ALBERTA INNOVATES

CLEAN RESOURCES: FINAL REPORT

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SECTION ONE: Executive Summary

EXECUTIVE SUMMARY:

Alberta is faced with water quality and scarcity issues driven by population growth, aging infrastructure and climate change. Stormwater and rainwater harvesting are alternative water sources that can supplement growing water demands. By decreasing dependency on potable supplies and reducing costs associated with infrastructure expansion of water treatment plants, these water sources also provide opportunities for drought control, long-term supply management and can simultaneously reduce environmental footprints (i.e., carbon emissions, pollutant loading, excessive scour and erosion). However, alternative water use strategies are plagued by: i) an aging stormwater infrastructure in fair, poor or very poor condition, with an estimated national replacement cost of around $31 billion across Canada; ii) design features pre-empting the harvest of good quality water for various purposes (e.g., combined sewer outfalls that mix sewage with rainwater collection during intense storms), iii) the potential for illicit cross-connections, leaky sewerage systems, and overland septic/agricultural/urban drainage that contribute pollutants to receiving systems; iv) an absence of international agreement on a definition of safe water quality for stormwater or rainwater use and the associated monitoring and reporting requirements; v) a lack of scientific data on pollutant sources and concentrations impacting water quality (including microbes) in these systems and interpreted within the context of human health risks; and vi) a conflicting regulatory policy framework in Alberta (e.g., *Water Act*, *Environmental Protection and Enhancement Act*, *Safety Codes Act*, and the *Public Health Act*). These challenges impede provincial and national adoption of alternative water use as part of an overall water management strategy.

Microbial pathogens represent the dominant acute health risk with the use of alternative water sources. Storm events have been linked to an increased incidence of waterborne diseases, demonstrating effective mobilization and transport of microbial pathogens under storm conditions. Importantly, human fecal contamination is common in stormwater, even in systems separate from sanitary sewers – a result of illicit cross connections, damaged/leaking sewage lines, intrusion, and overland seepage/drainage. Animal fecal wastes from domestic pets and wildlife (birds) can also contribute significant fecal loading to stormwater and rainwater. Environmental pathogens, such as *Legionella* spp., *Pseudomonas aeruginosa*, and the non-tuberculous mycobacteria [NTM](i.e., *Mycobacterium avium* complex) are also prominent microbial constituents of rainwater and stormwater systems. Future urban planning must incorporate public health risk assessments associated with microbial threats, for which Water Reuse Safety Plans (WRSP) have been encouraged, and are used in countries such as Australia for managing risks from alternative water sources. From an Alberta context, and prior to commencing this project there was virtually no data on the microbial water quality of stormwater/rainwater, the occurrence of pathogens in these systems, or an understanding of the fecal sources of contamination in these systems in Alberta. This significant knowledge gap precluded the development of effective stormwater policies or frameworks to protect public health.

The goal of this proposed study was to work with municipalities (Cities of Calgary and Airdrie) and provincial/national policy regulators in filling the knowledge gaps regarding microbial contamination sources and risks associated with stormwater/ rainwater reuse applications, and assist in the translation of this knowledge into development of an evidence-based risk management framework in Alberta. To this end we sought to: a) e*valuate microbial water quality, pathogen occurrence and treatment efficacy in stormwater and rainwater systems in urban municipalities in Alberta*, b) *use quantitative microbial risk assessment approaches to strategically identify water-fit-for purpose reuse options for stormwater and rainwater*, c) *develop process-based and probabilistic models of microbial contamination in urban*

stormwater ponds; d) *develop a Stormwater Use Management Plans (SUMP) Framework to support rainwater and stormwater use in Alberta*. The ultimate goal of this research was to support the development of regulatory frameworks centered around microbial risk assessment for 'water-fit-for-purpose' uses and incorporated into a water safety plans suitable for risk management, in order to support sustainable use of Alberta's precious water sources.

Microbial stormwater quality was monitored in three stormponds in the City of Calgary over the course of this study (McCall Lake, Country Hills Stormwater Facility, and the Inverness Stormpond). Additional work was done on the Elbow River (Calgary) and Nose Creek (Airdrie) to understand the impact of stormwater effluents on receiving waters. Microbial water quality was generally poor in stormponds, often failing current standards. Quality was variable both spatially (within a pond and between ponds) and temporally (within a pond and between ponds), suggesting that future water quality monitoring programs account for this variability. Stormwater also contributed to poor water quality in urban rivers. The fact that water quality often violated current standards suggests that the simple adoption of existing water quality standards for stormwater reuse (e.g., recreational water quality standards) would negate the use of these resources for many different purposes. This data supported the concept that Alberta aim to adopt microbial risk-based standards as a means of regulating the reuse of these alternative water resources.

Not surprisingly, human sources of fecal pollution were found to commonly impact water quality in all stormponds and in urban rivers receiving stormwater effluent – a finding noted in many other international jurisdictions. In most cases, human sources of fecal pollution were sporadic, but in one case a persistent human signature was observed at one site in a stormpond. The pattern of contamination at this site was interesting in that the levels of human fecal pollution at this site always decreased after long-weekends, and suggested a cross connection in an industrial/commercial area of the City of Calgary. In conjunction with City staff, we tracked the source of this pollution to a commercial area of the city, and investigations are ongoing. Based on microbial source tracking, birds (seagulls) were also commonly identified as a dominant source of fecal pollution in stormponds. Other sources of fecal pollution identified included dogs and geese, albeit far less than the levels contributed by human and seagull feces.

Pathogens such as *Arcobacter butzleri*, shiga-toxin producing *E. coli* (STEC), *Campylobacter* spp., and *Salmonella* spp. were also routinely detected in stormwater ponds or effluents (with decreasing prevalence in the order listed), emphasizing the importance of understanding public health risks associated with alternative water reuse. Remarkably, as many as 75% of stormwater samples analyzed contained culturable pathogenic *A. butzleri*, raising concerns that this 'under-appreciated' pathogen could contribute significantly to public health risks associated with stormwater reuse, and that risk assessments should incorporate this pathogen into future models.

The data collected above supported the project team's proposal to develop risk-based standards for stormwater reuse in Alberta. To this end, quantitative microbial risk assessment (QMRAs) was used as a framework for developing Log Reduction Targets (LRTs) for a variety of uses of stormwater, rainwater, grey water and wastewater. LRT values set the treatment and/or management options needed to reduce public health risks to an acceptable level for society, and help in expanding the range of water reuse applications for these alternative water sources. LRT values were derived for viruses, protozoan parasites and enteric bacterial pathogens in stormwater, rainwater, greywater and wastewater intended for reuse under a variety of scenarios, including: agri-food irrigation, car/truck washing, clothes washing, temperature control (cooling towers and evaporative condensers), dust control, street cleaning, non agri-food irrigation, recreation, aesthetic water features (indoor and outdoor), and toilet/urinal flushing. This strategic approach to water resource management focuses on a 'water-fit-for-purpose' concept, allowing for innovation in the water industry to achieve these targets. In collaboration with Government of Alberta regulatory agencies (Alberta Environment and Parks, Alberta Health/Alberta Health Services) these approaches formed the basis for the development of a new regulatory guidance policy framework

for water reuse in Alberta, and supported by two documents developed by these agencies and in participation with team members: a) *Public Health Guidance for Water Reuse and Stormwater Use* (Alberta Health/Alberta Health Services); and b) *Alberta Water Reuse and Stormwater Use Guidebook* (Alberta Environment and Parks). These documents form Alberta's proposed regulatory framework for water reuse in Alberta.

In conjunction with Alberta Health and Alberta Health Services we also developed an EXCEL-based Water Reuse Safety Plan (WRSP) template that encompasses many of the elements of these policy guidance documents. This resource tool allows industry and municipalities to perform all planning and management of these systems using a single, easy-to-complete program. It is intended that the WRSP act as both the planning document and the application for seeking approval by regulators. We continue to collaborate with the various government agencies in finalizing the guidance documents on water reuse, with the goal of adopting this framework in Alberta to create wise and sustainable water reuse management strategies.

Given the complexity of microbial water quality in stormwater, members of our team also developed an integrated computational flow dynamic (CFD) model to assist municipalities/industry in estimating bacterial water quality in stormwater ponds during and after storm events, and in order to promote the effective use of these resources during periods of good water quality. These integrated CFD models were shown to be valuable in identifying the best locations (i.e., cleanest) within a pond to extract water for reuse purposes, thereby further reducing the public health risks associated with water reuse extraction from highly contaminated areas, and the need for additional treatment. The models can also help understand flow fields in a pond (i.e., dispersion plumes), useful in developing stormwater designs that can limit the spread and dispersion of bacteria in a pond (i.e., engineered forebay designs to sequester bacterial loading). These tools will greatly assist in the practical management of stormwater reuse applications and the wise-use of these resources.

SECTION TWO: Table of Contents

2.1: TABLE OF CONTENTS

2.2: LIST OF TABLES

2.3: LIST OF FIGURES

SECTION THREE: Introduction

3.1: PROJECT DESCRIPTION

Background, Scope of Problem and Knowledge Gaps

The harvesting of alternative water sources, such as stormwater and rainwater, provides immediate opportunity for addressing the growing non-potable and potable water demands of Alberta's municipalities. Safe utilization of roof-harvested rainwater and ground-collected stormwater includes: i) irrigation for non-consumptive (i.e., recreational fields) and consumptive agronomy (i.e., food gardens), ii) toilet/urinal flushing and clothes washing, iii) aesthetic water features, iv) fire-fighting, v) industrial process water, and even vi) recreation (i.e., recreational ponds). An evaluation of the predominant stormwater use applications in Australia[1] noted that 44% of supplies were used for irrigation of gardens or public spaces, 15% for toilet/urinal flushing, 15% for outdoor uses (car washing, ornamental water features), 8% for fire-fighting, and about 8% as a source of drinking water. Approximately 10% was returned to catchments to meet environmental flow demands[1].

Although stormwater/rainwater use represents an important trajectory for future sustainable development in Alberta, a number of challenges persist, including: i) an aging stormwater infrastructure in fair, poor or very poor condition, with an estimated national replacement cost of around $31 billion across Canada[2]; ii) design features pre-empting the harvest of good quality water for various purposes (e.g., combined sewer outfalls [CSOs] where raw sewage and storm precipitation are combined and directly discharged into the environment), iii) the potential for illicit cross-connections, leaky sewerage systems, and overland septic/agricultural/urban drainage that contribute pollutants to receiving systems; iv) an absence of international agreement on a definition of safe water quality for stormwater or rainwater use and the associated monitoring and reporting requirements; v) a lack of scientific data on pollutant sources and concentrations impacting water quality (including microbes) in these systems and interpreted within the context of human health risks; and vi) in Alberta, a conflicting yet deficient regulatory policy framework for pathogen control. These challenges impede provincial and national adoption of alternative water use as part of an overall water management strategy.

Microbial pathogens represent the dominant acute health risk with the use of alternative water sources[3]. Storm events have been linked to an increased incidence of waterborne enteric diseases[4-7], largely due to effective mobilization and transport of pathogens under storm conditions. Importantly, human faecal contamination is common in stormwater, even in systems separate from sanitary sewers[8,9] – a result of illicit cross connections, damaged/leaking sewage lines, intrusion, and overland seepage/drainage. Animal faecal wastes from domestic pets[10,11] and wildlife[12,13] also contribute significant faecal loading to stormwater. Similarly, rainwater systems can be subject to faecal contamination from birds and arboreal mammals (squirrels, opossum)[14,15]. Environmental saprozoic pathogens, such as *Legionella* spp., *Pseudomonas aeruginosa*, and the non-tuberculous mycobacteria [NTM](i.e., *Mycobacterium avium* complex) are also prominent microbial pathogens of rainwater and stormwater systems[16].

The public health risks associated with alternative water uses, is largely dependent on the intended application. Studies have reported a high prevalence of *Shigella* spp., *Campylobacter* spp., *Yersinia* spp., *Salmonella enterica*, and *Giardia* spp.[15,17,18] in rainwater collection systems, and of potential human health risk. Ashbolt & Kirk[4] and Kirk et al.[19] identified rainwater collection systems as the source of

several waterborne disease outbreaks (*Campylobacter* and *Salmonella*) in long-term care facilities associated with exposure to inadequately treated rainwater used for drinking. Employing quantitative microbial risk assessment (QMRA) methods, Lim & Jiang[20] observed that health risks associated with routine consumption of garden produce, such as lettuce irrigated from overhead sprinklers with harvested rainwater, resulted in risks greater than the one case per 10,000, the threshold level deemed acceptable by the United States Environmental Protection Agency (U.S. EPA). In a combined rainwater/stormwater collection system in the Netherlands used for recreational purposes (i.e., a water plaza), *Campylobacter* risks for children exceeded acceptable levels[16]. In 2003 in Clinton, Utah, an outbreak of *E. coli* O157 was associated with spray irrigation of secondary water from a community stormwater reservoir[21]. Pathogens such as *Giardia* spp., *Campylobacter* spp., *Shigella* spp., norovirus, rotavirus and adenovirus are common in stormwater and can occur at relatively high concentrations[16,22,23], the levels of which are often unacceptable for certain purposes[8]. A study in China found rates of typhoid fever (*Salmonella* Typhi) decreased with distance from both food markets and stormwater canals, suggesting that irrigation of food crops from stormwater canals led to increased disease transmission in the nearby communities[24]. *Salmonella* was also found on the vegetables sold in local markets[24], and stormwater contaminated with human wastes [24] was identified as the potential cause of this outbreak. When human sewage is present, viruses represent a dominant health risk. Norovirus concentrations have been reported to be as high as 10^6-10^7 viruses/L in stormwater collected from residential and agricultural runoff in the U.S.[25], implying significant contamination of these sources with raw human sewage – a finding that is also common in studies in Australia and Europe[8,26]. Rotavirus levels have been shown to exceed 10^8 viruses/L in raw sewage, and impacting stormwater quality in systems contaminated with human sewage. Using QMRA, Lim *et al*.[8] demonstrated that the use of untreated stormwater was deemed unacceptable for applications associated with irrigation of food crops based on the levels of human enteric viruses reported in stormwater. Studies examining water quality in stormwater systems in South Carolina also reported significant levels of antibiotic-resistant bacteria[27,28], raising further concerns for certain water reuse applications. The incidence of Legionnaires' disease has been linked to increased precipitation[30], and since *L. pneumophila* is a respiratory pathogen transmitted through aerosols, re-used for toilet and urinal flushing, aesthetic water spray features, irrigation, and vehicular washing could play an important role in transmission of this agent. An outbreak of *L. pneumophila* serogroup 1 in Auckland, New Zealand, was linked to rainwater collection systems as well as water blasters used to clean boats[31]. *L. pneumophila* concentrations in rainwater collected for recreational purposes, such as spray parks, has also been shown to represent an unacceptable health risk[16,32-34]. In one report from Italy, two patients who were regular users of car washes contracted *L. pneumophila* (based on serology), and although the bacterium was not cultured from patient samples, *L. pneumophila* was able to be cultured from the hot water nozzles at the car washes used by the patients (Baldovin et al., 2018). A second report from the Netherlands documented a patient presenting with legionellosis for which the strain *of L. pneumophila* identified from the patient was identical to a strain isolated from a car wash used by the patient (Euser et al., 2013).

It is well known that faecal indicator bacteria (FIB [*E. coli* and faecal coliforms]) represent poor surrogates of pathogen occurrence in stormwater/rainwater, largely due to the fact that enteric viruses, parasitic protozoa, and helminth ova are, by comparative standards, more environmentally-resistant than FIB[36]. In addition, FIB are completely unrelated to the presence of environmentally-derived pathogens[36,37]. Parasitic worm eggs can survive and persist in the water/soil environment for years [38,39], and are highly resistant to both environmental desiccation and UV- inactivation [40]. Some enteric viruses (norovirus, astrovirus) show no appreciable loss of infectivity when seeded into water after several months, and can also survive desiccation for long periods of time[23]. Consequently, these pathogens can bioaccumulate in the receiving environment under repeated application (e.g. irrigation). Public health

risks may be dynamic, and change depending on the water demand, climatic cycles, and sources of pollution.

Various watershed models have been employed over the past two decades to attempt to physically model the fate and transport of microorganisms. Ferguson *et al*. [47] review issues relevant to microbial contaminant transport, listing notable uses of existing watershed hydrological models that were modified to consider microbial transport. The models vary widely in scale, applicability and capacity and are generally limited in predictive function because fundamental research is still required to answer key questions related to inactivation and transport. As the literature shows, many knowledge gaps still remain when using process-based methods for predicting the fate and transport of microbial contaminants in watersheds because of the level of complexity due to soil heterogeneity, land use characterization and the inherent variability in migration pathways as affected by the environment[58]. Bradford *et al*.[58] also noted that little is known about how to quantify transport and survival parameters at *the scale* of agricultural fields or watersheds. They suggest that modeling efforts need improvement in order to achieve a realistic assessment of the risk of waterborne disease transmission. In addition, many of these models do not represent conveyance through pipe systems that comprise part of the minor drainage system and thus, cannot effectively model cross-connections from sewer systems**.** He *et al*.[59] studied FIB levels in the pipes leading to a stormwater retention pond in Calgary and identified further complexities, such as contamination that appears during later rainfall events that is different than the original sources. To assess the feasibility of using stormwater for irrigation purposes in Calgary, Canada, both field and modeling studies were conducted to characterize the microbial indicators in stormwater runoff as well as the water in a major retention pond[59,61,62]. He *et al*.[62] demonstrated that artificial neural network (ANN) models out-performed multiple linear regression in predicting stormwater runoff quantity; suggesting that ANNs are promising tools to simulate event-based stormwater runoff quality for various physicochemical water quality parameters using hydro-meteorological variables as inputs. For example, Lin *et al*.[63] successfully adopted ANNs to predict near-shore coliform bacteria concentrations using rainfall, river flow, sunlight and tidal condition as inputs. He *et al*.[61] concluded that intermittent rain events contribute to elevated microbial levels in a stormwater pond and this demonstrated the potential influence of climate on microbial indicators. The potential linkages between microbial contamination and hydro-meteorological variables and the use of ANNs for modelling physicochemical water quality parameters in stormwater runoff and stormwater imply that ANNs and other data-driven models, which capture nonlinear relationships between inputs and output, are promising in modeling microbial contamination in stormwater if the datasets required are available. However, few works exist that provide a critical evaluation of the state-of-the art for microbial contaminant transport. Pachepsky *et al*.[66] critically evaluated the approaches used for various modeling components and identified the following serious challenges to progress: i) the paucity of experimental data about the transport of pathogenic microorganisms; ii) the uncertainty in background concentrations of indicator microorganisms; iii) understanding the uncertainty of the stream sampling data; and iv) up-scaling techniques to use local and small-scale measurements in watershed scale modeling. According to Ferguson *et al*.[47], knowledge gaps related to the microbial component of the process based models include (but not exclusively): a) inactivation kinetics of pathogens in soil and fecal matter and b) characterization of pathogen properties and watershed characteristics that affect transport and attenuation. Thus, there are many obstacles to successfully implementing a process-based transport model and many of these revolve around the uncertainty that exist in the data, the small data sets typically available, and the lack of knowledge of pathogen sources/transport mechanisms, and the scale at which they dominate. These issues largely prohibit accurate microbial risk assessments.

Overall, urban planning must incorporate public health risk assessments associated with microbial threats, for which Water Reuse Safety Plans (WRSP) have been encouraged, and are used in countries such as Australia for managing risks[35]. From an Alberta context, there is virtually no data on the

occurrence and concentrations of human enteric viruses, anthropogenic/zoonotic pathogenic bacteria/parasites, and environmentally-acquired bacteria (i.e., *Legionella*) in stormwater/rainwater systems. In addition, we have little or no understanding of the faecal sources of contamination in these systems. This significant knowledge gap precludes the development of stormwater policies or frameworks that can effectively protect public health. Reluctance towards implementation of an alternative water use framework rests largely in the knowledge gaps associated with pathogen occurrence in these systems[8], and the lack of risk assessment and transport modeling that supports various water-fit-for-purpose scenarios[8]. Challenges also relate to the explicit choice of a tolerable risk-based target, the efficacy of contaminant removal barriers, and the effect of system failures on health [45]. In addition, the inability to quantify transport and loading of pathogens impinges on effective risk assessment.

Another challenge relates to the plethora of faecal inputs contributing to contamination of water sources such as stormwater. The recent development of microbial source-tracking tools in the field of environmental microbiology, has provided more nuanced quantification of faecal loading from multiple sources during stormwater hydrographs[42]. Proportioning the range of faecal source mixtures in stormwater enables one to also proportion the pathogen component, which, when combined as inputs into a stochastic QMRA, enables the estimation of human exposure risks for a range of scenarios and among different pathogens[43]. Treatment requirements and monitoring targets can then be estimated to keep waterborne exposure risks under the WHO and Health Canada annual risk benchmark of one disability adjusted life year per million (1 µDALY). This approach is currently used in Australia and represents the best management practice internationally for water reuse, feeding into site-specific water reuse management plans (WRMP)[35].

Key Drivers

It is estimated that the City of Calgary's population will grow by a staggering 1.3 million people (1) over the next 50-60 years raising concerns regarding long-term sustainability of water quantity and quality within the Bow River Basin. The City of Calgary has invested significant resources in examining future economic sustainable development options through the use of reclaimed wastewater. A report commissioned by the City of Calgary in 2012 examined opportunities for water reuse for toilet/urinal flushing and irrigation. The report focused on infrastructure requirements necessary to support the development of two residential communities in the Calgary area, housing as many as 276,000 people and creating 85,000 local jobs for the province of Alberta (2). The projected demand for reused wastewater for toilet/urinal flushing and irrigation in these new communities alone was estimated at 7.5 billion liters per year.

Existing regulatory impediments jeopardize these future growth opportunities. For example, due to the excessive demands on current water resources, a moratorium on water extractions from the Bow River is currently in place. Consequently, future municipal growth is dependent upon development of a water reuse framework, particularly in Southern Alberta. It is anticipated that within the next 10-15 years the City of Calgary will also require the construction of additional waste treatment facilities to meet its growing population (2), and thus, the research carried out on this project affects multi-million dollar decisions regarding the most effective ways to manage water stewardship through implementation of water reuse strategies. Delays, or implementation of a poorly developed regulatory frameworks, could result in major economic burdens and liabilities to Albertans in the future, whereby all economic gains are lost due to a lack of water quantity and increased burden of disease/health care costs resulting from reuse of inappropriately treated or managed alternative water sources.

Original Goals/Objectives and Subsequent Changes

ORIGINAL GOALS & OBJECTIVES: The original goal of this proposed study was to work with municipalities and provincial/national policy regulators in filling the knowledge gaps regarding microbial contamination sources and risks associated with water re-use (stormwater/ rainwater [and wastewater]). The ***ultimate goal*** of our research was to translate this knowledge into an evidence-based policy framework provincially (and nationally) and in order to support the use of alternative water sources in Alberta (and Canada) while ensuring the protection of the public's health. The original objectives of our *three-year* research program, were to:

1. *Evaluate microbial water quality, pathogen occurrence and treatment efficacy in stormwater and rainwater systems in urban municipalities in Alberta.*
2. *Use quantitative microbial risk assessment approaches to strategically identify water-fit-for purpose reuse options for stormwater and rainwater.*
3. *Develop process-based and probabilistic models of microbial contamination in urban stormwater ponds.*
4. *Develop a Stormwater Use Management Plans (SUMP) Framework to support rainwater and stormwater use in Alberta*

CHANGES TO THE ORIGINAL PROJECT: It is important to note that the *four* objectives noted above, along with the ultimate goal of developing a water reuse regulatory framework for Alberta, remained our key strategic deliverables for this project. All extraneous pressures and challenges (including co-funding) were judged against these key objectives and the ultimate goal of the project. Changes to the original grant included the following:

- Removing the City of Edmonton as one of partnering municipalities – at the time of writing the original proposal to Alberta Innovates, the City of Edmonton was included as a participating member and for which the city was providing research funds to support the work ($75,000 cash [plus addition in-kind]). However, the Drainage Services Branch (i.e., agency responsible for stormwater management) from the City of Edmonton was acquired by EPCOR shortly after the grant was submitted to Alberta Innovates, and for which the city could no longer commit cash funding to this project.
- We originally proposed to match the Alberta Innovates grant with co-funding support from City of Calgary ($255,000 cash [and in-kind]), City of Airdrie ($60,000 in-kind) and City of Edmonton ($75,000 cash [and in-kind]) with an NSERC (Natural Sciences and Engineering Research Council Grant) Collaborative Research and Development Grant (CRD [$435,000]). The original proposed project to Alberta Innovates requested $1.435 million in cash funding (i.e., total from Alberta Innovates and all co-funding from partners). Since the City of Edmonton could no longer commit funding, the research scope was changed, and for which the NSERC grant was re-written to focus on research needs in Calgary and Airdrie as contributing partners.
- An NSERC-CRD grant was eventually submitted in June 2017 by the project team, but as a team, we were not notified of its success until June 25, 2018. Typically, NSERC-CRD grants are awarded on 3-month review turn-around-time, but unfortunately, this round of competitions took NSERC a full year

of review before decisions on funding were finally made. Upon announcement of the award, NSERC required that a single legal contract be signed by all agencies participating in the research (City of Calgary, City of Airdrie, University of Calgary, University of Victoria and University of Alberta) and before any funds could be released. NSERC provided a 6-month 'period-of-notice' to the project team to negotiate the terms of the contract (i.e., NSERC deadline set for December 25, 2018). The project team submitted a signed copy of the legal contract to NSERC on December 23, 2018. NSERC approved the legal contract in February, 2019. Full release of funds from NSERC was not secured by researchers until April 2019, once all additional approvals were in place (e.g., university /environmental health approvals, trust accounts, etc.). As such, funding for the NSERC-CRD portion of this project runs from September 1, 2018, to August 31, 2021. It is important to note the 'off-setting' of these deadlines from the Alberta Innovates grant (which run from May 1, 2016 to February 29, 2020). As such, our work continues on this project, even though funding has expired rom Alberta Innovates.

- Since NSERC-CRD grants require matching dollars from municipal/industry agencies (cash and in-kind), the funding from the City of Calgary was also not accessible to the project team until the NSERC was approved. Thus, funding from the City of Calgary is directly linked to the time frames set out by NSERC-CRD (September 1, 2018, to August 31, 2021), and thus, are offset from the Alberta Innovates time frames.
- Alberta Innovates originally required that all co-funding be in place by the project team before their funding could be accessed. Given the challenging funding issues described above, Alberta Innovates released their funding in the fall of 2016 (allowing the team to begin work on the project), and in good faith that the team would secure the additional funding sources as outlined.
- The overall level of cash funding originally proposed to Alberta Innovates ($1.435 million) is close to the final cash funding secured by the project team ($1.437 million), albeit the funding timelines are now offset. As such, work continues on the project (through NSERC-CRD and City of Calgary funding) on aspects originally proposed in the Alberta Innovates grant. Herein, we report on the work done to date on the original proposal.
- Given the challenges and uncertainty in funding, members of the team secured additional funding resources during the course of this project to help support elements that were originally proposed in the Alberta Innovates grant. Drs. Ashbolt, Neumann and Ruecker secured a *Canadian Institutes of Health Research (CIHR) Grant* entitled, "Developing a Framework for Water Reuse in Canada: using quantitative microbial risk assessment (QMRA), risk communication, and community engagement for evaluating water-fit-for-purpose" ($1,999,495 [2016-2021]). This grant allowed for some activities originally proposed in the Alberta Innovates grant to be directed onto the CIHR grant (e.g., risks associated with *Legionella* spp. and the saprozoic microbes).
- Due to the 'offsetting' of the funding cycles between granting agencies, the team needed to prioritize certain research elements in the early phases of funding (2016/2017/2018) in order to meet the objectives and ultimate goals outlined in our Alberta Innovates proposal. The objectives for the original grant were modified as follows:

- *Objective 1: Evaluate microbial water quality, pathogen occurrence and treatment efficacy in stormwater and rainwater systems in urban municipalities in Alberta*
    - Research on this objective focused on developing an in-depth examination of bacteriological water quality in stormwater systems in Calgary (with some work in Airdrie), and in alignment with current provincial testing protocols. Pathogen monitoring focused on the occurrence of enteric bacterial pathogens in stormwater (as opposed to viruses or parasites), largely due to the simplicity of adapting these parameters to our bacteriological water quality monitoring programs. The team also focused on identifying sources of fecal pollution impacting water quality at these sites, with the intent to identify those sites impacted by human fecal pollution and for which follow up testing could be directed (i.e., viruses and parasites). For example, we have identified certain stormwater outfalls/ponds that are consistently impacted by human waste, and therefore are targeting these sites in the upcoming field season (2020) for virus and parasite sampling (through the NSERC-CRD grant). Samples will also be tested for antibiotic resistant microorganisms (*E. coli*)
    - Although some work was done on Nose Creek stormwater outfalls in partnership with Airdrie, for the 2020 field season we are developing a stormwater monitoring program for the Nose Creek as well as stormwater ponds intended for reuse. A planning meeting with Airdrie occurred on February 20, 2020, to discuss the results of the project to date and begin the planning for the 2020 field season.

- *Objective 2. Use quantitative microbial risk assessment approaches to strategically identify water-fit-for purpose reuse options for stormwater and rainwater.*
    - This objective is largely completed. The work done represents the backbone of the current legislative policy framework being developed by Alberta Environment and Parks and Alberta Health/Alberta Health Services. This is important to note, as it does represent the achievement of the *ultimate goal* of the proposed research project to Alberta Innovates.
- *Objective 3. Develop process-based and probabilistic models of microbial contamination in urban stormwater ponds.*
    - This work has been ongoing and there is little change to the overall plans laid out in the original Alberta Innovates proposal, except for continuance of the work through NSERC-CRD and City of Calgary funding. For example, we have completed critical field work in 2019 (i.e., autosamplers triggered for sampling) to understand mobilization and transport of pathogens in stormwater, and for which models of transport developed by the project team on historical data will be important in the evaluating the temporal robustness of these integrated computational flow dynamic models.
- *Objective 4. Develop a Stormwater Use Management Plans (SUMP) Framework to support rainwater and stormwater use in Alberta*
    - This work is largely completed, and represents a major achievement by the project team in fulfilling the ultimate goal of the project – i.e., to translate this knowledge into an evidence-based policy framework provincially to support the use of alternative water sources in Alberta while ensuring public health protection. Objectives 2 and 4 form the fundamental framework for provincial guidance policies being developed *by Alberta Environment and Parks* and *Alberta Health* (see project results section), and for which members of the research team were actively involved.

SECTION FOUR: Project Results

4.1: PROJECT ACCOMPLISHMENTS/RESULTS

The milestones laid out for this Alberta Innovates project are linked to each of the 4 key objectives laid out in the original proposal. Critical tasks represent the specific aims within the original grant, and our research progress/results on each of these objectives/milestones are discussed individually in each of the sections below.

OBJECTIVE 1

Evaluate microbial water quality, pathogen occurrence and treatment efficacy in stormwater and rainwater systems in urban municipalities in Alberta. The critical tasks for this project included:

- Selection of urban stormwater and rainwater systems.
- Evaluate microbial water quality of stormwater and rainwater systems.
- Use MST to identify sources of pollution in stormwater and rainwater systems.
- Identify and characterize fecal pathogen occurrence and prevalence in stormwater and rainwater systems.
- Identify and characterize the occurrence and prevalence of environmentally-derived pathogens in stormwater and rainwater.
- Evaluating treatment efficacy for stormwater and rainwater systems.

RESULTS AND PROGRESS (Objective 1)

The results and progress on each of the milestone are presented below, and compiled as excerpts from the graduate thesis of Megan Beaudry, an MSc student at the University of Alberta (Thesis Title: *From Nuisance to Resource: Understanding Microbial Sources of Contamination in Urban Stormwater-Impacted Bodies of Water Intended for Water Reuse Activities*).

Stormwater Ponds (2017)

WATER QUALITY CHARACTERISTICS. To determine the microbial quality of stormwater, the sources of fecal contamination, and the pathogens present, stormwater samples in 2017 were collected semi-weekly over 20 weeks, with an additional sample collected on the 21st week. Grab sampling began as soon as stormwater ponds were fully thawed (i.e., May 9th, 2017), and ended just before freezing (September 25th, 2017). These ponds were chosen for sampling due to the potential for water reuse implementation (i.e., irrigation) at these sites. Samples were collected at three stormwater ponds in Calgary, Alberta, Canada, and included: a) McCall Lake, b) Country Hills Stormwater Facility, and c) the Inverness Stormpond. At each pond, we sampled four (i.e., McCall Lake and Inverness) or five (i.e., Country Hills) locations (**Table 2**, **Figure 1**, **Figure 2**, and **Figure 3**). Each site was sampled 41 times. In 2019, samples were once again collected from McCall Lake (grab samples) and from the Inverness

Stormpond (grab samples and autosamplers triggered for sampling during storm events). Land characteristics of the drainage network (i.e., stormshed) are also presented in **Table 2** .

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

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

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

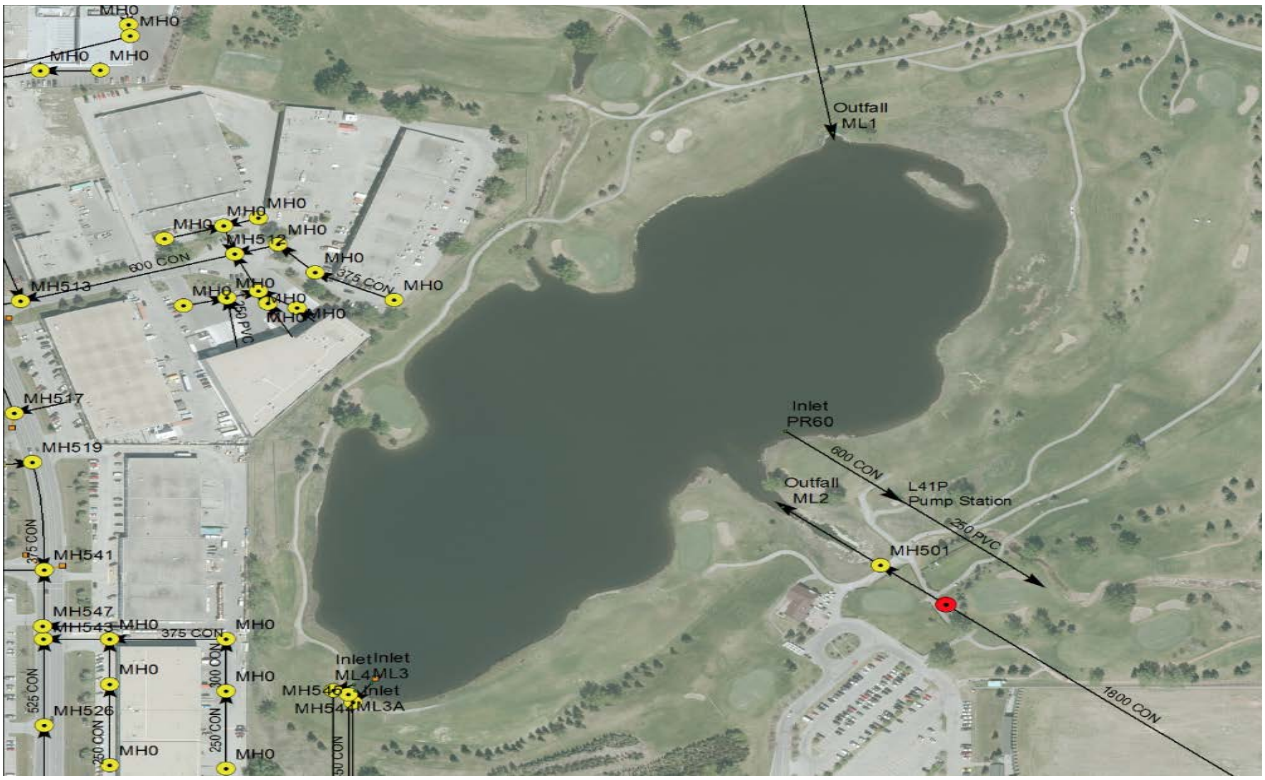


Figure 1. *Arial photo of McCall Lake. The yellow circles represent storm manholes, the orange squares represent catch basins, the black arrows indicate the direction which storm drains flow, and the black lines are storm pipes [provided by The City of Calgary].*

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

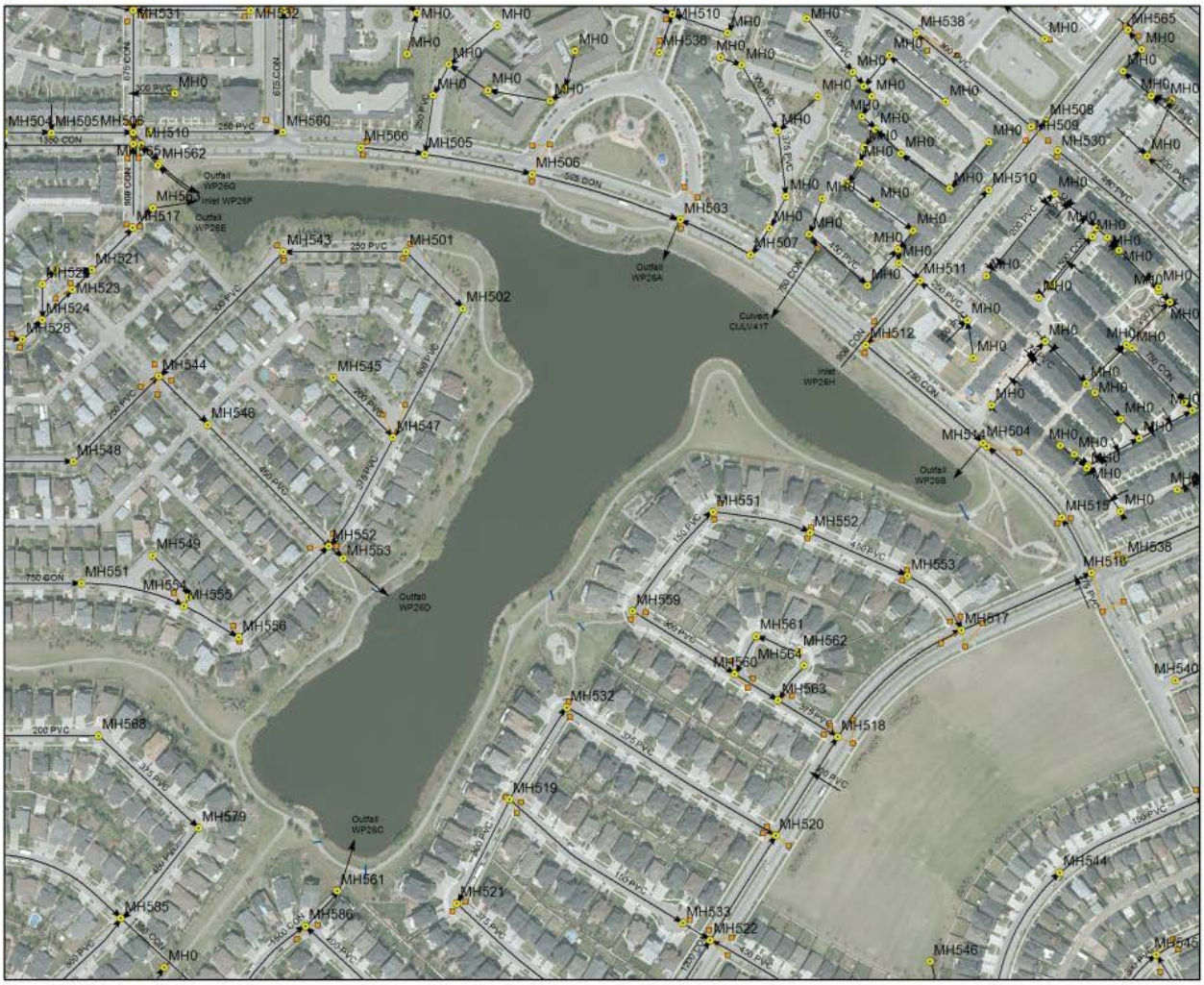


Figure 3. *Arial photo of Inverness Storm Pond. The yellow circles represent storm manholes, the orange squares represent catch basins, the black arrows indicate the direction which storm drains flow, and the black lines are storm pipes [provided by The City of Calgary].*

***Table 2**: Stormshed characteristics for the urban stormwater ponds (provided by City of Calgary)*

Stormwater Pond Facility	**Sampling Site**	**Hydrological positioning of inlet/outlet (above grade, below grade, or equal grade)**	**Catchment size in hectares (Overland Drainage size in Parentheses)**	**Land Use Characteristics for Catchment Area (Land Use Characteristics for Overland Drainage in parentheses)**					
				Residential	***Industrial***	***Infrastructure/ Transportation***	***Parks and Institutions***	***Commercial***	***Future Development***
McCall Lake	ML2	Above	275.99	4	69	4	11	12	0
	PR60[a]	Below	-	-	-	-	-	-	-
	ML1	Equal	1464.35	37	20	23	10	7	3
	Inlet 3/4[a]	Below	-	-	-	-	-	-	
	Total		**1830 (89.09)**	**30 (0)**	**26.8 (6)**	**19.4 (0)**	**14 (94)**	**7.3 (0)**	**2.6 (0)**
Country Hills	WP31A	Below	38.96	95	0	0	5	0	0
	WP31B[a]	Below	-	-	-	-	-	-	-
	WP31C	Below	28.49	5	0	17	28	50	0
	WP31D	Below	172.28	75	0	1	15	8	0
	WP31E	Below	10.63	100	0	0	0	0	0
	Total		**267**	**68 (20)**	**0 (0)**	**3 (2)**	**18 (77)**	**10 (1)**	**0 (0)**
Inverness	Inlets/outlets	Below	31.54	76	0	1	9	14	0
	WP26B	Below	89.52	72	0	4	20	4	0

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

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

Table 3. *Microbial water quality in the stormwater ponds based on the percentage of samples failing existing standards of water quality.*

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

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

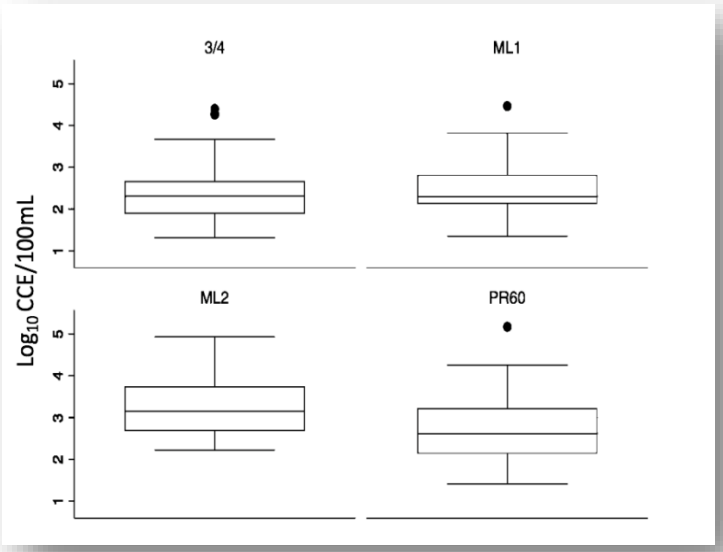
Variation in bacteriological water quality was also observed among sampling sites within a single pond. The most contaminated site across all stormwater ponds examined was site ML2 at McCall Lake, with upwards of 65% of all samples failing the US EPAs guidelines for recreational water quality for *E.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

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

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

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

It is important to note that in most cases for *Enterococcus* and *E. coli* at sites other than ML2, there were several outliers in the data set (**Figure 4**). In the context of this study, outliers were defined as a data point greater or less than 1.5*interquartile range (i.e., whiskers). Although outliers may reflect recent localized contamination events not necessarily reflective of overall water quality in the stormwater pond (e.g., aquatic birds in one area of the pond), their occurrence could also reflect the periods of peak contamination in stormwater ponds, and for which this effect may be contingent on temporal variables associated with water quality (e.g., first flush from storms, to be discussed later). Specifically, outliers for *Enterococcus* concentrations were represented by values higher than ~3.5 $\log_{10}$ for Inlet 3/4, ~3.75 $\log_{10}$ for ML1, and ~4 $\log_{10}$ for PR60. Similarly, outliers for *E. coli* concentrations occurred in Inlet 3/4 above ~2.5 $\log_{10}$ and in PR60 above ~3.25 $\log_{10}$. In some cases, the outliers were at an equal level of contamination of that observed in the ML2 range of values (i.e., 2-5 $\log_{10}$ CCE/100 mL for *Enterococcus* concentrations and 1-3.5 $\log_{10}$ CFU/100mL for *E. coli* concentrations) (**Figure 4**). The single greatest concentration of *Enterococcus* observed during the study period was observed at site PR60. The greatest concentration of *E. coli* observed was at ML1. Consequently, although ML2 represented the most consistently contaminated sampling site at McCall Lake, the other sampling sites in the stormwater pond appeared to be at risk for significant levels of periodic bacterial contamination. This observation warranted a closer examination of the temporal variance of bacteriological water quality in each of the ponds and at each of the sites within the ponds.



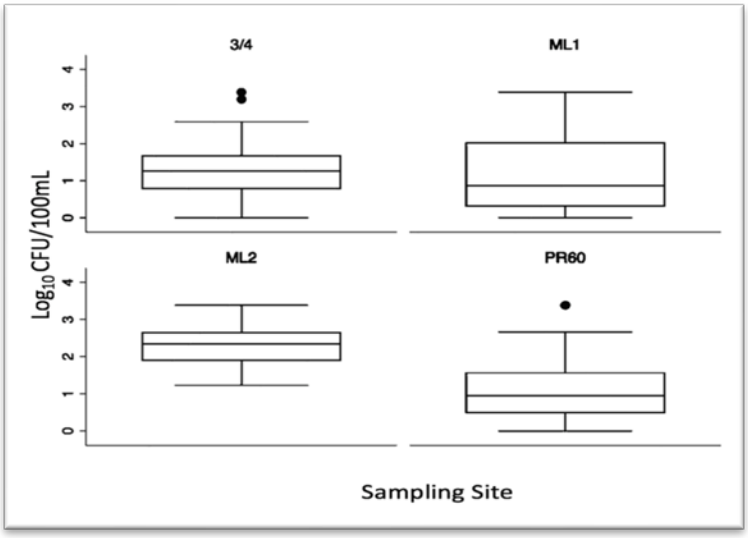


Figure 4. *Box and Whisker plot of Enterococcus log_{10} values (top) and E. coli log_{10} (bottom) values in McCall Lake over 21 weeks broken down by sampling site (i.e., ML2, ML1, Inlet ¾, and PR60). The outer edges of the box represent the 25th and 75th percentiles (i.e., interquartile range), and the line within the box represents the median. The location of median indicates the skew of the data. The whiskers represent the interquartile range*1.5. The outliers are determined by being greater or less than 1.5 times the upper of lower interquartile ranges as represented by circles.*

Significant temporal fluctuations in bacteriological water quality were observed between the stormwater ponds, and among the sampling sites within a stormwater pond (**Figure 5**, **Figure 6**). In the context of McCall Lake, sampling site ML1 showed the greatest fluctuations of the microbial water quality indicator *E. coli* from one sampling date to the next (**Figure 5**). For example, within one week, and in three consecutive samples from August 9th – 16th, water quality varied from below the statistical threshold value (STV) at 0.3 $\log_{10}$ CFU/100mL to above the STV at 3.5 $\log_{10}$ CFU/100mL then back below the STV at 1.7 $\log_{10}$ CFU/100mL (**Figure 5**). This high variability represented a significant fluctuation in water quality in a single week's time, raising potential concerns about the sampling frequency needed for water quality monitoring programs. In consideration of the STV threshold where we sampled biweekly, five water quality violations were due to extreme fluctuations in water quality indicators at ML1 (**Figure 5**). In fact, between any two sequential samples, variations in *E. coli* concentrations at ML1 could go from 0 $\log_{10}$ CFU/100mL to 4 $\log_{10}$ CFU/100mL (e.g., July 6th to July 10th). Although ML2 had a more consistent baseline contamination level (i.e., a higher median) than all other sites at McCall Lake, occasionally, water was of equally poor quality at some of the other sites, warranting a closer examination of the temporal patterns of occurrence associated with these failures across all sites in McCall Lake.

Interestingly, all of the sampling sites in McCall Lake failed the STV for *E. coli* on May 25th, July 10th, August 7th, and September 13th. Similarly, low values of *E. coli* were observed in all sampling sites at McCall Lake on May 16th, June 27th, July 6th, and September 5th, suggesting a common variable linking contamination among all sampling sites within that pond. Since it is well-known that precipitation can lead to pathogen transport, we examined the amount of precipitation to see the effects on the levels of fecal indicator bacteria. For simplicity, we evaluated potential relationships between antecedent rain (i.e., rain within 72 hours) and bacterial indicator values, noting that the highest values of antecedent rain occurred on May 25th, August 7th, and September 13th along with the highest values of bacterial indicators; whereas, the lowest values of antecedent rain occurred on May 16th, June 27th, July 6th, July 10th, and September 6th (i.e., no rain in the past 72 hours) and correlated with lower bacterial indicator values. The data suggests that on these dates, storm precipitation led to mobilization of fecal sources within the stormshed and affecting water quality at all sites.

As with the *E. coli* results, there was considerable temporal variability for *Enterococcus* in each of the stormwater ponds and between each of the sampling sites at each stormwater pond. *Enterococcus* levels at the McCall Lake sampling sites could be highly variable from week-to-week. A temporal change of ~2.5 $\log_{10}$ CCE/100mL with the resulting value being above ~4 $\log_{10}$ CCE/100mL was observed multiple times at each McCall Lake sampling site though usually on different sampling dates (e.g., ML2 on September 13th, ML1 on August 14th, PR60 on May 25th, and Inlet ¾ on July 17th) (**Figure 6**). This data overall suggested that microbial water quality indicators (i.e., *Enterococcus* and *E. coli*) could be highly variable (i.e., greater than 2.5 $\log_{10}$) in a relatively short period of time (i.e., two-to-five days).

Spatial-temporal variability in water quality was analyzed by the use of a 5-sample running geometric mean between stormwater ponds and among sampling sites within a single stormwater pond. Similar to what has been stated above regarding the trend of higher levels of microbial water quality indicators, sampling site ML2 at McCall Lake also had the highest 5-sample running geometric mean during the 21-week sampling season, the trends of which are presented in **Figure 5** and **Figure 6**. Site ML2 violated the 5-sample running geometric mean standard for *E. coli* (i.e., 2.1 $\log_{10}$ CFU/100mL) for all sampling dates, except for a three-week stretch of the 21-week sampling season (i.e., June 15th through July 6th) (Figure-3-2). Further, within McCall Lake, all other sampling sites (i.e., ML1, Inlet ¾, and PR60) did not violate the 5-sample running geometric mean for *E. coli* during the entire 21-week sampling season (**Figure 5**). A comparable pattern was reflected in the concentration of *Enterococcus* between sampling sites at McCall Lake: ML2 however violated the 5-sample running geometric mean for *Enterococcus* throughout all 21 weeks of the sampling season, with the geometric mean being above the standard of

2.48 $\log_{10}$ CCE/100 mL (**Figure 6**). In contrast, at all other McCall Lake sampling sites (i.e., ML1, Inlet ¾, and PR60), the 5-sample running geometric mean for *Enterococcus* had less violations than ML2 (**Figure 6**). However, the 5-sample running geometric mean for *Enterococcus* reflected more water quality failures than for *E. coli*. Overall, this data suggested that ML2 had poorer water quality throughout the duration of the 21-week sampling season in comparison to the other McCall Lake sampling sites.

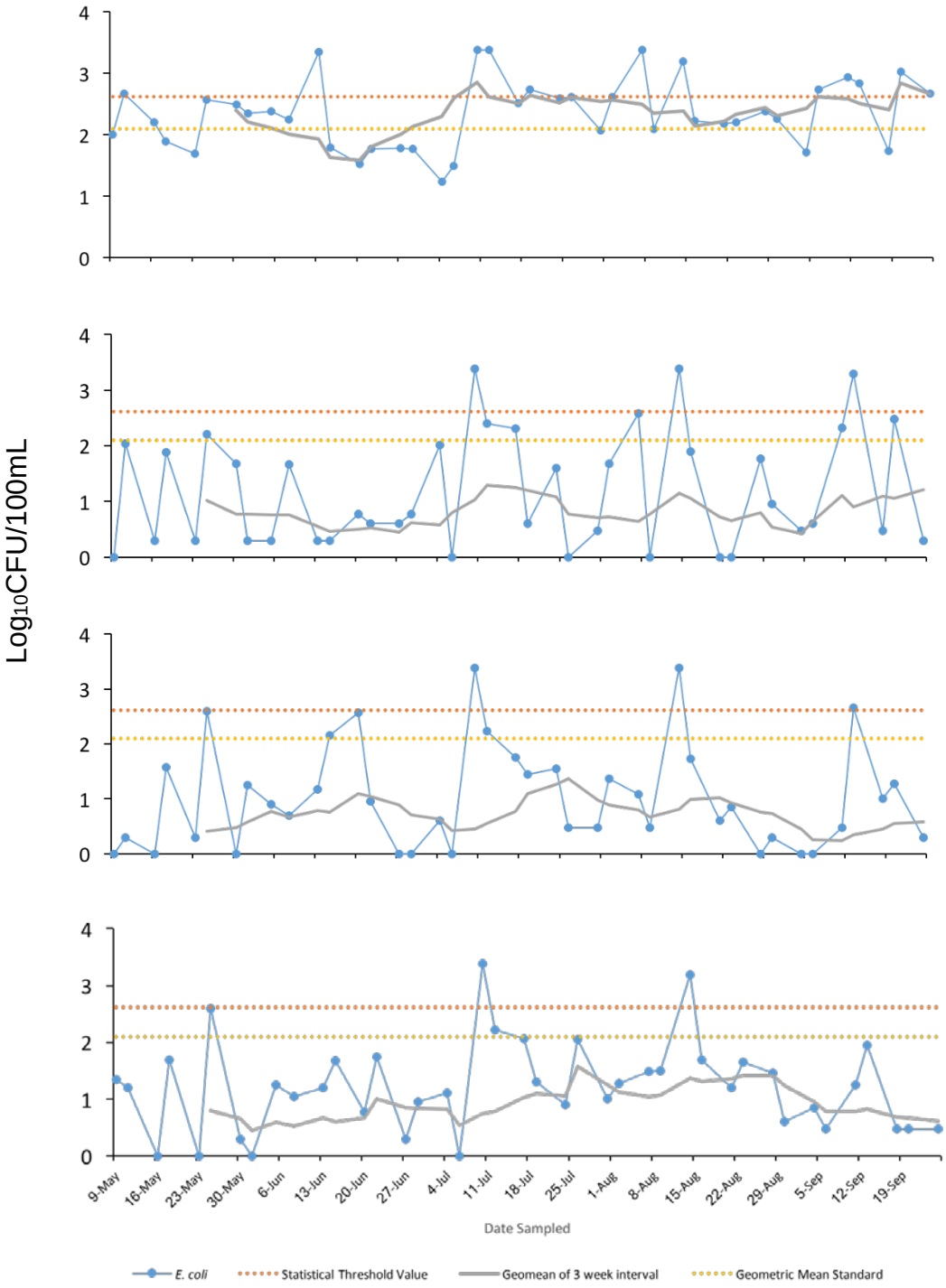


Figure 5. *Temporal pattern of occurrence of E. coli log$_{10}$ concentrations at sampling site ML2 (top), ML1 (second from the top), PR60 (third from the top), and Inlet ¾ (bottom) in McCall Lake over 21-weeks. The* US EPA Guidelines for Recreational Water Quality *geometric mean standard of >126 CFU/100mL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

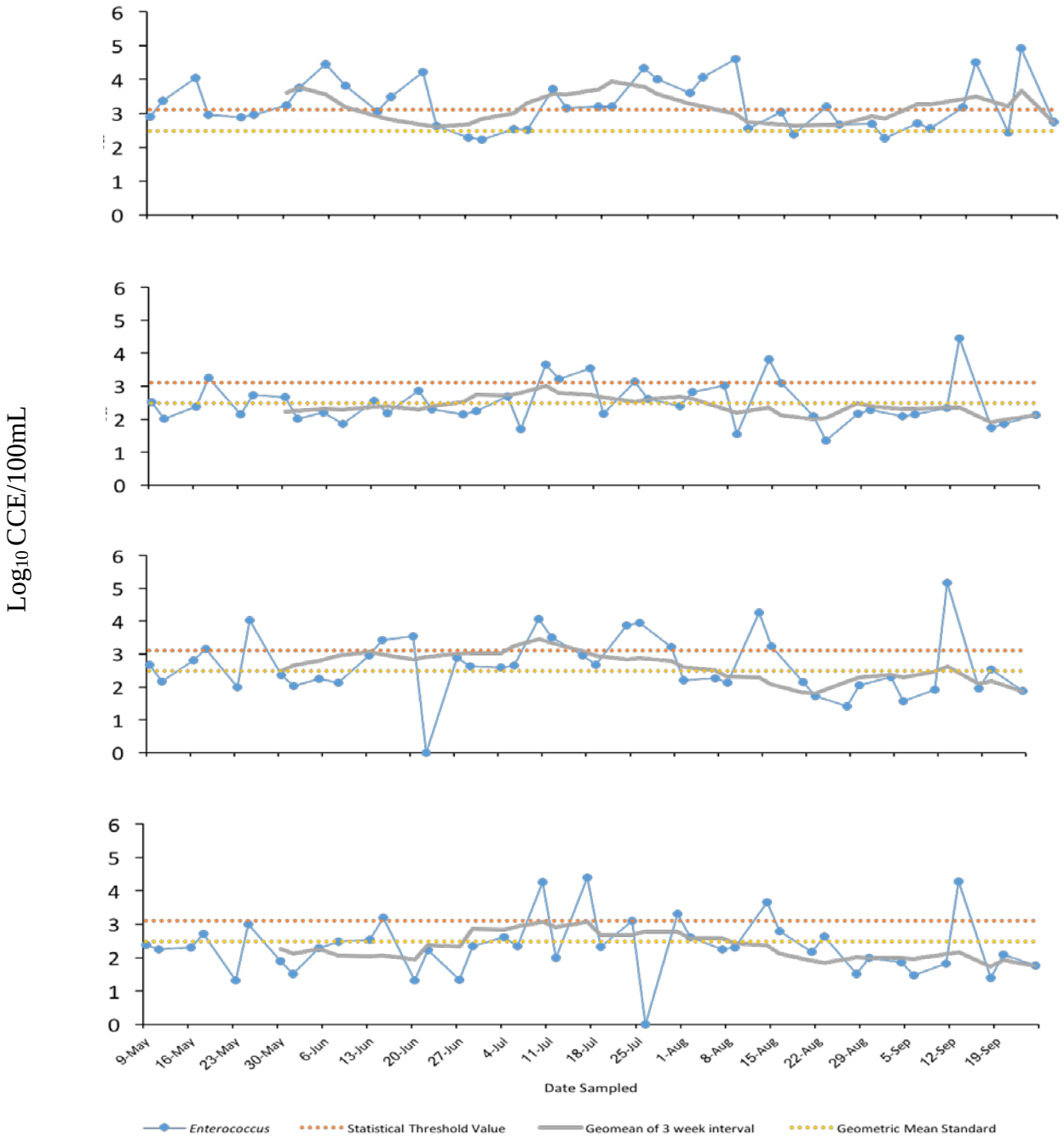


Figure 6. *Temporal pattern of occurrence of Enterococcus log$_{10}$ concentrations at sampling site ML2 (top), ML1 (second from the top), PR60 (second from the bottom), and Inlet ¾ (bottom) located in McCall Lake over 21 weeks. The* US EPA Guidelines for Recreational Water Quality *geometric mean standard of >300 CCE/100mL (yellow dotted line) and a single sample threshold value of >1280 CCE/ 100mL (red dotted line) are also provided. The 5-sample running geometric mean of the water samples is in gray, and the individual water sample concentrations of Enterococcus are in blue.*

MICROBIAL SOURCE TRACKING. A high-level descriptive overview of the frequency of occurrence of microbial source tracking markers in each of the Calgary stormwater ponds, and at each of the sites, is provided in **Table 4**. Calgary stormwater ponds were shown to be predominantly impacted by human and gull feces (**Table 4**). The human specific markers, HF183 and HumM2, were detected at 27% and 10%, of all samples, respectively (**Table 4**). The gull specific marker (i.e., LeeSg) was found in 9% of samples (**Table 4**). Of these, the more dominant source of fecal pollution was from humans (**Table 4**). All other host-specific markers (i.e., dog, Canada geese, muskrat, and ruminants) were detected in ≤2% of pond samples (**Table 4**).

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

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

The highest levels of gull fecal contamination were also observed at McCall Lake, with 15% of samples possessing the seagull microbial source tracking marker [LeeSg] (**Table 4**). Spatial variability was evident when comparing the stormwater ponds, as 10% of samples were positive for LeeSg in Country Hills Stormwater Facility, and only 4% of samples were positive for this same marker in the Inverness Stormpond.

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

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

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

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

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

Based on the variation of human fecal contamination markers among: a) the different stormwater ponds, and b) sites within a single stormwater pond, we examined the spatial and temporal characteristics of human fecal contamination in each of the stormwater ponds and at each of the sites within a single stormwater pond using detection of the two human markers (i.e., HF183 and HumM2). In congruence with the finding that ML2 at McCall Lake was the most frequently contaminated site with human feces, this site also had the greatest median concentration of the human fecal marker HF183 (i.e., 4.0 $\log_{10}$ copies/100 mL) observed across all three stormwater ponds and sampling sites in these ponds (compare Figure 4-1 [McCall Lake]). In comparison, all other McCall Lake sampling sites had a median concentration of HF183 at ~3.4 $\log_{10}$ copies/100 mL (i.e., close to the quantification limit of the assay) (**Figure 7**).

As was indicated previously, outliers in the data may reflect localized contamination events/conditions (e.g., recent undiluted deposition of feces, infrastructure failure, as a break in a sewer line) representing times of peak contamination in the urban stormwater ponds. Specifically, at ML2, there was a single outlier in the data set for HF183, represented by a value of 6.0 $\log_{10}$ copies/100 mL (**Figure 7**). However, although ML2 represented the most consistently contaminated sampling site with human fecal contamination at McCall Lake, all other sites appeared to be at risk for human fecal contamination.

A spatiotemporal pattern of contamination was noted regarding the detection of human fecal source tracking markers at McCall Lake. On at least three occasions, HF183 at McCall Lake was detected concurrently at all sampling sites (i.e., PR60, ML2, ML1, and Inlet ¾), suggesting a potentially common environmental variable associated with transport of these contaminants into the ponds.

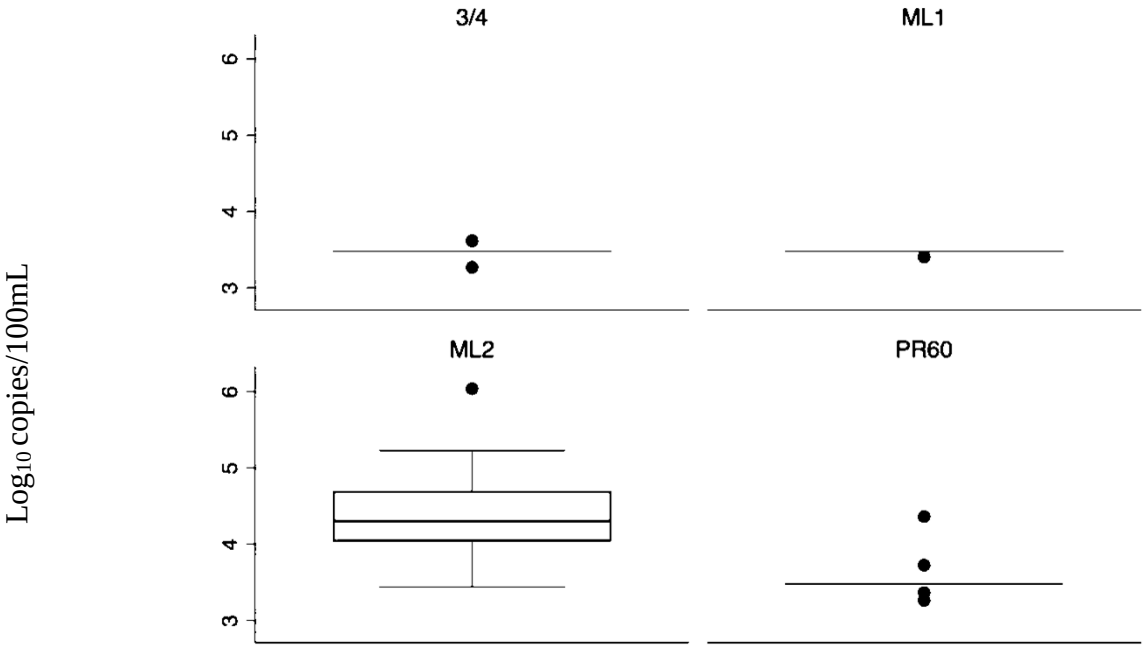


Figure 7. *Box and Whisker Plot of HF183 levels by sampling site in McCall Lake (ML2 n=38, ML1 n=6, PR60 n=13, Inlet ¾ n= 5). The outer edges of the box represent the 25th and 75th percentiles (i.e., interquartile range), and the line within the box represents the median. The location of median indicates the skew of the data. The whiskers represent the interquartile range*1.5. The outliers are determined by being greater or less than 1.5 times the upper of lower interquartile ranges as represented by circles.*

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

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

HumM2 was detected less frequently and at lower concentrations in all of the urban stormwater ponds tested. ML2 had the highest occurrence of HumM2 detections of all McCall Lake sampling sites, which corresponded with the findings with the human fecal contamination marker HF183 (**Figure 8**). Furthermore, Inlet ¾ had the lowest occurrence of HF183 in McCall Lake, and was also tied for the lowest occurrence of HumM2 in McCall Lake.

In addition, the data showed that detections of HumM2 did not always occur with detections of HF183. For example, on July 4th, at Inlet PR60, 4.3 $\log_{10}$ copies/100mL of HF183 was detected, while no HumM2 was detected. Conversely, HumM2 was detected on July 17th at Inlet ¾, whereas HF183 was not detected. This is likely due to: a) the observation that HF183 is a more sensitive marker than HumM2; or b) variable carriage rates of HF183 versus HumM2 in the human population.

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

One explanation for the variation between these results may be antecedent rainfall (i.e., rainfall within the previous 72 hours), and so, antecedent rainfall greater than 10 mm was also examined to see if it had any effect on the variability of human fecal pollution (**Figure 8**). Only three dates (i.e., May 25th, June 8th, and September 13th) had greater than 10 mm of rain. On September 13th, the highest values of HF183 (i.e., 6.0 $\log_{10}$ copies/100mL) and HumM2 (i.e., 5.0 $\log_{10}$ copies/100mL) were detected at ML2. The other two sampling dates corresponding to these rain events (i.e., May 25th and June 8th) did not result in any apparent increases in human fecal contamination markers.

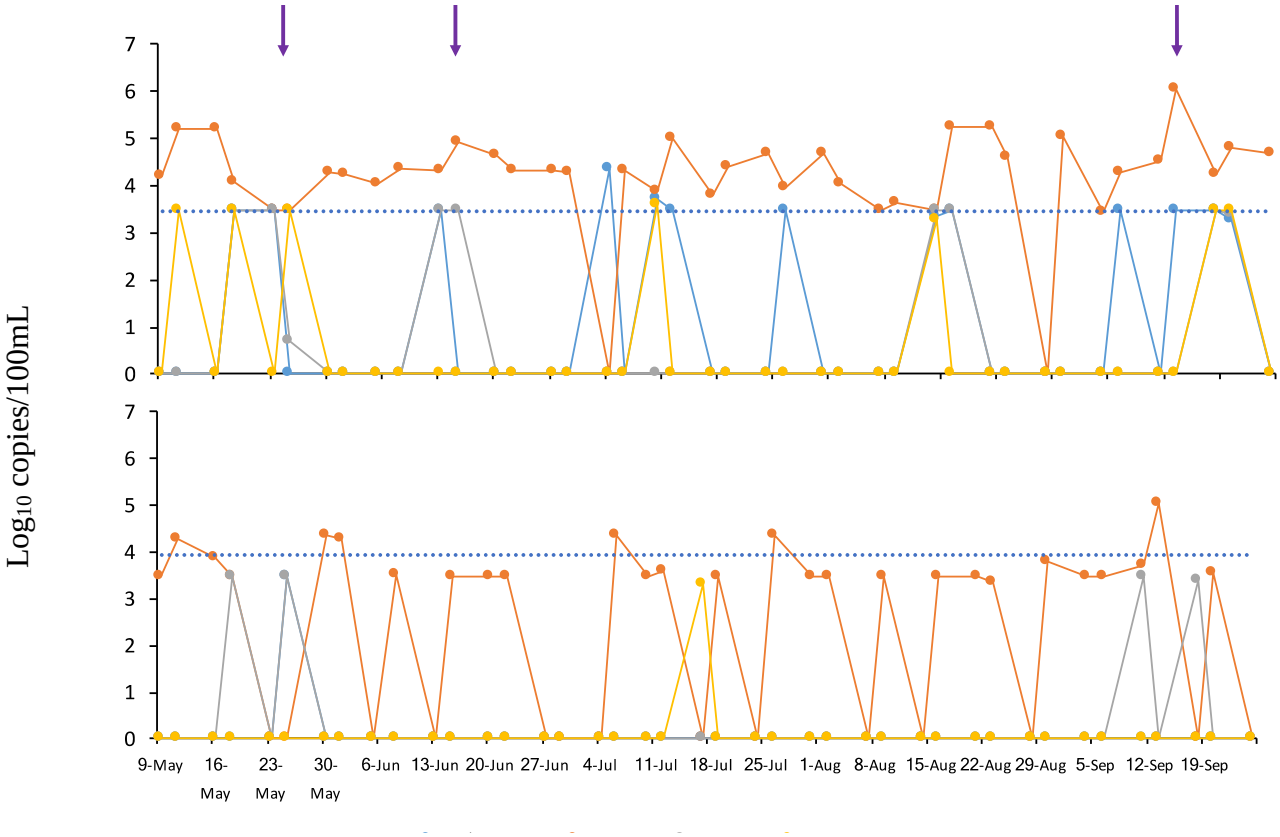


Figure 8. *Temporal pattern of occurrence HF183 log_{10} (upper panel) and HumM2 (lower panel) concentrations at all sampling sites in McCall Lake over the 21-week sampling season. Sampling site PR60 is in blue, ML2 in red, ML1 in gray, Inlet ¾ in yellow, and the limit of quantification$_{95}$ (LOQ_{95}) as a blue dotted line. The purple arrows represent greater than 10 mm of rain in the previous 72 hours.*

In terms of seagull fecal contamination, the occurrence and levels of this marker were observed between: a) the different stormwater ponds, and b) sites within a single stormwater pond (**Table 4**), and so we examined the spatial and temporal characteristics of seagull fecal contamination in each of the stormwater ponds and at each of the sites within a single stormwater pond.

Seagull fecal contamination was the second most common source of fecal contamination. The gull fecal marker (LeeSG) was detected at all sites but in only 9% of all samples. Analogous to what was noted above for human fecal contamination, McCall Lake had the highest occurrence of seagull fecal contamination of all urban stormwater ponds tested.

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

Temporal fluctuations in seagull fecal contamination were observed between the urban stormwater ponds, and at sampling sites within an urban stormwater pond. In McCall Lake, seagull fecal contamination was considered to be a sporadic, highly variable, source of pollution. Seagull contamination was first noted in McCall Lake at the end of June, and tended to be episodic (**Figure 9**). For example, at ML2, seagull fecal contamination was detected on July 12th and at a level of 4.1 $\log_{10}$ copies/100 mL, and then it was not detected at quantifiable levels again until August 8th (i.e., 3.7 $\log_{10}$ copies/100 mL) (**Figure 9**). Although ML2 was most frequently positive for detection of seagull fecal contamination among all sampling sites, this pattern of sporadic, highly variable findings was also noted at the other McCall Lake sites (i.e., PR60, ML1, and Inlet ¾).

There was some similarity in the patterns of seagull contamination observed across the sampling sites in McCall Lake, and which was noted for two key reasons. Firstly, there were three instances where seagull fecal contamination occurred concurrently at three or more McCall Lake sites (i.e., July 10th, August 14th, and September 13th). Secondly, on the aforementioned sampling dates, the levels of seagull fecal contamination detected were all within one order of magnitude of each other. These patterns suggested that a potential environmental variable may be a common component associated with fecal contamination at McCall Lake (i.e., precipitation, roosting by large flocks in early and late summer, etc.). One potential environmental variable examined was antecedent rainfall. Only three dates (i.e., May 25th, June 8th, and September 13th) had greater than 10 mm of rain. Seagull fecal contamination was detected on only one of the sampling dates (September 13th), though at three sampling sites on this date.

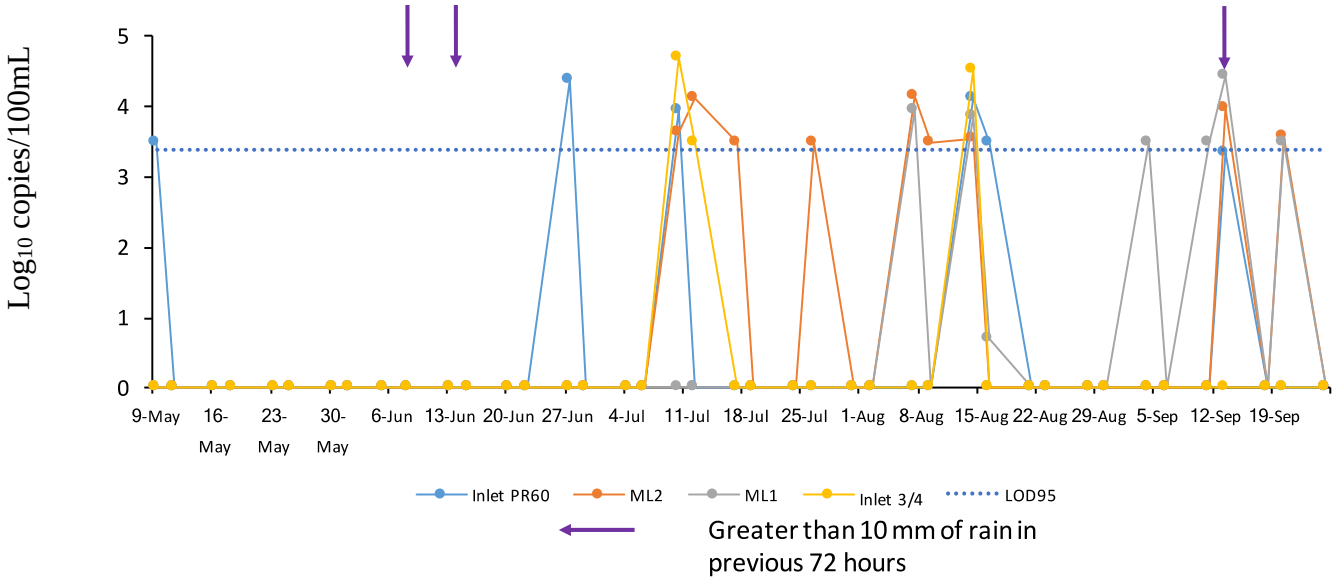
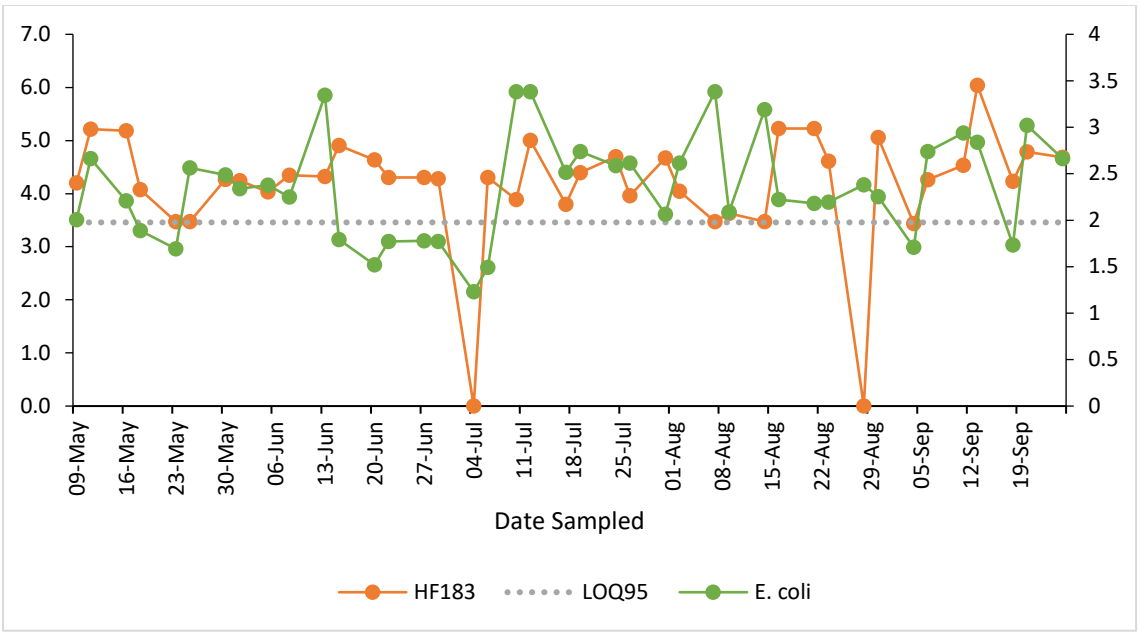


Figure 9. *Temporal pattern of seagull fecal contamination (LeeSg) at all sampling sites in McCall Lake over 21 weeks. The blue line represents PR60, red line ML2, gray line ML1, yellow line Inlet ¾, and the blue dotted line is the LOQ_{95}. The purple arrows represent greater than 10 mm of rain in the previous 72 hours.*

In order to better understand how the occurrence of bacterial indicators of water quality related to sources of fecal pollution, we examined the patterns of occurrence between *E. coli* and *Enterococcus* spp. and the levels of human and seagull fecal markers at ML2 in McCall Lake (***Figure 10***). During the 21-week sampling season at ML2, there were two sampling dates (i.e., May 23rd and July 4th) when the levels of human fecal contamination decreased, as did the levels of *Enterococcus* spp. (Figure 4-4). On August 7th and August 14th, spikes in *Enterococcus* spp. occurred in the absence of high levels of human fecal contamination. On June 5th, high levels of *Enterococcus* spp. and *E. coli* occurred, while low levels of human fecal contamination were detected.

Temporal fluctuations of bacterial water quality indicators (i.e., *Enterococcus* spp. and *E. coli*) and the microbial source tracking marker for seagull fecal contamination (i.e., LeeSg) were also examined at McCall Lake sampling sites ML2 and ML1 (**Figure 11**, **Figure 12**). ML2 was chosen because it was the site most heavily impacted by seagull fecal contamination, and it was also impacted by human fecal contamination. Conversely, ML1 was chosen because it was not as heavily impacted by human fecal contamination, though it was the second most contaminated site with seagull contamination. During the 21-week sampling season at ML2, there were four sampling dates (i.e., July 12th, August 7th, August 14th, and September 13th) when the levels of seagull fecal contamination increased, as did the levels of *Enterococcus* spp. and *E. coli* concentrations (**Figure 12**), and at two of these dates (August 7th and August 14th) the spikes in *Enterococcus* spp. occurred in the absence of high levels of human fecal contamination. This led us to believe that seagull fecal contamination could be attributed to these spikes. For ML1, the three sampling dates with detectable levels of seagull contamination (i.e., August 7th, August 14th, and September 13th) all corresponded to increases in *Enterococcus* spp. and *E. coli* concentrations, suggesting that these sources contribute to the overall loading of microbes (including pathogens) into these systems.



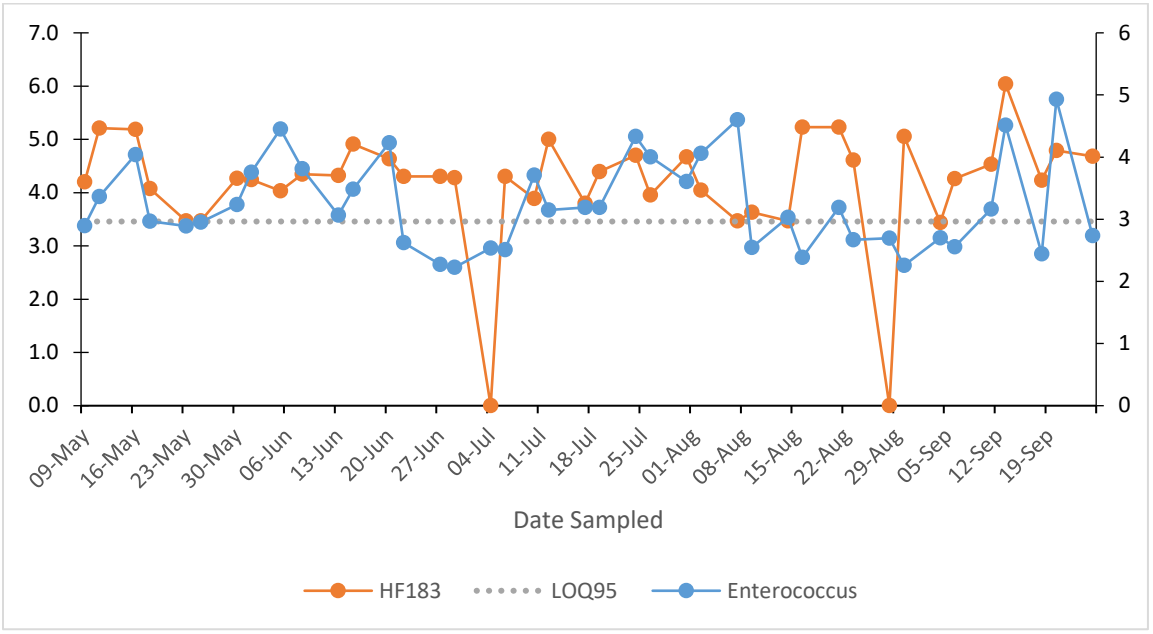


Figure 10. *Temporal pattern of occurrence of E. coli log$_{10}$ concentrations (upper panel) [green line], and Enterococcus spp. (lower panel) [blue line] and the human fecal marker HF183 (orange line), and referenced against the Limit of Quantitation with 95% confidence (LOQ$_{95}$) as a grey dotted line. Samples were taken at the ML2 site in McCall Lake over 21 weeks. MST Marker concentrations are on the primary axis and E. coli and Enterococcus spp concentrations are on the secondary Y-axis.*

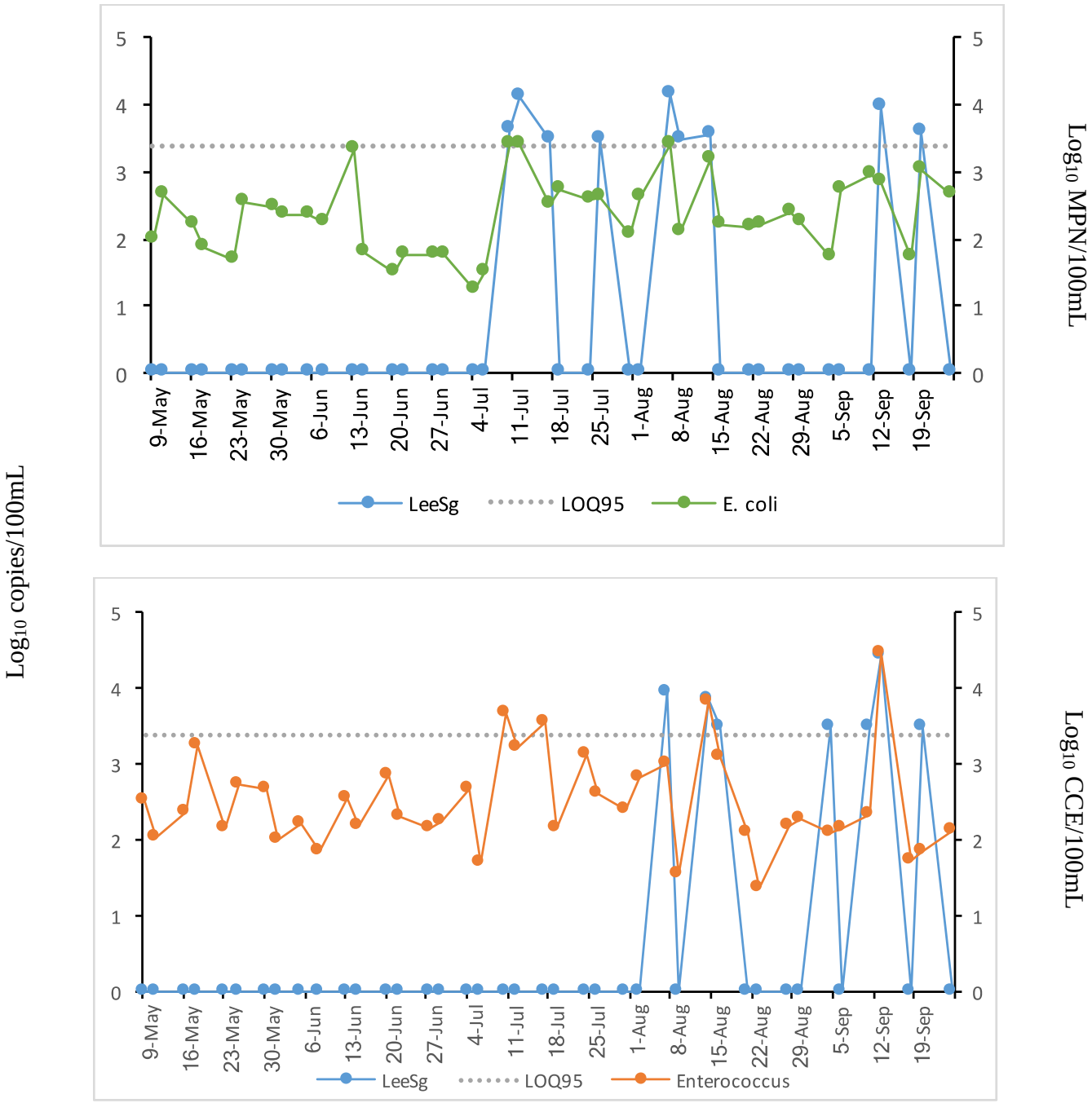


Figure 11. *Temporal patterns in the occurrence and concentrations of E. coli (upper panel) [green line] and Enterococcus (lower panel) [orange line] with seagull fecal contamination (LeeSg, blue line), and referenced against the Limit of Quantitation with 95% confidence (LOQ_{95}) as a grey dotted line. Data represents occurrence at the ML1 site in McCall Lake over 21 weeks. MST Marker concentrations are on the primary axis and E. coli and Enterococcus spp concentrations are on the secondary Y-axis.*

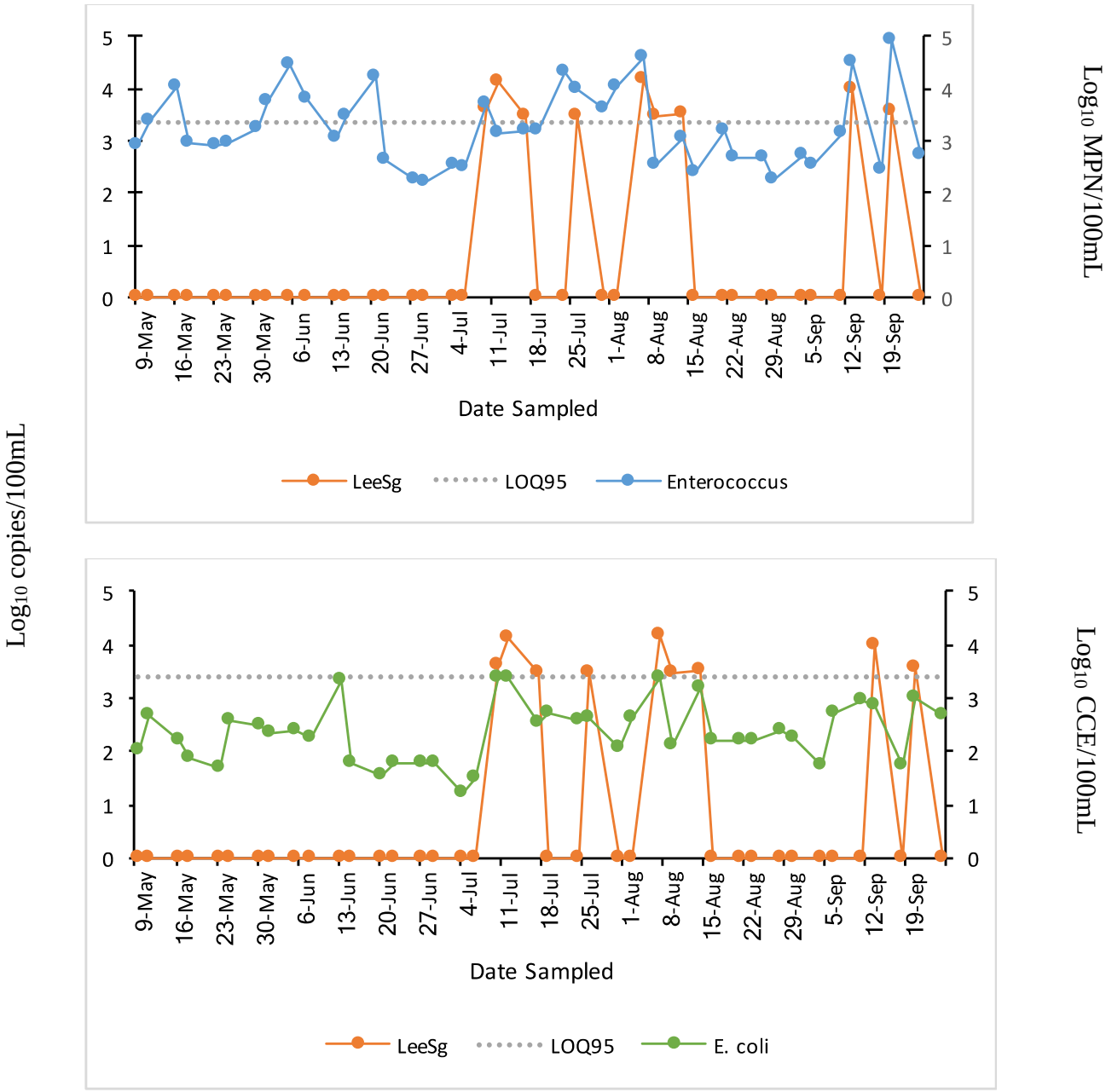


Figure 12. *Temporal patterns in the occurrence and concentrations of E. coli (upper panel) [green line] and Enterococcus (lower panel) [blue line] with seagull fecal contamination (LeeSg, orange line), and referenced against the Limit of Quantitation with 95% confidence (LOQ_{95}) as a grey dotted line. Data represents occurrence at the ML2 site in McCall Lake over 21 weeks. MST Marker concentrations are on the primary axis and E. coli and Enterococcus spp. concentrations are on the secondary Y-axis.*

WATERBORNE PATHOGEN ANALYSIS. A high-level descriptive overview of the frequency of several enteric bacterial pathogens (i.e., *A. butzleri*, *Campylobacter* spp., *Salmonella* spp., and Shigatoxin producing E. coli [STEC]) in each of the Calgary urban stormwater ponds, and at each sampling site within the ponds is provided in **Table 5**. The most frequently detected bacterial pathogen, based on DNA detection methods (quantitative polymerase chain reaction [qPCR]) found in stormwater ponds was *A. butzleri*, detected in 25% of samples (**Table 5**). The second most common pathogen detected was STEC, in 8% of samples, and followed by *Campylobacter* spp. (4%) and *Salmonella* spp. (1%).

As was observed with microbial fecal indicators and source tracking markers, considerable spatial variation was observed with respect to the occurrence of enteric bacterial pathogens. *A. butzleri* was the pathogen most frequently detected in all stormwater ponds; however, the frequency of detection varied among the ponds. In McCall Lake, *A. butzleri* was detected in 38% of samples; whereas, in the Inverness Stormpond and Country Hills Stormwater Facility, *A. butzleri* was detected in 22% of samples (**Table 5**). In addition, *A. butzleri* contamination varied between sampling sites within a single urban stormwater pond. Interestingly, the Inverness Stormpond had the highest frequency of *A. butzleri* even though it was considered to have the best microbial water quality, with 49% of samples testing positive for *A. butzleri* at the site designated as 'Outfalls/Inlets'. *A. butzleri* occurrence at this sampling site exceeded the other sites at Inverness Stormpond (i.e., WP26B, WP26C, WP26D), where the frequency of occurrence of *A. butzleri* detections ranged from 10-17% (**Table 5**). In McCall Lake, *A. butzleri* was observed in 47% of samples at ML2. By comparison, Inlet 3/4 at McCall Lake had the lowest frequency of detection of *A. butzleri,* occurring in only 29% of samples (**Table 5**).

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

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

		Percent of samples positive for bacterial pathogens			
Pond	Sampling Site	*A. butzleri*: (*HSP60* gene)	*Campylobacter* spp.: (Van Dyke16S gene target)	*Salmonella* spp.: (*invA* gene)	*E. coli* shigatoxin: (*stx*1 & *stx*2 genes)
McCall Lake	ML2 (n=41)	47	7	4	15
	PR60 (n=41)	41	4	0	15
	ML1 (n=41)	34	7	0	12
	Inlet ¾ (n=41)	29	7	0	10

	McCall Lake Total n=164	38	7	1	14
Country Hills	WP31A (n=41)	14	2	0	5
	WP31B (n=41)	34	0	0	7
	WP31C (n=41)	20	7	0	15
	WP31D (n=41)	20	10	2	5
	WP31E (n=41)	22	2	0	7
	Country Hills Total n=205	22	4	1	7
Inverness	Outfalls/Inlet (n=41)	49	2	2	5
	WP26B (n=41)	12	0	0	7
	WP26C (n=41)	10	0	0	5
	WP26D (n=41)	17	2	0	2
	Inverness Total n=164	22	1	1	5
Total n=533		25	4	1	8

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

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

The project team also examined whether molecular testing corroborated whether viable pathogens could be isolated from stormwater. This was done using culture-based algorithms (most probable number [MPN] qPCR) against both *Campylobacter* and *A. butzleri*. MPN-qPCR assays were performed for *Campylobacter* spp. and *A. butzleri* on stormwater samples from all sampling sites in McCall Lake (i.e., ML2, ML1, Inlet ¾, PR60) on sampling dates starting from mid-August through the end of the sampling season (i.e., August 21st - September 25th) [**Table 6**]. Based on culture, the average highest concentration of *A. butzleri* observed during the research study was at site ML2 at 18 bacteria/300 mL (**Table 6**). The single highest concentration of *A. butzleri* measured through culture-

based methods occurred at sampling site ML2 on September 13th, in which 93 bacteria/300mL were observed (**Table 6**).

To better understand temporal variation, we further examined patterns of occurrence based on molecular qPCR results. Notable temporal fluctuations in *A. butzleri* were observed between the urban stormwater ponds, and among the sampling sites within a pond (**Figure 13**). At Inlet ¾, in McCall Lake, considerable temporal fluctuations were detected in the levels of *A. butzleri* between sequential sampling dates. Within a two-week time period (i.e., four sequential sampling dates, June 20th – June 29th), the concentration of *A. butzleri* varied from being not detected (i.e., below the limit of quantification of 3.5 $\log_{10}$ copies/100 mL) on June 20th, then spiking to 3.9 $\log_{10}$ copies/100 mL on June 22nd, to be not detected on June 27th, and spiking again to 4.3 $\log_{10}$ copies/100 mL on June 29th.

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

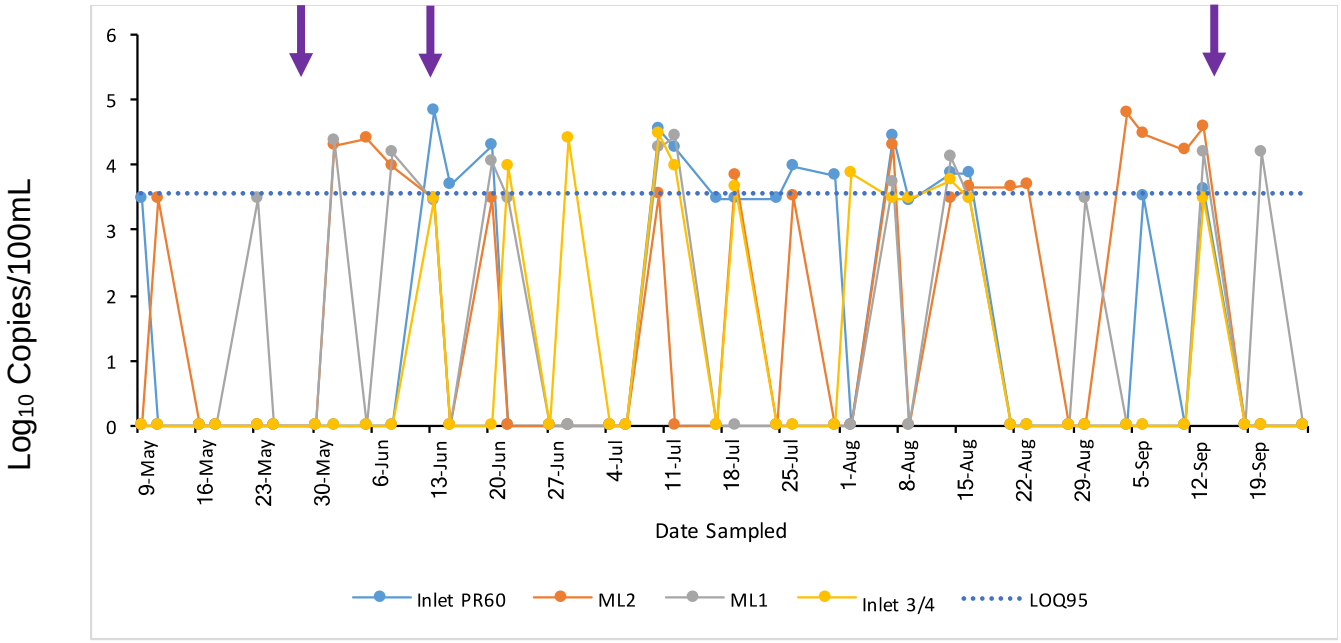


Figure 13. *Levels of A. butzleri, represented as $\log_{10}$ copies/100mL, in all sampling sites at McCall Lake over 21 weeks. The blue line represents inlet PR60, the orange line is ML2, gray line is ML1, yellow line is Inlet ¾, and reference against the the limit of quantification with 95% confidence (LOQ_{95}) (blue dotted line). Purple arrows denote sampling date with rainfall >10 mm in the previous 72 hours.*

Pathogen assays were carried out on split samples in order to determine if molecular-based methods were comparable to results obtained by culture. Based on the limited number of samples collected for comparison (n=32), no *Campylobacter* spp. were detected by either method (i.e., MPN-qPCR

assay or qPCR screen assay) (**Table 7**) suggesting that molecular screen testing and culture-based testing methods led to similar results, and overall, that *Campylobacter* concentrations were below detection limits.

However, this was not the case for *A. butzleri*, with 24 of 32 samples (75%) testing positive for *A. butzleri* by culture-based methods, but only 6 of these same samples were also positive by molecular-based methods [18.75%] (**Table 8**). Eighteen samples positive for *A. butzleri* by culture were negative by molecular-based screening methods (**Table 8**). These results suggest that the molecular screen results presented in **Table 7** and **Table 8** may underestimate the true occurrence of *A. butzleri* in stormwater samples, an effect possibly explained by the relatively low concentration of *A. butzleri* observed in stormwater samples (i.e., ~10^1bacteria /100mL, [**Table 6**]). Although molecular assays are highly sensitive, a major limitation rests in the overall sample volume examined during analysis, due to the extra processing steps that are required to prepare the template, and the small template/analysis volumes used during PCR amplification (i.e., 5.0 uL). When these volume corrections are taken into account, the PCR assay only examines the occurrence of a pathogen target within a 5.0 mL volume of the original stormwater sample. Consequently, in samples where only 10^1 *A. butzleri* /100mL exist (or 0.1 bacteria/mL) the likelihood of detecting this concentration by PCR is low, and this effect is particularly relevant for a single copy gene such as the *hsp60* gene used to detect *A. butzleri*.

Due to the prevalence and abundance of *A. butzleri* contamination, we sought to determine the potential sources of this pathogen contamination. Water samples were analyzed by identifying which microbial source tracking markers occurred most often with *A. butzleri* detections. We found that the most common source of pollution co-occurring with *A. butzleri* detection was when human fecal pollution was present. The human marker HF183 was present in 43% of *A. butzleri* positive samples, while the human marker HumM2 was detected in 10% of *A. butzleri* positive samples (**Table 9**). The second most dominant source of fecal pollution was seagull (i.e., LeeSg), which corresponded to *A. butzleri* detection in 10% of *A. butzleri* stormwater positive samples (**Table 9**). The only other markers found in conjunction with *A. butzleri* were for Canada geese (i.e., CGO1) and ruminants (i.e., Rum2Bac), which were detected in 2% and 1% of positive samples, respectively (**Table 9**).

Table 6. *Assessment of the concentrations of culturable A. butzleri using an MPN-qPCR assay on McCall Lake water samples (i.e., ML1, ML2, PR60, Inlet ¾) collected on eight different sampling dates (between August 21st - September 25th, 2017).*

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

Table 7. *Comparison of culture-based and molecular-based methods for Campylobacter spp. Detection and represented in a positive-negative two-by-two table.*

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

Table 8. *Comparison of culture-based and molecular-based methods for A. butzleri represented in a positive-negative two-by-two table.*

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

Human fecal contamination and *A. butzleri* co-occurred in many water samples throughout this study (**Figure 14**). Amongst the Calgary urban stormwater ponds, *A. butzleri* and human fecal contamination occurred most often in McCall Lake in 51% of aggregate samples from all sampling sites analyzed for HF183. In addition, high simultaneous occurrences of the two markers (i.e., HSP60 and HF183) occurred at individual sampling sites within a stormwater pond. Within McCall Lake at ML2, *A. butzleri* and HF183 co-occurred in 78% of *A. butzleri* positive samples, which was the highest simultaneous co-occurrence observed of any microbial source tracking marker. In addition, ML2 also had the highest simultaneous occurrence of HumM2 and *A. butzleri*, which co-occurred in 50% of all *A. butzleri* positive stormwater samples. In comparison, HF183 and HumM2 were only detected in 13% and 0% of samples at Inlet ¾. In order to better understand the co-occurrence of human fecal material and *A. butzleri*, temporal patterns of the qPCR markers were analyzed. During the 21-week sampling season at ML2, there were six sampling dates (i.e., June 1st, June 8th, August 16th, August 21st, August 28th and September 13th) when human fecal contamination and *A. butzleri* both reached quantifiable levels (**Figure 14**). On five of those dates (i.e., June 1st, June 8th, August 16th, August 21st, August 28th) the only microbial source of fecal contamination detected was human. This finding suggests that human fecal contamination may be a factor contributing to *A. butzleri* loading. In comparison, at sampling site ML1, HF183 and *A. butzleri* were only quantified together once (i.e., September 20th).

Patterns of co-occurrence were not limited to human fecal contamination, as seagull fecal contamination also occurred simultaneously with *A. butzleri* (**Table 9**). Between the three urban stormwater ponds tested, *A. butzleri* and seagull fecal contamination occurred most often in McCall Lake (i.e., 16% of samples) [**Table 9**]. The most contaminated site across all stormwater ponds examined for seagull fecal contamination and *A. butzleri* was sampling site ML1 at McCall Lake, where 40% of samples detected seagull fecal contamination and *A. butzleri*. It should be noted that ML1 was not heavily impacted by human fecal contamination, though it was the second most contaminated site, with seagull contamination. In comparison, at sampling site ML2, seagull fecal contamination was detected with *A. butzleri* in 21% of samples.

In order to better understand the co-occurrence of bird fecal material (i.e., LeeSg and CGO1) and *A. butzleri*, temporal patterns of the qPCR markers were analyzed. This analysis revealed that there were three sampling dates at ML1 when *A. butzleri* was detected in conjunction with seagull fecal contamination LeeSg (i.e., August 7th, August 14th, and September 13th) (**Figure 15**). Furthermore, on August 7th and September 13th at sampling site ML1, no human fecal contamination was detected. In addition, there was one sampling date (i.e., June 20th) when the Canada Goose marker (i.e., CGO1) was detected along with *A. butzleri*, but no human fecal contamination detected. This suggests that *A. butzleri* contamination may occur in the absence of human fecal contamination, and therefore may be influenced by another fecal source such as birds. In comparison, although much more heavily contaminated with human fecal contamination, sampling site ML2 had three *A. butzleri* detections occurring with seagull contamination (i.e., July 10th, August 7th, and September 13th). On all of these dates human fecal contamination was also detected at sampling site ML2.

There were also several instances when *A. butzleri* was detected in the absence of human and animal microbial source tracking markers (**Figure 14** and **Figure 15**). For example, at sampling site ML1 on July 10th and July 12th *A. butzleri* was detected, however, on these two dates no microbial source tracking markers were detected (**Figure 14** and **Figure 15**). This finding suggests that there could be another source of *A. butzleri* in the urban stormwater ponds, or that *A. butzleri* may be more persistent in the environment than the markers used for microbial source tracking.

Table 9. *Co-occurrence of microbial fecal source tracking markers and molecular-methods for A. butzleri in the Calgary urban stormwater ponds.*

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

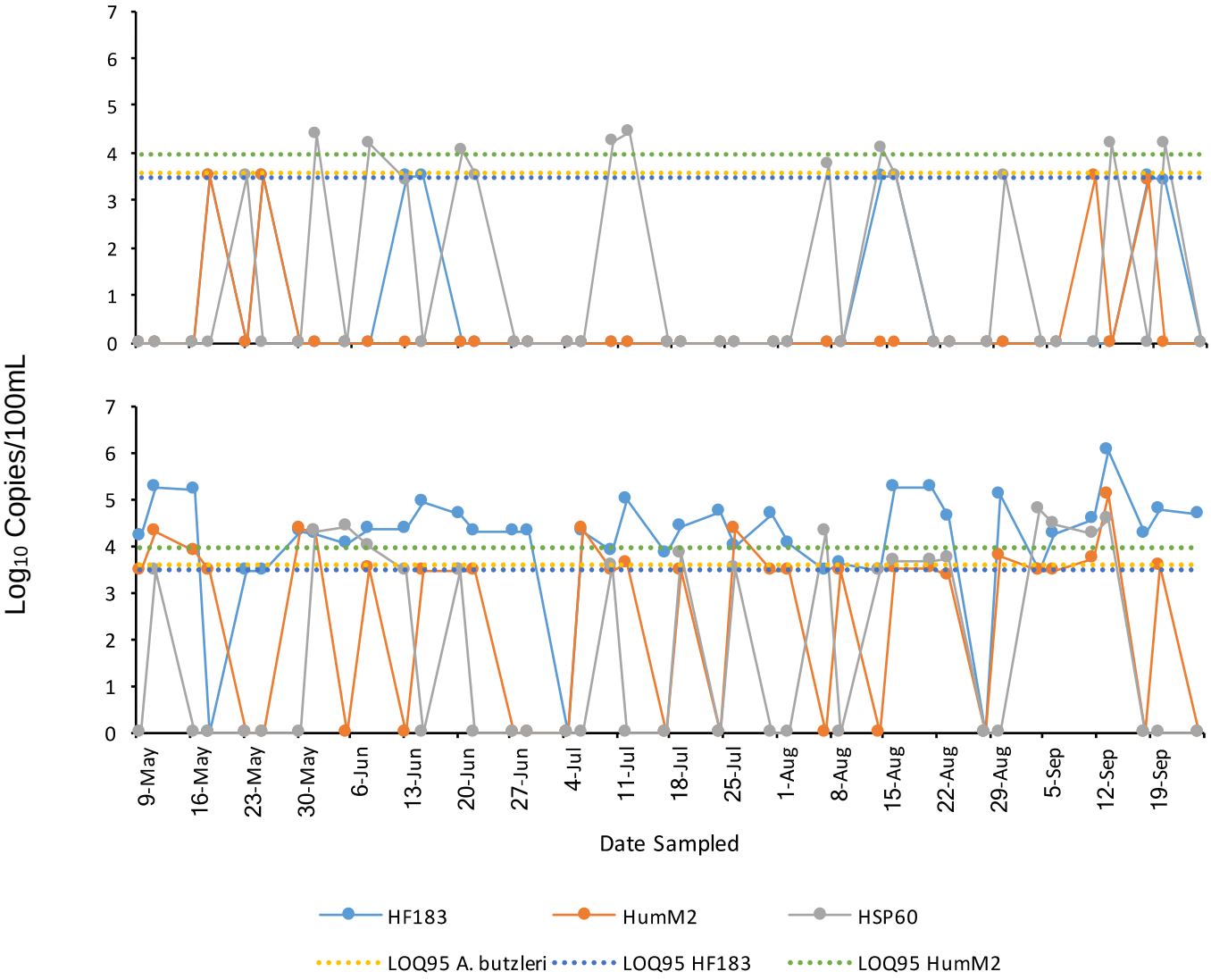


Figure 14. *Association between A. butzleri and human microbial source tracking markers (HF183 [blue line] and HumM2 [orange line]) at ML1 (upper panel) and ML2 (lower panel) sites over 21 weeks and represented on a scale of log_{10} copies/100mL. The gray line represents A. butzleri concentrations (hsp60). All concentrations are referenced against the LOQ_{95} for A. butzleri (yellow dotted line), the LOQ_{95} for the human marker HF183 (blue dotted line), and the LOQ_{95} for HumM2 (green dotted line).*

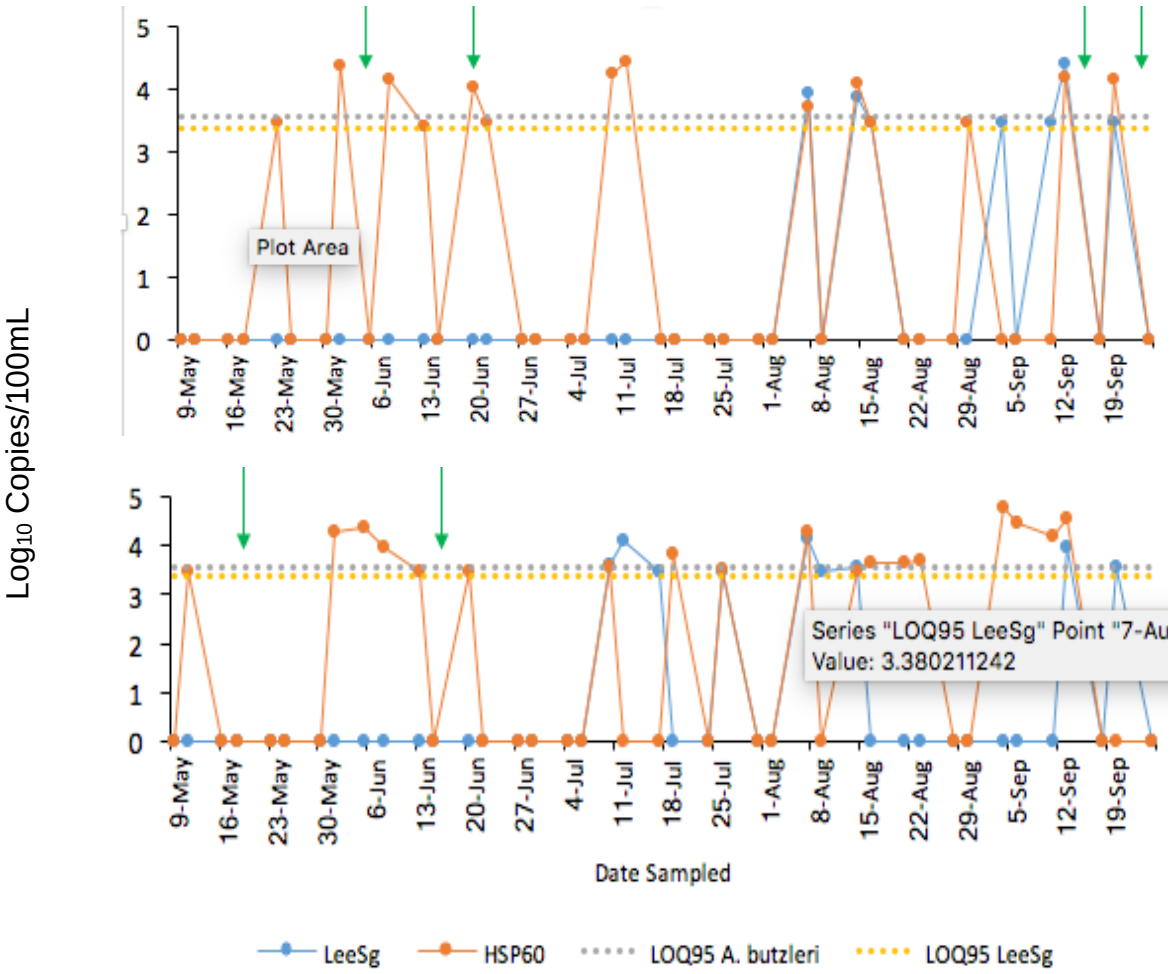


Figure 15. *Association between A. butzleri and seagull fecal contamination (LeeSg) over 21 weeks at sites ML1 (upper panel) and ML2 (lower panel). The oranges lines represent A. butzleri (HSP60), the blue lines represent seagull marker (LeeSg), the gray dotted line is the LOQ_{95} of A. butzleri, and the yellow dotted line is LOQ_{95} for LeeSg. The green arrows represent sampling dates when the Canada goose marker (CGO1) was detected.*

To determine the pathogenic potential of *A. butzleri* found in stormwater samples in McCall Lake, *A. butzleri* isolates were screened for: a) genetic variability through ERIC-PCR [a method of bacterial fingerprinting], and b) the presence of virulence genes characterized based on homologs of virulence genes found in *Campylobacter* spp. ERIC-PCR bacterial fingerprints were analyzed for similar DNA banding patterns by comparing all 85 *A. butzleri* stormwater isolates against each other (**Figure 16**). Genetic similarity was further assessed through the corresponding capillary electropherograms where peaks in relative fluorescence units and size were assessed against each of the *A. butzleri* stormwater positive isolates (**Figure 17**). Genetic similarity was based on visual assessment when banding patterns differed by more than two bands. For example, in **Figure 16** and **Figure 17**, two *A. butzleri* isolates (i.e., isolates D1 and D2) originated from the same water sample and were deemed to be genetically similar based on their bacterial fingerprint and electropherogram, while a third isolate from the same water sample was deemed genetically distinct based on its bacterial fingerprint and electropherogram (i.e., isolate C12). These analyses from ERIC – PCR reflected that only 12 *A. butzleri* isolates were genetically similar to others within the original collection of 85 isolates. Thus, there was a considerable and remarkable number of genetically diverse *A. butzleri* stormwater isolates (73 in total) collected from the McCall Lake stormwater pond alone.

All 73 genetically distinct isolates were screened for the virulence genes *ciaB* and *cadF* initially. The putative virulence marker, *ciaB,* was found in 100% (i.e., all 73) of the genetically distinct *A. butzleri* stormwater isolates (**Table 10**). In addition, *cadF* was detected in 91% of these stormwater isolates (**Table 10**). Since all

genetically distinct isolates reflected the presence of *cadF* or *ciaB*, further screening was initiated on an additional seven putative virulence genes (i.e., *mviN, pldA, tlyA, irgA, hecA, hecB*, and *cj1349*) on all 73 isolates. Three out-of-seven of these new virulence genes (i.e., *cj1349, tlyA, pldA*) tested positive in 90% or more of the McCall Lake *A. butzleri* isolates (**Table 10**). Finally, the frequency of occurrence of all virulence genes tested was at least 50%. Not only was a number of genetically distinct isolates collected from McCall Lake, many of the isolates were characterized as having multiple virulence genes (**Figure 18**). Importantly, 21 of the 73 *A. butzleri* isolates contained all 9 virulence genes, and 89% of the strains possessed 6 or more virulence genes. The data strongly suggests that most *A. butzleri* strains observed in stormwater are pathogenic to humans.

A high-level descriptive overview of each of the isolates was performed, in order to see if there was a relationship between the presence of virulence markers and positive detections of the dominant sources of microbial fecal pollution (i.e., HF183, HumM2, and LeeSg) in those water samples. As outlined previously, the occurrence of *A. butzleri* in stormwater was more associated with human sources of fecal pollution followed by seagull feces. The fact that there appeared to be an association of *A. butzleri* with human fecal contamination also supported the virulence gene data, as one would assume that human-derived *A. butzleri* are more likely to pose a greater risk to human health, and thereby possess more virulence genes than strains derived from birds. However, there was little spatial variability with respect to the frequency of virulence marker detections between sampling sites at McCall Lake (i.e., ML2, ML1, PR60, and Inlet ¾). This result was true regardless of the virulence marker (**Figure 19** and **Figure 20**). However, notably, *cadF* was positive for 100% of samples at ML1 (**Figure 20**), as was *pldA*, ciaB, cj1329, tlvA, These same virulence genes occurred in 100% of *A. butzleri* isolates collected at PR60 as well (**Figure 19**).

There did not appear to be a consistent relationship between fecal source (i.e., human or seagull) and virulence markers. The median number of virulence genes associated with *A. butzleri* isolates obtained from water samples where the human fecal marker (i.e., HF183) was observed was 6.5. The median number of virulence genes associated with *A. butzleri* isolates obtained from water samples where the seagull marker (i.e., LeeSg) was observed was 8, suggesting that strains associated with bird feces maybe equally pathogenic to humans. Water samples taken at ML2 on August 22nd were positive for both microbial source tracking markers for human fecal contamination (i.e., HF183 and HumM2), however *ciaB* was the only virulence marker that was found in every genetically unique isolate on that date. Further, one sample from ML2 on August 22nd contained two other virulence markers (i.e., *cadF* and *mniV*). In addition, a series of samples taken from ML1 on September 12th, in which a seagull signature (i.e., *LeeSg*) had been detected, tested positive for 7/9 of the virulence markers (i.e., *hecA, irgA, ciaB, cadF, cj1349, tlvA*, and *pldA*). With that said, it needs to be noted that in many of the water samples in which potentially pathogenic *A. butzleri* were isolated, there was no corresponding microbial source tracking marker observed (i.e., human, dog, ruminant, seagull or Canada goose), raising the possibility that: a) other fecal sources of pollution may be contributing to stormwater contamination and the presence of *A. butzleri*, b) that environmental sources of *A. butzleri* may exist and replicate in the environment, as reported by others, or c) that *A. butzleri* may persist in stormwater longer than the MST markers used to track sources of fecal pollution. Regardless, the ability to culture viable and potentially pathogenic A. butzleri within stormwater samples may be a concern, albeit the overall concentrations are low (**Table 6**). This data is valuable in terms of generating a quantitative microbial risk assessment (QMRA) for human health risks related to *A. butzleri* in stormwater, and we are recommending that this bacteria be used as a surrogate for all future QMRAs for stormwater.

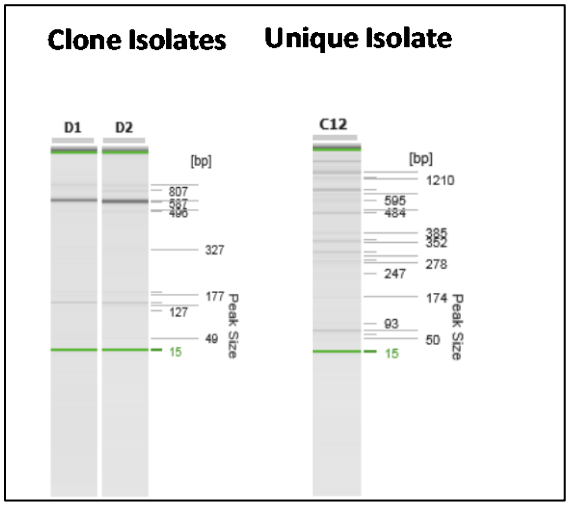


Figure 16. *Comparison of ERIC-PCR gel images of samples taken from Inlet 3/4 at McCall Lake on August 8th. The two samples on the left (i.e., D1 and D2) were determined to be genetically similar, while the sample on the right was determined to be genetically unique (i.e., C12).*

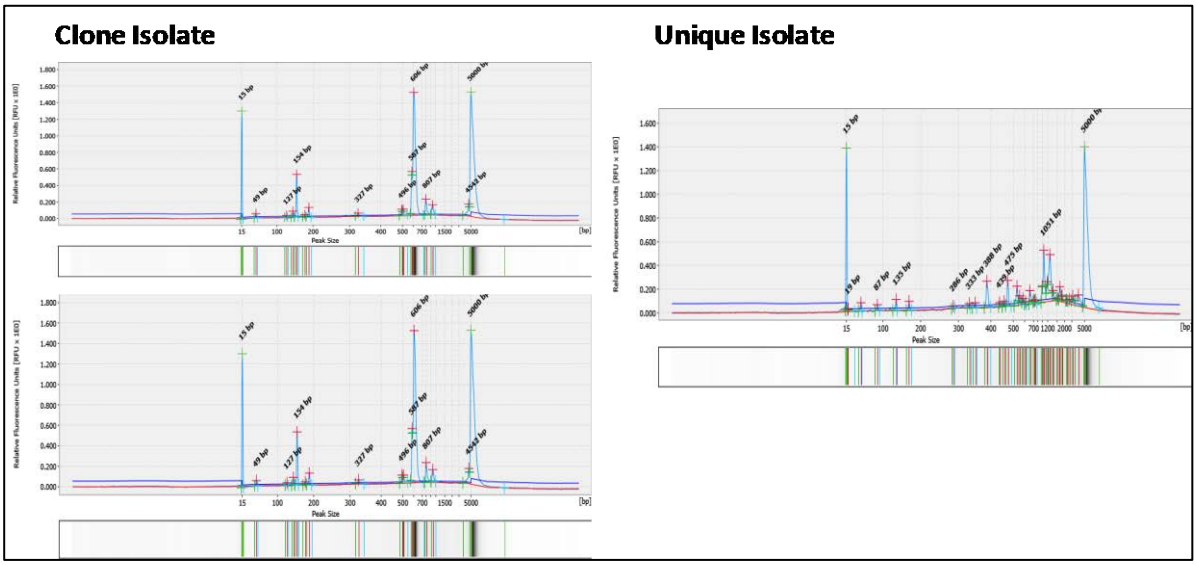


Figure 17. *Comparison of ERIC-PCR electropherograms of two isolates determined to be clones (top, D1 and D2) and a unique isolate (bottom, C12). The peaks at 15 bp and 5000 bp are from size markers. Comparisons were made by looking at the peaks in the isolates. All samples were taken from Inlet 3/4 at McCall Lake on August 8th.*

Table 10. *Percent of A. butzleri strains (n=73) possessing putative virulence genes known to be important in determining pathogenicity of isolates.*

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

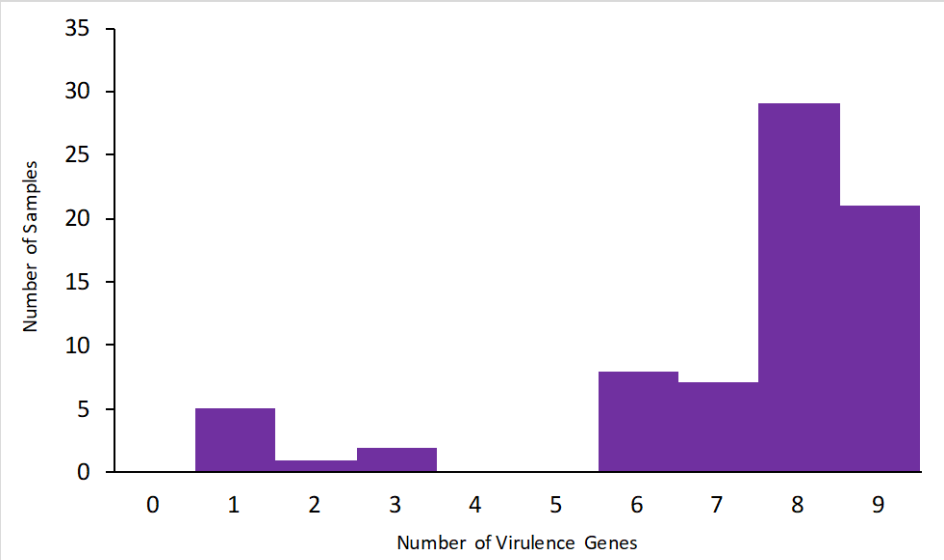


Figure 18*. Histogram representing the number of virulence genes carried by genotypically-distinct A. butzleri isolates collected from stormwater (total n=73).*

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

Inlet 3/4

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

Inlet PR60

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

Outfall ML2

Figure 19. *Association between sampling location, date, select microbial source tracking markers (i.e., HF183, HumM2, LeeSg,) and virulence genes (i.e., cadF, ciaB, cj1349, hecA, hecB, mniV, irgA, tlyA, pldA) in the 73 A. butzleri isolates (indicated by column labelled 'Isolate ID'). Stormwater samples were collected (by date) at Inlet ¾ (top panel), Inlet PR60 (middle panel) and the ML2 Outfall (bottom panel) at McCall Lake. Targets that were not detected (microbial source tracking marker or virulence genes) are represented by white boxes, whereas detectable levels of the microbial source tracking markers are shown as blue boxes, and the presence of the virulence genes shown with yellow boxes. Note that in Inlet ¾ and Inlet PR60 that A. butzleri strains were isolated in the absence of detection of human or seagull sources of fecal pollution, and yet possessed a significant number of virulence genes.*

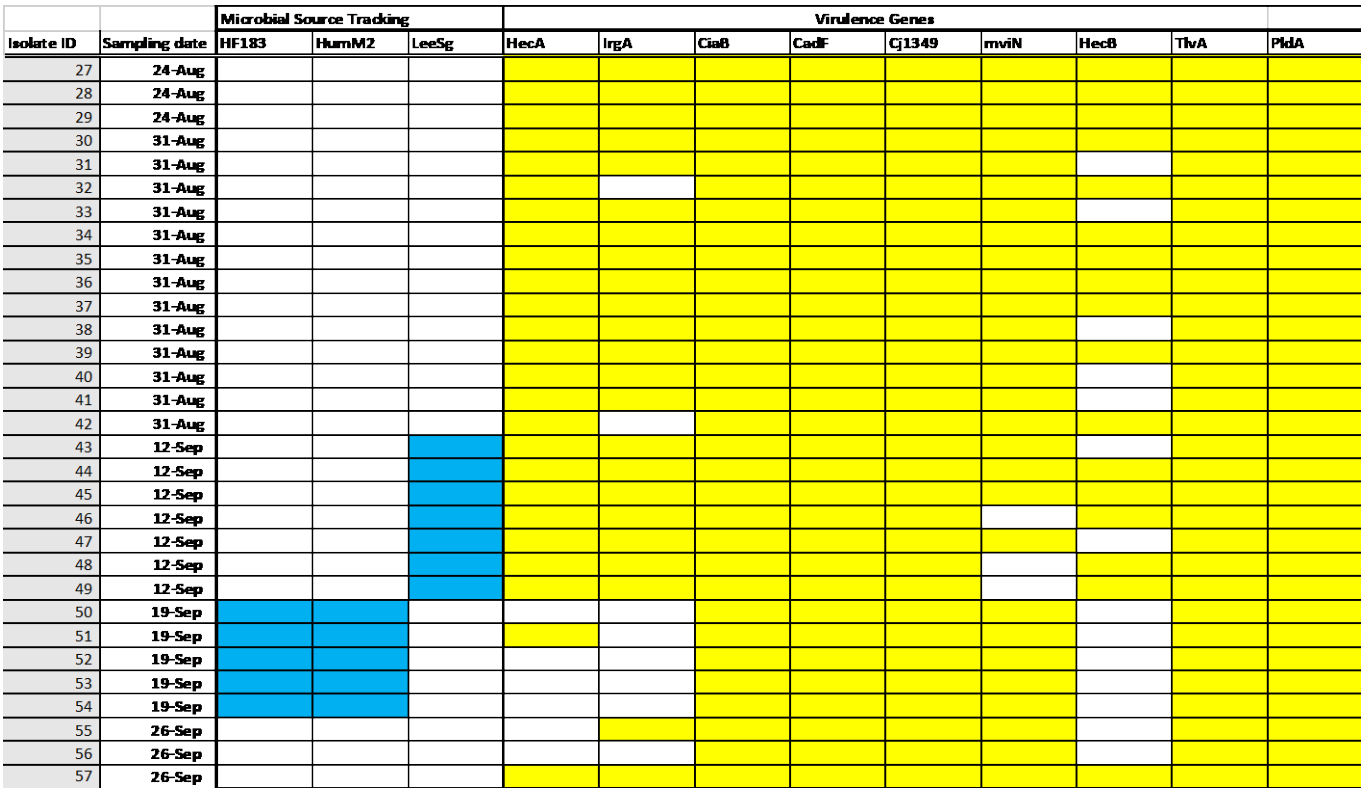


Figure 20. *Association between sampling location, date, select microbial source tracking markers (i.e., HF183, HumM2, and LeeSg,) and virulence genes (i.e., cadF, ciaB, cj1349, hecA, hecB, mniV, irgA, tlyA, and pldA) in A. butzleri isolates collected from the representative stormwater samples (by date) at outfall ML1 in McCall Lake. Targets that were not detected (microbial source tracking marker or virulence genes) are represented by white boxes, whereas detectable levels of the microbial source tracking markers are shown as blue boxes, and the presence of the virulence genes shown with yellow boxes.*

Stormwater Ponds (2019)

In 2019, we once again sampled stormwater ponds in Calgary, this time focusing on McCall Lake (grab samples) and the Inverness Stormpond (grab samples, autosamplers). The intent was to examine whether the stormwater quality trends in the sampling season of 2019 were similar to those observed in 2017. For purposes of this report, we have focused our discussion on the McCall Lake samples, largely due to the fact that the Inverness Stormpond data is currently been analyzed in terms of modeling the loading, transport and fate of microorganisms in this stormwater pond (work being continued under the NSERC-CRD and City of Calgary funds).

Four sites at McCall lake were sampled over 13 sampling dates. The sites were the same as those sampled in 2017 (i.e., outfalls ML1 and ML2 and Inlets ¾ and PR60) and tested for; *E. coli* (culture) and *Enterococcus* cell calibrator equivalents (CCE) based on quantitative polymerase chain reaction (U.S. EPA Method 1611). In addition, a panel of microbial source tracking (MST) qPCR markers were run on each sample.

With respect to *Enterococcus* levels for the 4 sites, inlet ¾, Inlet PR60 and Outfall ML1 all had similar mean values of ~10^3 CCE/100 mL for the 2019 season, while Outfall ML2 continued to have a mean value that was ~1

log_{10} higher (**Table 11**) as was noted in the dataset collected in 2017. In terms of failure rates based on recreational water guidelines, *E. coli* failure rates were 38.5% across all sites, which was similar to that observed in 2017 (36%, **Table 12**). *Enterococcus*, on the other hand, had an overall failure rate of 75% based on the 1280 CCE single sample threshold (STV) level. This was significantly higher than the failure rate observed in 2017 (29.3%).

Table 11. *Summary of the levels of Enterococcus in cell calibrator equivalents (CCE) at 4 sampling sites in McCall Lake in 2019 (n = 13 for each site).*

Location	Mean ($x10^3$)	Min ($x10^3$)	Max ($x10^3$)
Inlet ¾	1.66	0.04	5.46
Inlet PR60	5.04	0.213	33.6
Outfall ML1	6.53	0.120	37.5
Outfall ML2	3	2.02	298

Table 12. *Summary of bacteriological indicator failures based on single sample maximums in 2019 versus 2017 at McCall Lake (ENT = Enterococcus CCE; EC = E. coli)*

	2019 (*n* = 13 / site)		2017 (*n* = 41 / site)	
Location	ENT >1280 CCE (%)	EC >100 MPN (%)	ENT >1280 CCE (%)	EC >100 MPN (%)
Inlet ¾	7 (53.8)	3 (23.1)	6 (14.6)[a]	7 (17.1)
Inlet PR60	9 (69.2)	2 (15.4)	12 (29.3)[b]	9 (22.0)
Outfall ML1	10 (76.9)	6 (46.2)	7 (17.1)	13 (31.7)
Outfall ML2	13 (100)	9 (69.2)	21 (51.2)	30 (73.2)
total	39 (75.0)	20 (38.5)	48 (29.3)	59 (36.0)

[a] One sample inhibited; no PCR result obtained
[b] One sample inhibited; no PCR result obtained

In the 2017 sampling campaign, we observed that the ML2 outfall consistently showed a human *Bacteroides* signature (both HF183 and HumM2), and that samples collected at the ML2 site immediately after a long weekend resulted in HF183 levels were lower than in those weeks were there was no holiday on the Monday (all samples were collected on Tuesdays) [**Figure 21**]. We hypothesized that an industrial/commercial business may be responsible for sewer cross connection within the stormshed, and for which the business may be closed on long weekends. In collaboration with City of Calgary staff, a partial investigation of the ML2 storm sewer drainage network was done and the pollution source localized to a commercial/industrial area with the stormshed. Further investigations with the City of Calgary are under discussion to use MST and bacteriological water quality analysis to pinpoint the exact location of this persistent contamination.

In 2019 a similar trend in human contamination of the McCall Lake Stormpond was observed (**Figure 21**). On the samples immediately following the three statutory holidays during the sampling period (August 5, September 2 and October 14) HF183 decreased at least one order of magnitude from the sample preceding it. Molecular *Enterococcus* values do not appear to show this trend, potentially explained by the fact that other known sources of faecal pollution (e.g., birds) likely contribute to the overall *Enterococcus* values observed at this site, but do not the human marker values. This data demonstrates the utility of using MST to identify infrastructure problems that impacting stormwater quality.

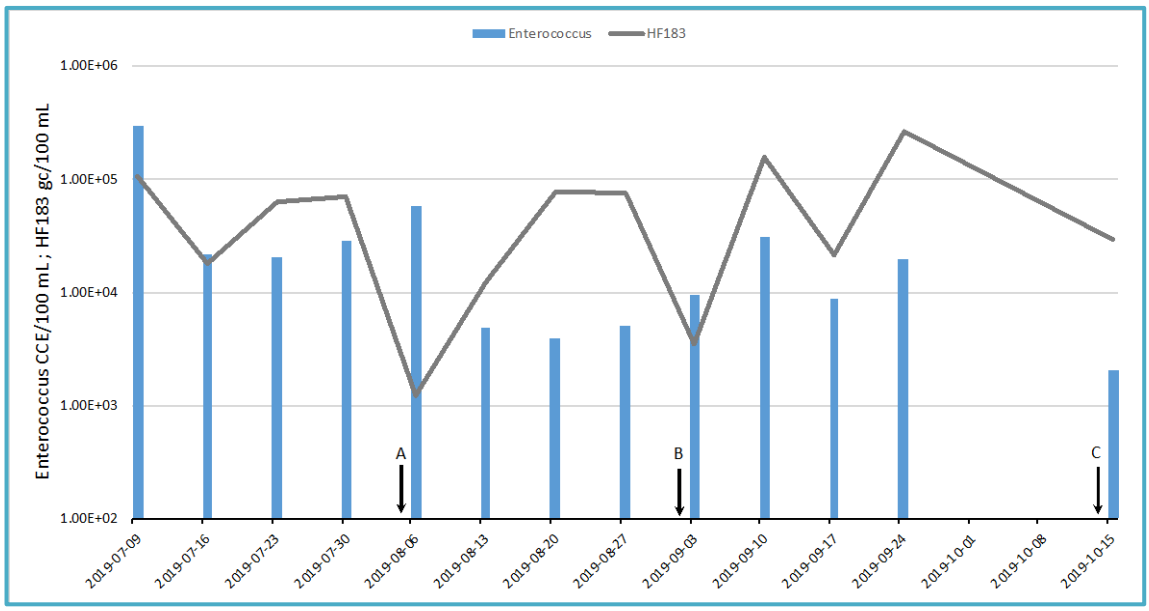


Figure 21. Enterococcus *and HF183 levels at ML2 outfall in McCall Lake during summer 2019. All samples were collected on Tuesdays. Three statutory holidays falling on Mondays are highlighted (A – Heritage Day; B – Labour Day; C – Thanksgiving).*

As observed in the 2017 dataset, the ML1 outfall displayed slightly lower levels of *Enterococcus* as compared to ML2 (**Figure 22**). Additionally, as was observed in 2017, ML1 did not appear to have the same persistent human signature as outfall ML2, albeit it was still detected on occasion. Unlike site ML2, there is no correlation between the levels of the human HF183 marker and their reduced levels on statutory holidays (**Figure 22**). The human HF183 marker was only observed on 5/13 sampling dates at ML1 (similar to the observed frequency at the two other inlet sites), whereas it was present in 100% of samples in ML2. Inlets ¾ (**Figure 23**) and PR60 (**Figure 24**) displayed similar results to that of Outfall ML1 – i.e., having background levels of *Enterococcus* ~10^3 CCE/100 mL with sporadic HF183 detections, the trends of which were similar to data collected in 2017. Similarly, there was no association between levels of HF183 detected and occurrence of statutory holidays at these inlets.

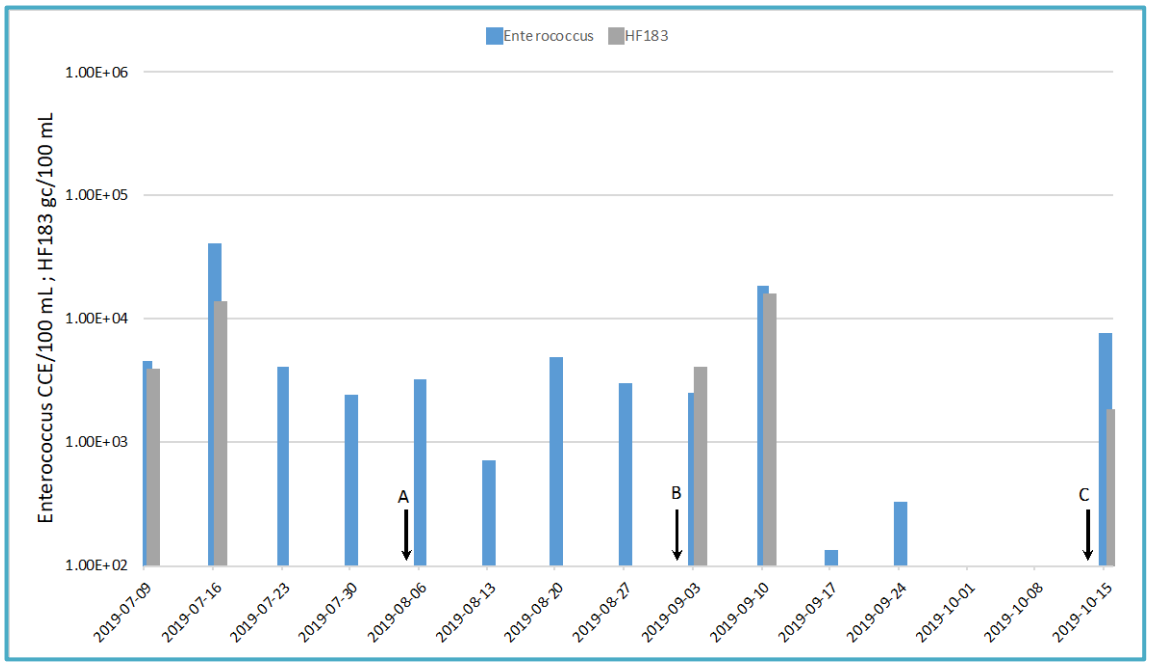


Figure 22. *Enterococcus* and HF183 levels at ML1 outfall in McCall Lake during summer 2019. All samples were collected on Tuesdays. Three statutory holidays falling on Mondays are highlighted (A – Heritage Day; B – Labour Day; C – Thanksgiving).

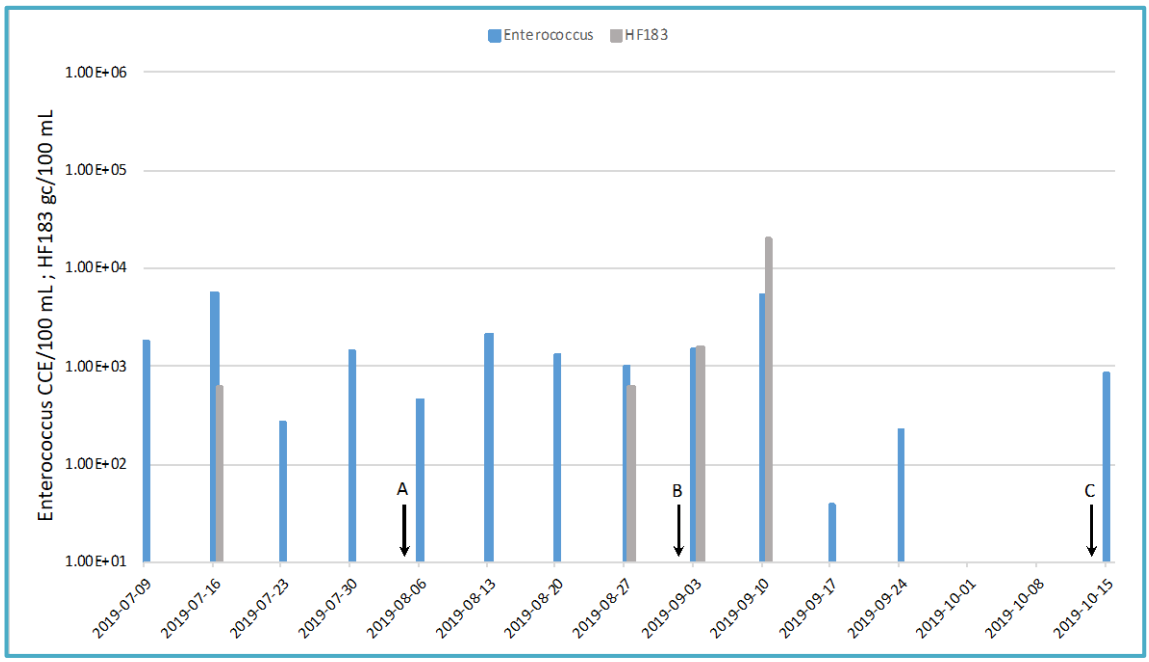


Figure 23. Enterococcus *and HF183 levels at Inlet 3/4 in McCall Lake during summer 2019. All samples were collected on Tuesdays. Three statutory holidays falling on Mondays are highlighted (A – Heritage Day; B – Labour Day; C – Thanksgiving).*

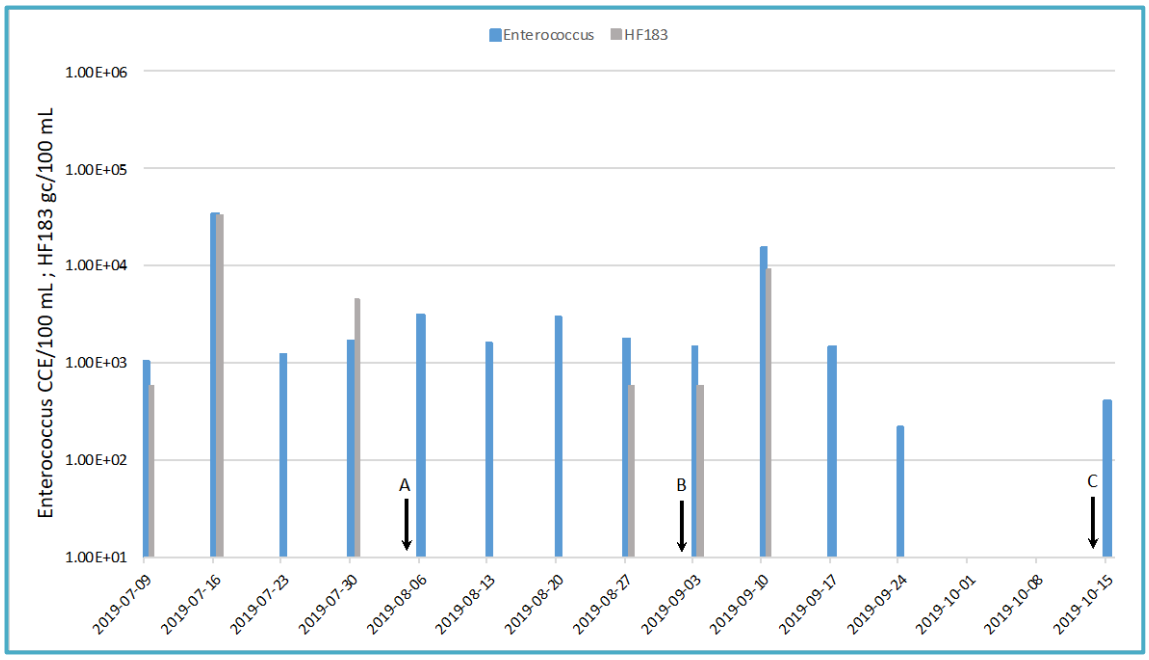


Figure 24. *Enterococcus* and HF183 levels at Inlet PR60 in McCall Lake during summer 2019. All samples were collected on Tuesdays. Three statutory holidays falling on Mondays are highlighted (A – Heritage Day; B – Labour Day; C – Thanksgiving).

In terms of overall fecal source influences affecting stormwater quality, the MST data collected in 2019 was consistent with the data collected in 2017. Humans and gulls were the predominant sources of fecal pollution observed at McCall Lake. Gull markers were observed at a low frequency at all four sites (**Table 13**) consistent with a mode of bird fecal deposition directly into the lake or entering via stormwater runoff. Both of the human fecal markers (HF183 and HumM2) were observed at all 4 sites, but the frequency of detection was much higher at the ML2 site (100% detection rate) as compared to the other sites.

Table 13. Number (and percent) of microbial source tracking marker observations made at McCall lake sites in the 2019 sampling period (*n* = 13; each site) [HF183 = human; HumM2 = human; Rum2Bac = ruminants; LeeSG = seagulls; CG01 = Canada goose; Dog3 = dogs; MuBac = muskrat).

Location	HF183	HumM2	Rum2Bac	LeeSG	CGO1	Dog3	MuBac
Inlet ¾	4 (30.8)	1 (7.7)	0 (0)	3 (23.1)	0 (0)	0 (0)	0 (0)
Inlet PR60	6 (46.2)	1 (7.7)	1 (7.7)	4 (30.8)	0 (0)	0 (0)	0 (0)
Outfall ML1	5 (38.5)	2 (15.4)	0 (0)	4 (30.8)	1 (7.7)	0 (0)	0 (0)
Outfall ML2	13 (100)	10 (76.9)	0 (0)	2 (15.4)	0 (0)	0 (0)	0 (0)
total	28 (53.8)	14 (26.9)	1 (1.9)	13 (25.0)	1 (1.9)	0 (0)	0 (0)

Stormwater-Impacted River Sampling

NOSE CREEK (AIRDRIE). Synoptic sampling was also carried out on the Nose Creek in Airdrie, Alberta, Canada, following rain events. Five rain events occurred over the course of the study period: May 25, June 9, August 14, September 13, and September 21, 2017. There were 10 sampling locations along the Nose Creek, and stormwater was directly collected from stormwater effluent discharges going into the Nose Creek.

Molecular-based methods for *Enterococcus* densities often exceeded the standard of 1280 CCE/100 mL, with 79% of samples failing in the Nose Creek stormwater samples in Airdrie (**Table 14**). Four Nose Creek sampling sites had a 100% failure rate for *Enterococcus* STV. Additionally, thermotolerant coliforms from the Nose Creek sampling sites had a high failure rate, with 56% of samples exceeding the Alberta Recreational Water Quality Standard for thermotolerant coliforms of >400 CFU/100 mL in the Nose Creek.

Table 14. *Microbial water quality in stormwater effluents flowing in Nose Creek based on the percentage of sample failing existing standards of water quality.*

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

Stormwater samples collected along the Nose Creek were tested for seven microbial source tracking indicators (i.e., HF183, HumM2, Rum2Bac, MuBac, LeeSg, CGO1, and Dog3) (**Table 15**). There were two dominant sources of fecal pollution in the Nose Creek, the most dominant source being human fecal contamination with 57% of samples containing detectable levels of HF183 (**Table 15**). The second most dominant source of pollution was ruminants (i.e., Rum2Bac), which was detected in 34% of samples from the Nose Creek (**Table 15**).

Table 15. *Occurrence of microbial source tracking markers in the Nose Creek in Airdrie, based on the percentage of samples that detected each microbial source tracking marker (i.e., HF183, HumM2, LeeSg, CGO1, Dog3, Rum2Bac, and MuBac).*

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

In terms of pathogens, *A. butzleri* was detected in 27% of samples and STEC in 23% of samples and based on molecular DNA testing (**Table 16**). *Campylobacter* spp. were observed in only 2% of samples and *Salmonella* was not detected in any samples.. Spatial variability was also observed at specific sampling sites (storm drains) discharging into the Nose Creek. Although the overall prevalence of *Campylobacter* was low (i.e., 2%) all detections of this pathogen occurred at a single sampling site, 25814 (**Table 16**). Spatial variability occurred between samplings sites for *A. butzleri*, as 66% of samples tested positive at site 25817, which was the highest frequency of occurrence for *A. butzleri* at any of the outfalls tested. However, *A. butzleri* was not detected in any samples taken from site 25804. As for STEC, 40% of samples at three sampling sites (i.e., 25756, 25807, and

25855) were positive, whereas STEC was not detected in any samples taken from three other sampling sites (i.e., 25841, 25847, and 25793) [**Table 16**].

Table 16. *Frequency of occurrence of positive samples by pathogen-specific qPCR screening of stormwater samples flowing into the Nose Creek from Airdrie, Alberta.*

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

ELBOW RIVER (CALGARY). In Calgary, Elbow River samples came from the area between the Glenmore Dam and downstream to the Bow River. This section of the river contains 88 stormwater outfalls and 13 sanitary sewer crossings beneath the river, and this waterway is utilized for summer recreational activities (e.g., swimming, canoeing, tubing, fishing, etc.). For the purposes of our study, ten sampling sites, several of which are accessible recreational points along the river, were studied. Each of the ten sites along the Elbow River was sampled once a week 13 times from June 5th to August 28th in 2017. Sampling was also carried out in 2018, but data not presented in this report. A total of 117 samples were taken from ten sampling sites along the Elbow River in Calgary.

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

In the Elbow River, eight sampling sites (i.e., Stanley Park, Rideau Pedestrian Bridge, 26th Ave SW, 25th Ave SW, 1 St SE, Stampede Grandstand, Enmax Park, and 9th Ave SE) occasionally failed the Alberta recreational water quality standardsf or thermotolerant coliforms of >400 CFU/100 mL (**Table 17**). Four of the sampling sites (i.e., 26th Ave SW, 25th Ave SW, 1 St SE, and 9th Ave SE) had a failure rate of 23% (**Table 17**). The percentage of samples failing recreational water quality based on *Enterococcus* were higher than those failing by thermotolerant coliforms (**Table 17**).

Table 17. *Microbial water quality in the Elbow River based on the percentage of samples failing existing standards of water quality in the Elbow River.*

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

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

Considerable spatial variation in water quality was observed among the sampling sites in the Elbow River. Average concentrations of *Enterococcus* varied only slightly among the ten sampling sites along the Elbow River, with median values ranging from 2.5 $\log_{10}$ CCE/100mL to 3 $\log_{10}$ CCE/100mL (**Figure 25**). However, the site with the highest range of values was Stanley Park, with *Enterococcus* values ranging from 2.1 $\log_{10}$ CCE/100mL to 5.4 $\log_{10}$ CCE/100mL, and demonstrating how drastic water quality could vary within one sampling site.

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

CCE/100mL) on July 10th. As represented by the outliers at the Elbow River sampling sites, bacterial water quality overall appeared to be highly variable, and therefore at risk for significant levels of periodic bacterial contamination (**Figure 25**).

In contrast to the Elbow River, a box and whisker plot for the rural river control showed median values of ~2.2 $\log_{10}$ CCE/100mL at all three sampling sites tested (**Figure 25**). The highest range of values was observed at sampling site 'Rural River C', with *Enterococcus* values ranging from 1.6 to 2.6 $\log_{10}$ CCE/100mL. In addition, only two sampling sites had outliers (i.e., 'Rural River A' and 'Rural River B'), which occurred below the lower whisker. These ranges reflected the consistency of water quality in the rural river control. This data suggested that urban stormwater may be a significant source of microbial fecal loading coming into riverine systems. This observation justified a closer examination of the temporal variance of bacteriological water quality at each of the sites in the Elbow River and the rural river control.

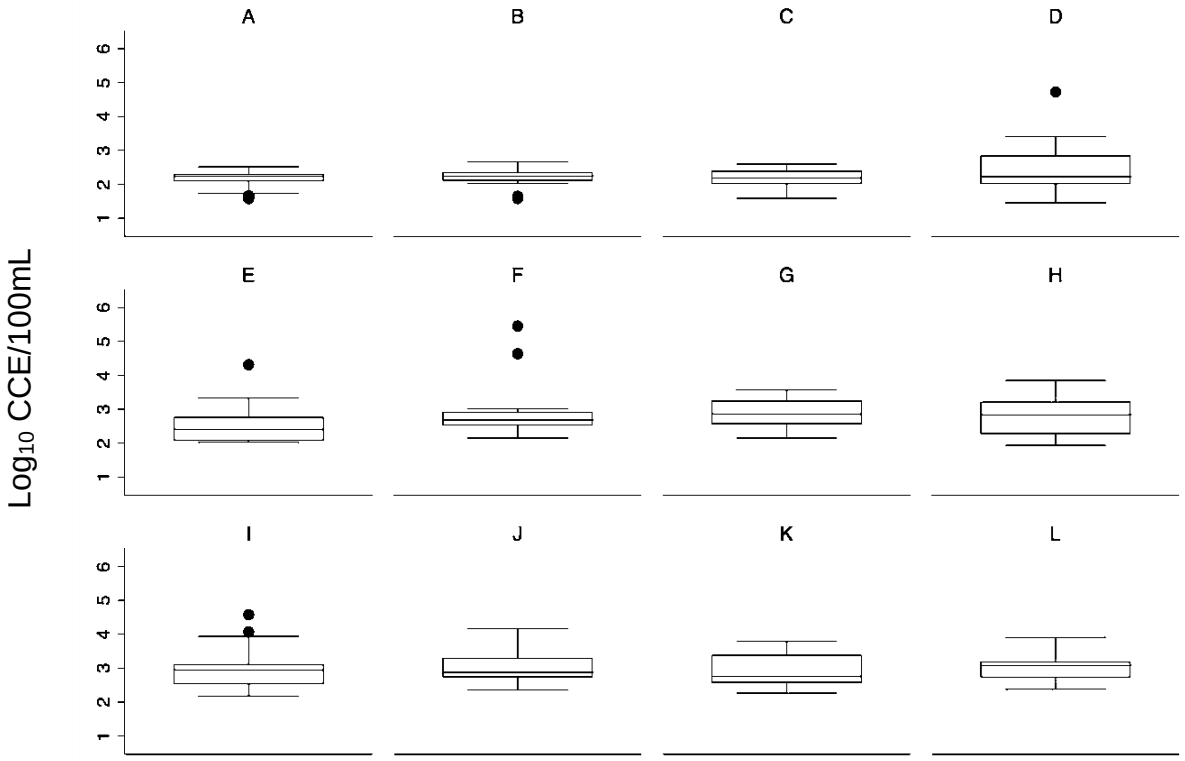


Figure 25. *Box and Whisker plot of Enterococcus spp. values in the Elbow River and Rural River broken down by sampling site. Three sampling sites in the rural river: "A" – Sampling site A; "B" – Sampling site B; and "C" – Sampling site C. Each site in the rural river had 18 data points for analysis.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

Two Elbow River sampling sites (i.e., Stanley Park and Sandy Beach) (**Figure 26**) were compared to a rural river control (**Figure 27**) throughout the sampling season. The Elbow River sampling sites were chosen for comparison due to one sampling site having the widest range of *Enterococcus* spp. concentrations (i.e., Stanley Park), and the other, which was farthest upstream, being the least contaminated (i.e., Sandy Beach). With respect to the rural river control, the most contaminated sampling site was chosen (i.e., 'Sample Site B'). *Enterococcus* spp. levels appeared to be significantly higher in the urban river (i.e., the Elbow River) impacted by stormwater than the rural river control, with the rural river control never exceeding the U.S. EPA's recreational water quality standard for *Enterococcus* spp. at >1280 CCE/100mL (**Figure 27**). This finding suggested that water quality of the rural river control was relatively more stable than the water quality of the urban stormwater-impacted Elbow River, and that stormwater likely contributes to significant fecal microbial loading in urban centers.

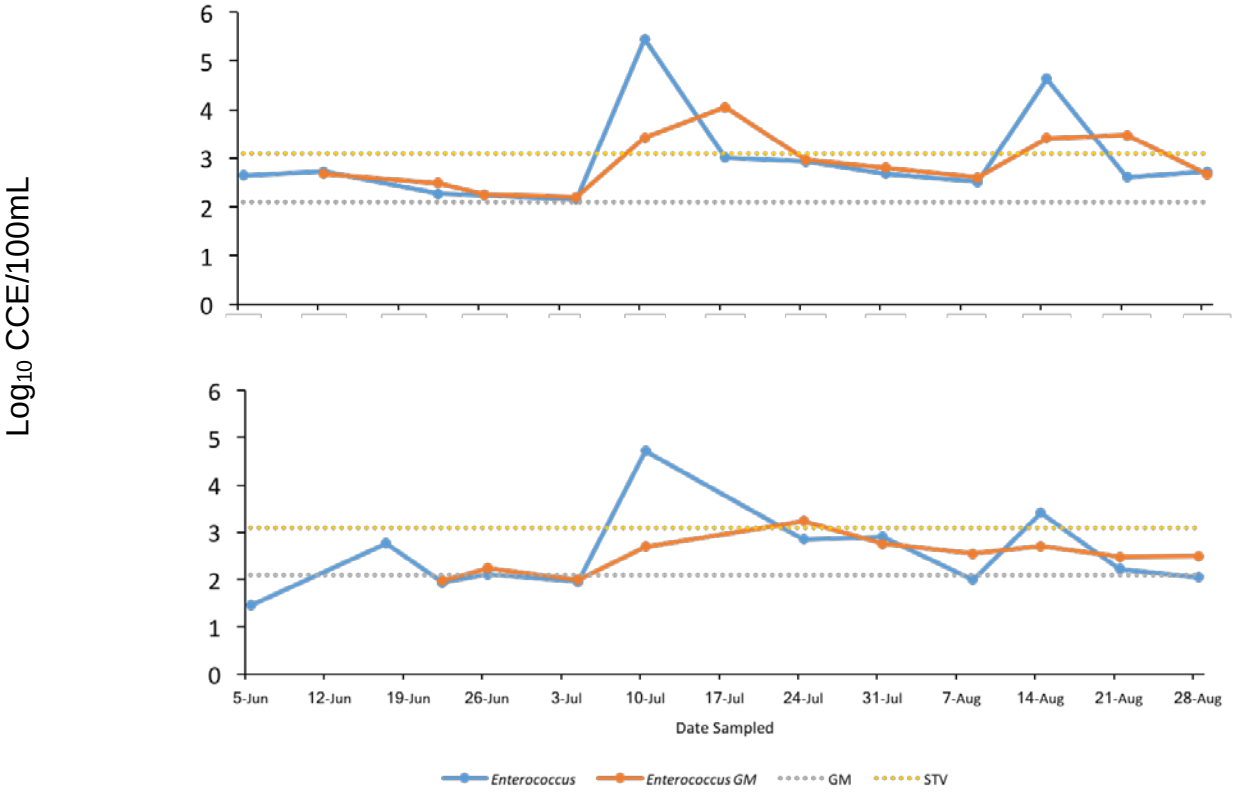


Figure 26. *Temporal pattern of occurrence of Enterococcus spp. log$_{10}$ CCE concentrations at sampling site Stanley Park (top) and Sandy Beach (bottom) located in the Elbow River. The USEPA Recreational Water Quality Standard geometric mean standard of >300 CCE/100mL is in yellow; the USEPA Recreational Water Quality Standard statistical threshold value of >1280 CCE/100mL is in red; the 5-sample running geometric mean of the water samples is in gray; and the single sample concentration of Enterococcus spp. are in blue.*

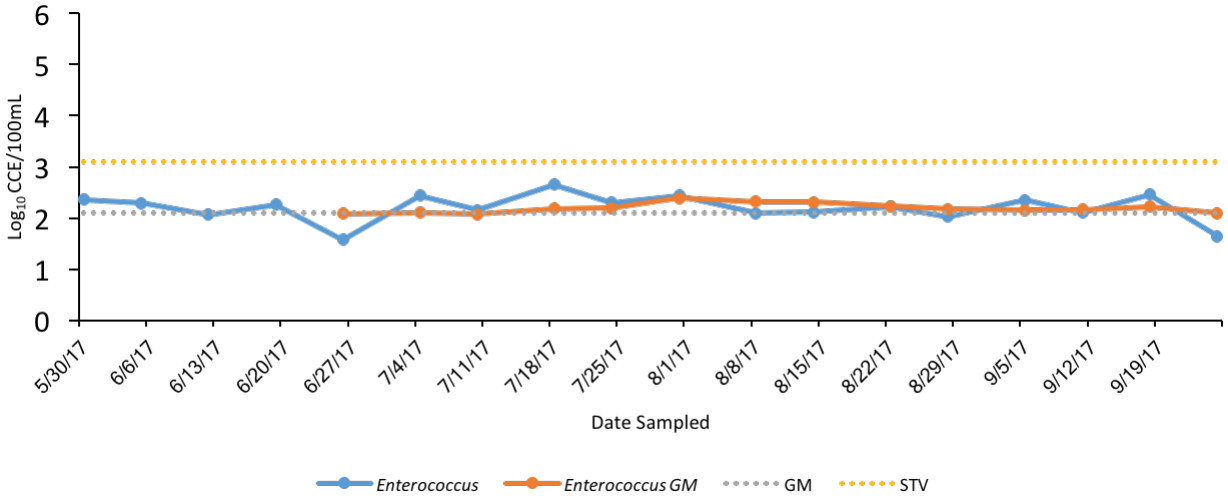


Figure 27. *Temporal pattern of occurrence of Enterococcus spp. log$_{10}$ CCE concentrations at a rural river control. The USEPA Recreational Water Quality Standard geometric mean standard of >300 CCE/100mL is in yellow; the USEPA Recreational Water Quality Standard statistical threshold value of >1280 CCE/100mL is in red; the 5-sample running geometric mean of the water samples is in gray; and the single sample concentration of Enterococcus spp. are in blue.*

OBJECTIVE 2

Use quantitative microbial risk assessment approaches to strategically identify water-fit-for purpose reuse options for stormwater and rainwater. The critical tasks for this project included:

- Interpret pathogen data, surrogates and reference organisms characterizing potential risks at points of exposures based on quantitative microbial risk assessment (QMRA) and water-use fit-for-purpose
- Describe critical performance targets to ensure microbial reductions meet safe use of reclaimed water.

RESULTS AND PROGRESS (Objective 2)

Our progress on this objective has been substantial. We have been actively involved in working with Alberta Environment and Parks (AEP) and Alberta Health (AH)/Alberta Health Services (AHS) in performing a large number of QMRAs across a broad range of water-fit-for-purpose reuse activities. Much of the data used for developing these risks assessments originated from the work presented in Objective 1 (above) and through a previous grant we held from Alberta Innovates (*Expanding Wastewater Reuse in Alberta Through Application of a Quantitative Microbial Risk Assessment Framework* [funding from 2013-2016]). Unlike approaches used in many other jurisdictions that rely on a range of published datasets, we have incorporated both published data and our empirical datasets in developing these QMRAs. Moreover, our studies represent the most comprehensive datasets on stormwater quality and wastewater treatment in terms of pathogen removal and occurrence, allowing for contextual understanding unique to Alberta, but applicable nationally and internationally.

Two provincial guidance documents have been developed and include: a) *Public Health Guidance for Water Reuse and Stormwater Use* (Alberta Health/Alberta Health Services); and b) *Alberta Water Reuse and Stormwater Use Guidebook* (Alberta Environment and Parks). **The documents being developed in partnership with these Government of Alberta agencies, but are CONFIDENTIAL and these ministries have requested an embargo on these documents for public release until they have been formally approved and released by the Government of Alberta.** Nevertheless, as evidence of the work being done by the project team, we have extracted specific examples of Log Reduction Targets (LRT) from these documents and being proposed for the various water reuse scenarios in Alberta. A probabilistic quantitative microbial risk assessment (QMRA) model was used to derive the pathogen $\log_{10}$ reduction targets that corresponded with a tolerable risk levels of exposure to pathogens per person per year ($1x10^{-2}$ and $1x10^{-4}$). The log reduction targets are a minimum value that must be achieved through treatment or exposure and management controls. LRT targets for viruses, protozoan parasites and pathogenic bacteria have been developed for wastewater reuse (**Table 18**), greywater (**Table 19** and **Table 20**), stormwater (**Table 21** and **Table 22**), and rooftop collected rainwater (**Table 23**).

The policy guidance documents being developed by these ministries provides a comprehensive explanation of navigating the regulatory processes for approval of water reuse systems in Alberta and contains detailed information of how to achieve the different LRTs, including infrastructure management (i.e., drainage ponds vs direct use, drip irrigation vs. spray irrigation), treatment (filtration or active disinfection), and management strategies (restricted access irrigation, irrigation at night vs. daytime).

As an example of how Objective 1 connects with Objective 2, in Objective 1 we demonstrated that stormwater or stormwater ponds are often contaminated with human wastes. Although this occurs, the level of human sewage contamination of stormwater observed in these systems (Calgary and Airdrie) falls within the lower source risk categorization for LRTs - i.e., human wastes are dilute ($\geq 10^{-3}$ dilution [1 litre of sewage for every 999 litres of water]), and therefore these stormwater systems would be subject to the LRT targets set out in **Table 22** as opposed to **Table 21**. The MST targets for human waste (HF183 and HumM2) can be used to evaluate the loading of human wastes in these systems. For example, the level of the human HF183 marker is typically around 10^8 copies/100mL in raw municipal sewage. Thus, at McCall Lake, although we observed persistent levels of human waste contamination at the ML2 site, the levels were typically around 10^4-10^5 copies/100 mL, suggesting that these human wastes were diluted within the drainage network by more than 3 orders of magnitude by the time it reached the outfall. The ability to track and quantify these sources of

pollution allows municipalities to identify and respond to water quality issues that might compromise reuse activities for stormwater, yet still allow the use of this water as long as it meets the LRT criteria. As such, it does not necessarily limit the use of the water, but sets public health criteria for use. It also sets a precedence for industry/municipalities to be more actively involved in routine monitoring of stormwater systems, since those facilities that choose not to monitor for human contamination will automatically fall into the higher risk category of human sewage pollution (**Table 21**) as opposed to the lower risk category (**Table 22**), ensuring public health protection even in systems not monitored for human sewage intrusion.

Table 18. Log_{10} reduction targets for untreated domestic wastewater[a]

Water End Use		Log Reduction Target for Viruses[b]	Log Reduction Target for Protozoa[b]	Log Reduction Target for Bacteria[b]
Aesthetic water features	Indoor	6.0	5.0	5.5
	Outdoor	4.0	3.0	3.5
Agri-food irrigation [c]		7.0	6.5	7.0
Car/truck washing [d]		5.5	4.5	5.5
Clothes washing		4.5	3.5	4.5
Cooling towers and evaporative condensers		4.5	4.0	4.5
Dust control/street cleaning		4.0	3.5	4.0
Non-agri food irrigation		4.0	3.5	4.0
Recreational		4.0	3.5	4.0
Toilet and urinal flushing		5.5	5.0	5.5

[a] Log reduction targets were rounded to the nearest 0.5 units, given that there will be probable errors in estimating performance in field experiments (Refer to Schoen et al., 2017 and Li et al., *in prep*, for individual pathogen LRT estimates).
[b] The reference pathogens for viruses, protozoa, and bacteria are Norovirus, Giardia, and Campylobacter, respectively.
[c] Log reduction credits are available to meet the LRT through food processing. See Appendix G Log_{10} reduction credits for exposure controls and management practices.
[d] Assumes a wand wash with exposure to water spray.

Table 19. Log_{10} reduction targets for greywater from a single family residential system[a]

Water End Use		Log Reduction Target for Viruses[b]	Log Reduction Target for Protozoa[b]	Log Reduction Target for Bacteria[b]
Aesthetic water features	Indoor	4.0	0	0
	Outdoor	2.0	0	0
Agri-food irrigation[c]		6.5	0	0
Car/truck washing[d]		4.5	0	0
Clothes washing		3.5	0	0
Cooling towers and evaporative condensers		3.0	0	0
Dust control/street cleaning		3.0	0	0
Non-agri food irrigation		3.0	0	0
Recreational		2.0	0	0
Toilet and urinal flushing		5.0	0	0

[a] Log reduction targets were rounded to the nearest 0.5 units, given that there will be probable errors in estimating performance in field experiments (Refer to Schoen et al., 2017 and Li et al., *in prep*, for individual pathogen LRT estimates).
[b] The reference pathogens for viruses, protozoa, and bacteria are Norovirus, Giardia, and Campylobacter, respectively.
[c] Log reduction credits are available to meet the LRT through food processing. See Appendix G Log Reduction Credits for Exposure Controls and Management Practices.
[d] Assumes a wand wash with exposure to water spray.

Table 20. Log_{10} reduction targets for greywater (from a community system)[a]

Water End Use		Log Reduction Target for Viruses[b]	Log Reduction Target for Protozoa[b]	Log Reduction Target for Bacteria[b]
Aesthetic water features	Indoor	5.0	3.0	3.0
	Outdoor	3.0	1.0	1.0
Agri-food irrigation[c]		6.5	4.5	4.5
Car/truck washing[d]		5.0	2.5	3.0
Clothes washing		4.0	2.0	2.0
Cooling towers and evaporative condensers		4.0	2.0	2.0
Dust control/street cleaning		3.5	1.5	1.5
Non-agri food irrigation		3.5	1.5	2.0
Recreational		3.5	1.5	1.5
Toilet and urinal flushing		5.0	3.0	3.5

[a] Log reduction targets were rounded to the nearest 0.5 units, given that there will be probable errors in estimating performance in field experiments (Refer to Schoen et al., 2017 and Li et al., *in prep*, for individual pathogen LRT estimates).

[b] The reference pathogens for viruses, protozoa, and bacteria are Norovirus, Giardia, and Campylobacter, respectively.

[c] Log reduction credits are available to meet the LRT through food processing. See Appendix G Log Reduction Credits for Exposure Controls and Management Practices.

[d] Assumes a wand wash with exposure to water spray.

Table 21. Log_{10} reduction targets for stormwater (10^{-1} dilution or 10% contribution from wastewater)[a]

Water End Use		Log Reduction Target for Viruses[b]	Log Reduction Target for Protozoa[b]	Log Reduction Target for Bacteria[b]
Aesthetic water features	Indoor	5.0	4.0	4.5
	Outdoor	3.0	2.0	2.5
Agri-food irrigation[c]		6.0	5.5	6.0
Car/truck washing[d]		4.5	3.5	4.5
Clothes washing		3.5	2.5	3.5
Cooling towers and evaporative condensers		3.5	3.0	3.5
Dust control/street cleaning		3.0	2.5	3.0
Non-agri food irrigation		3.0	2.5	3.0
Recreational		3.0	2.5	3.0
Toilet and urinal flushing		4.0	4.0	4.5

[a] Log reduction targets were rounded to the nearest 0.5 units, given that there will be probable errors in estimating performance in field experiments (Refer to Schoen et al., 2017 and Li et al., *in prep*, for individual pathogen LRT estimates).

[b] The reference pathogens for viruses, protozoa, and bacteria are Norovirus, Giardia, and Campylobacter, respectively.

[c] Log reduction credits are available to meet the LRT through food processing. See Appendix G Log Reduction Credits for Exposure Controls and Management Practices.

[d] Assumes a wand wash with exposure to water spray.

Table 22. Log_{10} reduction targets for stormwater (10^{-3} dilution or 0.1% contribution from wastewater)[a]

Water End Use		Log Reduction Target for Viruses[b]	Log Reduction Target for Protozoa[b]	Log Reduction Target for Bacteria[b]
Aesthetic water features	Indoor	2.5	2.0	2.5
	Outdoor	0.5	0	0.5
Agri-food irrigation[c]		4.0	3.5	4.0
Car/truck washing[d]		2.5	1.5	2.5
Clothes washing		1.5	0.5	1.5
Cooling towers and evaporative condensers		1.5	1.0	1.5
Dust control/street cleaning		1.0	0.5	1.0
Non-agri food irrigation		1.0	0.5	1.0
Recreational		1.0	0.5	1.0
Toilet and urinal flushing		2.5	2.0	2.5

[a] Log reduction targets were rounded to the nearest 0.5 units, given that there will be probable errors in estimating performance in field experiments (Refer to Schoen et al., 2017 and Li et al., *in prep*, for individual pathogen LRT estimates).
[b] The reference pathogens for viruses, protozoa, and bacteria are Norovirus, Giardia, and Campylobacter, respectively.
[c] Log reduction credits are available to meet the LRT through food processing. See Appendix G Log Reduction Credits for Exposure Controls and Management Practices.
[d] Assumes a wand wash with exposure to water spray.

Table 23. Log_{10} reduction targets for roof-top collected rainwater [a]

Water End Use		Log Reduction Target for Viruses[b]	Log Reduction Target for Protozoa[b]	Log Reduction Target for Bacteria[b]
Aesthetic water features	Indoor	Not applicable (NA)[e]	NA	3.0
	Outdoor	NA	NA	1.0
Agri-food irrigation[c]		NA	NA	2.0
Car/truck washing[d]		NA	NA	3.0
Clothes washing		NA	NA	2.0
Cooling towers and evaporative condensers		NA	NA	3.0
Dust control/street cleaning		NA	NA	1.5
Non-agri food irrigation		NA	NA	1.5
Recreational		NA	NA	1.5
Toilet and urinal flushing		NA	NA	3.0

[a] Log reduction targets were rounded to the nearest 0.5 units, given that there will be probable errors in estimating performance in field experiments (Refer to Schoen et al., 2017 and Li et al., *in prep*, for individual pathogen LRT estimates).
[b] The reference pathogens for viruses, protozoa, and bacteria are Norovirus, Giardia, and Campylobacter, respectively.
[c] Log reduction credits are available to meet the LRT through food processing. See Appendix G Log Reduction Credits for Exposure Controls and Management Practices.
[d] Assumes a wand wash with exposure to water spray.
[e] Not applicable because human viruses and protozoa are not common in rooftop sources (Sharvelle et al., 2017).

OBJECTIVE 3

Develop process-based and probabilistic models of microbial contamination in urban stormwater ponds. The critical tasks for this project included:

- Develop process-based modeling parameters from existing models (SWAT05, HSPF or others) for simulating microbial contamination in stormwater runoff but incorporate new knowledge on microbial fate and transport (i.e., MST)
- Investigate appropriate probabilistic methods and combine them with the process-based model.

RESULTS AND PROGRESS (Objective 3)

Results presented in this portion of the project are extracted from our latest published manuscript relevant to this project:

- Allafchi et al, 2019. An Integrated Hydrological-CFD Model for Estimating Bacterial Levels in Stormwater Ponds. *Water,* 11, 1016; doi:10.3390/w11051016

Although stormwater ponds are built with the primary objective of reducing runoff quantities in order to protect urban areas against flooding, they also improve the quality of stormwater. As demonstrated in Objective 1, microbial stormwater quality within a pond varies both spatially and temporally and is not only a function of the quality of the influent but also a function of local hydrological conditions and on the pond's design. Thus, stormwater recycling with pond water often requires continuous or intermittent water quality monitoring of the pond water in order to remain compliant with local regulations on reuse (i.e., irrigation standards). Highly distributed water quality sampling in stormwater ponds is often impractical due to the sizes of these ponds and the cost. In addition, most ponds are not designed with reuse in mind and the extraction point is often located in an ad hoc fashion and possibly in a region of the pond which has higher pollution levels relative to the rest of the pond because of local hydrodynamic conditions. If retrofitting a pond with the intent to recycle the water was a goal, the municipality could undertake a water quality sampling program that collected samples distributed throughout the pond over a period of time in order to identify the optimum location for extracting the "cleanest" water in the pond (assuming there is no treatment of this water). However, a more cost-effective alternative is to develop a physical model to estimate the bacteria level in the pond that incorporates the factors leading to bacterial contamination of stormwater in retention ponds.

A comprehensive model was developed during this study that integrates a hydrological model for a catchment draining to the Inverness Stormpond, with a computational fluid dynamics (CFD) model simulating the pond's hydrodynamics. The results of the hydrological model were used as inputs to the CFD simulation. The model results were validated against data collected at the pond. The overall goal was to enhance knowledge of bacterial fate and transport in stormwater ponds. Furthermore, the developed modeling approach leading to this enhanced understanding may ultimately be used as a tool to evaluate the capacity for a stormwater pond as a candidate for reuse and/or the need for the modification/retrofit. In addition, it may provide designers and planners with guidance to define standards for stormwater reuse.

The rationale for using the Inverness Stormpond relates to availability of historical microbial water quality datasets that we have collected on this pond over the years. The intent was to use these historical datasets to develop models that could be used to assess their temporal robustness. Data collected in 2007-2009 examined storm event loading, incorporating autosamplers at distinct inlets into the pond, and allowed for an interrogation of these events and their affect on water quality. Thus, data collected in 2007-2009 was used in developing the models and for which we will subsequently be use these to characterize validity of these models from the 2017 and 2019 datasets (under continued funding from NSERC-CRD and City of Calgary funds) at the Inverness Stormpond and other stormponds in this study.

The simulation results for data collected on a storm event on 26 August 2007 are detailed and discussed in this section as an example. The concentrations of *E.coli* on the surface of the pond at different time steps

after the rain event are shown in **Figure 28**. As time passes, bacteria entering from the inlets redistribute in the pond. The flow field was affected by the inlet velocities for the first few hours after the events, but afterwards, the wind was the only parameter affecting the flow field

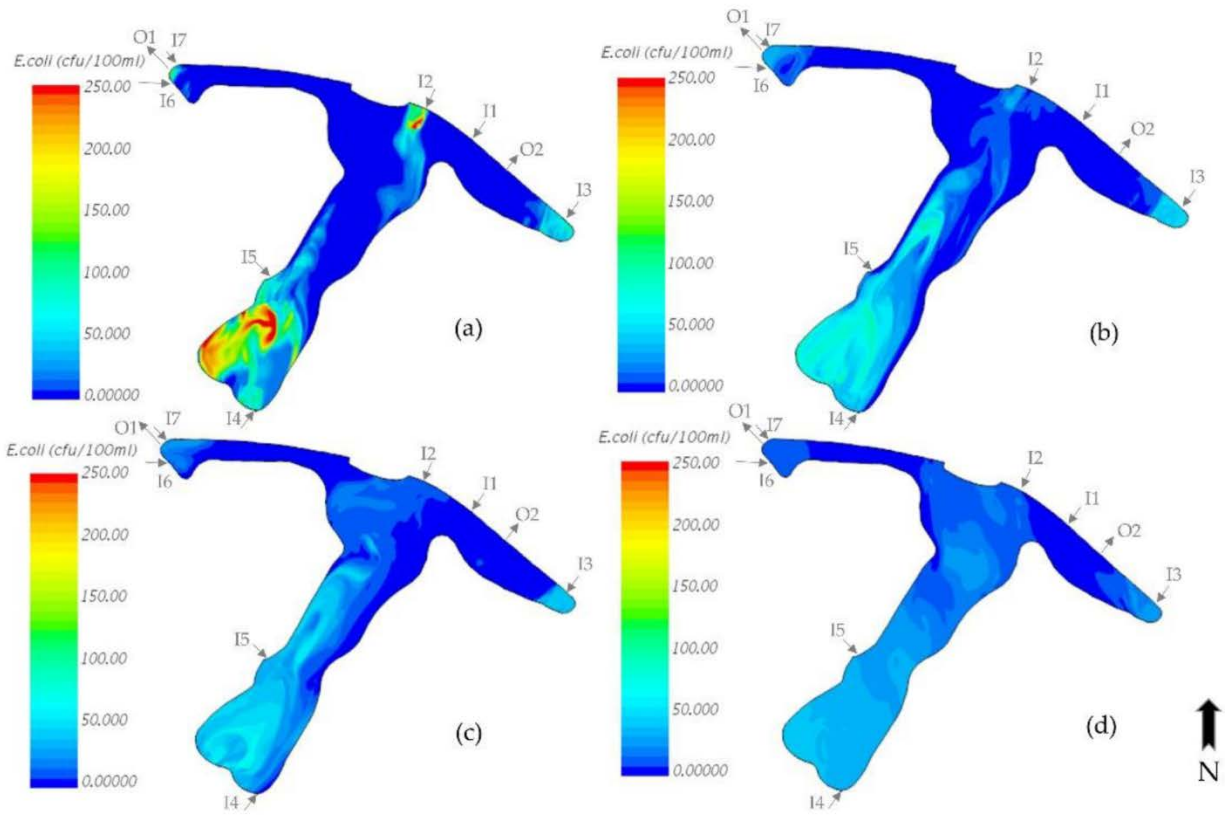


Figure 28. Contours of *E. coli* concentration (cfu/100 mL) on the surface of the Inverness pond on (**a**) 26 August 2007 at 5 p.m., (**b**) 26 August 2007 at 11 p.m., (**c**) 27 August 2007 at 5 a.m., and (**d**) 27 *August 2007 at 11 a.m.*

In 2007, the bacteria concentrations in the south wing of the pond were greater as compared with the other two wings (the east and west wings). There are two inlets (I5 and I4) in the south wing. The *E. coli* concentration was higher in the stormwater runoff entering the pond from inlet I5 than all other inlets; while bacteria loading from I4 was highest as it drains the largest area. For example, the *E. coli* concentration in the storm runoff from I5 on 26 August 2007 at 2 p.m. (during the storm) was 2100 cfu/100mL, while the storm runoff from I4 had a concentration of 1038 cfu/100 mL. However, the flow rates of the inlets at that time were 0.045 m3/s and 0.39 m3/s for the I5 and I4 inlets, respectively. Therefore, more bacterial mass entered the pond from inlet I4 even though the concentration of bacteria was greater in I5. In addition, a relatively large amount of bacteria entered the pond from inlets I3, I7 and I6, but most of the bacteria that came from I7 and I6 immediately exited the pond through outlet O1, because these two inlets are in proximity to the outlet. Therefore, they do not have a significant effect on the bacteria level in the pond. In general, inlets I4, I3 and I5 had the most significant effect on the bacteria levels in the pond. **Figure 29** shows the vertical profile of *E. coli* distribution in the pond at 6 h after the end of the storm. It reveals that the bacteria concentration also changes with depth. As illustrated in **Figure 29**, the maximum concentration of *E. coli* on the surface barely reached 90 cfu/100 mL. However, the maximum *E. coli* concentration was more than 120 cfu/100 mL at 2 m depth. In addition, the bacteria in the middle of the pond (where the three wings join) was less distributed at the bottom compared to the surface. The reason is that the direction of the wind had been NE and NNE for the last few hours, so the bacteria escaping from the sediment forebay of inlet I2 could reach the other side where the south

wing and the west wing join. In contrast, there was current flow toward the NE and NNE directions at the bottom simply because of mass conservation. This current potentially brought clean water close to the I2 sediment forebay. There was also a downwelling near the bank of the middle of the pond where the south and the west wings join. Generally, the wind causes differences in *E. coli* distribution in different layers of the water column. *E. coli* data collected at different depths on five random days between the year 2006 and 2007 revealed that bacterial concentration changed with depth. However, these data did not show any specific trend, and this is likely an indication of the influence of multiple environmental conditions on the bacteria distribution at various depths.

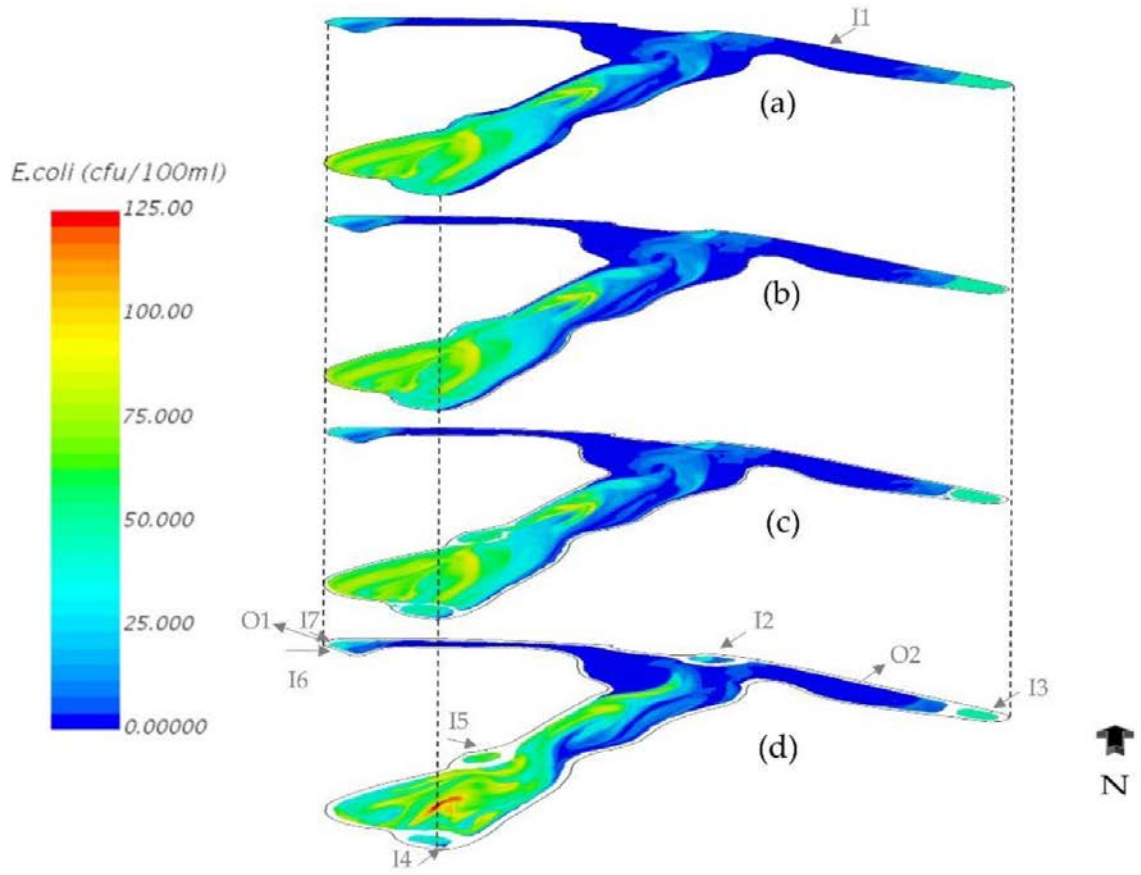


Figure 29. The vertical profile of E. coli concentration on 26 August 2007 at 11 p.m. (**a**) on the surface, ***(b)*** *at 0.5 m below the surface,* ***(c)*** *at 1 m below the surface, and* ***(d)*** *at 2 m below the surface.*

The sediment forebay corresponding to I3 was full of bacteria at all depths after the event (**Figure 30**). The sediment forebay was able to retain bacteria many hours after the event, which resulted in keeping The east wing relatively clean as compared with the south wing. **Figure 30** magnifies the contour of *E. coli* concentration near the sediment forebays at the surface on 27 August 2007 at 2 a.m. It also confirms that the sediment forebay of I3 outperforms the forebay of other inlets. As illustrated in **Figure 30**, the concentration of *E. coli* in the sediment forebays of inlets I4 and I5 appears to be similar or even slightly less than that in the region outside the forebays. This reveals that the bacteria entering these forebays during the storm were promptly discharged out of the forebays and consequently increased the *E. coli* concentration in their nearby regions.

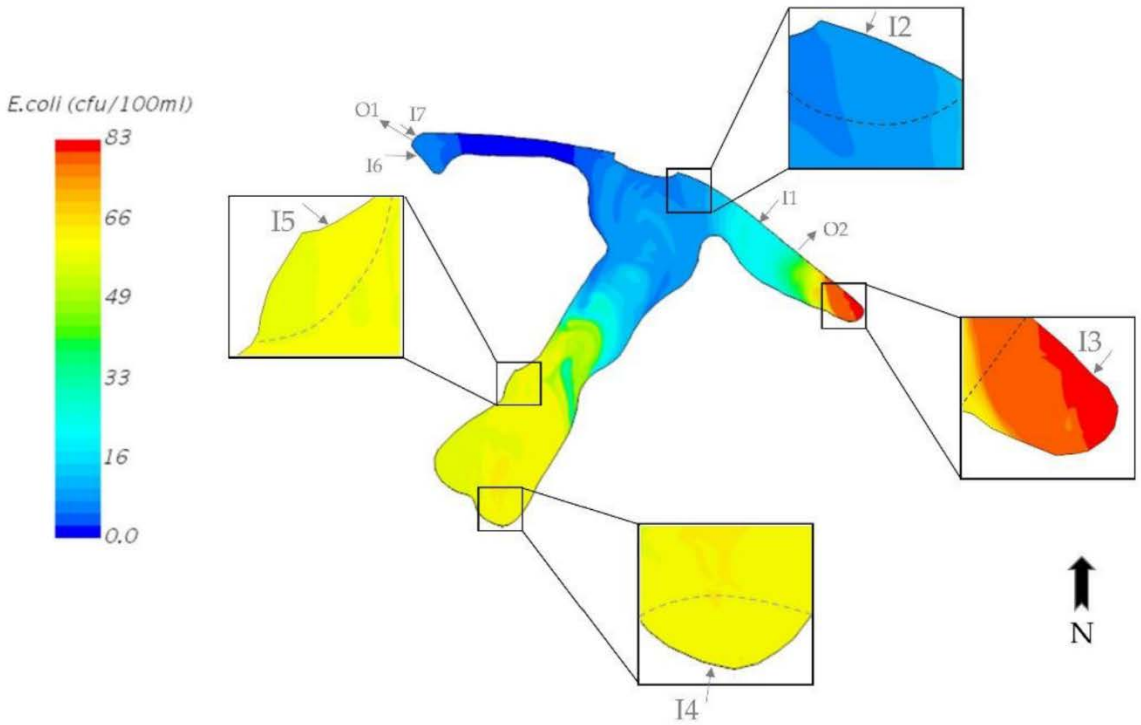


Figure 30. *E. coli distribution on the surface 27 August 2007 at 2 a.m. (9 h after the end of the storm)*

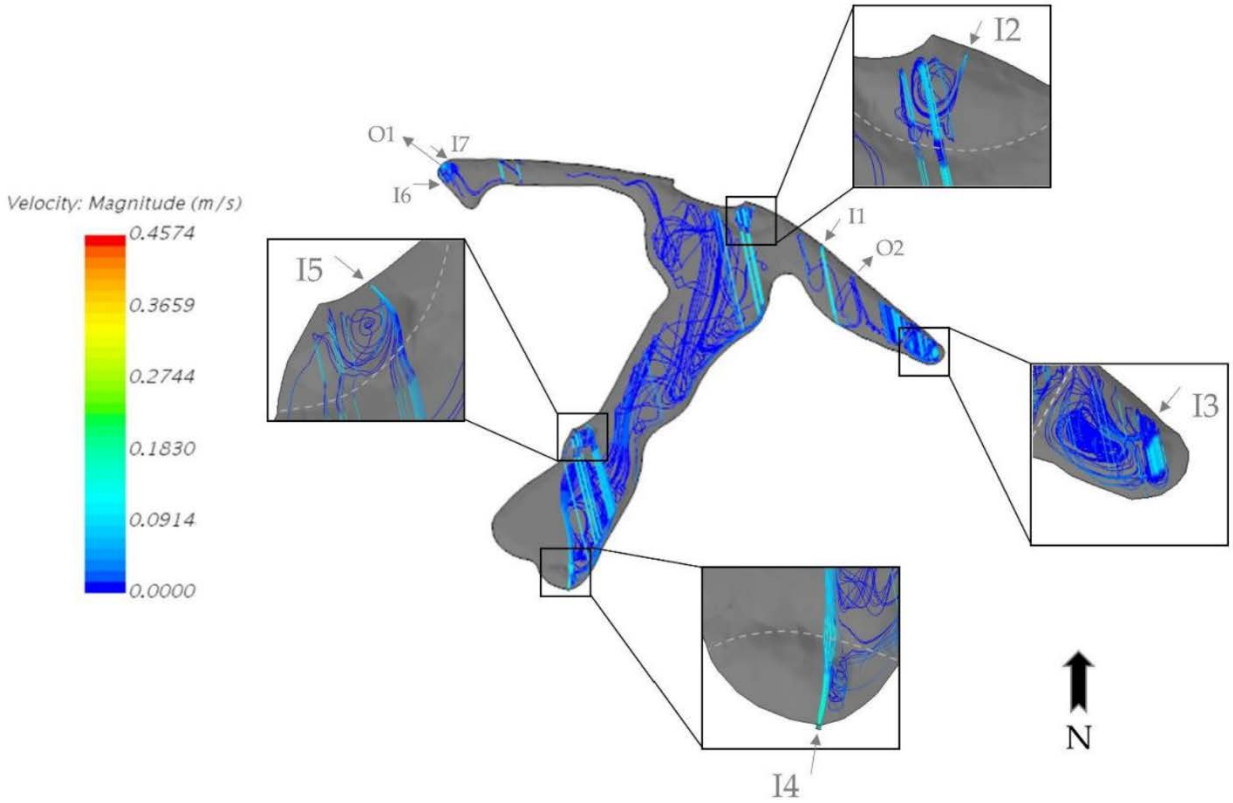


Figure 31. *Velocity streamlines on 26 August 2007 at 3 p.m. (during the storm).*

The design of sediment forebays, such as their configuration and size, determines their efficiency in trapping bacteria. **Figure 31** shows streamlines coming out from the inlets and spreading throughout the pond during the storm. The streamlines from I4 continue straight out of the forebay, which means the bacteria were directly transported into the pond. One reason is that the size of the forebay was not large enough to fit a circulation proportional to the high flow rate of the I4 inlet. Another reason may be the configuration of the inlet and sediment forebay. The direction of the streamline from I4 and the corresponding sediment forebay were perpendicular to each other (i.e., they were in front of each other), so the water jet coming out of the inlet easily escaped the forebay without circulating. The situation was the same in the sediment forebays corresponding to the inlets I2 and I5. However, their size was proportional to their flow rate. The sediment forebay corresponding to the inlet I3 had the best configuration since the direction of streamlines coming out of the inlet was parallel to the forebay. Thus, the bacteria coming from the inlet had no way but to circulate and in the long term, are retained and are likely to die off, which kept the east wing relatively clean. In addition, the size of the forebay was large enough to fit two large eddies. During the data collection campaign, surface grab samples were collected from six different sites at the pond over several days. All of the samples were collected at an average depth of 15 cm. One of the sample collection days was after a day with heavy rain. On 10 September 2005, 68 mm of rain fell and on the next day, water samples were collected from the six sites. Although much greater rain fell on 10 September 2005 than in the simulated events from 2007, it provided an interesting case for validation due to the dominant effect of rain on the bacteria distribution. The initial bacteria level in the pond before an event can influence the final bacteria levels after an event, particularly for small, low rainfall events. However, for high rainfall events, the majority of bacteria is transported into the pond with the storm runoff, and the initial bacteria level is a far smaller proportion of the total bacteria level.

Although it was noted that due to a lack of data it was difficult to validate the process-based FIB methods thoroughly, a comparison was done between the different events using a non-dimensional number. The non dimensional number was computed as the ratio of *E. coli* concentration at a site to the maximum E. coli concentration among all of the six sites at a certain time. Thus, the non-dimensional number at the site where *E. coli* concentration was a maximum was one. The number was calculated for the individual modeled events, and then the average was taken for each site. **Figure 32** shows the non-dimensional number at the six sites for the measured data and the average of the simulated events with its variation range. Simulation results were in good agreement with the measured data in recognizing hot spots (spots with a high concentration of bacteria) and spots with the lowest level of bacteria. The variation in the non-dimensional number in the east and west wings were higher than that in the south wing and in the middle of the pond. This may be due to a stronger influence from meteorological factors such as wind and rain at the tip of the east wing and the tip of the west wing. On the other hand, the low variation in the calculated data in the south wing and their high values show that the south wing always had the highest bacteria levels in the pond. Therefore, it is not recommended to extract water for reuse from the south wing.

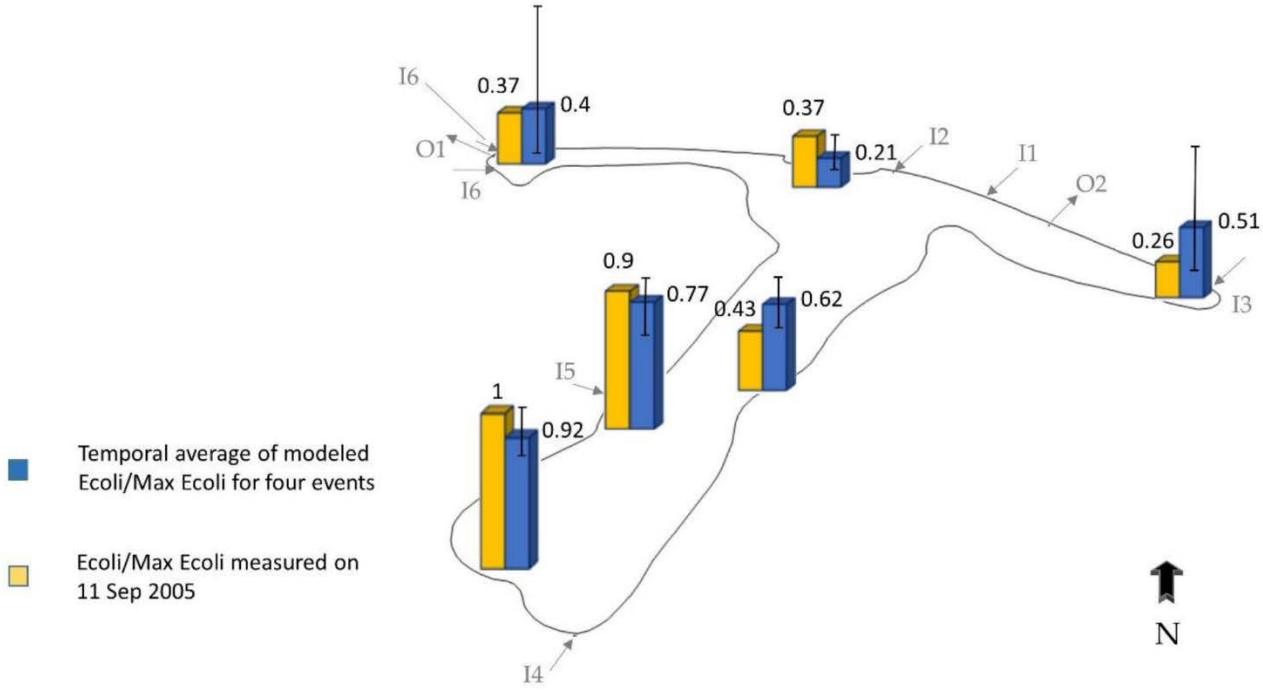


Figure 32. *Non-dimensional E. coli concentrations 15 cm below the surface based on measured and modelled concentrations.*

OBJECTIVE 4

Develop a Stormwater Use Management Plan (SUMP) Framework to support rainwater and stormwater use in Alberta.

RESULTS AND PROGRESS (Objective 4)

Our work on this objective has been extensive, entailing the collaborative development of provincial Water Reuse Safety Plans with various Government of Alberta ministries, in particular Alberta Health and Alberta Health Services. This risk-based tool encompasses aspects of the policy document described in Objective 2 (*Public Health Guidance for Water Reuse and Stormwater Use*), and consolidated into a Microsoft EXCEL program. The intent behind the development of this application solution was to create a simplified tool that could be used by industry/municipalities to navigate the regulatory framework and provide all the necessary details pertaining to water reuse projects, including a microbial risk assessment and with continuous management oversight of any proposed water reuse project. For example, the EXCEL database contains all the necessary QMRA-based Log Reduction Targets (i.e., those described in Objective 2) embedded in the locked down data files in the EXCEL program, as well as treatment or management strategies/targets to meet these Log Reduction Target requirements. The program is designed around the collection of simple data inputs such as box-embedded scripts and drop-down menus to capture information about reuse project. This information is then used in the background programming to determine whether the project meets the necessary risk targets. It is also intended that completion of these Water Reuse Safety Plans will act as one of the requirements for approval of these projects. **An e-version of this EXCEL program is appended to this report as evidence of its development, but we request that the application remain confidential and embargoed against public release until all supporting guidance documents have been released by the government (i.e., *Public Health Guidance***

for Water Reuse and Stormwater Use **[Alberta Health/Alberta Health Services] and the** ***Alberta Water Reuse and Stormwater Use Guidebook*** **[Alberta Environment and Parks]).**

We are currently working with Alberta Health in drafting a new research proposal to move this tool into industry/municipal planning circles. The goal is to partner with select municipalities/developers to 'test-drive' the tool in specific case studies in Alberta, and to help refine the tool for full release. We are in current discussions with our partnering municipalities (i.e., Calgary and Airdrie) to develop these case studies, and which will be supported by the offset funding from NSERC-CRD, City of Calgary funds, and any additional funding resources provided by Alberta Health through their funding allocations under the Water for Life Strategy.

4.2: LESSONS LEARNED/OUTCOMES OF RESULTS DURING PROJECT

Key Lesson Learned and Current Status of Project

Several key lessons were learned and an overview of each of these lessons is contextualized in terms of the objectives and milestone that we set out to accomplish in this project. The current status of these milestones is also included, and reflective of the work that will be continuing on this project through co-funding that we received from our NSERC-CRD and City of Calgary (and other funding). The funding from Alberta Innovates was instrumental in completing this work, and for which these additional funding sources will be used to fill any gaps remaining.

Objective 1 : *Evaluate microbial water quality, pathogen occurrence and treatment efficacy in stormwater and rainwater systems in urban municipalities in Alberta.*

Lessons Learned:

- Microbial quality of stormwater is often poor, and generally does not meet current microbial water quality criteria deemed safe for public health. Adopting a simple requirement for stormwater to meet current microbial water quality standards for reuse will not advance the sustainable use of this alternative water source in Alberta. A risk-based framework is required to support the use of these vital water resources in Alberta. This observation was true for the current microbial indicators used for evaluating water quality (i.e., *Enterococcus*, *E. coli*, or thermotolerant coliforms).
- Microbial water quality is highly variable spatially and temporally. Water quality in some stormponds is better than others, albeit all stormponds appear to be subject to major fluctuations in bacteriological water quality. These fluctuations are dependent on the sources of fecal pollution impacting the ponds.
- As has been demonstrated in studies carried out in the U.S., Europe, Australia and other international jurisdictions, stormwater ponds in urban environments in Alberta are highly subject to human fecal contamination. Human feces was a dominant source of stormwater pollution in urban environments, even in systems separate from sewerage. This represents a significant risk to human health for stormwater use, and requires Alberta to adopt a risk-based approach to manage these alternative resources. Human contributing sources can include cross connections, sewer infrastructure failures, and homeless/vulnerable/displaced populations.
- Human sources of pollution can be persistent or sporadic, but its occurrence was observed in every stormpond. This variability requires an overall management approach that is based on precautionary principles and risk-assessment based on these high-risk sources.

- Human source tracking tools were useful in identifying drainage networks contributing to fecal pollution levels in stormwater, and could be used by municipalities to help mitigate these risks to their stormwater.
- Other major sources of fecal pollution include aquatic birds (seagulls, geese). Although the influx of these sources of fecal pollution were often noted during precipitation events, significant contamination can also occur in the absence of precipitation events (i.e., aggregations of birds on stormponds in late summer).
- Other sources of fecal pollution included dogs and aquatic mammals [muskrat]) but overall, these levels appeared to low and sporadically observed.
- Bacterial enteric pathogens, such as *Arcobacter butzleri*, shiga-toxin producing *E. coli*, *Campylobacter* spp., and *Salmonella* spp. could be routinely detected in stormponds, with prevalence reflective of the order of these pathogens listed. Interestingly, pathogenic *A. butzleri* could be cultured from 75% of stormwater samples, albeit the maximal concentrations observed peaked at 93 bacteria/300 mL of stormwater.
- The presence of *A. butzleri* was most often associated with detection of human sources of pollution in stormwater, suggesting that its origin may be coming from human sources. However, birds feces (i.e., seagulls) was also associated with *A. butzleri* prevalence, suggesting the potential for multiple host reservoirs contributing to the loading of this pathogen in stormwater systems.
- Given its dominance in stormwater, *A. butzleri* should be considered the most appropriate pathogen for development of QMRAs around stormwater.

Current Status:

- Our work has focused on stormwater (as opposed to rainwater) for a couple of reasons. Firstly, and most importantly, stormwater represents the most immediately available alternative water resource for water reuse activities in southern Alberta. Thus, it became a priority for the project team. Secondly, although rainwater systems exist in the province, several have also been decommissioned due to the lack of reuse standards and impeding adoption of these system. Consequently, the focus of the team was to work with regulators to develop these guidance documents to help facilitate a more broad uptake of reuse activities. As an example, although the project team planned to work with the Calgary Airport Authority for evaluation performance of their large rainwater collection system, the system was decommissioned until further regulation/validation process were in place.
- Work in 2020, under funding from our NSERC-CRD/City of Calgary co-funding, will include an assessment of microbial water quality and pathogen occurrence in select rainwater systems.
- In 2020, we are planning to include the targeted testing of specific pathogens (viruses and parasites) at highly contaminated sites (i.e., Site ML2 at McCall Lake), in order to verify levels and occurrence of these pathogens. A major point of validation relates to whether these pathogens can *bioaccumulate* in receiving bodies and landscapes, and therefore whether levels/risks may be higher than anticipated. It is generally believed that bacterial indicators of water quality are poor surrogates for viruses and parasites, albeit they may be more stable indicators of fecal pollution compared to viruses/parasites in context of their persistent output by all host sources – i.e., as opposed to virus/parasite occurrence which is largely contingent on infection rates in the community.
- In the 2020 field season we will also be including antibiotic resistant microbes (*E. coli* and *Enterococcus*) in our analysis. We, and others, have recently demonstrated that certain strains of antibiotic resistant *E. coli* (i.e., extended spectrum beta-lactamase

producing *E. coli* [ESBL – *E . coli*] may be persistent in aquatic environments and appear to be resistant to water treatment.

2. *Use quantitative microbial risk assessment approaches to strategically identify water-fit-for purpose reuse options for stormwater and rainwater.*

Lessons Learned:

- As outlined above given the water quality issues associated with stormwater, Alberta needs to adopt a risk-based approach to the use and management of alternative water supplies.
- The work performed to date represents the most comprehensive approach to development of reuse standards (wastewater and stormwater), and is based on scientific evidence collected in Alberta and relevant to our situation and needs in a cold climate.

Current Status:

- The project team continues their collaboration with various government agencies in finalizing the guidance documents on water reuse, and adoption of this framework in Alberta for water reuse related to wastewater, grey water, stormwater and rainwater. This includes performing new QMRAs for other water reuse innovations not currently and explicitly covered in the existing guidance documents. We are now focusing on publishing the approaches and guidance criteria in the scientific literature.

3. *Develop process-based and probabilistic models of microbial contamination in urban stormwater ponds.*

Lessons Learned:

- An integrated computational flow dynamic (CFD) model could be used to accurately estimate bacterial levels at various points in the stormwater ponds during and after storm events.
- The integrated CFD models could be used to identify the best locations (i.e., cleanest) within a pond to extract water for reuse purposes, thereby further reducing the public health risks associated with reuse extraction from highly contaminated areas. The models could also be used to predict the fate of bacteria so as to identify when water quality is most suitable for reuse.
- Wind plays a crucial role in forming the flow fields in a stormwater pond and consequently the dispersion of bacterial levels within the lake.
- An understanding of flow fields can help in developing stormwater designs that can limit the spread and dispersion of bacteria in a pond (i.e., forebay designs to sequester bacterial loading).

Current Status:

- We are currently assessing whether the CFD models developed using data collected in 2007 are temporally robust in terms of predicting levels seen in the 2019 field season at the Inverness Stormpond.
- The project team continues to work on improving the integrated CFD models and apply these to other stormwater ponds (i.e., McCall Lake) and in order to assess whether they can accurately predict microbial water quality in different ponds. This work is currently being carried out using the 2019 field season dataset.

4. *Develop a Stormwater Use Management Plans (SUMP) Framework to support rainwater and stormwater use in Alberta*

Lessons Learned:

- In conjunction with Alberta Health and Alberta Health Services we developed an EXCEL-based Water Reuse Safety Plan template that encompasses elements of policy guidance documents

Current Status:

- The project team continues their collaboration with various government agencies in finalizing the guidance documents on water reuse, and adoption of this framework in Alberta for water reuse related to wastewater, grey water, stormwater and rainwater. This includes performing new QMRAs for other water reuse innovations not currently and explicitly covered in the existing guidance documents. We are now focusing on publishing the approaches and guidance criteria in the scientific literature.

SECTION FIVE: Project Impacts

5.1: STRATEGIC OBJECTIVES

Project Impacts: Economic, Environmental and Social Benefits

As outlined in Section 4.1, the impact of this research project is enormous. It is estimated that the City of Calgary's population will grow by a staggering 1.3 million people over the next 50-60 years[1] raising concerns regarding long-term sustainability of water quantity and quality within the Bow River Basin. A report commissioned by the City of Calgary in 2012 examined opportunities for water reuse for toilet/urinal flushing and irrigation. The report focused on infrastructure requirements necessary to support the development of two residential communities in the Calgary area, housing as many as 276,000 people and creating 85,000 local jobs for the province of Alberta [2]. The projected demand for reused water for toilet/urinal flushing and irrigation in these new communities alone was estimated at 7.5 billion liters per year.

Our current provincial framework around water management jeopardize these future growth opportunities. For example, a moratorium on water extractions from the Bow River is currently in place, and future municipal growth is dependent upon development of a water reuse framework, particularly in Southern Alberta. It is anticipated that within the next 10-15 years the City of Calgary will also require the construction of additional waste treatment facilities to meet its growing population (2), and thus, the research carried out on this project today affects multi-billion dollar decisions regarding the most effective ways to manage water stewardship in the future, the success of which is contingent on the implementation of water reuse strategies by municipalities. Delays in implementation of effective policies, or conversely, poorly developed regulatory frameworks, could result in major economic burdens and liabilities to Albertans in the future. The adoption of ill-conceived regulatory standards could be costly, whereby all economic gains are lost due to a lack of water quantity, costly retrofitting, or increased burden of disease/health care costs resulting from reuse of inappropriately treated alternative water sources.

The risk-based regulatory framework being developed through this research addresses these very threats to Alberta's economy and society (i.e., the public's health), and supports environmental management and stewardship of our water resources. The research also provides a framework to support and foster innovation in

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1 http://www.calgary.ca/PDA/LUPP/Pages/Municipal-Development-Plan/Municipal-Development-Plan-MDP.aspx

2 City of Calgary, Document File Number: 110773276. *Preliminary Design of a Dual Pipe Water System Volumes 1 and 2*. May 2012. Stantec Consulting Ltd.

water reuse designs and technologies for industry, providing a competitive edge to Alberta-based industries on national and international markets. Collectively, the research supported by this Alberta Innovates project elevates academic institutions, municipalities, government regulators and industry professionals in Alberta as recognized industry and regulatory leaders in water reuse and in the sustainable economic development of water resources.

SECTION SIX: Project Outlook

6.1: BUILDING INNOVATION CAPACITY

Table 24: Developing Talent – HSP generation during the course of the project

Employment Type	Existing HSP	New Hires	Person-Years of Employment
Academic HSP*	3	5	11.2
Industry HSP**			
Other HSP			
Construction and Trades			
Other Non-HSP			

**This includes: Faculty, Post-Doctoral Fellows, Graduate Students, Research Associates, Undergraduates etc.*

***This includes: Professional, Management, Technologists, as well as Operations and Maintenance Personnel etc.*

6.2: PROJECT COMMUNICATIONS AND MEDIA

WEBINARS

- Neumann, N. F. 2018. Resistant microbes and VBNC – what might they mean to food producers. Webinar series: Does Water Matter: Part 2 - What could be in your municipal water source. Sponsored by the International Association for Food Protection's Water Safety and Professional Development Group. April 30, 2018.
- Ashbolt, N. 2018. Drinking water sampling and what it means – what might they mean to food producers. Webinar series: Does Water Matter: Part 2 - What could be in your municipal water source. Sponsored by the International Association for Food Protection's Water Safety and Professional Development Group.

CONFERENCE ABSTRACTS, PRESENTATIONS, PROCEEDINGS

- Ruecker, N.J., and N. F. Neumann. 2016. When uncertainty affects public health action: Case Studies. Annual Conference for the Canadian Institute for Public Health Inspectors, Edmonton, Alberta. September 25-28, 2017.
- Neumann, N. F. 2016. Case studies: risks and opportunities for reuse. Conference for the Canadian Institute for Public Health Inspectors, Edmonton, Alberta. September 25-28, 2017.
- Bichai, F., and Ashbolt, N. 2017. Public Health and Water Quality Management in Low-Exposure Stormwater Schemes: a Critical Review of Regulatory Frameworks and Path Forward. *Sustainable Cities and Society*, 28: 453-465. http://dx.doi.org/10.1016/j.scs.2016.09.003

- Allafchi, F., C. Valeo, J. He, N. Neumann. 2018. CFD modeling of fate and transport of bacteria in stormwater ponds." Presentation to the Canadian Water Resources Annual Conference, Victoria, BC, May 28 to June 1, 2018.
- Neumann, N. F., and N. Ashbolt, N. 2018. Understanding the public health risks associated with alternative water sources: a comparative policy review. *Alberta Low Impact Development Conference*, Calgary, Alberta, Canada. March 12-13, 2018.
- Neumann, N. F., M. Beaudry, N. Ashbolt, C. Valeo, and J. He. 2018. Evaluating microbial risks and performance criteria for safe management of stormwater and rainwater reuse in Alberta. *Alberta Low Impact Development Conference*, Calgary, Alberta, Canada. March 12-13, 2018.
- Neumann, N. F (Invited Speaker). 2018. Emerging pathogen issues related to water reuse. *International Potable Reuse Symposium*, Austin, Texas. January 22-23, 2018.
- Neumann, N. F., S. Zhi and G. Banting. 2017. Emerging pathogen issues related to water reuse. *Water Quality and Technology Conference*, American Waterworks Association (AWWA), Portland, Oregon. November 12-17, 2017.
- Sharvelle S, Ashbolt N, Clerico E, Holquist R, Levernz H, Olivieri A. 2017. In *Risk-based framework for the development of public health guidance for decentralized non-potable water systems*, 11th IWA International Conference on Water Reclamation and Reuse, July 23-27, Long Beach Convention Center, California.
- Beaudry, M., G. Banting, C. Scott, N. Ashbolt, C. Valeo, J. He, N. Ruecker, B. van Duin, B. Jeon and N. Neumann. 2017. Understanding sources of contamination in stormwater ponds to promote water reuse in Alberta. Water and Health Conference, Chapel Hill, North Carolina. October 16-20.
- Beaudry, M., G. Banting, B. Jeon, N. Ashbolt, J. He, C. Valeo, N. Ruecker, B. van Duin, and N. Neumann. 2017. Assessing occurrence of bacterial pathogens in urban stormwater ponds in Alberta. *Campus Alberta Student Conference on Health*, Edmonton, Alberta, Canada. September 15-16, 2017.
- Rempel, B., N. Ruecker, E. Camm, G. Banting, N. F. Neumann. 2019. A survey of microbial contaminants discharged during storm events to the source water supply of the City of Calgary. *International Symposium on Waterborne Pathogens of the American Water Works Association*, Tampa Bay, Florida. April 29-30, 2019.
- Neumann, N.F. 2018. Extremely heat-resistant *E. coli* in the food-water nexus. Annual conference for the International Association for Food Protection, Salt Lake City, Utah. July 8-11, 2019.
- Atanasova, N., Dey, R., Li, Q., Scott, C., Leifels, M., Pang, X.-L., Ashbolt, N.J. (2018). Persistence of infectious coxsackievirus B5 in free-living amoebae. May 22, In: *Water Microbiology 2018*, Friday Center, University of North Carolina, NC.
- Ashbolt, N.J. (2018). Pathogens of regulatory and health concern. In *Expert Panel Working Meeting: Canada's Needs and Opportunities to Address Contaminants in Wastewater, January 16*, Canadian Water Network: Inn at the Forks, Winnipeg, Manitoba.
- Ashbolt, N.J. (2018). Managing key hazards for water reuse applications: Rationale for performance-based targets. In *Safety Codes Council Conference, May 3*, Rimrock Resort in Banff AB.
- Ashbolt, N.J. (2018). Developing a mechanistic QMRA model to aid control of *Legionella* levels. May 10, In: *Managing Legionella and Other Pathogens in Building Water Systems*, American Water Works Association: Hilton Baltimore.
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- *Alberta Water Reuse and Stormwater Use Guidebook* (CONFIDENTIAL DRAFT attached in Appendix C).
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PEER-REVIEWED MANUSCRIPTS, BOOK CHAPTERS, ETC.

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INTERNATIONAL RESEARCH LINKAGES

- (Ashbolt) EU Project, Resist'eau'me : Project advisory member (August 2015-2018) for the EU project investigating antibiotic-resistance genes in the water cycle using metagenomics approaches. Project team is lead by Dr. Jean-François LORET (Suez Environnement, France), Dr. Gilbert Greub (University of Lausanne, Switzerland), and Dr. Gerjan MEDEMA (KWR, the Netherlands).
- (Ashbolt) Water Research Foundation (WRF), WateReuse Research Foundation, and Water Environment Research Foundation (WERF): Stakeholder Advisory Committee Member (with 12 others, August 2015-2018) working with Paula Kehoe, Research Manager of the San Francisco Public Utilities Commission and Jeff Mosher, Executive Director, National Water Research Institute (California), providing oversight and direction for developing technical guidance for public health standards to address non-potable applications, including water quality criteria, monitoring regimes, and permitting strategies for onsite water systems.
- (Ashbolt) National Water Research Institute (NWRI), Independent Advisory Panel for technical requirements for public health standards for onsite water systems (WERF Project No. SWIM10C15).