this water may pose. As such, a targeted monitoring approach would be recommended first and foremost for water bodies earmarked for higher risk activities (i.e., recreation), and where human fecal pollution is particularly abundant, such as in the case of Nose Creek as described above. In this case, weekly monitoring of outfalls using Alberta Health's '*Alberta safe beach protocol*' water quality guidelines, in conjunction with MST monitoring would be deemed appropriate (Alberta Health, 2021). However, monthly monitoring of these parameters in stormwater-impacted water bodies may be more appropriate if they have previously displayed little, if any human sewage contamination (such as the Canals and King's Heights South stormpond), and/or have not been designated for water use or known to cause exposure via local recreational activity. It would be therefore less important to assess the drainage network upstream of these sites via MST for point sources of pollution. Conversely, in receptor water bodies (such as the Nose Creek) where many stormwater ponds drain, more extensive MST investigations of drainage networks may be warranted in order to study cumulative fecal pollution impacts and more effectively isolate drainage trunks and areas of a municipality that are particularly problematic. It should be noted, however, that stormwater ponds, urban creeks, and outfalls of interest to a municipality may first need to have a scoping study performed as described in this thesis, in order to construct a baseline understanding of human (and animal) fecal impacts before the above reccomendations can be put in place. Lastly, jurisdictions might consider implementing these tools into current municipal cross-connection programs for both newer sub-developments and older sub-developments by using the 'toolbox' approach as outlined in this thesis. Occassional monitoring of major drainage trunks can also be used to initiate the process of pinpointing sources of human fecal pollution, as described in this thesis.

Several gaps remain to be filled by future studies. Firstly, the number of routine samples taken in the current study were limited in terms of the number of weeks sampled (7 weeks in 2020 and 4 to 5 weeks in 2021). This was particularly problematic in trying to resolve temporal trends of FIB, human sewage contamination, and pathogens in base-line versus wet weather conditions. This may be particularly important when considering that FIB and human sewage (i.e., HF183) distributions have especially been found to differ under wet-weather versus under dry-weather (Ahmed et al., 2020; Hart et al., 2020; Lee et al., 2020; Olds et al., 2018; Steele et al., 2018), with it being assumed that differing transport dynamics occur under differing conditions and may change levels of contamination in stormwater ponds and stormwater-impacted creeks. Future studies focused on using auto-samplers triggered by high flow and low flow sampling could be used to more closely understand the dynamics of fecal contamination of stormwater systems and risks to human health.

Aquatic waterfowl are common on urban stormponds and can represent a significant source of fecal pollution in these systems. The high concentrations and high sensitivity of the gull fecal marker (LeeSG) suggested that this was a potentially sensitive indicator of gull fecal pollution when found in stormwater (such as in Airdrie), whereas the low concentrations and sensitivity in goose feces of the goose marker (CGO1) suggested that this marker may be less useful as an accurate indicator of goose fecal contamination. An important observation was that high concentrations of *Enterococcus* were found in both bird hosts, suggesting that potential spikes of this FIB found in stormwater could be indicative of bird fecal contamination in the absence of human sewage indicators such as HF183. Importantly, while bacterial pathogen carriage in gulls and geese could be low (with the exception of *Campylobacter* spp. in geese), high concentrations of *Campylobacter* spp. and *Salmonella* spp. (i.e., up to 5-6 $\log_{10}$ MPN/g)

occasionally present in aquatic avian feces suggests that "super-spreaders" exist in these populations of birds and that may shed substantial amounts of pathogens into stormwater. However, it should be noted that just as *Campylobacter* spp. and *Salmonella* spp. were infrequently detected in Albertan stormwater and at low concentrations (if at all), the majority of gulls also did not test positive for either of these pathogens. While risk-assessment studies suggest that avian (particularly gull) fecal contamination of environmental waters may be less risky than human sewage contamination (Brown et al., 2017a; Schoen & Ashbolt, 2010; Soller et al., 2010), this risk is still underassessed and must be further studied in light of the above results and other similar studies (Antilles et al., 2021; Broman et al., 2002; Keller & Shriver, 2014; Kinzelman et al., 2008; Lévesque et al., 2000, and Lu et al., 2011a; Rutledge et al., 2013; Van Dyke et al., 2010).

In conclusion, the use of stormwater as an alternative source water must be handled with some caution and care, taking into consideration the hazards and sources of contamination assessed herein in order to use this source water in the most safe and effective way. Furthermore, the data collected herein supports the QMRA approach ratified in the *Public Health Guidelines for Water Reuse and Stormwater Use* recently ratified by Alberta Health and Alberta Health Services.

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