Characterizing Sources of Fecal Pollution at Four Urban Public Beaches in the Halifax Regional Municipality

by

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Abstract

Within the last few years several beaches in the Halifax Regional Municipality have closed frequently due to increased levels of *E. coli* within the beach waters. Enumeration, microbial enrichment, and genetic microbial source tracking methods were used to enumerate *E. coli* levels and detect the presence of select pathogens and host-specific fecal contamination markers within four local freshwater urban beaches. *E. coli* levels mostly remained below the maximum allowed concentration throughout the sampling season. Tested pathogen and fecal contamination markers displayed a low prevalence. *E. coli* levels were influenced by measured water quality parameters and were shown to fluctuate on a day-to-day basis. However, *E. coli* were unable to predict the presence of enteric pathogens or fecal contamination markers. The beaches do not appear to be heavily contaminated and should generally be safe for public use. The use of *E. coli* as a fecal indicator needs to be further assessed in future studies.

List of Abbreviations Used

95% CI – 95% confidence interval

BB – Bolton broth

BPW – Buffered peptone water

CD – Conditional density

CDC- Centre for Disease Control and Prevention

CFU – Colony forming units

Ct – Cycle threshold

DO – Dissolved oxygen

EPA – Environmental Protection Agency

FB – Fraser broth

FC – Fecal coliforms

FIB – Fecal indicator bacteria

GI – Gastrointestinal illness

HRM – Halifax regional municipality

HRMC – High resolution melt curve

LD-MST – Library-dependent microbial source tracking

LEB - Listeria enrichment broth

LI-MST – Library-independent microbial source tracking

LOD – Limit of detection

LOQ – Limit of quantification

MST – Microbial source tracking

mtDNA – Mitochondrial DNA

OR – Odds ratio

PCR – Polymerase Chain Reaction

PS – Peptone saline

qPCR – Quantitative polymerase chain reaction

RVS – Rappaport-Vassiliadis Salmonella Enrichment Broth

spp. – Species

TC – Total coliform

TSB – Tryptic soy broth

TSS – Total suspended solids

VBNCS – Viable but not culturable state

WHO – World health organization

WQP – Water quality parameters

ρ^2 – McFadden's pseudo R^2

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Chapter 1 – Introduction

1.1 – Thesis Context

Recreational waters are composed of surface water and include both freshwater and marine bodies such as lakes, ponds, and quarries. Due to their close proximity to humans, recreational waters can become contaminated by chemicals, toxins, and microbial pathogens. Health Canada provides provincial jurisdictions, and their corresponding entities, a strict set of water quality guidelines to ensure safe use of recreational waters. The effective monitoring and maintenance of recreational water quality requires joint participation from the public, government, and businesses.

Pathogenic organisms can become introduced into surface waters via fecal matter from numerous sources including humans, wild animals, and domesticated pets. Possible human pathogens include microbes (e.g., *Escherichia coli* O157:H7, *Listeria monocytogenes*), viruses (e.g., Hepatitis A virus, Coronavirus), protozoa (e.g., *Cryptosporidium parvum*, *Giardia lamblia*), and parasites (e.g., tapeworms). As recreational waters remain untreated, pathogens can remain passively within the water column. Therefore, coming into direct contact with contaminated recreational water sources can result in pathogenic infection. For example, between 2009-2010 a total of 81 recreational water-related outbreaks occurred in the United States, causing 1,326 cases of illness and 62 hospitalizations (Centre for Disease Control and Prevention (CDC), 2014a). Furthermore, research shows a high correlation between illness and the presence of waterborne pathogens or fecal contamination indicators, as highlighted by Prüss (1998).

Government bodies in charge of managing water quality use a multi-barrier approach to ensure the safety of its users through the development of monitoring programs. Monitoring programs are retrospective in nature but allow for constant review of water quality, leading to the verification of current monitoring methods and more informed decision making. Most monitoring programs will determine possible fecal contamination through the use of fecal indicator bacteria (FIB). FIB, such as *Escherichia coli* or *Enterococcus* species (spp.), signal the presence of fecal contamination, as they are associated with the gut of warm blooded organisms, but are not capable of determining the fecal contamination source. A more recent group of

methodologies, labeled microbial source tracking (MST) methods, focus on tracking the source of fecal contamination directly by identifying host-specific indicator microbes or genes. In conjunction, these procedures form a powerful toolbox of methods that allow for effective management and monitoring of recreational waters.

The Halifax Regional Municipality (HRM) currently manages twenty-three beaches that are open to the public between July 1st and August 31st. The closure of beaches are determined by measuring *E. coli* levels in water samples taken within beach limits. In accordance with Health Canada Guidelines, beaches become closed to the public in two instances: i) A single water sample yields more than 400 *E. coli*/100 mL of water or ii) the geometric mean of at least five different water samples is greater than 200 *E. coli*/100 mL (Health Canada, 2012a). Within recent years, several of the beaches under HRM control have closed frequently throughout the beach open season due to increased levels of *E. coli*.

The methods used to determine *E. coli* counts are not instantaneous and in fact can take over a day to process, ultimately leading to delays in beach closures. A high frequency of beach closures can result in wasted resources and negative perception of local recreational departments. The current beach monitoring protocol focuses solely on the enumeration of *E. coli* and is therefore not providing the information necessary to better manage resources and eliminate fecal contamination. This study aims to both monitor *E. coli* and coliforms levels in four HRM freshwater beaches before, during, and after the beach open season, and determine potential sources of fecal contamination within these beaches using novel molecular methods.

1.2 – Research Questions and Objectives

This study aims to answer the following research questions:

1. What are potential sources of fecal contamination associated with four HRM freshwater beaches?
2. Can commonly measured water quality and weather parameters be used to predict *E. coli* levels within the four beaches?
3. Can *E. coli* be used as a valid indicator of fecal contaminant and pathogen presence within the four tested freshwater recreational beaches?

The objectives of this study are as follows:

1. Monitor levels of *E. coli* and total fecal coliforms (FC) before, during, and after the beach season.
2. Determine the prevalence of selected human pathogens and fecal contamination markers within the four HRM beaches.
3. Produce a predictor model of beach water *E. coli* levels based on use of common water quality and weather parameters.
4. Evaluate *E. coli* as an indicator of fecal contamination and pathogen presences within Nova Scotia recreational waters.

Chapter 2 – Literature Review

2.1 - Water Quality

2.1.1 – Chemical and physical parameters of water quality

The quality of recreational surface waters are determined through the measurement of numerous biological, chemical, and physical factors, referred to as water quality parameters (WQP). Chemical parameters, such as water temperature, pH, and dissolved oxygen (DO), can indicate the presence of pollutants and toxins. DO is a measure of free oxygen present within water while pH measures the acidity and alkalinity of water samples. Health Canada recommends that recreational waters have a pH between 5 and 9 to avoid eye and skin irritation (Health Canada, 2012a). Physical WQP include colour, odor and most importantly for this study turbidity and total suspended solids (TSS). Turbidity is an optical characteristic that measures water clarity (EPA, 2012a) and by extension the quantity of particles within the water column. TSS is a direct measure of the total abundance of particles, organic and inorganic, present within the water column (EPA, 2012b). An increase in Turbidity and TSS within normally clear water can represent an influx of contamination, indicating a degradation of water quality that can affect human health (Fondriest Environmental, 2014).

2.1.2 – Microbial quality of water

Microbes are ubiquitous within aquatic environments but the vast majority are not harmful to humans. Exposure of humans to waterborne pathogens can lead to infection, ranging in severity from asymptomatic to lethal. The World Health Organization (WHO) estimated that in 2012, waterborne diseases represented 3.6% of the daily burden of disease with 1.5 million deaths worldwide (WHO, 2015). Gastrointestinal illness (GI) comprises the majority of waterborne disease and typically runs its course in a few days with little health effects. However, infection from waterborne pathogens in weaker populations, such as immunocompromised or pregnant women, poses more serious health risks. The waterborne pathogens included in this study are *E. coli* O157:H7*, Salmonella* spp.*, Campylobacter* spp.*, L. monocytogenes, G. lamblia,* and *C. parvum*.

E. coli is a large group of Gram-negative bacteria found naturally within the gastrointestinal tract of warm-blooded animals. The majority of *E. coli* are not pathogenic but several strains, including the enterohemorrhagic *E. coli* O157:H7, can be detrimental to human health. *E. coli* O157:H7 was responsible for the Walkerton tragedy in Walkerton, Ontario, in which 2300 people contracted serious cases of GI and seven people died due to consumption of contaminated water (Hrudey *et al.,* 2002). Infection by *E. coli* O157:H7 can cause abdominal cramps, diarrhoea, vomiting, and fever but in severe cases can lead to the development of the potentially fatal haemolytic uraemic syndrome (WHO, 2011a). Propagation of *E. coli* O157:H7 infection mainly occurs through contact with contaminated food consumables, contaminated water, animals, and interaction with infected individuals or surfaces (Karch *et al.,* 2005). Farm animals such as cattle, pigs, sheep, and poultry are natural reservoirs of *E. coli* O157:H7 (Hancock *et al.,* 1994; Kudva *et al.,* 1996; Bielaszewska *et al.,* 1997).

Salmonellosis is a GI caused by several species of *Salmonella*. *Salmonella* is a broad group of Gram-negative motile rod-shaped bacteria. *Salmonella* spp. occur exclusively within the feces of warm blooded animals and should not be found naturally within the environment (Winfield & Groisman, 2003**)**. Although Salmonellosis is transmitted mainly through contaminated food, poor factory conditions, and poor kitchen hygiene, *Salmonella* spp. have been observed surviving in aquatic sources (Cherry *et al.,* 1972; Winfield & Groisman, 2003). *Salmonella enterica* subspecies *enterica* is responsible for the majority of Salmonellosis cases (Olsen *et al*., 2001; Graziani *et al*., 2013) and is composed of numerous pathogenic serovars including Enteritidis, Typhi, and Typhimurium. *S.* Typhimurium and *S.* Enteritidis cause enteritis GI (Santos *et al*., 2001), characterized by bloody diarrhea, abdominal cramps, fever, and vomiting. Symptoms usually pass in 1 – 3 days but severe cases can lead to dehydration and sepsis (Acheson & Hohmann, 2001; Ramalho & Correia, 2013). *S.*Typhi is responsible for typhoid fever, a much more serious infection characterized by intestinal haemorrhagic bleeding, encephalitis, and metastatic abscesses (Wali, 1991; Ezzat *et al*., 2010; Jorge *et al*., 2014). *Salmonellosis* is more prevalent in developing countries but the CDC estimates that each year in the USA there will be 1.2 million *Salmonella* spp. related illnesses, 19,000 of which resulted in hospitalizations and 380 are deaths (CDC, 2014b).

Campylobacter, a group of Gram-negative bacteria, are the most common bacterial cause of gastroenteritis in humans globally (WHO, 2011b). Campylobacteriosis infection can cause mucal/bloody diarrhea, abdominal pain, fever, nausea, and vomiting. *Campylobacter jejuni and Campylobacter coli* account for a large portion of human Campylobacteriosis infection (Horrocks *et al*., 2009), although *Campylobacter* spp. *lari* and *upsaliensis* have also been noted to cause GI to a lesser extent (Kaakoush *et al*., 2015). Animals that are raised for food (cattle, pigs, chicken; Wilson *et al*., 2008) and pets (Workman *et al.,* 2005) act as the main reservoirs for *Campylobacter* spp.. Once introduced, *Campylobacter* spp. can survive in aquatic environments by undergoing physical changes into a viable but not cultural state (VBNCS) or by incorporation into natural biofilms (Murphy *et al*., 2006)**.** *Campylobacter* spp. played a role in the Walkerton tragedy (Clark *et al.,* 2003) and has been linked to Nordic water-related gastrointestinal outbreaks (Rautelin *et al.,* 1990; Melby *et al.,* 1991), including an outbreak due to contaminated water in Northern Finland that made 2700 people ill (Kuusi *et al*., 2005).

Infection with *L. monocytogenes* may cause Listeriosis, which predominantly affects pregnant women, elderly individuals, the immunocompromised, and newborns. Infected individuals can display symptoms of septicemia, meningitis, stillbirths, and miscarriages (Jackson *et al.,* 2010; Silk *et al*., 2012). Unlike the other pathogenic bacteria presented in this study, *L. monocytogenes* are ubiquitous throughout the environment, with natural reservoirs including soil and water (Sauders *et al*., 2012; Linke *et al.,* 2014). Transmission of *L. monocytogenes* is mainly through food products (Hof, 2003) although research has shown a capability to survive within aquatic systems (Budzińska *et al*., 2012; Stea *et al*., 2015b). No major waterborne outbreaks of *L. monocytogenes* have been identified but numerous food-borne outbreaks have been reported throughout the last few years.

Surface waters can also be contaminated with unicellular eukaryotic protozoans, the two most common of which are *G. lamblia* and *C. parvum*. Baldursson & Karanis (2011) reported that these two organisms were responsible for 95% of 190 studied protozoa-based global outbreaks. These protozoans exist within water as (oo)cysts that are highly resistant to environmental and chemical stress, allowing them to survive for longer periods of time in natural environments. Upon infection, *G. lamblia* causes "beaver fever" marked by vomiting, chills, diarrhoea, and intestinal gas (Gardner & Hill, 2001). Cryptosporidiosis, caused by

Cryptosporidium spp., leads to watery diarrhoea, headaches, cramps, and nausea among infected individuals. The main sources of *Giardia* comes from rodents, beavers, cats, and dogs (Wallis *et al*., 1984; Bouzid *et al*., 2015) while *Cryptosporidium* primarily comes from cattle feces (Hunter & Thompson, 2005). During the spring of 1993, an estimated 400,000 people were affected by a waterborne outbreak of *Cryptosporidium* through failure of the Milwaukee public water supply (MacKenzie *et al*., 1994). Furthermore, *Giardia* was responsible for a 31-person waterborne outbreak due to consumption of contaminated drinking water in New Hampshire (Daly *et al*., 2010).

2.1.3 – Interaction between water quality parameters and microbial load within surface waters

Water quality cannot be determined solely through the singular effects of WQP but must also take into account the complex interactions between WQP, pathogens, and weather parameters. Water temperature is highly seasonal, with temperatures being the warmest during the summer and coldest during the winter. Water temperature shares a complex relationship with DO and TSS/turbidity such that suspended solids will absorb solar radiation (Fondriest Environmental, 2014), increasing water temperature which in turn will decrease DO as the solubility of oxygen decreases in warmer water (Fondriest Environmental, 2013). The abundance of pathogenic microbes in water is heavily dependent on the chemical and physical characteristics of the water system. The survival of pathogenic microbes within surface water systems increases with lower water temperatures (Korhonen & Martikaien, 1991; Obiri-Danso *et al*., 2001; Budzińska *et al*., 2012). Studies have yet to effectively determine any strong correlation between DO and/or pH and abundance of pathogenic microbes. Turbidity, and by extension TSS, are known to have a direct effect on the abundance of bacteria present within water systems. In more turbid waters, microbes are capable of growing by using floating particles as both food and protection (EPA, 1999; Huey & Meyer, 2010). Furthermore, there is a positive correlation between increased turbidity in water and the occurrence of GI (Morris *et al*., 1996; Schwartz *et al*., 1997). Storm events are positively correlated with the prevalence of FIB and pathogenic organisms. Heavy rainfall has been linked to increased *E. coli* levels that coincide with storm water runoff entering surface water systems (Ackerman & Weisberg, 2003; Haack *et al*., 2003). The influx of runoff from increased rainfall leads to an increase in

TSS/turbidity, which in turn has been correlated with an increase in FIB (Chen & Chang, 2014). It is therefore important to take into account WQP and weather parameters when monitoring FIB and pathogen levels in recreational waters.

2.2 – Contamination of Surface Waters

2.2.1 – Fecal contamination and waterborne illness in surface waters

The gastrointestinal tract of humans and other warm blooded animals contains a mix of non-pathogenic and pathogenic enteric microbes, which become introduced into water sources through fecal matter. Waterborne pathogens are likely to survive for short periods in recreational waters as they remain untreated, possibly leading to infection in beach goers. Several studies have shown the association between contaminated water and an increased chance of GI (Zmirou *et al*., 2003; Wade *et al*., 2006).

Fecal matter can become introduced into recreational surface water through numerous routes. Waterfowl release significant amounts of fecal matter and enteric pathogens into recreational waters (Weiskel *et al*., 1996; Meerburg *et al*., 2011). Wildlife, such as deer, and farm animals, and corresponding agricultural areas, contribute heavily to fecal contamination in aquatic systems (Somarelli *et al.,* 2007; Kumar *et al*., 2013; Guber *et al*., 2014). However, humans represent the largest source of fecal contamination (Whitlock *et al*., 2002), especially in urban areas and recreational beaches. Human fecal contamination in surface waters does not typically come directly from humans defecating in the water but instead through secondary sources such as leaky buried infrastructure, urban/sewage runoff, or storm drains (Boehm, 2003; Korajkic *et al*., 2010; Sauer *et al*., 2011). Due to the public health risks associated with contaminated recreational waters, it is crucial to constantly monitor for the presence and sources of fecal contamination.

2.2.2 – Indicators of fecal contamination

It is not realistic to test for the presence of every potential waterborne pathogen within a recreational water system. As a result, FIB are typically utilized. Indicator organisms are associated with fecal contamination and the potential presence of waterborne pathogens although

they are not pathogenic themselves (Wilkes *et al*., 2009). The indicators most commonly utilized in water quality monitoring include FC, *E. coli*, and *Enterococci* spp.. FC are a group of facultative anaerobic, rod-shaped, Gram-negative bacteria that originate in the intestines of warm blooded animals. In recent years FC have been phased out and replaced by the use of *E. coli* and *Enterococcus* spp.. In Canada, *E. coli* are used as indicators in freshwater while *Enterococci* are reserved for use in marine environments (Health Canada, 2012a). In accordance with Health Canada Guidelines, recreational waters will become closed to the public if a single water sample shows *E. coli* levels greater than 400 *E. coli*/100 mL or if the geometric mean of at least five water samples is greater than 200 *E. coli*/100 mL (Health Canada, 2012a). A combination of these indicators, and other methods, can be used in conjunction to provide a more complete assessment of fecal contamination in surface water systems.

2.2.3 – Limitations of fecal indicator bacteria

An ideal fecal indicator should be found exclusively within the enteric environment and should not be able to replicate or become naturalized outside this environment (Cabral, 2010). Furthermore, the presence of FIB should be correlated with the presence of enteric pathogens within the water system and should persist in the environment as long as the corresponding target pathogen (Field & Samadpour, 2007). However, recent research has brought into question the validity of FIB as indictors of fecal contamination. For example, persisting *E. coli* have been detected in non-fecal sources such as runoff from paper and pulp mills (Gauthier & Archibald, 2001) and tropical waters (Lopez-Torres *et al.,* 1987; Jiménez *et al.,* 1989). *Enterococcus* spp. have been found to persist and survive in areas including soil (Byappanahalli & Fujioka, 2004; Byappanahalli *et al*., 2012), surface sediments (Niewolak, 1998; Obiri-Danso & Jones, 2000; Anderson *et al*., 2005), and vegetation (Müller *et al*., 2001; Whitman *et al*., 2003; Badgley *et al*., 2010). FIB have also been thought capable of predicting the presence of human pathogenic microbes within water, however research surrounding this correlation displays varied results (Harwood *et* al., 2005; Wu *et al*., 2011; Ferguson *et* al., 2012).

2.3. – Microbial Source Tracking:

2.3.1 – Background and origins of microbial source tracking

FIB indicate the presence of fecal contamination but cannot differentiate between contamination sources. In recent years a group of methodologies termed MST have been developed to determine fecal contamination sources within water systems (Scott *et al*., 2002; Simpson *et al*., 2002). MST methods are diverse and include microbial, phenotypical, genotypical, and biochemical methods (Scott *et al*., 2002). MST methods have been used to determine water quality and track sources of fecal contamination in recreational beaches and water systems throughout the world. Gourmelon *et al*. (2007) utilized MST methods to track potential fecal contamination sources in three French estuaries used in shellfish harvesting. Moreover, bird droppings from Canada geese and gulls were determined as a heavy source of fecal contamination in a Lake Ontario freshwater beach (Edge and Hill, 2007). These methods can therefore be utilized to build effective recreational water quality protocols that allow for the identification and enumeration of pathogenic microbes along with the tracking of fecal contamination sources.

Section 2.3.2 – Library-dependent microbial source tracking

Library-dependent MST (LD-MST) methods are based around building a library of FIB isolates from known fecal contamination sources, identifying phenotypic and/or genotypic traits of FIB from each source, and then comparing them to environmental FIB isolates (Stoeckel & Harwood, 2007). This could, for example, entail collecting *E. coli* isolates from a beach and comparing them to a library of *E. coli* isolates taken from human sewage, animal feces, and sediment samples. Phenotypic methods rely on differences in characteristics between isolates and include biochemical tests (Kühn *et al*., 1998), antibody reactivity (Harwood *et al*., 2000), and susceptibility to bacteriophages (Payan *et al*., 2005). Conversely, genotypic methods rely on genetic differences between indicator organisms from different hosts (Casarez *et al*., 2007). Some genotypic LD-MST methods include rep-PCR (Dombek *et al.,* 2000) and ribotyping (Hartel *et al*., 2002).

Section 2.3.3 – Library-independent microbial source tracking

Library-independent microbial source tracking (LI-MST) methods target bacterial species, host genes, or chemicals that are specific to a certain fecal contamination source. For example, the 16S rRNA gene, a gene that codes for a small part of the prokaryotic ribosomes, is used extensively in LI-MST studies (Bonjoch *et al.,* 2004; Lee *et al.,* 2010). LI-MST methods use Polymerase Chain Reaction (PCR) or quantitative PCR (qPCR) to detect host-specific sources of fecal contamination. Detection of unculturable or difficult-to-culture microbes within fecal matter or water samples is possible with LI-MST methods. For example, *Bifidobacterium* and *Bacteroidales* are common gut microbes that are abundant in feces (Savage, 2001) but are difficult to detect in routine tests due to their fastidious nature. However, through the use of LI-MST these microbes have been used to source fecal contamination from both human and animal sources (Bonjoch *et al*., 2004; Savichtcheva *et al*., 2007; Walters *et al*., 2007; Lee *et al*., 2010). Furthermore, LI-MST methods have been developed to track sources of fecal contamination by targeting host-specific mitochondrial DNA (mtDNA) within shed epithelial cells (Kortbaoui *et al*., 2009; Ballesté *et al*., 2010; Baker-Austin *et al*., 2010), allowing for the detection of fecal sources by directly targeting the host.

Section 2.3.4 – Assumptions and limitations of LD-MST and LI-MST methods

LD-MST and LI-MST methods are very useful water quality tools but have their limitations. The size of isolate libraries and their representativeness of fecal contamination sources are two crucial factors of LD-MST (Ahmed *et al*., 2007). A library must be large enough to encompass all genotypic and phenotypic variations of the subgroups of the chosen indicator organism. If a library is too small it may misrepresent the fecal contamination sources present within the water due to missing isolates that represent specific fecal sources or geographic regions. Such a case is exhibited in Goto and Yan (2011) in which the number of *E. coli* isolates collected represented only a small portion of genetic diversity of *E. coli* found in a Hawaiian stream. LD-MST also assumes that the subgroups of the selected FIB are specific to one source or host. However this is not always the case as highlighted by Ahmed (2005), in which out of a library of 1056 *E. coli* and *Enterococcus* isolates, 488 were found in multiple host groups. Isolate

libraries need to be constantly updated to ensure that the chosen FIB is both temporally and geographically stable. Likewise, ideal FIB should display genotypic and phenotypic stability across geographical area (Stewart *et al*., 2003). If a marker is prone to geographic or temporal instability it needs to be validated before use in a specific geographical location.

Due to its recent conception LI-MST methods have a limited number of validated target genetic sequences available for the detection of host-specific microbes or genes. However new markers are constantly being produced, such as those for the detection of dog (Kildare *et al*, 2007; Sinigalliano *et al.,* 2010), cat (Caldwell & Levine, 2009), and waterfowl (Hamilton *et al.,* 2006; Green *et al.,* 2012; Ryu *et al*., 2012). It is very difficult to produce a genetic marker that is truly "specific" or stable in one host due to the ever-changing nature of gut flora (Hartel *et al.,* 2002; Hansen *et al.,* 2009) although this can be mitigated through the targeting of host-specific mtDNA. However mtDNA are less concentrated within host feces compared to *Bacteroidales* (Tambalo *et al*., 2012), leading to a decreased chance of detection in water samples. LI-MST methods allow for the detection of unculturable fecal indicators but the genetic sequence of the target gene must be known. Therefore, if a genetic marker has not yet been developed then it could remain undetected during analysis, leading to a misrepresentation of fecal contamination sources present within a water system.

2.4 – Genetic Markers for the Detection of Fecal Contamination in Surface Waters

2.4.1 – PCR and qPCR methods for detection of fecal-related genetic markers

LI-MST methods detect and quantify source tracking markers from specific fecal contamination sources through the use of (q)PCR. The amplification of target DNA through PCR or qPCR relies on a three step process: Denaturation, Annealing, and Elongation. During denaturation, high temperatures cause DNA to split into two single strands, allowing primers to attach to corresponding specific sequences on both strands. DNA polymerase will anneal with the primers and elongate the DNA sequence by adding nucleotides from the surrounding solution. This cycle continues for a pre-determined number of amplification cycles. As opposed to end-point PCR, which detects the amplification of the target gene at the end of the assay, qPCR detects the amplification of the target gene after each individual amplification cycle. qPCR requires the use of template DNA (from the target-source or microbe), a thermostable DNA

polymerase, target-gene specific primers, and a mastermix (containing deoxyribonucleotides, buffers, a fluorescent dye, and other molecules required by PCR).

qPCR quantifies the amount of target gene present in a sample by measuring the amount of light given off by reactions between target DNA and fluorescent dyes. A multitude of qPCR methods have been developed, of which two will be utilized in this study. The SYBR green method uses a non-specific fluorescent dye that intercalates into all double stranded DNA. When primers anneal to target DNA, SYBR Green fluorescent molecules will become intercalated into the dsDNA. As the DNA amplifies, more SYBR Green molecules will bind to the DNA thereby increasing the amount of florescence detected. The intensity of fluorescence given off in this reaction will be equal to the length of the amplified DNA. SYBR Green qPCR is typically used in conjunction with high resolution melt curve (HRMC) analysis to verify if the target gene was present/amplified. HRMC analysis works by detecting differences in the melting temperature of different amplified DNA strands. Ideally, the target DNA sequence will display a different melt curve then all other non-target DNA. TaqMan reactions use sequence-specific probes to detect the presence of target genes. TaqMan probes contain a fluorophore at the 5' end and a quencher at the 3' end. When the probe becomes attached to a target sequence, light will excite the fluorophore but the quencher will stop any florescence from being discharged. As the DNA amplifies, DNA polymerase will hydrolyze the DNA attached to the fluorophore, releasing it from the degraded probe thereby separating it from the quencher. Light is now able to excite the flurorphore, creating a fluorescence which is measured by the qPCR machine. In both methods the florescence will increase with each amplification cycle until it surpasses a pre-set threshold. The amplification cycle at which the florescence surpasses the threshold is referred to as the cycle threshold (Ct) value, which is used to determine the presence of and quantify a source tracking marker.

2.4.2 – Considerations when using genetic markers in MST studies

The ability of fecal markers to correctly differentiate the presence or absence of a genetic target is crucial when designing MST studies. In quantifiable terms, this is typically done through sensitivity and specificity measurements (equations can be observed in Appendix A). The ability of a marker to accurately detect its target is known as its sensitivity while specificity

is a marker's ability to differentiate between targets and non-targets. The rates of false positives, incorrectly identifying the presence of a marker (Fremaux *et al*., 2009), and false negative, incorrectly identifying the absence of a marker (Fremaux *et al*., 2009), influence sensitivity and specificity and must also be considered. Ideal markers should display high sensitivity and specificity with low rates of false positives and false negatives. Optimizing the sensitivity and specificity of markers will ensure accurate and precise target detection and data interpretation (Reischer *et al*., 2013).

The limit of detection (LOD) and limit of quantification (LOQ) of chosen fecal markers must always be considered when developing new assays. The LOD refers to the lowest possible concentration at which a specific marker can be detected (Armbruster & Pry, 2008). The LOQ represents a concentration at which quantitative results of the target marker can be reported with a high degree of certainty (Armbruster & Pry, 2008). An effective marker will display a low LOD and LOQ, allowing the target to be amplified in fecal samples containing very small amounts of target markers.

2.4.3 – Use of Bacteroidales and mitochondrial genetic markers for MST

Bacteroidales are an order of obligate anaerobic bacteria that make up a significant portion of the microbial load of fecal matter from warm-blooded animals (Matsuki *et al.,* 2004). Targeting the 16S rRNA genes of these *Bacteroidales* species has led to the development of assays for the detection of total fecal contamination and contamination from individual sources. Fecal matters from warm-blooded animals also contain epithelial cells that can be isolated from the feces (Kamra *et al*., 2005). Recently, some groups have utilized host-specific mtDNA gene targets within MST studies (Roslev & Bukh, 2011). As there are numerous copies of mtDNA present within each cell (Bogenhagen & Clayton, 1974) and little intra-species variation in mtDNA genes, these assays are a powerful tool for detection of fecal contamination in water.

2.4.4 – Universal markers of fecal contamination

Bacteroidales are prominent in the gastrointestinal tract of warm-blooded animals and can therefore be used to assess total fecal pollution in water samples. AllBac (Layton *et al*., 2006) and BacUNI-UCD (Kildare *et al.,* 2007) are two common universal *Bacteroidales* markers

that target specific 16S rRNA genes present in all *Bacteroidales.* Using these universal markers can be problematic however as several studies have shown that *Bacteroidales,* and corresponding universal markers, are abundant in non-fecal sources such as soils (Lauber *et al.,* 2009; Vierheilig *et al*., 2012) and the hindgut of insects (Ohkuma *et al*., 2002; Nakabachi *et al*., 2003). The detection of non-fecal species in water samples can lead to the misrepresentation of total fecal pollution within the water and subsequent inaccurate interpretation of results.

2.4.5 – Human markers of fecal contamination

Numerous human-specific fecal markers have been produced in response to the contribution of humans to the pollution of water systems. HF183 (Bernhard and Field, 2000a & 2000b) was one of the first human-specific markers constructed. A modified form of the HF183 assay, using SYBR green qPCR, was developed by Seurinck *et al* (2005) and has since been utilized in numerous MST studies (Kirs *et al.,* 2011; Sercu *et al*., 2011; Walters *et al*., 2011). A TaqMan-based HF183 assay was developed by Haugland *et al*. (2004 & 2010). Additionally, three more TaqMan-based qPCR human-specific assays have been developed: BacHum-UCD (Kildare *et al*., 2007), BacH (Reischer *et al*., 2007), and HuBac (Layton *et al*., 2006). BacHum-UCD displayed an original 100% sensitivity to wastewater samples but was not detected in all tested human stool samples and displayed some (12.5%) cross-reactivity with dog stool samples. Reischer *et al*. (2007) reported a sensitivity and specificity of 98% for their BacH marker. A 100% sensitivity rate was reported for the HuBac marker but appeared to moderately cross-react with swine fecal samples (Layton *et al*., 2006). A meta-analysis, by Layton *et al*. (2013), reported that the HF183 marker (with TaqMan qPCR) was the most effective marker when compared to BacHum-UCD and BacH.

2.4.6 – Dog markers of fecal contamination

Domesticated pets, such as cats and dogs, represent a potential source of fecal contamination in urban recreational beaches. In fact, dogs have previously been noted to contribute significantly to enterococci fecal indicator numbers in recreational beaches (Wright *et al*., 2009). Dick *et al*. (2005) were the first to try and develop an assay to target the 16S rRNA gene of dog-specific *Bacteroidales* spp.. Additionally, two other dog-specific 16S rRNA

markers, DogBact (Sinigalliano *et al.*, 2010) and BacCan (BacCan; Kildare *et al.*, 2007), have been developed for use. The sensitivity and specificity of these markers depend on the statistical methods used to analyze the data. However, in a study conducted by Schriewer *et al*. (2013) both markers displayed an average sensitivity of 100% but specificity was found to be 83% and 68% for DogBact and BacCan respectively. Furthermore, BacCan displayed a sensitivity and specificity >80% while DogBact displayed a sensitivity >80% but a very poor specificity in a meta-study by Boehm *et al* (2013). As a result, these markers are prone to cross-reactivity with other host-specific *Bacteroidales* from cows, humans, cats, deer, goose, and sewage (Kildare *et al*., 2007; Silkie & Nelson, 2009; Boehm *et al*., 2013).

MST assays have also been developed for the detection of dog mtDNA. Schill and Mathes (2008) developed a dog-specific TaqMan mtDNA assay that targeted the mitochondrial Cytochrome B gene, which displayed good sensitivity and specificity. Caldwell and Levine (2009) also developed TaqMan qPCR primer and probe sets, referred to as dogmt, for the detection of dog and cat cells using the NADH dehydrogenase subunit 5 gene present on mtDNA. This marker shows high sensitivity and specificity and can be detected in as little as 1 mg of feces/100 mL of water (Tambalo *et al*., 2012) or 10 copies/mL water (Caldwell and Levine, 2009). Due to the close proximity of domesticated dogs to humans and recreational waters, the addition of dog markers to MST toolboxes for fecal contamination detection is crucial.

2.4.7 – Avian markers of fecal contamination

Waterfowl may be significant sources of fecal pollution in marine and freshwater recreational beaches (Edge & Hill, 2007) and can also act as a reservoir for human pathogens such as *Salmonella* spp., *Campylobacter* spp., *Giardia*, and *Cryptosporidium* (Graczyk *et al*., 1998; Fallacara *et al*., 2004; Devane *et al*., 2005). Gulls have a significant abundance of FC present in their feces and contribute heavily to fecal contamination and *E. coli* presence in coastal and recreational waters (Lévesque *et al*., 1993; Alderisio and Deluca, 1999; Converse *et al*., 2012). Several MST assays have been constructed to detect total avian fecal contamination in water. Hamilton *et al*., (2006) developed hybridization probes, for suppression subtractive hybridization, specific to seven Canada goose-related *E. coli* strains. Combined, the seven

markers detected approximately 80% of the goose-specific *E. coli* isolates. Furthermore, a good portion of duck-specific *E. coli* isolates were also detected. Three assays (GFB, GFC, GFD), based on the 16s rRNA gene of *Fusobacterium*, *Catellicoccus marimammalium*, and *Heliobacter*, were developed by Green *et al* (2012) from gull-specific 16S rRNA gene markers that were capable of detecting gull, duck, Canada goose, and Chicken feces. Only the GFD assay was entirely specific to avian species while GFB and GFC showed some cross-reactivity with non-avian species.

2.4.8 - Use of MST studies in recreational waters

MST methods have already been extensively used to monitor fecal contamination in recreational waters. In Canada, Edge and Hill (2007) used LD-MST methods to determine that waterfowl played a big part in the contamination of a Lake Ontario freshwater beach. Through the utilization of LI-MST methods, McQuaig *et al*. (2012) tracked the HF183 marker at two California beaches to determine that human sewage was one of the main sources of fecal contamination. Furthermore, they determined that using a toolbox, containing FIB and MST methods, allowed for a more in-depth assessment of the beaches compared to FIB alone. Lee *et al*. (2012) utilized *Bacteroidales* markers to show that the density of pathogenic *Acrobacter* species, which can cause GI, in Lake Erie beaches was related to the abundance of the HuBac human fecal-matter marker, indicating that the presence of these pathogenic bacteria may be related to fecal contamination from humans. It is therefore apparent that both *Bacteroidales* and mtDNA MST methods offer powerful tools for the effective assessment and tracking of fecal contamination in surface waters.

Chapter 3 – Materials and Methods

3.1 – Sampling Beaches and Sample Collection

3.1.1 – Sampling beaches

Four freshwater urban beaches within the HRM, Nova Scotia were selected as study sites, including: Springfield Beach (44.819799°, -63.736401°), Kinsmen Beach (44.776634°, -63.673244°), Sandy Lake Beach (44.736521°, -63.695052°), and Birch Cove Beach (44.679278°, -63.561222°). Springfield, Kinsmen, and Birch Cove Beaches were chosen as experimental beaches due to a history of frequent beach closures. Sandy Lake Beach was chosen as a control beach due to a history of good water quality and location in a relatively unpopulated area. Springfield Lake (Figure 3.1) is directly surrounded by a sparsely populated residential area, although many of the houses have attached docks and boats. Springfield Lake is a headwater lake with Springfield Beach located near the water output (Figure 3.1). Kinsmen Lake is also a headwater lake, although the beach is located near the start of the lake (Figure 3.2). The lake itself is surrounded by a densely populated urbanized area including residential, commercial, and industrial land uses (Figure 3.2). It is important to note that Sackville Business Park is located near the output of the lake. Sandy Lake Beach is located near the middle of Sandy Lake (Figure 3.3). Although most of the surrounding area is undeveloped, containing mostly marshes and swamps, there is a sparsely populated residential area and industrial presence, including Atlantic Acres Business Park and Farmers Co-op factory, in close proximity to the lake (Figure 3.3). Lake Banook (Figure 3.4) is fed water from Lake Micmac and rivers running through Bedford Business Park and Dartmouth Crossing. Birch Cove beach is located near the output of the lake (Figure 3.3). Directly surrounding Lake Micmac and Lake Banook is a heavily urbanized area that includes residential, commercial, and industrial land uses (Figure 3.4).

3.1.2 – Sample collection

Water and sediment samples were collected from all four test beaches between May 20th, 2014 and October 20th, 2014. Information regarding sampling dates and samples collected can be observed in Table 3.1. Water samples were aseptically collected in accordance

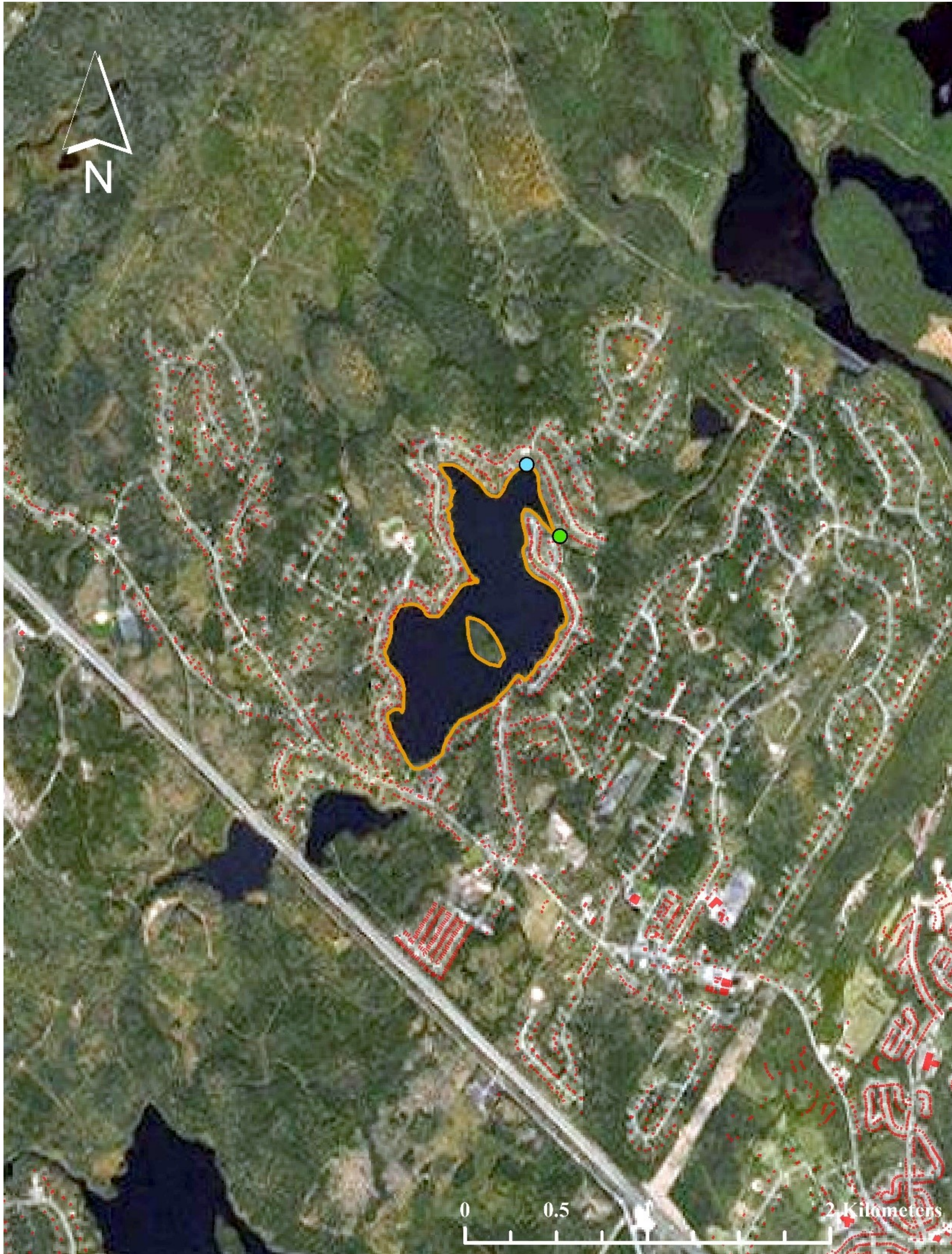


Figure 3.1: Springfield Beach (blue point) on Springfield Lake (outlined in orange) in Middle Sackville. Output point of water is represented by green dot while buildings surrounding Springfield Lake are represented by red polygons.

Figure 3.2: Kinsmen Beach (blue point) on First Lake (outlined in orange) in Lower Sackville. Output point of water is represented by green dot while buildings surrounding First Lake are represented by red polygons.

Figure 3.3: Sandy Lake Beach (blue point) on Sandy Lake (outlined in orange) in Bedford. Green point represent lake output while buildings surrounding Sandy Lake are represented as red polygons.

Figure 3.4: Birch Cove Beach (blue point) on Lake Banook in Dartmouth. Lake MicMac and Lake Banook are outlined in orange. Green point represents lake output. Buildings surrounding Lake Banook are represented as red polygons.

Table 3.1: Information regarding sampling events during, before, and after the beach season

Sampling Day	Sample Taken	Number of Sampling Events	Sampling Period[1]
May 20th	Water monitoring and pathogen enrichment	1	Before
June 2nd	Water monitoring and sediment collection	1	Before
June 17th	Water monitoring and pathogen enrichment	1	Before
July 7th	Water monitoring and sediment collection	1	During
July 15th	Water monitoring and pathogen enrichment	1	During
July 29th	Water monitoring and sediment collection	1	During
August 12th	Water monitoring and pathogen enrichment	1	During
August 30th, August 31st	Water monitoring and sediment collection	2	During
September 7th, September 8th, September 9th	Water monitoring and pathogen enrichment	3	After
September 22nd, September 23rd, September 24th	Water and Sediment	3	After
October 20th	Water monitoring, pathogen enrichment, and sediment collection	1	After

[1]Includes before (May 20th – June 17th), during (July 7th – August 31st), and after (September 7th – October 20th) the open beach season.

with HRM water collection protocol. Ethanol sterilized Nalgene water collection bottles were rinsed with sample water three times to wash out remaining ethanol. To collect samples, the bottle was inverted approximately a foot below the surface and then reverted to fill up. Filled bottles were kept in coolers with ice packs until they could be processed. At each beach, three water and sediment samples were collected within beach limits, although exact sampling sites were dynamic between sampling runs. However, samples were always taken from the right, centre, and left of each beach (Figure 3.5). At the time of sampling, several general water chemistry parameters were measured on site. Using a handheld 600R Sonde device (YSI, Yellow Springs, OH, USA) pH, DO (in mg/L and %), and water temperature (°C) were measured by placing the sonde approximately a foot below the surface of the water and allowing subsequent readings to stabilize before recording. On alternating sampling runs surface sediment samples were collected directly below each water sampling site (Table 3.1). Sediment samples were kept on ice until they could be processed. Precipitation levels three days and seven days before each sampling date were collected from the Government of Canada's Historical Climate Database (http://climate.weather.gc.ca/).

3.2 - Sample Processing

Water samples were fully processed in a microbiology laboratory at Dalhousie University, Halifax, Nova Scotia, Canada. A flowchart of the processing protocol can be observed in Figure 3.6.

3.2.1 – Water and sediment sample filtration

For each sample, 500 mL of water was filtered using an EMD Millipore Microfil vacuum filtration system (EMD Millipore, Billerica, MA, USA). Water was filtered through 47 mm Millipore S-Pack white-gridded filters (0.45 µm pore size; EMD Millipore, Billerica, MA, USA). Before filtration, and in between samples from different beaches, the surface of the filtration equipment was sterilized by ethanol flaming. Filtration of sediment samples required an extra processing step. Ten grams of sediment samples were placed into separate sterile dilution bottles containing 90 mL of sterile peptone saline (PS), producing a 1/10 dilution.

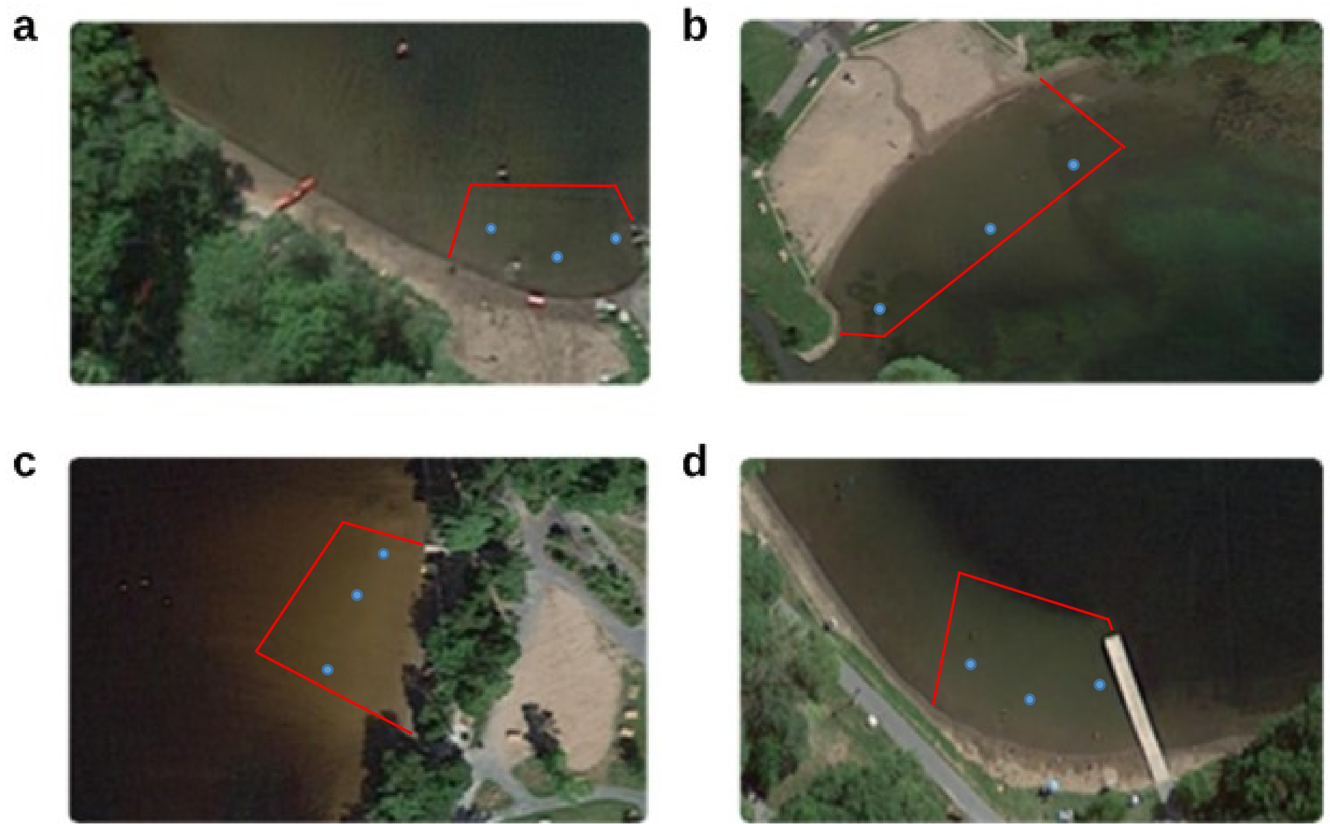


Figure 3.5: Beach boundaries and water sampling sites at tested beaches. Red lines represent beach limits while blue dots represent sampling sites at Springfield Beach (a), Kinsmen Beach (b), Sandy Lake Beach (c), and Birch Cove Beach (d).

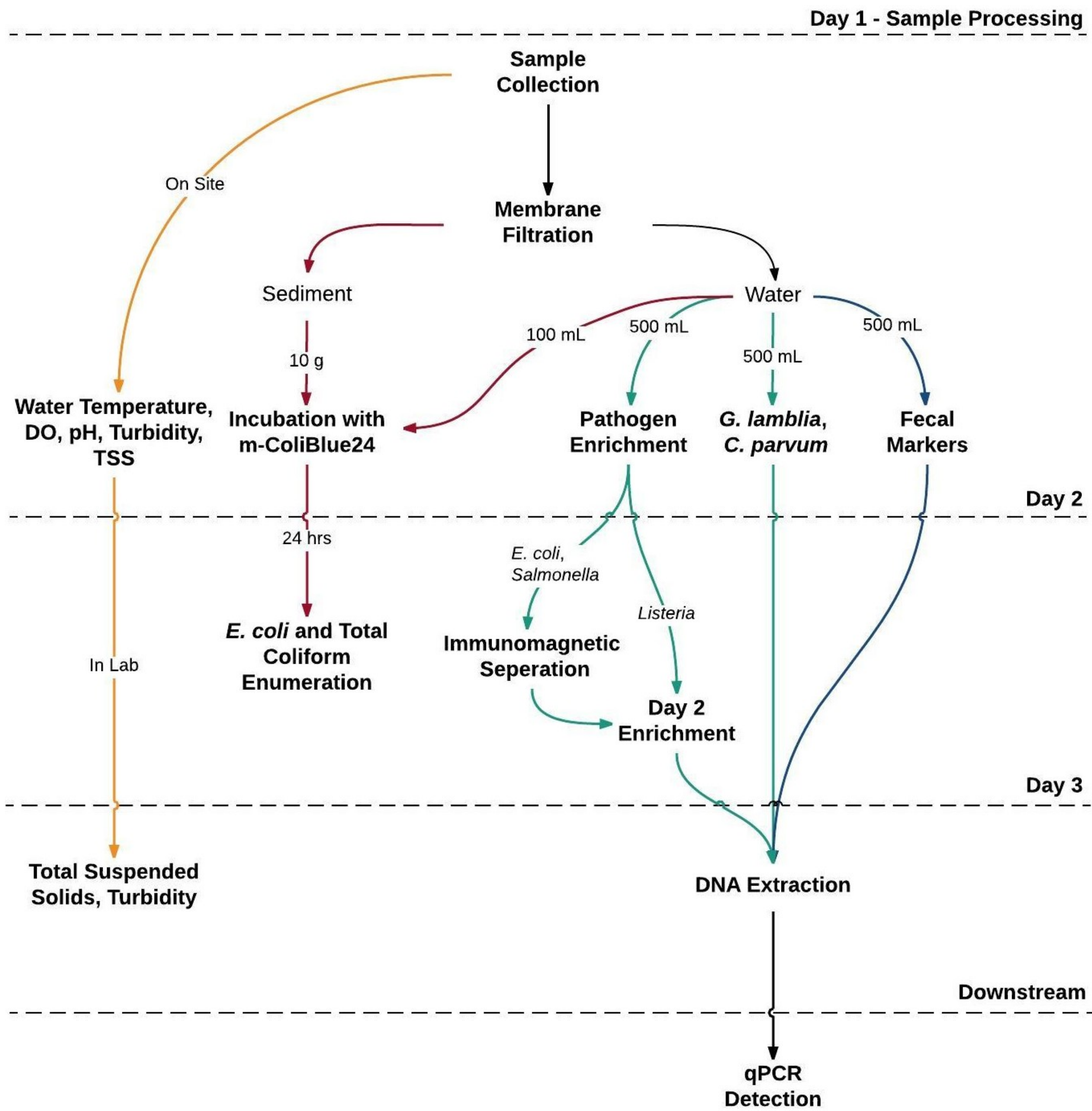


Figure 3.6: Workflow for the processing of water and sediment samples.

The 1/10 solution was further diluted to 1/100 and 1/1000 solutions by placing 10 mL of the 1/10 dilution into 90 mL of sterile PS and 1 mL of the 1/10 dilution was placed into 99 mL of sterile PS respectively. Before filtration, the 1/100 and 1/1000 solutions were shaken for a minute and allowed to settle for at least ten minutes. After settling, the 1/100 and 1/1000 dilutions (100 mL) were filtered through the Millipore system as outlined above.

3.2.2 – Total suspended solids and turbidity

A 934-AH Whatman Glass Microfibre Filter (with a pore size of 1.5 µm; Sigma-Aldrich, Oakville, ON, Canada) was dried in an oven at 106°C for 24 hours. Prior to filtering, the dried filter was placed in an aluminum weighing dish and weighed. Five hundred mL of water sample was filtered and the filter was placed into the oven to dry for at least 24 hours. After drying, the weighing dish and filter were weighed and TSS was calculated (Equation can be observed in Appendix A). Water turbidity was measured using a 2100 AN IS Laboratory Turbidimeter (Hach, Mississauga, ON, Canada). Cuvettes were rinsed three times with sample water prior to measuring. Turbidity readings were allowed to stabilize and three readings were recorded per sample. A deionized water blank was measured and subtracted from the average reading of all samples, as shown in Appendix A.

3.3 – Pathogen Enrichment

3.3.1 – Enrichment media and incubation

Water samples on alternating sampling runs (Table 3.1) were enriched for the detection of *E. coli* O157:H7, *L. monocytogenes, Salmonella* spp., *and Campylobacter* spp. (Figure 3.6). A total of six different enrichment media were used in this study, including buffered peptone water (BPW), tryptic soy broth (TSB), *Listeria* enrichment broth (LEB), Fraser broth (FB), Bolton broth (BB), and Rappaport-Vassiliadis *Salmonella* enrichment broth (RVS) (Oxoid, Nepean, ON, Canada). Five hundred mL of water from each sampling site was filtered, as described above, and placed into the appropriate enrichment media aseptically (Table 3.2; Appendix B). All media was produced according to manufacturer's instructions (Appendix B). *L. monocytogenes, Salmonella,* and *E. coli* O157:H7 incubation took place in an aerobic environment while *Campylobacter* enrichment took place in an anaerobic environment. Anaerobic environments

Table 3.2: Conditions for the enrichment of *E. coli* O157:*H7, Listeria monocytogenes*, *Salmonella* spp., and *Campylobacter* spp..

Target Pathogen	Enrichment Step #1	Incubation Step #1	Enrichment Step #2	Incubation Step #2
E. coli	Filter placed in 100 mL BPW	37°C for 24 hrs aerobically	Immunomagnetic separation; 1 mL of sample in 9 mL TSB	42°C for >8 hrs aerobically
Salmonella spp.	Filter placed in 100 mL BPW	37°C for 24 hrs aerobically	Immunomagnetic separation; 1 mL of sample in 9 mL RVS	42°C for >8 hrs aerobically
Campylobacter spp.	Filter placed in 40 mL BB	42°C for 48 hrs, anaerobically	N/A	N/A
Listeria monocytogenes	Filter placed in 10 mL LEB	37°C for 24 hrs aerobically	Place 1 mL LEB in 9 mL FB	37°C for 24 hrs aerobically

were created through the use of a BD GasPak EZ anaerobic chamber (BD, Mississauga, ON, Canada) and BD GasPak EZ Campy Sachets (BD, Mississauga, ON, Canada). Enrichment conditions utilized for each pathogen can be observed in Table 3.2.

3.3.2 – Immunomagnetic separation of E. coli O157:H7 and Salmonella spp.

E. coli O157:H7 and *Salmonella* spp. were separated from each other in the BPW media through the use of immunomagnetic separation. Dynabeads anti-*E. coli* O157 beads were used for *E. coli* isolation while *Salmonella* isolation utilized Dynabeads anti-*Salmonella* beads (Dynabeads, Oslo, Norway). The following process was completed twice, once for *E. coli* O157:H7 and once for *Salmonella*. Ten mL of day 1 BPW media was vortexed for 5 minutes at 3200x g and the supernatant was poured off. The pellet was re-suspended in 500 µL 0.05% PBS-Tween and transferred to a sterilized eppendorf tube. Twenty µL of corresponding beads were added to the mixture and placed in a magnetic particle concentrator without the magnetic base, and rotated for twenty-five minutes at approximately 1.5 rpm. The samples were then rotated for another three minutes, with the magnetic base attached. Without disturbing the pellet the supernatant was removed and 1 mL of PBS-Tween was added; this process was completed two more times. After the final rotation, the pellets were re-suspended with 1 mL of corresponding media and further enriched according to Table 3.2.

3.4 – Monitoring of *E. coli* and Total Coliform Levels at Beaches

E. coli and total coliform (TC) levels were monitored in both the water column and sediment of each beach before, during, and after the beach season. Water and diluted sediment samples (100 mL) were filtered as described above. All filters were incubated on m-ColiBlue24 (EMD Millipore, Billerica, MA, USA; MCB) plates for 24 hours at 37°C. After incubation, blue colonies were counted as *E. coli* while red colonies represent coliforms. TC include the total number of both blue and red colonies. The numbers of *E. coli* and TC were reported as colony forming units (CFU) per 100 mL (Equation can be observed in Appendix A). Near the end of the sampling season several consecutive sampling trips were made (Table 3.1) to measure the day-to-day variation in *E. coli* levels. Sampling Run 9 included two consecutive sampling trips

(August 30th-31st, 2014) while sample runs 10 (September 7th – 9th, 2014) and 11 (September 22nd – 24th, 2014) included sampling on three consecutive days (Table 3.1).

3.5 – qPCR Detection of Select Pathogens and Fecal Contamination Markers

3.5.1 – DNA extractions from water samples

DNA was extracted from beach water samples on the third day of the water processing protocol (Figure 3.6) for use in pathogen and fecal marker detection assays. A 2 mL aliquot from BPW, TSB, FB, BB, and RSV media were combined and centrifuged at 3200x *g* to pellet pathogens. The supernatant was discarded and the pellet resuspended in any residual media. DNA was then extracted from the resuspended pellet (~ 250 µL) using MoBio PowerSoil DNA extraction kit (VWR, Mississauga, ON, Canada) as per manufacturer's instructions. For the extraction of DNA from water samples for the *G. lamblia*, *C. parvum*, and fecal marker assays the filters were vortexed at top speed for three minutes. The filter was then aseptically removed and the remaining sample water was centrifuged at 3200x *g* for ten minutes. The supernatant was discarded and DNA was extracted with the Zymo Fecal DNA MiniPrep DNA extraction kit (Zymo Research, Irvine, California, USA) using 150 µL of resuspended pellet. All DNA samples were stored frozen at -20°C until use.

3.5.2 – Pathogen presence/absence experiments

3.5.2.1 – *qPCR detection of pathogens*

The presence of pathogens within water samples were tested through the use of TaqMan qPCR presence/absence experiments. The DNA sequences of the primers and probes utilized within this study and their corresponding qPCR assays can be observed in Table 3.3 and Table 3.4 respectively. All reactions were carried out in Bio-Rad Hard-Shell PCR plates (BIO-RAD, Ontario, Canada) with each well containing 23 µL of reaction mixture. The reaction mixture contained 4 µL of template DNA and 19 µL of mastermix mixture. This solution was mixed lightly before being dispensed into wells of PCR plate and contained: 1) Bio-Rad Sso Advanced Universal Probes Supermix, 2) Corresponding primers/probes, and 3) DNase free water.

Table 3.3: Primers and probes utilized for pathogen detection assays

Target Pathogen	Primer/ Probe	Sequence (5' – 3')	Reference
E. coli O157:H7	EaeP[1]	AAATGGACATAGCATCAGCATAATAGGC TTGCT[3]	Ibekwe *et al.*, 2002
	EaeF[2]	GTAAGTTACACTATAAAAGCACCGTCG	
	EaeF[3]	TCTGTGTGGATGGTAATAAATTTTTG	
Listeria monocytogenes	HlyQP	CGCCTGCAAGTCCTAAGACGCCA[4]	Rodriguez-Lazaro *et al.*, 2004
	HlyQF	CATGGCACCACCAGCATCT	
	HlyQR	ATCCGCGTGTTTCTTTTCGA	
Salmonella species	invAP	TGGAAGCGCTCGCATTGTGG[3]	Cheng *et al.*, 2008
	invAF	AACGTGTTTCCGTGCGTAAT	
	invAR	TCCATCAAA TTAGCGGAGGC	
Giardia lamblia	Gl18s-P	CCCGCGGCGGTCCCTGCTAG[4]	Verweij *et al.*, 2003
	Gl18s-F	GACGGCTCAGGACAACGGTT	
	Gl18s-R	TTGCCAGCGGTGTCCG	
Cryptosporidium parvum	JVAG2-p	ATTTATCTCTTCGTAGCGGCG[3]	Jothikumar *et al.*, 2008
	JVAG2-F	ACTTTTTGTTTGTTTTACGCCG	
	JVAG2-R	AATGTGGTAGTTGCGGTTGAA	

[1]Sequence names with a "p/P" at the end represent probes
[2]"F" indicates forward primer sequences while "R" represent reverse primer sequences
[3]Probe uses the [FAM] fluorophore and [BHQ1] quencher
[4]Probe used the [FAM] fluorophore and [TAMRA] quencher

Table 3.4: qPCR assays utilized for pathogen detection in tested water samples.

Pathogen	Target Gene	Amplicon (bp)[1]	qPCR protocol	Detection Limit
E. coli O157:H7	*eae*	106	95°C for 6 min; 40 cycles of 95°C for 20 sec, 55°C for 30 sec, 72°C for 40 sec	$7.9*10^{-5}$ pg/mL 6.4E3 CFU/mL[2]
Listeria monocytogenes	*Hly*	64	95°C for 10 min; 40 cycles of 95°C for 20 sec, 56°C for 30 sec, 72°C for 1 min	8 Genome Molecules[3]
Salmonella spp.	*invA*	262	95°C for 3 min; 40 cycles of 95°C for 3 min, 95°C for 15 sec, 60°C for 1 min	1E3 CFU/mL[4]
Giardia lamblia	18s rRNA	N/A[5]	95°C for 3 min; 40 cycles of 95°C for 15 sec, 57°C for 30 sec, 68°C for 30 sec	DNA from 0.5 *G. lamblia* cysts[6]
Cryptosporidium parvum	18s rRNA	N/A	95°C for 3 min; 40 cycles of 95°C for 15 sec, 50°C for 30 sec, 68°C for 20 sec	1 oocyst / 300 µL stool sample[7]

[1]Amplicon refers to the length of the amplified product produced by each assay, represented in base pairs
[2]Ibekwe *et al*., 2002
[3]Rodriguez-Lazaro *et al*., 2004; Approximately equal to 25 fg of pure DNA
[4]Cheng *et al*., 2008
[5]N/A represents information that was not provided by assay developers.
[6]Verweij *et al*., 2003
[7]Jothikumar *et al*., 2008

3.5.2.2 – *Detection of C. jejuni, C. lari, and C. coli in beach water*

Water samples were tested for the presence of *C. jejuni, C. lari,* and *C. coli* by using a two-step process. All water samples were run through a general qPCR assay that detected the presence of any *Campylobacter* spp. (Table 3.5). An end-point triplex PCR and gel electrophoresis assay was used to determine if *Campylobacter*-positive water samples were *C. jejuni*, *C. coli*, *C. coli*, or a less common *Campylobacter* spp.. Gel electrophoresis was run at 100 volts for an hour using 1.5% agarose gels. Samples showing clear bands were compared to positive controls and a 100 bp DNA ladder to determine if the band represented *C. jejuni* (349 bp amplicon), *C. lari* (279 bp amplicon), or *C. coli* (72 bp amplicon). Samples that did not show any bands represented samples with no *Campylobacter* or *Campylobacter* belonging to less common *Campylobacter* species such as *C. upsaliensis* or *C. hyoinstensalis*. All protocols and primers/probes used in the above assays can be observed in Table 3.5 and Table 3.6.

3.5.3 – Detection of fecal contamination in beach water

3.5.3.1 – Fecal sample collection

Fresh fecal samples were required for use in the formation of standard curves and to act as positive controls. All fecal sources were collected within the HRM and surrounding area. Samples were collected and put into sterile 15 mL or 50 mL falcon tubes or unused Ziploc bags and put on ice until they reached the lab. The majority of the animal samples were collected from Shubenacadie Wildlife Park. Samples were collected by park staff using a plastic scoop that was washed briefly with snow in between collection of samples from different species. Dog samples were collected from the SPCA Provincial Animal Centre by staff using doggie bags. Fecal samples were stored on ice between collection and arrival at the lab. Plastic sterile disposable inoculation loops were used to split the fecal samples into smaller portions, which were then placed in sterile falcon tubes and frozen at -20°C until DNA was extracted. DNA was extracted using Zymo Fecal DNA MiniPrep DNA Extraction Kit (Zymo Research, Irvine, CA, USA) as per the manufacturer's instructions.

Table 3.5: Assay conditions for the general detection of *Campylobacter* and the triplex detection *of C. jejuni*, *C. lari*, *C. coli* in water samples.

Assay	Primer /Probe	Sequence	Reference
Campylobacter spp.	CampF2	CACGTGCTACAATGGCATAT	Lund *et al*., 2004
	CampR2	GGCTTCATGCTCTCGAGTT	
	CampP2	[1]CAGAGAACAATCCGAACTGGGACA	
Campylobacter triplex	J-UP[2]	CTTAGATTTATTTTTATCTTTAACT	Khan & Edge, 2007
	J-DN[2]	ACTAAATGATTTAGTCTCA	
	L-UP[3]	CTTACTTTAGGTTTTAAGACC	
	L-DN[3]	CAATAAAACCTTACTATCTC	
	C-UP[4]	GAAGTATCAATCTTAAAAAGATAA	
	C-DN[4]	AAATATATACTTGCTTTAGATT	

[1]Probe sequencer has FAM fluorophore and BHQ1 Quencher
[2]J-UP and J-DN primers used to detect *C. jejuni*
[3]L-UP and L-DN primers used to detect *C. lari*
[4]C-UP and C-DN primers used to detect *C. coli*

Table 3.6 – Information regarding *Campylobacter* detection and differentiation assays.

Assay	Target Gene	Amplicon (bp)[1]	qPCR Protocol	Detection Limit
Campylobacter spp.	16s rRNA	108	95°C for 6 min; 40 cycles of 95°C for 15 sec, 60°C for 1 min	100 – 150 CFU mL[2]
Campylobacter triplex	***C. jejuni*** 16S-23S rDNA internal transcribed spacer	349	95°C for 3 min; 35 cycles of 95°C for 30 sec, 47.2°C for 30 sec, 68°C for 45 sec; 68°C for 5 min	N/A[3]
	C. lari 16S-23S rDNA internal transcribed spacer	279		
	C. coli 16S-23S rDNA internal transcribed spacer	72		

[1]Amplicon refers to the length of the amplified product produced by each assay, represented in base pairs
[2]per mL of chicken feces suspension; Lund *et al*., 2004.
[3] N/A represents information that was not provided by assay developers

3.5.3.2 – Formation of fecal marker standard curves

For each fecal contamination marker a standard curve was produced. Three DNA samples per corresponding fecal source were run through end-point PCR and gel electrophoresis to determine if the correct PCR products were present. Any impurities remaining in the PCR reactions were removed by running the samples through MoBio Ultraclean PCR cleanup kit (MO BIO, Carlsbad, CA, United States) as per the manufacturer' s instructions. To construct the standard curve, each fecal marker was cloned into a pCR 2.1-TOPO vector using Invitrogen's TOPO TA Cloning Kit (Invitrogen, Burlington, ON, Canada). Plasmids, containing the appropriate insert, were transported into chemically competent TOP 10 One Shot *E. coli* cells (Invitrogen, Burlington, ON, Canada). *E. coli* cells were spread onto Luria-Bertani agar plates containing 40 µL of a 40 mg/mL X-gal-Dimethylformamide solution and incubated overnight at 37°C. Following blue-white screening, light blue and white colonies were picked and grown in 1 mL TSB overnight at 37°C. As per the manufacturer's instructions, DNA was extracted from these colonies using Invitrogen's PureLink Quick Plasmid Miniprep Kit (Invitrogen, Burlington, ON, Canada). Using the picogreen and nanophotometer methods the quality and concentration of the purified plasmids were determined. For each marker, the plasmid that displayed the highest picogreen reading, and best 260:280 ratio, was used to construct the corresponding standard curve. Serially diluted plasmid samples were then run through corresponding qPCR conditions (Table 3.7). Pure plasmid samples were sent away to the Innovation Centre at McGill University for sequencing to ensure that the plasmids used to construct the standard curves contained the correct sequence.

3.5.3.3 – Detection of fecal contamination in beach water

At each beach, the presence of human, dog, and avian species were detected using SYBR Green and TaqMan qPCR. Information regarding the fecal markers utilized in this study can be observed in Table 3.7 and Table 3.8. Human feces was targeted using an updated TaqMan assay targeting the HF183 cluster of human-specific *Bacteroidales*. BacCan and dogmt assays were both utilized to detect the presence of dog contamination, the former targeting 16s rRNA of dog-specific *Bacteroidales* and the latter targeting the ND5 (NADH Dehydrogenase 5) gene on the mtDNA of dog gut cells shed during fecal excretion. The GFD marker is a general avian marker

Table 3.7: Primers and probes utilized for the detection of fecal contamination markers in water samples.

Target	Primer	Sequence (5' – 3')	Reference
Human[1]	HF183-F[3] HF183-R[3] HF183-p[4]	ATCATGAGTTCACATGTCCG CTTCCTCTCAGAACCCCTATCC CTAATGGAACGCATCCC[5]	Haugland *et al*., 2010; Layton *et al*., 2013
Dog[1]	BacCan-F BacCan-R BacCan-p	GGAGCGCAGACGGGTTTT CAATCGGAGTTCTTCGTGATATCTA TGGTGTAGCGGTGAAA[5]	Kildare *et al*., 2007; Tambalo *et al*., 2012
Dog mtDNA[1]	dogmt-f dogmt-r dogmt-p	GGCATGCCTTTCCTTACAGGATTC GGGATGTGGCAACGAGTGTAATTATG TCATCGAGTCCGCTAACACGTCGAAT[6]	Caldwell & Levine, 2009; Tambalo *et al*., 2012
Avian species[2]	GFD-F GFD-R	TCGGCTGAGCACTCTAGGG GCGTCTCTTTGTACATCCCA	Green *et al*., 2012

[1]The human and dog fecal detection assays use TaqMan qPCR chemistry
[2]The general avian fecal detection assay uses Sybr Green qPCR chemistry
[3]"F" represents the forward primer sequence while "R" represents the reverse primer sequence
[4]"P/p" represents the probe sequence
[5]Probe utilized [FAM] fluorophore and BHQ1 quencher
[6]Probe utilized [FAM] fluorophore and BHQ quencher

Table 3.8: qPCR protocols and detection limits of fecal marker assays utilized in this study.

Target	Assay	Amplicon (bp)[1]	qPCR Protocols	Detection Limit
Human	HF183	126	6 min @ 95°C; 30x 30 sec @ 95°C, 30 sec @ 58°C; 30 sec @ 72°C, final 15 min @ 72°C	10 marker copies / 100 mL water[3]
Dog	BacCan	145	6 min @ 95°C; 30x 30 sec @ 95°C, 30 sec @ 60°C; 30 sec @ 72°C, final 15 min @ 72°C	1 gene copy/reaction[4]
Dog mtDNA	dogmt	102	6 min @ 95°C; 30x 30 sec @ 95°C, 30 sec @ 60°C, 30 sec @ 72°C; final 15 min @ 72°C	10 Copies/mL[5] 1 mg feces / 100 mL water[6]
Avian	GFD	123	6 min @ 95°C; 30x 30 sec @95°C, 30 sec @ 57°C; 30 sec @ 72°C; 15 min @ 72°C; Melt step[2]	0.1 mg chicken feces 87 coliform MPN/100 mL 13 *E. coli* MPN/100 mL[7]

[1]Amplicon refers to the length of the amplified product produced by each assay, represented in base pairs
[2]Melt step was used to create a melt curve (65°C to 95°C increments of 0.5°C for 5 seconds.
[3]Layton *et al*., 2013.
[4]Kildare *et al*., 2007.
[5]Caldwell and Levine, 2009.
[6]Tambalo *et al*., 2012.
[7]Green *et al*., 2012.

that will detect the presence of gull, duck, Canadian goose, and chicken fecal contamination. Human and dog markers used TaqMan chemistry while the GFD is a SYBR green assay. All qPCR reaction mixtures contained 20 µL per sample, containing 4 µL of template DNA and 16 µL of mastermix. The mastermix consisted of primers and probes (if necessary), Bio-Rad SsoAdvanced Universal Probes Supermix (BIO-RAD, Mississauga, ON, Canada) (for BacCAn, dogmt, and HF183) or Bio-Rad SsoAdvanced Universal SYBR Green Supermix (for GFD), and Nuclease-free water in varying quantities. A Bio-Rad CFX96 Real-Time PCR Detection System (BIO-RAD, Mississauga, ON, Canada) was used for all qPCR reactions. Each water sample was run in triplicates and went through forty cycles of amplification.

3.5.4 – Assay Controls

Controls were utilized in this study to ensure that all assays were completed without external contamination. A complete description of controls utilized in this study can be found in Appendix C. Two controls, labeled as “negative filter” and “negative media”, were produced during the filtration step of water processing (Table 3.1). The negative filter control was utilized in the pathogen and fecal marker detection assays and was produced by filtering 500 mL of sterile dH_2O through a 0.45 µM pore membrane filter and placed in appropriate media according to corresponding assay. The negative media control was utilized during pathogen enrichment/detection and consisted of sterile enrichment media that was run through the corresponding enrichment protocol. A “negative bead” control was utilized during the immunomagnetic separation of *E. coli* O157:H7 and *Salmonella* spp.. Sterile dH_2O was run through the immunomagnetic separation process of both *E. coli* O157:H7 and *Salmonella* and then run through the secondary enrichment step for both species. Each time DNA was extracted from a group of samples a “negative extraction” control was run simultaneously. Following the manufacturer’s instructions, sterile dH_2O was run through the same DNA extraction kit as the other samples.

3.6 – Statistical Analysis

All graphs were produced and statistics completed using RStudio and Graphpad Prism (version 5.0). A Kruskal-Wallis non-parametric test, followed by a post-hoc Dunn’s multiple

comparison test, was used to determine if *E. coli* counts differed with sampling time (before, during, and after the sampling season) and between beaches (Springfield, Kinsmen, Sandy Lake, and Birch Cove). A significant p-value (α=0.05) in Kruskall-Wallis indicates that the sampled populations have a different distribution and are therefore significantly different. The Dunn's multiple comparison test determines the statistical difference between groups that make up a certain population. GraphPad Prism reports significance of this test using p-value >0.05, indicating non-significance, and <0.05 if groups are significantly different. To supplement statistical analysis the distribution of *E. coli* counts from each period (regardless of beach) and each beach (regardless of sampling period) were graphed against one another.

Binary logistic regression was used in this study to compare the probability of pathogen/markers prevalence, *E. coli* levels, and WQP measurements. All variables were transformed into binary variables based on specific cut-off values including the presence/absence of pathogens/markers, whether levels of *E. coli* surpassed >100/>200 CFU/100 mL, or if measured WQP were higher than a set value. Statistical output included the odds ratio (OR), 95% confidence interval (95% CI), McFadden's pseudo R^2 (ρ^2), and the p-value ($\alpha = 0.05$). OR values lower than 1.5 were considered weak while OR values greater than 3.0 were considered strong. The ρ^2 is a measure of how well the sample data explains the regression outcome, such that values between 0.2 – 0.4 indicate a strong goodness of fit. Conditional density (CD) plots were produced for significant results. These plots indicate the probability of each level of a binary outcome occurring at a specific value of a continuous variable. However, it is important to note that these plots should only be used to further explore the relationship between significant variables and not for making concrete conclusion.

A stepwise regression, in both directions, was performed to explore how measured WQP affected measured *E. coli* levels within the beaches. Stepwise regression adds or removes variables from an input regression equation and returns a formula that has the best fit to the sample data. All single variable and first level interactions were included into the input formula. The adjusted R^2, which only increases if added or removed variables improve the model more than is expected by chance, was utilized as a goodness of fit measure with values closer to 1.0 representing better quality models.

Chapter 4 – Results

4.1 – Monitoring of Sample Beaches before, during, and after beach open season

4.1.1 – Summer E.coli and coliform monitoring data

Within the HRM, the annual beach season runs from July 1st to August 31st. To capture the water quality before, during, and after the beach season, water samples were collected between May 20th, 2014 and October 20th, 2014. Turbidity, water temperature, and 7-day precipitation measurements obtained during this study can be observed in Figure 4.1 while *E. coli* levels from water collected throughout the sampling season can be observed in Figure 4.2. Water temperatures were highest during the beach open season (Figure 4.1a). Kinsmen beach displayed consistently high turbidity levels, surpassing measurements from the other beaches during most of the sampling runs (Figure 4.1b). On July 15th, there was a small spike in *E. coli* levels at Springfield and Kinsmen Beaches but levels did not surpass 200 CFU/100 mL (Figure 4.2a), although coliform levels at all beaches were greater than 1000 CFU/100 mL (Figure 4.2b). *E. coli* levels surpassed 200 CFU/100 mL on four separate occasions, twice at Kinsmen Beach and twice at Sandy Lake Beach, during the September 22nd -24th and October 20th sampling runs (Figure 4.2a). During the September 22nd-24th sampling run, all beaches displayed elevated *E. coli* levels, corresponding to a large storm event with high winds and precipitation levels (average rainfall of 72 mm 3 days and 162 mm 7 days before sampling; Figure 4.1b). *E. coli* levels at Kinsmen Beach on September 23rd displayed a geometric mean of 1068 CFU/100 mL with each individual water sample surpassing the 400 CFU/100 mL limit set by Health Canada (Health Canada, 2012a; Figure 4.2a**).** On October 20th both Kinsmen and Sandy Lake Beach had *E. coli* levels surpassing 300 CFU/100 mL (Figure 4.2a). Simultaneously, turbidity levels at these beaches were the highest observed during the entire sampling season (Figure 4.1b) and coliform levels were also elevated (Figure 4.2b).

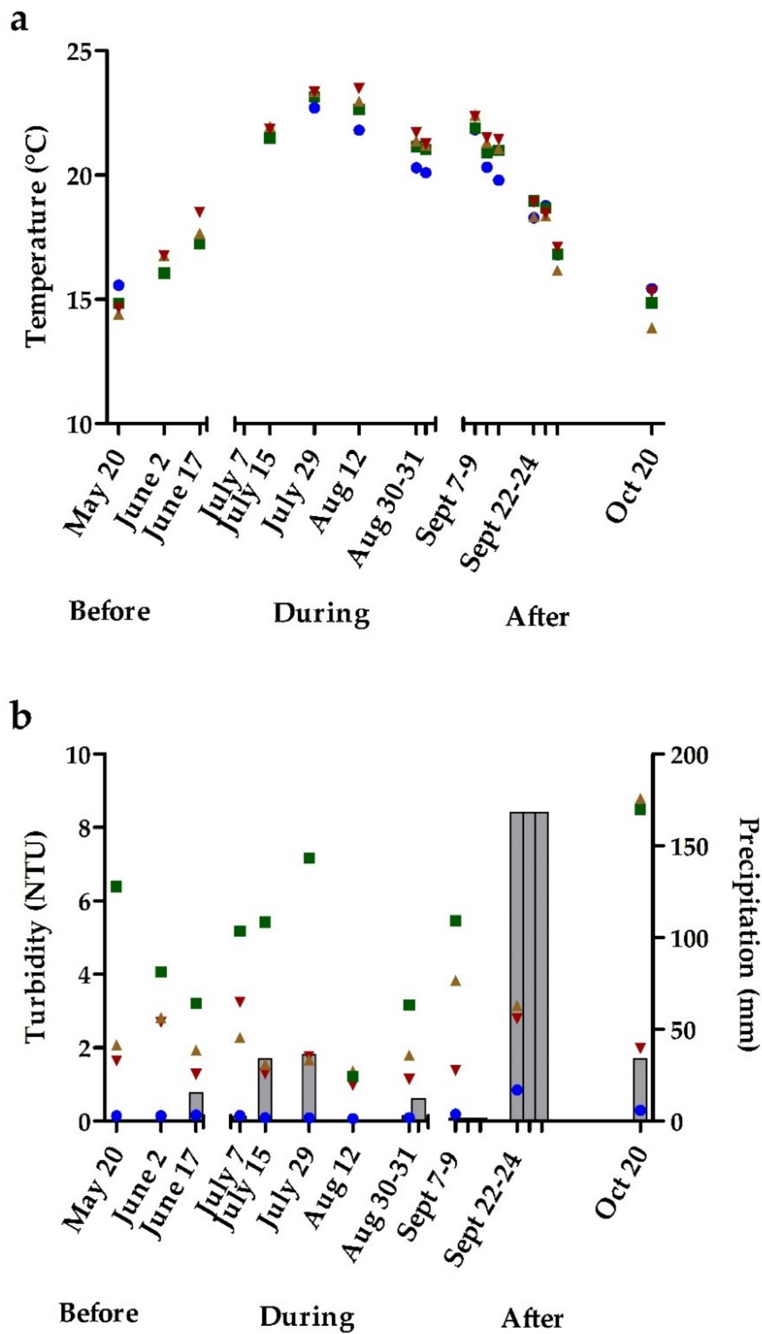


Figure 4.1: Water temperature (a) and turbidity and 7-day precipitation (b) measurements observed throughout the sampling season. Blue circles represent Springfield, green squares represent Kinsmen, brown upwards triangles represent Sandy, and red downward triangles represent Birch Cove. In Figure 4.1b, colored points represent turbidity measurements while grey bars represent 7-day precipitation values.

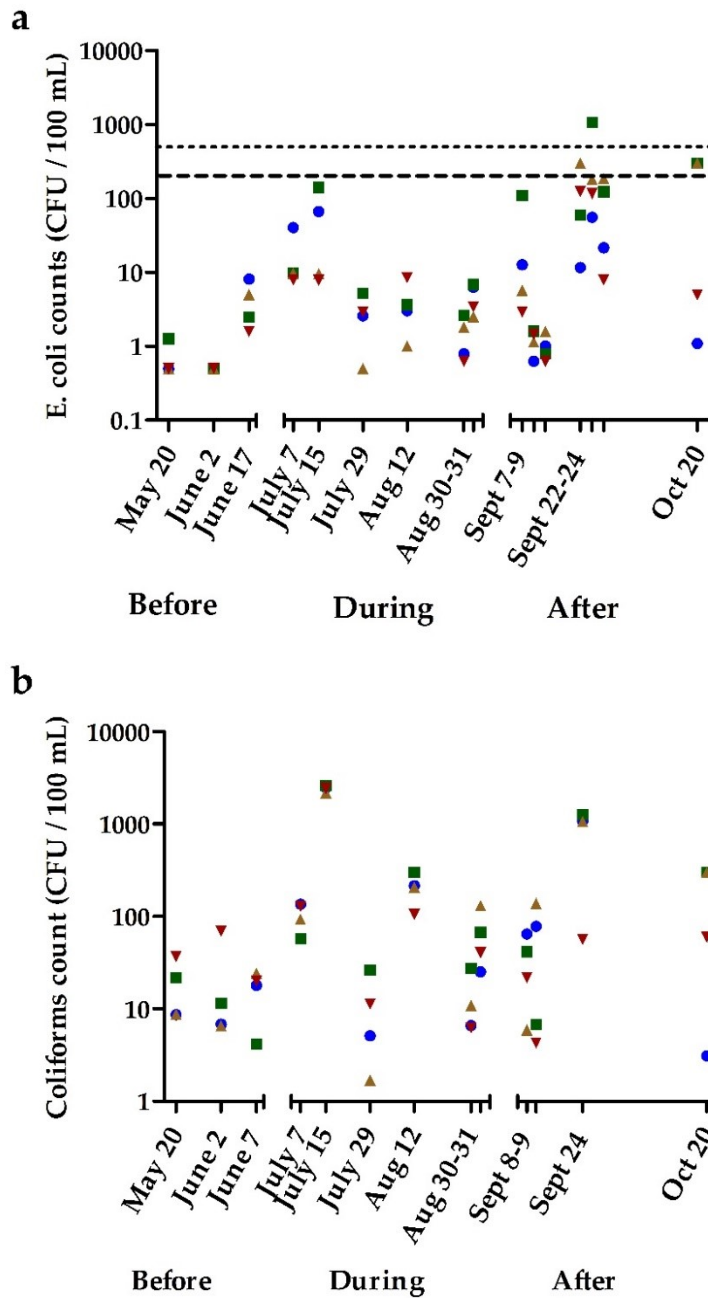


Figure 4.2: ***E. coli*** **(a) and TC (b) levels in the water column of all beaches throughout the sampling season.** Blue circles represent Springfield, green squares represent Kinsmen, brown upwards triangles represent Sandy Lake, and red downward triangles represent Birch Cove. The dashed line represents 200 CFU/100 mL while the dotted line represents 400 CFU/100 mL.

A Kruskal-Wallis non-parametric test was run in order to determine if *E. coli* counts differed between sampling beaches and sampling periods. Geometric mean *E. coli* levels did not significantly differ ($p>0.05$) between sampling beaches (Table 4.1). Kinsmen beach displayed the highest levels of *E. coli* with a geometric mean of 9 CFU/100 mL (Table 4.2; Figure 4.3). Springfield, Sandy Lake, and Birch Cove Beaches displayed similar levels of *E. coli* with levels of 4, 5, and 3 CFU/100 mL respectively (Table 4.2). It is important to note that the distribution of *E. coli* counts at Kinsmen and Sandy Lake beach displayed two clusters, one with approximately 10 CFU/100 mL and below and the other approximately 100 CFU/100 mL and above (Figure 4.3). There was a significant difference ($p = 0.0004$) in *E. coli* levels between the different sampling periods (Table 4.1) Furthermore, mean counts obtained from both during (5 CFU/100 mL) and after (13 CFU/100 mL) the beach season were significantly higher ($p<0.05$) than those obtained before (1 CFU/100 mL) the beach season (Table 4.1; Table 4.2). However, there was not a significant difference in *E. coli* levels during and after the beach season (Table 4.1).

Table 4.1: Kruskal-Wallis and post-hoc Dunn's multiple comparisons test to determine if *E. coli* counts differ between beach sites and sampling times.

	Test	Comparison	p-value	Significant?
Sampling Sites	Kruskal-Wallis	Difference between beaches	0.607	No
Sampling Period	Kruskal-Wallis	**Difference between period**	**0.000400**	**Yes**
	Dunn's multiple comparisons	**Before vs During**	**< 0.05**	**Yes**
		Before vs After	**< 0.05**	**Yes**
		During vs After	> 0.05	No

Table 4.2: Statistical information obtained from Kruskal-Wallis test used to determine if *E. coli* levels differ between sampling periods and sampling sites.

	Site / Time	Geometric Mean (100 CFU/100 mL)	Range[2] (CFU/100 mL)	95% CI[4]
Sampling Sites	Springfield	3.77 (4)[1]	0.500[3] – 66.7	1.51 – 9.36
	Kinsmen	9.03 (9)	0.500 – 300	2.81 – 28.9
	Sandy	5.14 (5)	0.500 – 300	1.47 – 17.8
	Birch Cove	3.02 (3)	0.500 – 124	1.34 – 6.82
Sampling Period	Before	1.03 (1)	0.500 – 8.08	0.543 – 1.98
	During	4.87 (5)	0.500 – 140	2.73 – 8.68
	After	13.1 (13)	0.630 – 300	5.42 – 31.9

[1]Numbers in brackets represents the rounded geometric mean used to report *E. coli* levels.
[2]Range displays the lowest and highest number in each sampling set.
[3]0.5 CFU/100 mL is half of the detection limit of enumeration method which is 1.0 CFU/100 mL
[4]Displays the 95% CI of the geometric mean (Equation in Appendix A).

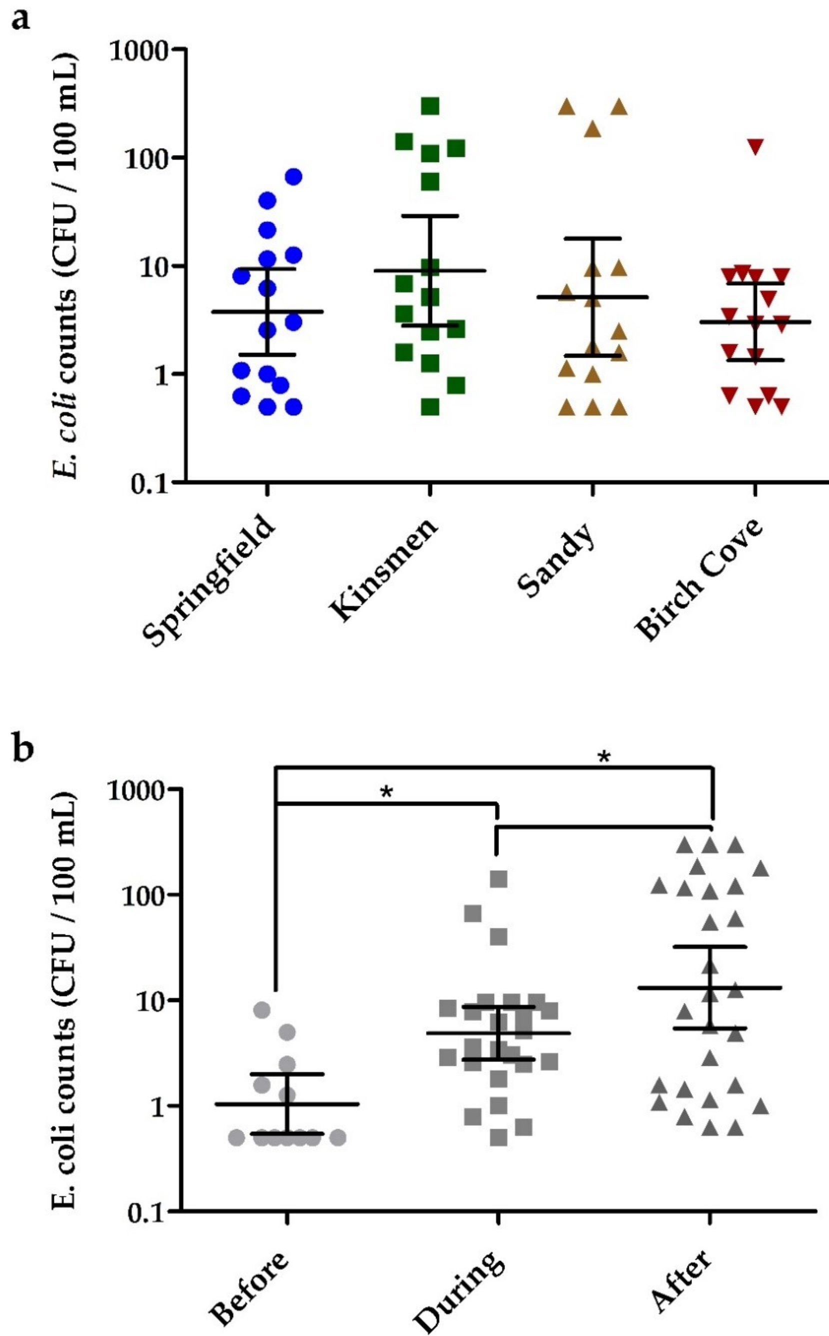


Figure 4.3: Kruskal-Wallis analysis to determine if measured *E. coli* counts differ between sampling sites (a) and sampling periods (b). Solid points represent individual *E. coli* counts. For each sample the geometric mean (middle bar) and 95% CI (whiskers) are shown. Significantly different (p <0.05) geometric *E. coli* levels are denoted by an asterisk.

4.2 – Prevalence of Pathogens and Fecal Contamination Markers in the Beaches Before, During, and After the Beach Open Season

4.2.1 – Prevalence of selected human pathogens throughout the sampling season

Overall prevalence and distribution of the selected pathogens can be observed in Table 4.3. *L. monocytogenes* and *Salmonella* spp. were detected in similar numbers, with a prevalence rate of 25.0% and 29.1% respectively. *L. monocytogenes* was detected almost exclusively after the beach season, with only one positive sample at Springfield during the sampling season (Table 4.3). Half of the *L. monocytogenes* samples occurred at Springfield Beach with the remaining three occurring once at the other three beaches (Table 4.3). *Salmonella* prevalence was much more stable across the beaches with two positive samples at Springfield and Birch Cove and one each at Kinsmen and Sandy Lake beaches (Table 4.3). However, five out of the seven positive samples occurred during the beach season (Table 4.3). *E. coli* O157:H7 was only detected once in twenty-four water samples (4.16%), corresponding to a water sample from Kinsmen Beach during the beach season (Table 4.3). Eleven total water samples tested positive for the presence of *Campylobacter* spp. using the general qPCR assay (Table 4.3). However, only one of these positive samples were identified with the *Campylobacter* PCR triplex, corresponding to *C. jejuni*.

Table 4.3: Pathogen detection frequency in water samples collected from test beaches before, during, and after the beach season.

Pathogen[1]	Sampling Period	Springfield	Kinsmen	Sandy	Birch Cove
	Total Prevalence: 6/24 (25.0%)[2]				
L. monocytogenes	Before	0/2	0/2	0/2	0/2
	During	1/2	0/2	0/2	0/2
	After	2/2	1/2	1/2	1/2
	Total Prevalence: 7/24 (29.1%)				
Salmonella spp.	Before	0/2	0/2	0/2	1/2
	During	2/2	1/2	1/2	1/2
	After	0/2	1/2	0/2	0/2
	Total Prevalence: 1/24 (4.16%)				
E. coli O157:H7	Before	0/2	0/2	0/2	0/2
	During	0/2	1/2	0/2	0/2
	After	0/2	0/2	0/2	0/2
	Total Prevalence: 1/24 (4.16%)				
Campylobacter spp.	Before	0/2	0/2	0/2	1/2[1]
	During	0/2	0/2	0/2	0/2
	After	0/2	0/2	0/2	0/2
G. lamblia	**Not Detected (0/24)[2]**				
C. parvum	**Not Detected (0/24)[2]**				

[1]Pathogens were tested for in 500 mL of water sample.
[2]Percentage of positive samples (Number of positive samples/number of tested samples).
[3]Detected *Campylobacter* spp. was identified as *C. jejuni*.

4.2.2 – Relation of pathogen prevalence and WQP at the tested beaches

Logistic regression was utilized in this study to examine the relationship between pathogen presence and WQP in the tested beaches. The results from this logistic regression can be observed in Table 4.4. At water temperatures > 20°C *Campylobacter* spp. were ten times more likely to be present ($p = 0.0197$, $\rho^2 = 0.200$; Table 4.4). Figure 4.4 displays a CD plot of this relationship, indicating that as the temperature approaches 20°C, the probability of *Campylobacter* being present within the water increases sharply. No other regression pairs were found to be statistically significant ($p > 0.05$; Table 4.4).

Table 4.4: Logistic regression to determine relationship between prevalence of selected pathogens and measured WQP

Pathogen	WQP	95% CI	OR	ρ^2	p-value
	Temperature >20°C	0.148 – 6.74	1.00	0.00	1.00
	Turbidity > 2.5 NTU	0.479 – 27.4	3.14	0.0522	0.248
L. monocytogenes	Turbidity >5 NTU	0.262 – 21.5	2.50	0.0289	0.392
	3-day Precipitation	0.0438 – 10.1	1.00	0.00	1.00
	7-Day Precipitation	1.00 – 66.7	7.00	0.148	0.0601
	Temperature >20°C	0.582 – 30.3	3.57	0.0642	0.189
	Turbidity > 2.5 NTU	0.00550 – 0.892	0.116	0.149	0.0703
Salmonella	Turbidity >5 NTU	0.0247 – 4.74	0.541	0.00937	0.616
	3-day Precipitation	0.0345 – 7.63	0.777	0.00142	0.841
	7-Day Precipitation	0.862 – 4.68	0.733	0.00353	0.751
	Temperature >20°C	**1.66 – 89.7**	**10.0**	**0.200**	**0.0197**[2]
	Turbidity > 2.5 NTU	0.257 – 7.05	1.33	0.00367	0.729
Campylobacter	Turbidity >5 NTU	0.102 – 6.84	0.916	2.22	0.932
	3-day Precipitation	N/A[1]	2.69E+08	0.250	0.995
	7-Day Precipitation	0.293 – 9.73	1.66	0.0104	0.560

[1]N/A displays values that were extremely high or extremely low due to small sample bias
[2]Bolded values represent statistically significant regressions ($p < 0.05$)

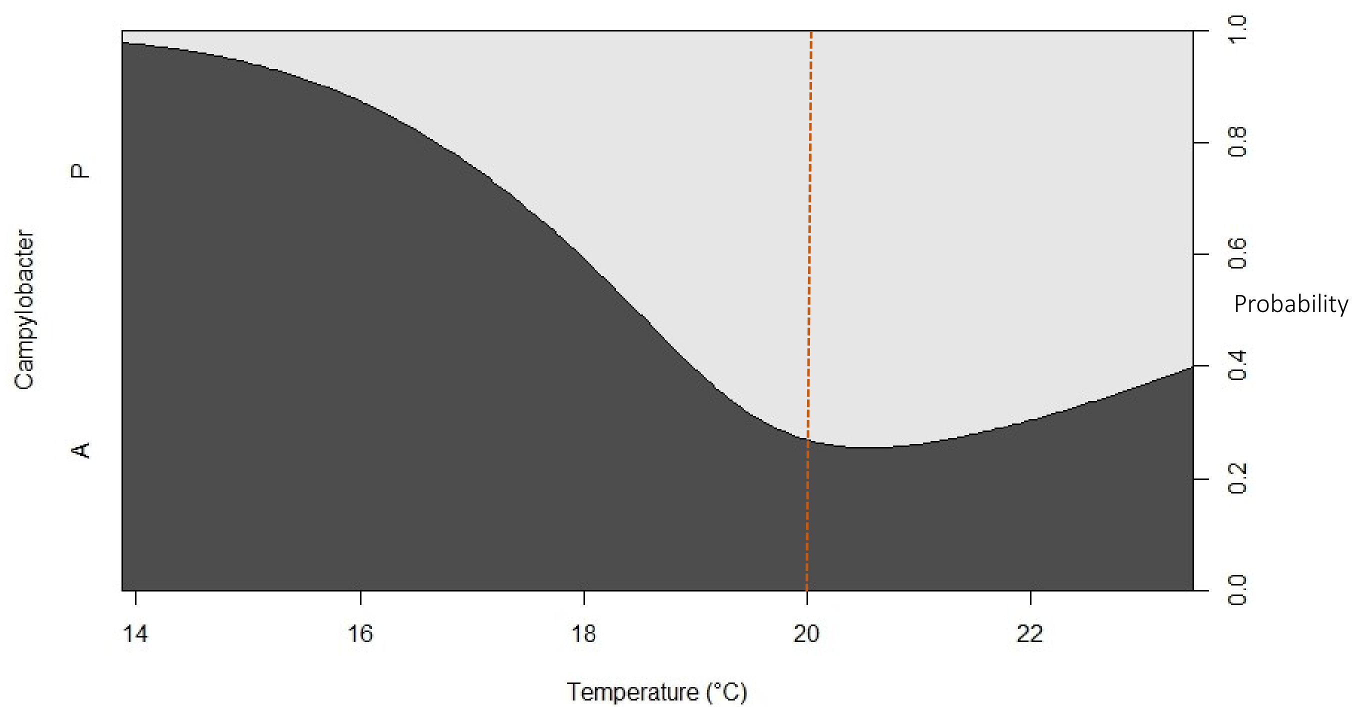


Figure 4.4: Probability of *Campylobacter* being detected along continuous measurements of water temperatures observed in test beaches. *Campylobacter* is a binary factor, such that it is either absent (A) or present (P). The orange dotted line represents the cut- off temperature used in the logistic regression (20°C).

4.2.3 – Prevalence of fecal contamination markers throughout the sampling season

Water samples from each beach were tested regularly for human, dog, and general avian fecal contamination markers. Prevalence and distribution of detected markers can be observed in Table 4.5. The human HF183 showed the highest prevalence, being detected in 13.6% of all tested water samples (Table 4.5). Four of the six positive samples occurred during one sampling run corresponding to May 20th, 2014 (table 4.5). The remaining two positive samples occurred during and after the sampling season at Sandy and Birch Cove Beaches respectively (Table 4.5). The BacCan marker was detected in three samples (10.7%) while the dogmt marker was not detected at all (Table 4.5). All three positives occurred during the beach season, once at Springfield Beach and twice at Kinsmen Beach (Table 4.5). The GFD marker was utilized to detect the presence of total avian (duck, gull, chicken) fecal contamination. Out of forty-four water samples the GFD marker was only detected once, corresponding to a water sample from Springfield Beach before the beach season (Table 4.5). It is important to note that ducks were consistently seen at Kinsmen Beach during sampling. Although all three markers were detected at Springfield beach, the HF183 and BacCan markers had a higher prevalence in Kinsmen Beach (Table 4.5). Additionally, logistic regression showed no significant relationship between the prevalence of HF183 marker and human enteric pathogenic microbes ($p > 0.05$; Table 4.6).

Table 4.5: Prevalence and distribution of fecal marker detection at Springfield, Kinsmen, Sandy Lake, and Birch Cove Beaches before, during, and after the sampling season.

Marker[1]	Sampling Period	Springfield	Kinsmen	Sandy	Birch Cove
HF183[3]	**Total Prevalence: 6/44 (13.6%)[2]**				
	Before	1/3	1/3	1/3	1/3
	During	0/5	0/5	1/5	0/5
	After	0/3	1/3	0/3	0/3
Dogmt[4]	**Not Detected (0/28)**				
BacCan[4]	**Total Prevalence: 3/28 (10.7%)**				
	Before	0/3	0/3	0/3	0/3
	During	1/4	2/4	0/4	0/4
GFD[3]	**Total Prevalence: 1/44 (2.27%)**				
	Before	1/3	0/3	0/3	0/3
	During	0/5	0/5	0/5	0/5
	After	0/3	0/3	0/3	0/3

[1]Fecal markers were tested for in 500 mL of water sample.

[2] Percentage of positive samples (Number of positive samples/number of tested samples).

[4]The HF183 and GFD marker were tested for in 44 water samples expanding across sampling period and sampling site.

[4]The dogmt and BacCan markers were tested for in water 28 samples from before and during the beach season.

Table 4.6: Evaluation of the relationship between HF183 fecal marker and enteric pathogen presence within tested water samples.

Marker	Pathogen	OR	95% CI	ρ^2	p-value
HF183	*L. monocytogenes*	0.520	0.0240 - 4.45	0.0117	0.591
	Salmonella	0.400	0.0186 – 3.30	0.0242	0.447
	E. coli O157:H7	N/A[1]	N/A[1]	N/A[1]	0.994
	Campylobacter	0.500	0.0581 – 3.27	0.0189	0.482

[1]N/A represents values which were very low, very high, or were infinity

4.2.4 – Logistic regression of E. coli levels and the presence of pathogens and fecal markers

Logistic regression was performed in order to determine if prevalence of fecal markers or enteric pathogen were associated with *E. coli* levels at the test beaches. Results from the logistic regression can be observed in Table 4.7. None of the associations were statistically significant ($p>0.05$; Table 4.7). It is important to note that *Salmonella* and the HF183 marker displayed a decrease in p-value from *E. coli* levels >100 CFU/100 mL to >200 CFU/100 mL (Table 4.7). With a p-value of 0.236 the regression pair of *Listeria* and *E. coli* levels >100 CFU/100 mL was the closest to being significant (Table 4.7). At both levels of *E. coli,* the BacCan fecal marker and *E. coli* O157:H7 pathogen displayed very high p-values (Table 4.7).

Table 4.7: Logistic regression to evaluate the relationship between *E. coli* levels and pathogen/ fecal marker presence.

E. coli level (CFU/100 mL)	Pathogen / Marker	OR	ρ^2	p-value
>100	*Listeria*	4.00	0.0661	0.226
	Salmonella	0.777	0.00191	0.841
	O157:H7	2.83E+08	0.176	0.996
	Campylobacter	1.50	0.00627	0.712
	HF183	1.32	0.00147	0.816
	BacCan	1.37e-07	0.0260	0.994
>200	*Listeria*	4.27	0.445	0.996
	Salmonella	2.66	0.0305	0.511
	O157:H7	2.46e-07	0.0129	0.996
	Campylobacter	1.90e-08	0.165	0.996
	HF183	3.59	0.0377	0.330
	BacCan	1.09e-07	0.0200	0.997

4.3 – *E. coli* as an Indicator of Fecal Contamination Within Test Beaches

4.3.1 – E. coli levels in surface sediments

E. coli counts from surface sediments were collected on five different sampling runs during and after the beach season, the results of which can be observed in Figure 4.5. *E. coli* levels were relatively low in collected samples with the highest counts occurring on August 30th at Kinsmen Beach (105 CFU/g) (Figure 4.5a). Elevated levels of *E. coli* were not observed during the September 22nd storm event (Figure 4.5a). The proportion of *E. coli* levels within water and sediment samples on select sampling runs can be observed in Figure 4.5b. A proportion of one occurs when water *E. coli* counts equal sediment *E. coli* counts. All beaches displayed higher *E. coli* counts in the water column on the July 7th sampling run (Figure 4.5b). Furthermore, Kinsmen, Sandy Lake, and Birch Cove Beaches displayed proportions greater than ten on September 22nd and October 20th, although levels at Springfield had proportions close to one. (Figure 4.5b). It is important to note the proportions observed at Sandy Lake Beach during these dates were elevated compared to the other beaches (Figure 4.5b). On July 29th all beaches displayed higher *E. coli* counts in the sediment except for Kinsmen which had equal levels of *E. coli* in both media. All beaches on August 30th displayed higher counts in the sediment with Kinsmen showing the highest proportion of 0.0249 (Figure 4.5). *E. coli* levels in Kinsmen, Sandy Lake, and Birch Cove Beach were higher in the water column on September 22nd and October 20th while Springfield displays proportions around 1. Counts in Sandy beach were especially high during these dates, with a proportion of 280 being calculated on October 20th (Figure 4.5b).

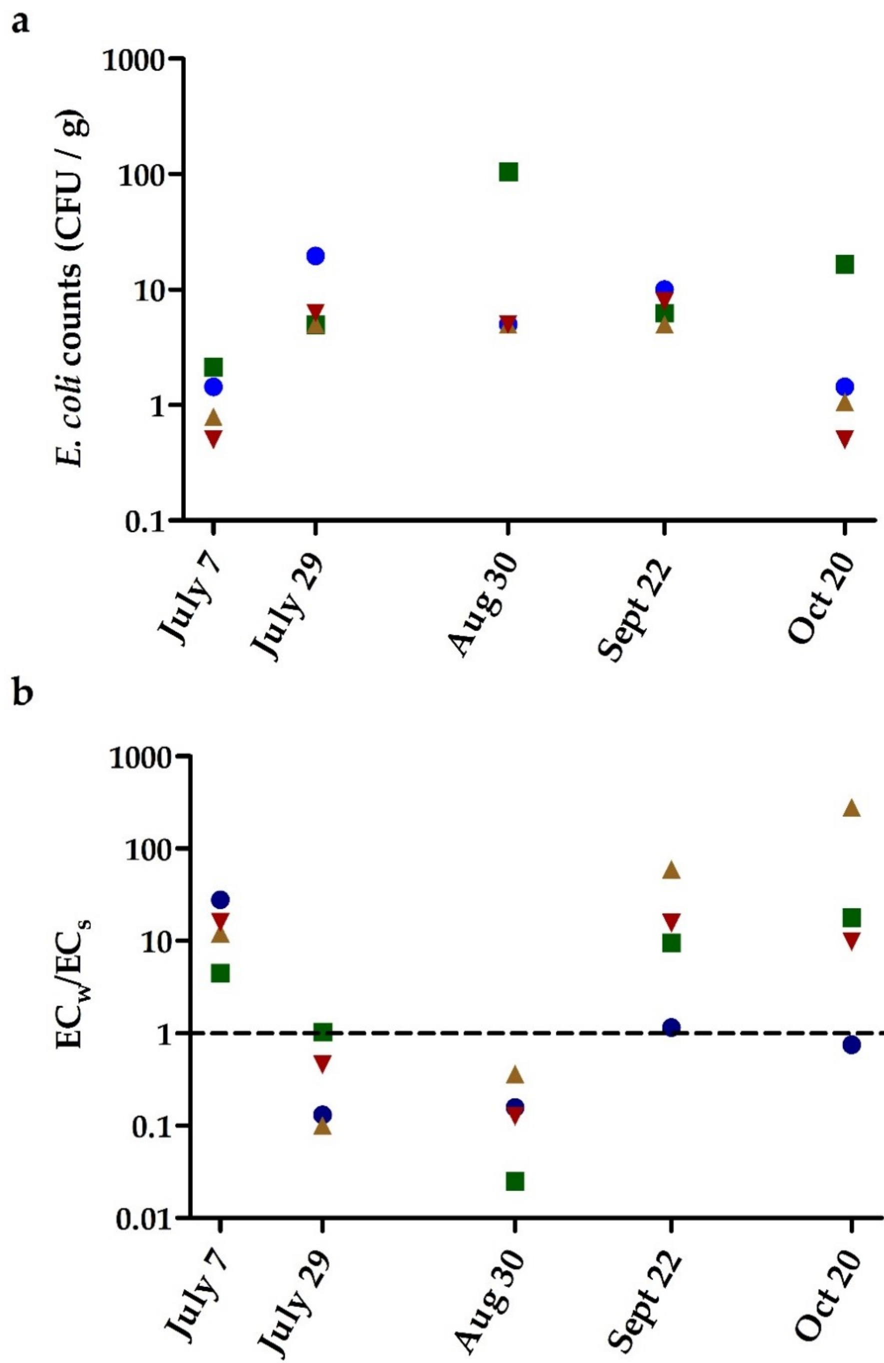


Figure 4.5: Sediment *E. coli* levels in tested beaches (a) and proportion of water and sediment *E. coli* levels (b). Points are color coded for each site with blue circles representing Springfield, green squares representing Kinsmen, brown upwards triangles representing Sandy Lake, and red downward triangles representing Birch Cove. Dotted line represents a proportion of water and sediment *E. coli* = 1.

4.3.2 – Day to day fluctuations in E. coli levels at the beaches

Near the end of the sampling season, consecutive sampling runs were undergone to assess fluctuations in *E. coli* on a day-to-day scale. Figure 4.6 shows geometric mean *E. coli* levels per beach per consecutive sampling runs with associated 95% CI. The 95% CI bars that do not overlap indicate a significant difference in geometric mean between consecutive days. *E. coli* counts at Kinsmen Beach were significantly higher ($p < 0.05$) on the 7th than on both the 8th and 9th (Figure 4.6b). *E. coli* levels on September 22nd at Birch Cove beach were significantly higher ($p < 0.05$) than those observed on September 24th (Figure 4.6c). Mostly all sample bars display a wide 95% CI. Geometric means and attached 95% CI for each day and beach can be observed in Appendix D.

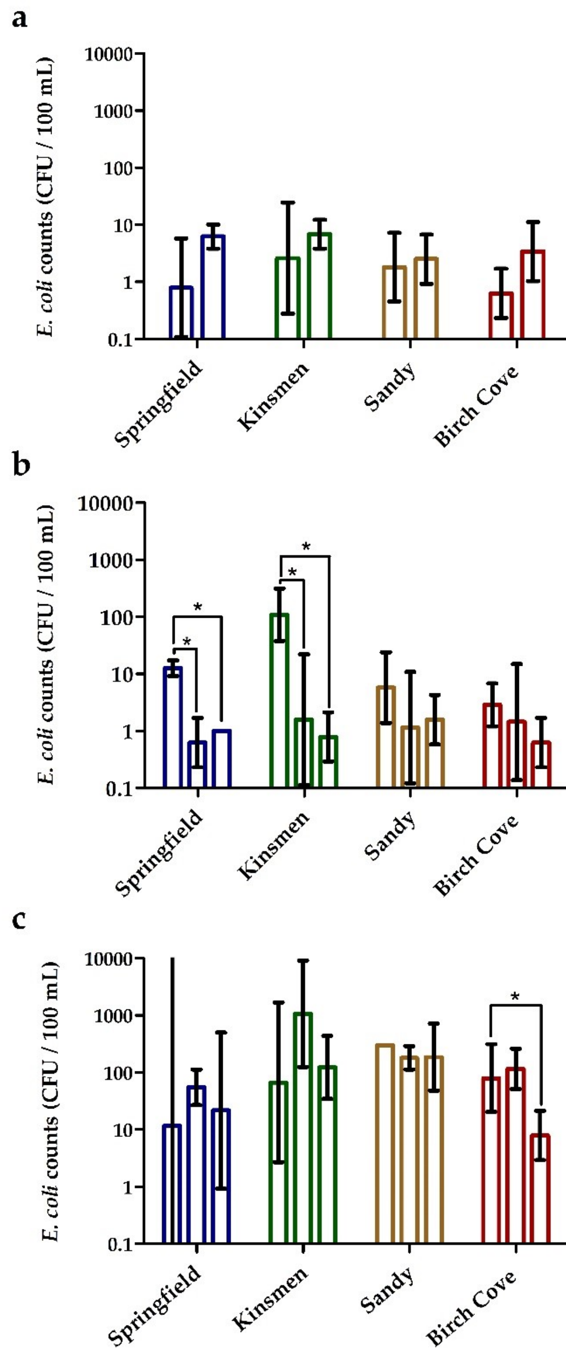


Figure 4.6: **Geometric means of *E. coli* counts from all beaches on consecutive sampling days to determine if *E. coli* counts differ between consecutive sampling days**. Consecutive sampling days include August 30th – 31st (a), September 7th – 9th (b), and September 22nd – September 24th (c). Bars represent geometric *E. coli* means and lines represent 95% CI. Statistically different *E. coli* counts ($p < 0.05$) are denoted by an asterisk.

4.3.3 – Predictor model of the interaction between water quality parameters and E. coli levels at tested beaches

Stepwise regression is commonly used for data exploration and to obtain a best-predictor model for a specific dependent variable, in this case *E. coli* counts. Table 4.8 shows all predictor models that were produced from stepwise regression, beginning with a full model that included all single variables and first-level interactions (Table 4.8). However, only the water temperature variable and the turbidity:7 day precipitation interaction were significant ($p< 0.05$) in this model (Table 4.8). Performing stepwise regression, in both directions, produced a model that included all single variables and Temp:DO, Temp:pH, Turb:pH, Turb:precip7 interactions (Table 4.8). Although this model attained the highest adjusted R^2 value (0.705), the individual pH variable and the Temp:pH interaction were not significant (Table 4.8). Removing these non-significant variables produced a formula that had a slightly lower adjusted R^2 value (0.702) but all variables and interactions were significant. Further removing the pH variable and all pH interactions lowered the adjusted R^2 value to 0.616 (Table 4.8). The final model obtained from the stepwise-regression is as follows: "*E. coli* = Temp + Turb + DO + pre7 + Temp:DO + Temp:pH + Turb:pH + Turb:pre7"(Table 4.8).

Table 4.8: Stepwise regression of measured WQP to produce a best predictor model for *E. coli* levels observed in sample beaches.

Model Formula[1]	Model Step	Significant Variables[2]	Model $R^{2, 3}$	Model p-value
E. coli = Temp + Turb + DO + ph + pre7 + Temp:Turb + Temp:DO + Temp:ph + Turb:Precip7 + Temp;pre7 + Turb:DO + Turb:ph + DO:ph + DO:pre7 + ph:pre7	Full model	Temp, Turb*Pre7	0.666	4.37E-05
E. coli = Temp + Turb + DO +ph + pre7 + Temp:DO + Temp:ph + Turb:ph + Turb:pre7	Stepwise Regression	Temp, Turb, DO, Pre7, Temp*DO, Turb*pH, Turb*Pre7	0.705	1.68E-07
***E. coli* = Temp + Turb + DO + pre7 + Temp:DO + Temp:ph + Turb:ph + Turb:pre7**	Removing insignificant variables	**Temp, Turb, DO, pre7, Temp:DO, Temp:ph, Turb:ph, Turb:pre7**	**0.702**	**8.43E-05**
E. coli = Temp + Turb + DO + pre7 + Temp:DO + Turb:pre7	Removing pH interactions	DO, pre7, Temp:DO, Turb:pre7	0.616	6.73E-07

[1]Abbreviations include: Temp = Water Temperature, Turb = Turbidity, DO = Dissolved Oxygen, pre3 = 3 day precipitation, pre7 = 7 day precipitation.
[2]Significant variables include those with p-values <0.05.
[3]The model R^2 represent the adjusted R^2 rather than typical multiple R^2

4.3.4– Factors affecting the levels of E. coli measured at test beaches

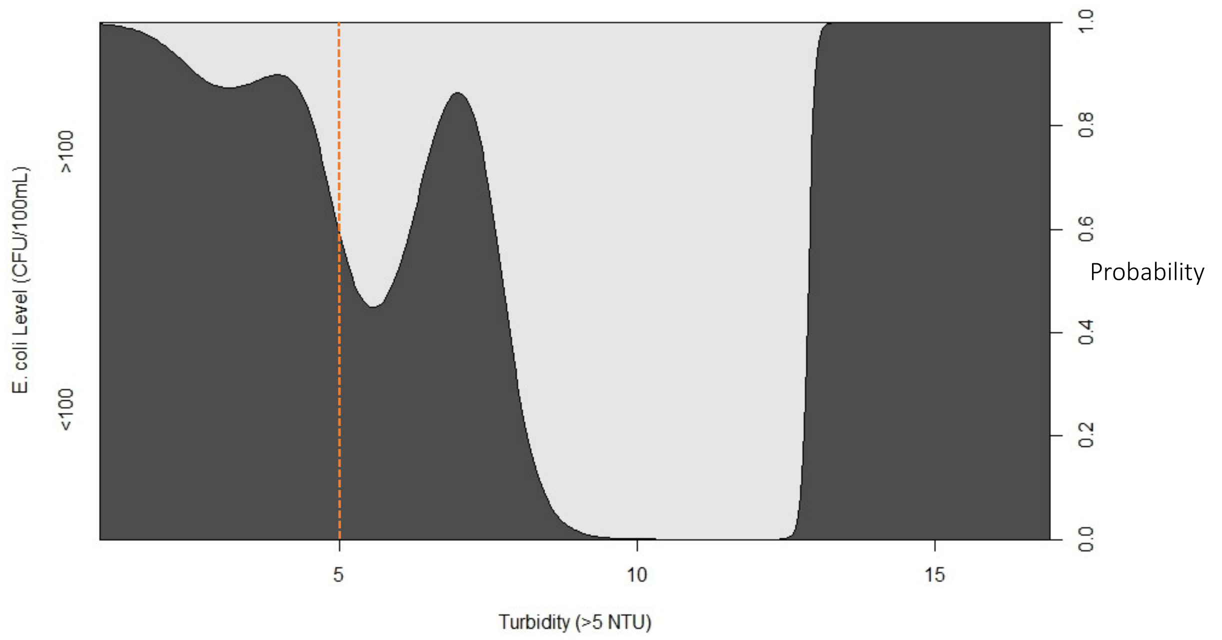
In order to determine if any of the WQP were associated with increased odds of *E. coli* levels >100 or 200 CFU/100 mL a logistic regression was performed. Subsequent results can be observed in Table 4.9 and corresponding CD plots for significant regressions can be observed in Figure 4.7. DO did not show a significant logistic relationship with *E. coli* levels >100 CFU/100 mL or >200 CFU/100 mL ($p>0.05$; Table 4.9). Although, turbidity levels >1/>10 NTU were not significant ($p >0.05$) *E. coli* levels were 13.16 times more likely to be >100 CFU/ 100 mL when turbidity levels were > 5 NTU (Table 4.9). As can be observed in Figure 4.7a, the probability of *E. coli* being >100 CFU/100 mL decreases as it approaches 5 NTU but increases shortly after passing 5 NTU (Figure 4.7a). As can be seen in Table 4.9, water temperature > 20°C, and total precipitation 3 and 7 days (>20mm) before sampling were associated with significantly ($p<0.05$) reduced and increased odds respectively, of detecting *E. coli* levels above 100 CFU/100 mL. At 15/20°C there is reduced odds of *E. coli* levels being greater than 100/200 CFU/100 mL as OR values were 0.0566 and 0.153 respectively (Table 4.9). On the corresponding CD plots, the probability of *E. coli* levels being greater than 100/200 CFU/100 mL is relatively low at the corresponding threshold temperatures (Figure 4.7b,c). Although the water temperature >20°C had a lower ρ^2 value (0.112) the water temperature >15°C variable displayed ρ^2 value of 0.186, indicating a better goodness of fit (Table 4.9). Total precipitation 3 and 7 days (>20mm) before sampling date increased the odds of detecting *E. coli* levels >100 CFU/100 mL, where 7 day precipitation (>20 mm) had a high OR of 27.8 compared to 9.11 for 3 day precipitation (>20 mm; Table 4.9). At the limiting value of 20 mm, the probability of *E. coli* >100 CFU/100 mL is approximately 20% for both CD plots Figure 4.7d,e). Within the 3 day precipitation plot there is an increase in probability at 50 mm, which plateaus and then slowly decreases as precipitation levels of 100 mm were passed (Figure 4.7d). Conversely, as precipitation levels of 100 mm on the 7 day precipitation plot were reached there is approximately a 20% chance that *E. coli* levels will be >100 CFU/100 mL, which then dropped to approximately 60% levels of 100 mm were surpassed (Figure 4.7e).

Table 4.9: Logistic regression to determine the influence of measured WQP on *E. coli* levels observed in test beaches.

Parameter[1]	*E. coli* level (CFU/100 mL)[2]	Limiting value[3]	95% CI	OR	ρ^2	p-value
Water Temperature (°C)	>100	>15	0.423 - 2.45	0.293	0.0248	0.212
		>20	**0.0217 - 0.672**	**0.153**	**0.112**	**0.0245**[5]
	>200	**>15**	**0.00493 - 0.596**	**0.0566**	**0.186**	**0.0135**
		>20	N/A[4]	7.31e-09	0.208	0.995
Turbidity (NTU)	>100	>1	N/A	2.53e+06	0.00847	0.995
		>5	**2.46 - 152**	**16.49**	**0.239**	**0.00574**
		>10	N/A	1.41e-07	0.0172	0.996
	>200	>1	N/A	3.19e+06	0.00652	0.997
		>5	0.823 - 227	9.71	0.150	0.0787
		>10	N/A	3.05	4.24e+182	0.996
Dissolved Oxygen (mg/L)	>100	Range (5.0<x>9.5)	0.285 - 4.17	1.11	4.31e-04	0.875
	>200	Range (5.0<x>9.5)	0.151 - 11.7	1.33	0.00261	0.781
3 day precipitation (mm)	>100	**>20**	**2.25 - 46.8**	**9.11**	**0.169**	**0.00332**
	>200	>20	0.263 - 20.7	2.33	0.0217	0.415
7 day precipitation (mm)	>100	**>20**	**4.71 - 535**	**27.8**	**0.286**	**0.00236**
	>200	>20	N/A	1.70	0.277	0.995

[1]The WQP were used as independent (x) variable
[2]The *E. coli* levels were used as the dependent (y) variable
[3]Value used as cutoff point for binary coding of independent variable
[4]N/A indicates values that are very low, very high, or infinity
[5]Bold values indicate a significant regression ($p < 0.05$).

a



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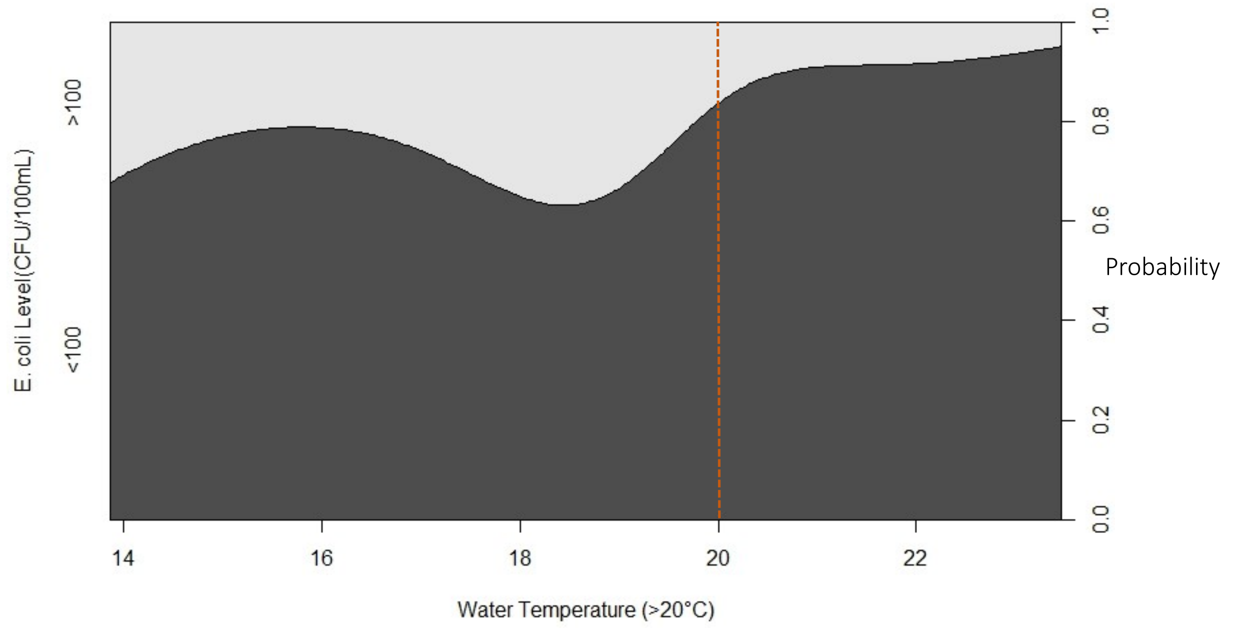

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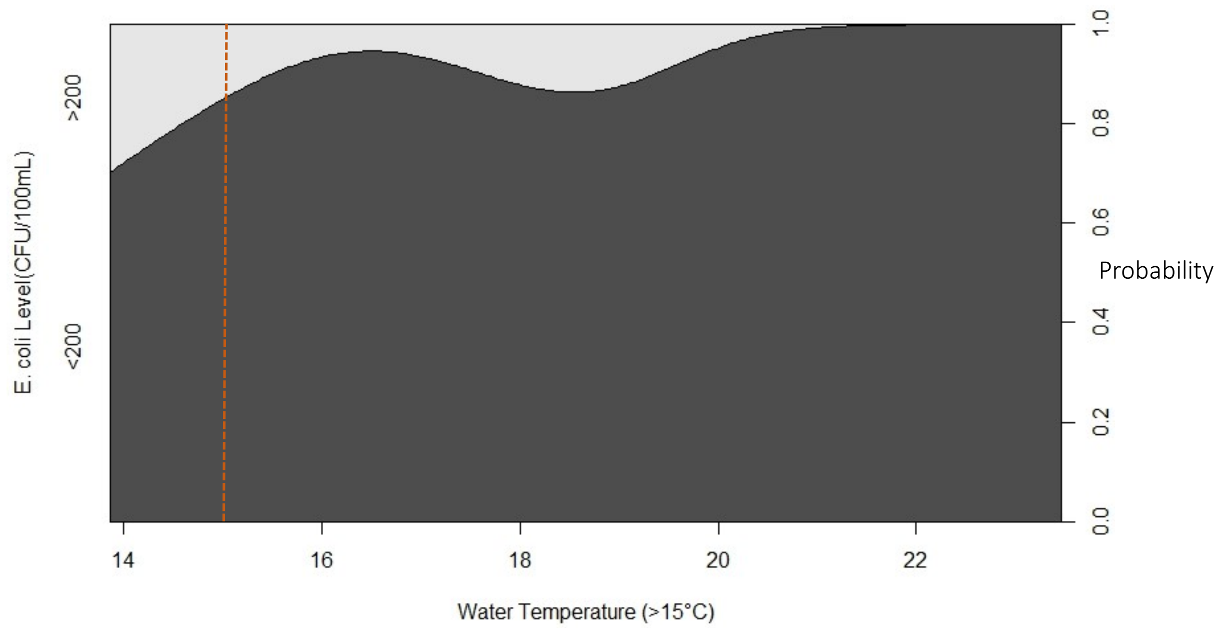

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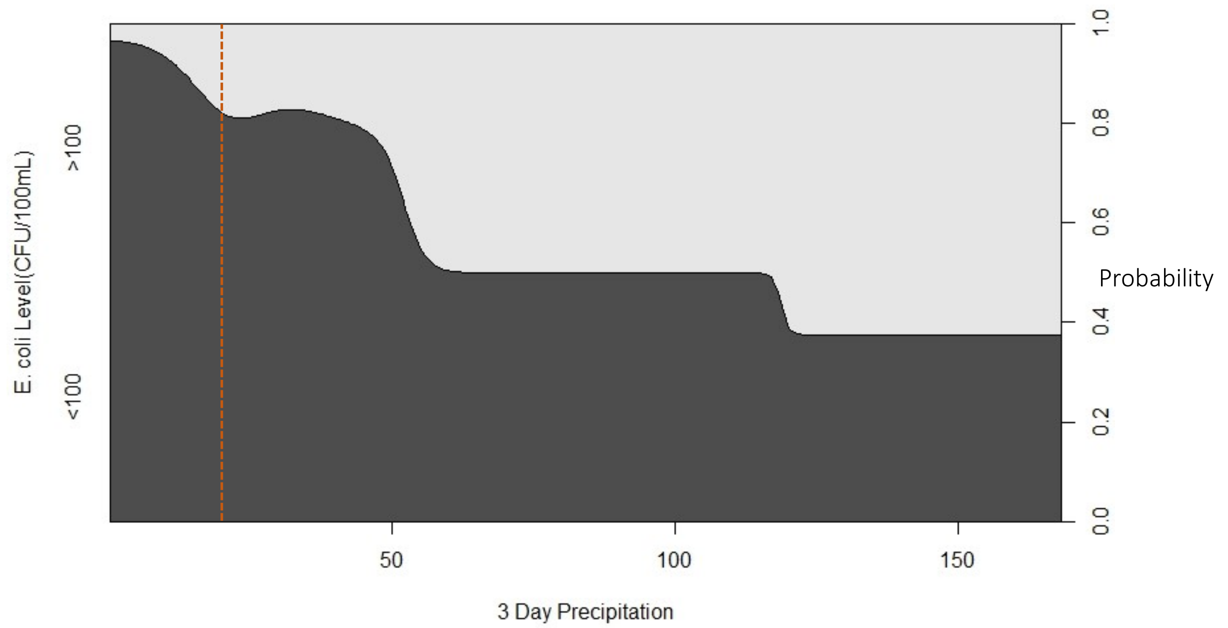

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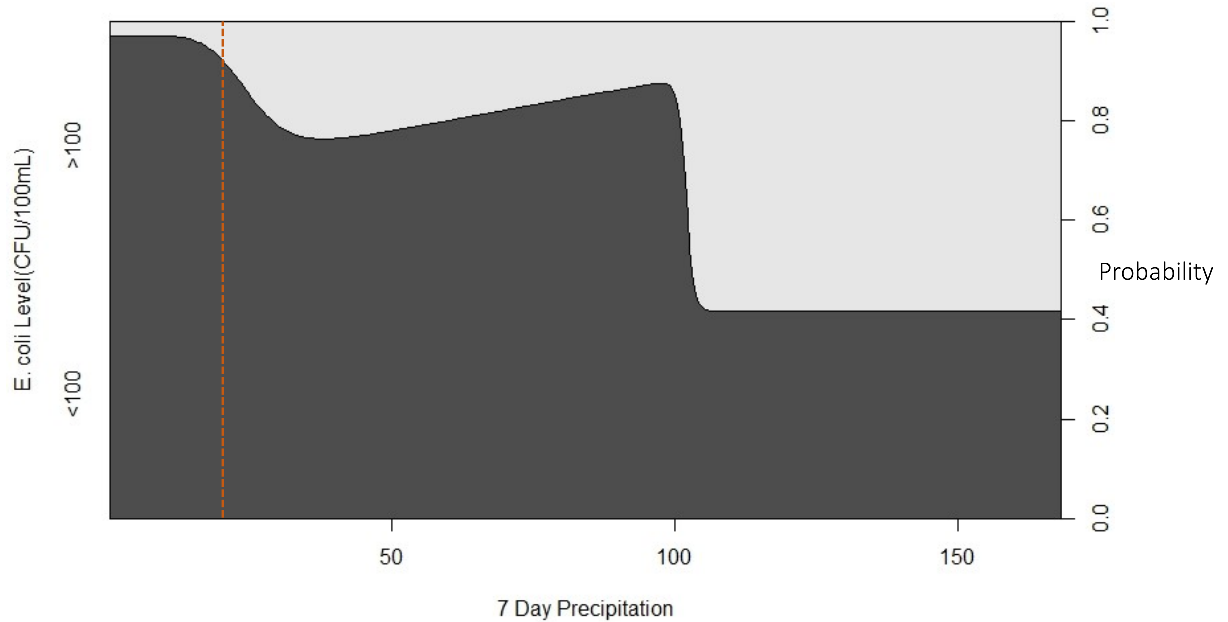

Figure 4.7: Probability of *E coli* counts being greater than >100/>200 CFU/100 mL along continuous measurements of turbidity (a) water temperature (b, c) and 3-day (d) and 7-day (e) precipitation. Dotted lines represent the cut-off value used in logistic regression.

Chapter 5 – Discussion

5.1 – Prevalence of FIB, Pathogens, and Fecal Markers at Test Beaches

5.1.1 – E. coli and coliform levels within test beaches

According to Health Canada Guidelines for Recreational waters, a beach must close to the public if *E. coli* levels surpass 400 CFU/100 mL for a single sample or if the geometric mean of five samples surpasses 200 CFU/100 mL. None of the tested water samples taken during the beach season surpassed the maximum allowed concentration of *E. coli* although levels at Kinsmen Beach on September 23rd surpassed 1000 CFU/100 mL. There was not a significant difference in *E. coli* levels between sampling sites within this study. The geometric *E. coli* mean at all sites was relatively low despite levels of ≥100 CFU/100 mL being observed on numerous occasions. However, *E. coli* levels ≤ 10 CFU/100 mL were much more common throughout the sampling season. Therefore, the final geometric *E. coli* mean of each beach would be skewed towards lower geometric means and have higher attached variances, as supported by the relatively wide 95% CI values observed.

E. coli levels were significantly ($p<0.05$) greater during and after the beach season compared to before the beach season. Geometric *E. coli* levels were highest after the beach season due to elevated *E. coli* levels caused by the September 22nd – 24th storm event and elevated turbidity levels on October 20th. Heavy rainfall will increase the level of *E. coli* within aquatic systems (Whitman *et al*., 2006; Kleinheinz *et al*., 2009). In fact, Ackerman and Weisberg (2003) noted large scale elevation in FC levels across the United States after storm events with just 25 mm of rain. Increased levels of turbidity are positively correlated with an increase in *E. coli* concentration, such that increasing turbidity will lead to increased *E. coli* levels (Francy *et al*., 2013; Marion *et al*., 2015). An increased concentration of particles, corresponding to increased turbidity, within surface waters can reflect an influx of *E. coli* from runoff (Jeng *et al*., 2005) or the release of sediment-bound *E. coli* into the water column (Whitman *et* al., 2006; Phillips *et al*., 2014). Extending the sampling period to include all four seasons would allow for a more in-depth analysis of *E. coli* levels within the tested beaches.

5.1.2 – Detection of selected pathogens within tested beaches

E. coli O157:H7 was only detected once out of twenty-four water samples. This bacterium is mostly associated with ruminants and agricultural watersheds (Walters *et al.*, 2007; Ferens & Hovde, 2011). The beaches tested in this study were located in urban settings with little agricultural influence. Furthermore, a study by Shelton and authors (2004) reported similar low *E. coli* O157:H7 detection in a mostly urban Maryland watershed. Therefore, the low prevalence of *E. coli* O157:H7 is not an unexpected result.

L. monocytogenes displayed a moderate prevalence with detection in 25% (6/24) of tested water samples. Low to moderate prevalences of *Listeria* spp. in urban environments are commonly reported within the literature (Lyautey *et* al., 2007; Sauders *et al*., 2012). Stea and others (2015b) reported a moderate prevalence of *L. monocytogenes* (35.4%) in a Nova Scotian Urban watershed, although a much higher prevalence of 72.1% was observed in an agricultural watershed. Within this study, five out of the six positive *L. monocytogenes* samples occurred after the beach season, when temperatures were cooler. Several groups have reported an increase in prevalence of *L. monocytogenes* at cooler water temperatures (Budzinska *et al*., 2012; Strawn *et al*., 2012). Furthermore, Cooley and others (2014) reported lowest *Listeria* spp. prevalence during the fall at a Californian agricultural watershed. Due to their ubiquitous nature however the presence of this pathogen could represent either new bacteria entering the beaches or natural reservoirs of the pathogen, as highlighted by Stea *et al*. (2015b).

Within this study, *Salmonella* displayed the highest prevalence among tested pathogens. *Salmonella* prevalence in freshwater systems is variable within the literature, as highlighted by (Levantesi *et al*., 2012). However, Stea *et al*. (2015a) displayed very similar prevalence in Nova Scotia with detection rates of 27.9% and 23.1% in an agricultural and urban watershed, respectively. Within this study, *Salmonella* was mostly detected during the beach open season. Seasonal variation of *Salmonella* has not been established within the literature, with varying seasonality effects being reported (Till *et al.,* 2008; Haley *et al*., 2009; Wilkes *et al*., 2009). Human interaction could influence *Salmonella* levels within the beaches. However, prevalence remained relatively stable across the four beaches despite varying levels of urbanization and swimmer density. Both water temperature and rainfall events have been linked to the prevalence

of *Salmonella* in freshwater systems (Schets *et al*., 2008; Haley *et al*., 2009; Wilkes *et al*., 2009), although such a result was not supported in this study. It is important to note that *Salmonella* was not tested for in relation to the two storm events that occurred during the sampling season.

Presumptive *Campylobacter* spp. were detected in eleven water samples by the general qPCR assay, although only one was identified by the triplex PCR assay as *C. jejuni*. *C. jejuni* and *C. coli*, and to a lesser extent *C. lari*, are the most common *Campylobacter* spp. in both humans and aquatic environment (Gillespie *et al*., 2002; Kemp *et al.,* 2005; Jokinen *et al*., 2010). Stea and others (2015b) reported higher prevalence of all three species in both an agricultural and urban watershed. Furthermore, Khan and Edge (2007) reported excellent reproducibility and high specificity/selectivity with the triplex PCR assay. The other ten positive *Campylobacter* species could be other less common *Campylobacter* species such as *C. helveticus* or *C.* upsaliensis. However, it is possible that the unidentified samples were false positives, as was observed in Stea *et al*. (2015a) in which unspecific products of *Erythrobacter* were detected as *Campylobacter* spp. by the same qPCR assay utilized in this study. It is important to note that due to the age of the *C. coli* primers, a primer dimer band covered the potential position of the *C. coli* band.

Neither *G. lamblia* nor *C. parvum* were detected in 500 mL any of the tested water samples. These enteric protist pathogens have been detected within recreational areas (Coupe *et al*., 2006; Ehsan *et al*., 2015) and act as parasites in drinking water (MacKenzie *et al*., 1994; Daly *et al*., 2010). However, in freshwater systems the prevalence of *C. parvum* and *G. lamblia* (oo)cysts has been consistently low (Schets *et al.,* 2008; Coupe *et al*., 2006; Galván *et al.,* 2014; Ehsan *et al*., 2015). It is important to note however that similar qPCR assays in the literature sample several liters of water (Guy *et al*., 2003; Helmi *et al*., 2011; Moss *et al*., 2014).. In the environment *C. parvum* and *G. lamblia* are found as hard to break (oo)cysts, which need to be broken and DNA released before molecular methods can detect the DNA. It is therefore possible that if these organisms were present within tested water sample but were not detected. However, low LODs were reported for both qPCR assays (DNA from 0.5 cysts for *G. lamblia* and 1 oocyst/300 µL stool sample; Verweij *et al*., 2003; Jothikumar *et al*., 2008). It is therefore more likely that these pathogens were not present at the tested beaches within 500 mL of tested water samples.

5.1.3 – Prevalence of fecal contamination markers at the test beaches

Within this study, the HF183 marker displayed the highest prevalence among tested fecal markers, with an overall prevalence of 13.6%. This prevalence is similar to results obtained by Stea and others (2015a) in which a prevalence rate of 9-10% was observed in two Nova Scotian watersheds. Four out of the six positive samples occurred at all beaches in the same sampling run, corresponding to May 20th, 2014. The low prevalence of the human marker during the beach season is unexpected as increased human traffic would logically lead to an increase of human contamination in recreational waters. A low LOD of 10 copies/100 mL and high sensitivity of the HF183 assay (Layton *et al*., 2013; Boehm *et al*., 2013; Green *et al*., 2014) makes it unlikely that any markers present within tested water samples remained undetected. Seurinck and others (2005) determined that at a temperature of 28°C the HF183 marker was capable of being detected up to 8 days after contamination and for up to 24 days at 4°C. Sampling runs occurred every two weeks, therefore it is possible that HF183 markers introduced into the water at any point between sampling runs did not survive long enough to be detected. Furthermore, an influx of human feces, and by extensions the HF183 maker, would be diluted by thousands of litres of lake water and water samples were only taken inside beach limits. As a result, the marker may be too dilute and dispersed to be detected. A larger scale study, including sampling outside the beach limits, is required to determine the presence of HF183 throughout the lakes.

A *Bacteroidales* (BacCan) and mitochondrial (dogmt) marker were utilized to test for the presence of dog-related fecal contamination within the beaches. Dogs have been shown to heavily impact microbial load and water quality at recreational beaches (Wright *et al.,* 2009; Wang *et al*., 2010; Zhu *et al*., 2011; Walker *et al*., 2015). However, in this study the *Bacteroidales* BacCan marker was detected in 10.7% of all water samples, occurring exclusively during the beach open season, while the mtDNA dogmt markers was not detected in any samples. Tambalo *et al*. (2012) determined that the dogmt and BacCan markers had comparable sensitivity but different specificity, with several groups reporting cross-reactivity of the BacCan marker with humans, deer, pigs, horses, and several other non-specific targets (Kildare *et al*., 2007; Tambalo *et al*., 2012; Boehm *et al*., 2013). Therefore, there is a possibility that the

detected BacCan markers did not originate from a canine source. *Bacteroidales* concentration have been shown to be higher in both fresh feces (Silke & Nelson, 2009) and water systems (Tambalo *et al*., 2012). Furthermore, the quantity of BacCan marker is higher in dog feces compared to the dogmt marker (Kildare *et al*., 2007; Caldwell & Levine, 2009; Boehm *et al*., 2013). As a result, dog contamination could be present within the tested water samples but were in the form of *Bacteroidales* rather than mtDNA. Overall, the low prevalence of the BacCan marker indicates that dogs may not be a large source of contamination at the tested beaches and the ban on dog's access to beaches imposed by the HRM is being obeyed.

Waterfowl have been identified as a major source of fecal contamination in both marine and freshwater recreational beaches (Converse *et al*., 2012). Based on the 16S rRNA fragment of an unclassified *Helicobacter*, the GFD marker is capable of detecting gull, duck, Canada goose, and chicken feces. This marker was only detected once in all of the water samples corresponding to Springfield before the sampling season, suggesting that avian species may not be a significant source of fecal contamination within beach limits. At Kinsmen Beach however there was a heavy presence of ducks observed consistently when collecting samples. As a result there should be a higher prevalence of this marker in Kinsmen across the entire sampling period. Green and authors (2012) reported excellent specificity and good sensitivity for this marker, which could be detected in as little as 0.1 mg of chicken feces. At this LOD it is likely that the GFD marker would be detected if it was present within the 500 mL of tested water samples. As avian species can defecate directly into the water as they fly over it, the lack of detection of the GFD marker within beach limits does not mean that the lakes are free from avian fecal contamination. Therefore, waterfowl may be contributing to the contamination at the beaches but the GFD markers are too dilute or sparse to be detected in water samples taken from within beach limits. Expanding the sampling sites to outside of beach limits would allow for more in-depth analysis of avian fecal contamination within these beaches.

5.1.4 – Public health risk associated with test beaches

The regular monitoring and tracking of beach water quality allows constant assessment of associated public health risks. Dwight and others (2004) reported that for each 2.5 hours of exposure of northern California surfers to the ocean, there was a 10% increase in GI-related

symptoms such as fever, nausea, stomach pain, and vomiting. Furthermore, children under the age of ten were shown to have increased risk of GI after swimming in four great lakes beaches (Wade *et al*., 2008). Therefore, the connection between untreated recreational waters and the onset of GI in swimmers is apparent.

The presence of fecal indicator *E. coli* within aquatic systems is not a risk to humans in itself but instead represents a risk due to the potential presence of harmful waterborne pathogens. Pruss (1998) reported an increased risk of GI in waters containing <30 indicators/100 mL. Additionally, in a study from Marion and others (2010) the percentage of swimmers in an inland U.S beach who displayed GI-related symptoms increased with water column *E. coli* density, even at relatively low levels. *E. coli* levels observed in this study therefore warrant additional study into the potential public health risks associated with *E. coli* levels at these beaches.

Enteric pathogenic microbes can be introduced into a water system several ways, including sewage or domesticated and wild animals (Walters *et al*., 2007; Jokinen *et al*., 2010; Van Dyke *et al*., 2010; Ferens & Hovde; 2011). Therefore, the presence of enteric pathogens, and by extension fecal contamination, will signal the degradation of water quality and increase the risk of swimmers contracting GI (Wong *et al*., 2009; Dorevitch *et al*., 2012). Interactions between beach goers and pathogen contaminated sediments have been shown to be associated with the incidence of GI (Bonilla *et al*., 2007; Heaney *et al*., 2009). The low prevalence of *L. monocytogenes* observed in this study may not represent a high risk to swimmers. The exact infectious dose of *L. monocytogenes* is not known but doses of 100,000 – 10 million CFU or 10 million – 100 million CFU have been estimated for immunocompromised and healthy individuals, respectively (Bortolussi, 2008). Furthermore, there has been no documented major waterborne outbreaks caused by *L. monocytogenes* to date. The presence of *Campylobacter* spp. and *Salmonella* spp. within the tested water samples potentially pose a health risk to the public. Both of these pathogens have been involved in serious waterborne outbreaks. Contamination of *E. coli* O157:H7 and C. *jejuni* in drinking water caused the large outbreak in Walkerton, Ontario while *Salmonella* caused a waterborne outbreak in Missouri that killed seven people (Angulo *et al*., 1997). Exact quantities of pathogens were not determined in this study but relatively low doses of tested pathogens have led to infection and illness in humans (Blaser & Newman, 1982; D'aoust, 1985; Black *et al*., 1988; Tuttle *et al*., 1999; Hara-Kudo & Takatori, 2011).

The presence of human, dog, and avian markers within the tested beach water indicates the presence of fecal contamination. Boehm *et al*. (2015) reported that at median concentrations of 4200 HF183/100 mL there were 30 instances of GI per thousand swimmers in California recreational waters. Little information is available describing how the presence of dog and avian markers influence GI rates in recreational waters. The presence of fecal contamination also potentially indicates the presence of enteric waterborne pathogens. For example, dog feces can contain *Giardia* and *Cryptosporidium* (Shukla *et al*., 2006; Olson *et al*., 2010), *Salmonella* (Bagcigil *et al*., 2007; Finley *et al*., 2007), *Campylobacter* (Rodrigues *et al*., 2015), and several harmful viruses (Sakulwira *et al*., 2003), all of which are capable of causing infection in humans. Furthermore, numerous different human pathogens occur within ducks (Murphy *et al*., 2005) and gulls (Kinzelman *et al*., 2008). Future study is required to fully assess the public health risk associated with the presence of fecal markers and waterborne pathogens within these beaches.

5.1.5 – Association between E. coli, pathogens, and fecal markers in recreational waters

E. coli are commonplace in water quality monitoring programs due to their apparent ability to indicate the presence of fecal contamination, and by extension pathogenic enteric bacteria, in water systems. None of the selected pathogens or fecal contamination markers displayed a significant relationship with the occurrence of *E. coli* levels greater than 100 and 200 CFU/100 mL of water, a result that has been observed in the literature. For example, in a meta-study by Wu and others (2011), non-enteric coliforms displayed a stronger correlation to the presence of a wide range of waterborne pathogens compared to FC/*E. coli*. Several groups have reported conflicting relationships between *E. coli* level and *Campylobacter* but positive relationships have still been relatively weak (Hörman *et al.* 2004; St-Pierre *et al*., 2009; Edge *et al*., 2013). A positive relationship between *Salmonella* and *E. coli* or FC levels has been reported on several occasions (Moriñigo *et al*., 1990; Polo *et al*., 1998; Wilkes *et al*., 2009; McEgan *et al*., 2013). Neither the human HF183 nor the dog BacCan marker displayed a significant relationship to *E. coli* levels greater than 100 or 200 CFU/100 mL. Contradictory relationships have been reported for the presences of human HF183 and indicator *E. coli* (Nshimyimana *et al*., 2014 Stea *et al*., 2015a). However, the prevalence of HF183 was discovered to increase at *E. coli* levels greater than 100 CFU/100 mL in Canadian watersheds (Fremaux *et al*., 2009, Stea *et al*., 2015a).

The microbes tested for in this study, with the exception of *L. monocytogenes*, are enteric and therefore should correlate with the presence of fecal markers. Within this study, a logistic regression was carried out to compare HF183 and pathogen prevalence. However, none of the tested regression were statistical significant. Research is limited, and sometimes contradictory, regarding the correlation between the presences of a fecal contamination marker and waterborne pathogen microbes. This is highlighted in a review by Green and others (2014) which shows that some groups have found positive correlation with human fecal contamination and the presence of *Salmonella* spp., *E. coli* O157:H7, and *Campylobacter* spp. while other groups found no such correlation. Human markers, including HF183, have been reported to positively correlate with the presence of *Campylobacter* spp. (Walters *et al.,* 2007). Conversely, Stea and others (2015a) reported that *Salmonella* spp. were 2.155 times more likely to be present in two Nova Scotia watersheds when the HF183 marker was present while no significant relationship was found between *Campylobacter* and HF183. Little information is available on the correlation between dog and avian markers and the presence of enteric pathogens but tested pathogens have been known to occur in dogs and ducks (Adesiyun *et al*., 1997; Beutin, 1999; Kuhn *et al*., 2002; Shukla *et al*., 2006: Lowden *et al*., 2015)

The deviation in *E. coli* levels and the presence of markers and pathogen results within this study can be explained several different ways. Rare events in logistic regression will skew the analysis towards insignificance (King & Zeng, 2001), as was observed in this study with the low prevalence of tested pathogens and fecal markers. Differential survival and decay rates between *E. coli*, pathogens, and fecal markers could have also influenced the lack of correlations within this study. The survivability of *E. coli* in water is heavily affected by solar radiation, water temperature, and a host of other physiochemical factors (Barcina *et al*., 1986; Rhodes & Kator, 1988; Whitman *et al*., 2004). Walters and Field (2009) reported that decay rates between HF183 and *E. coli* levels significantly differed however Dick and others (2010) describe a similar decay rate between the two. *Campylobacter* spp. are capable of surviving long periods in aquatic systems by transforming into a viable but non-cultural state or incorporating into biofilms (Murphy *et al*., 2006).If *E. coli* and enteric pathogens or fecal markers have differential survival it is possible for pathogens or fecal contamination to be present within the water but remained undetected by *E. coli* levels. It is recommend that the ability of *E. coli* to detect fecal

contamination, and by extension enteric pathogens, be further assess for use in Nova Scotian recreational waters.

5.2 – *E. coli* as an Indicator of Fecal Contamination in Recreational Waters

5.2.1 – E. coli in the surface sediment of tested beaches

Throughout the sampling period, *E. coli* levels obtained from surface sediment remained low, only surpassing 100 CFU/g once. Sediment *E. coli* levels observed at several different urban beaches, collected from the foreshore and at ankle/knee depth, display similar, although overall slightly higher, levels as those observed in this study (Boehm *et al*., 2009; Staley *et al*., 2015). Furthermore, Piorkowski and others (2014) obtained similar *E. coli* concentrations within the sediment of Thomas Brook, a Nova Scotian agricultural watershed. It therefore appears that the *E. coli* levels observed in this study are similar, albeit slightly lower, than what is reported within the literature. The presence of *E. coli* within the sand can indicate the presence of sand-based pathogenic microbes (Yamahara *et al*., 2012). Additionally, several groups have displayed a correlation between the presence of FIB in sediments and risk of contracting an enteric disease (Whitman *et al.,* 2009; Heany *et al.,* 2012).

As an indicator of fecal contamination, *E. coli* should not be found naturalized within the environment. However, *E. coli* have been demonstrated to not only survive in beach sand for up to a month (Staley *et al.,* 2016) but are able to replicate within the sediment (Beversdorf *et al*., 2006). Furthermore, several groups have shown that beach sediments can act as a sink of naturalized or persisting *E. coli* (Alm *et al*., 2006; Ishii *et al.,* 2007). Therefore, *E. coli* levels are capable of building up within sediments even without a fresh source of fecal contamination. Within this study, the proportion of *E. coli* in sediment and water fluctuated between sampling runs, such that neither medium displayed constantly higher levels. As highlighted by Whitman and Nevers (2003), there is constant movement of *E. coli* between surface sediment and water. Water does not constantly deposit *E. coli* into sediment without sediment *E. coli* leaching back into the water column. The leaching of sediment *E. coli* into the water column can lead to inflated levels of fecal indicators within the water column (Phillips *et al*., 2011; Phillips *et al*., 2014), which in turn could lead to unnecessary beach closures. Although levels were low, the

presence of *E. coli* within tested sediment samples calls into question the validity of *E. coli* as an FIB.

5.2.2 – Fluctuations in measured E. coli counts

The HRM reported several beach closures throughout the 2014 open beach season. Springfield beach was closed twice, from July 7th-9th and August 7th-11th, while Birch Cove Beach was closed from August 20th-22nd, yet no samples during the beach season displayed levels that would indicate the need for a beach closure. The HRM sends water samples to a commercial lab that uses m-FC media for enumeration of *E. coli* while m-ColiBlue24 broth was utilized in this study. Both media have varying sensitivity and false-positive rates (Ciebin *et al*., 1995; Grant, 1997; Jensen *et al*., 2001; McLain & Williams, 2008). Furthermore, McLain and Williams (2008) indicated that specificity of m-ColiBlue24 was highest during the summer and lowest during the fall and winter. Subsequent variation and inaccuracies in *E. coli* counts caused by enumeration media can therefore lead to unnecessary beach closures.

Small scale variances in sampling time, weather or hydrological factors also play a role in *E. coli* variability. Within this study there were several statistically significant differences in *E. coli* levels at the beach during consecutive sampling days. *E. coli* levels in water have been noted to vary on minute, hour, and day time scales (Whitman *et al*., 2004; Desai & Rifai, 2013; Amorim *et al*., 2014). Desai and Rifai (2013) reported that in a single 24-hour period *E. coli* levels in an urban watershed varied as much as five magnitudes from each other. Weather and hydrological factors also influence the temporal variability of *E. coli*. Sunshine, waves, currents, and wind have all been associated with temporal variability of *E. coli* counts (Whitman *et al*., 2004; Ge *et al*., 2012). The timing of sampling is therefore crucial in the monitoring of *E. coli* levels in recreational waters and should be standardized to ensure accurate and precise *E. coli* measurements.

5.2.3 – Effect of WQP on E. coli prevalence within the beaches

The levels of *E. coli* in an aquatic systems are constantly influenced by physical and chemical water parameters. A regression model was produced to further explore how measured

E. coli counts obtained in the four test beaches were influenced by selected WQP. Logistic regressions were completed while simultaneously building the predictor model in order to aid in the construction of the final regression model. The obtained model is as follows: E. coli = Water temperature + Turbidity + DO + 7 day precipitation + Water temperature: DO + Temperature: pH + Turbidity: pH + Turbidity: 7 day precipitation.

Water temperatures greater than 15°C and 20°C were significantly related to *E. coli* levels greater than >200 CFU/100 mL and >100 CFU/100 mL respectively. Both sets of regressions had OR values less than one, indicating that *E. coli* levels will likely be below these numbers at the tested temperatures. This supports the hypothesis that *E. coli* are known to have decreased survival at higher water temperatures (Flint *et al*., 1987; Sampson *et al*., 2006). Therefore, the presence of the water temperature variable within the final predictor model is expected. DO was not significantly related to *E. coli* levels during logistic regression but the individual variable was present and significant in the final predictor model. A weak negative correlation has been reported between *E. coli* levels and DO (Nevers & Whitman, 2005), suggesting that *E. coli* levels may decrease with increasing concentrations of DO (Curtis *et al*., 1992). The solubility of oxygen will decrease as water temperatures increase (Fondriest Environmental, 2013), indicating that the positive interaction term between DO and water temperature in the model is to be expected.

The large storm event that occurred during September 22nd – 24th increased *E. coli* levels at all beaches. *E. coli* levels were 9.11 and 27.8 times more likely to be greater than 100/200 CFU/100 mL when there was more than 20 mm of rain 3 and 7 days prior to sampling, respectively. *E. coli* levels will increase with increasing rainfall (Ackerman & Weisberg, 2003; Kleinheinz *et al*., 2009) such that some municipalities preemptively close recreational beaches based on rainfall amounts. It should be noted however that the correlation between previous days of rainfall and *E. coli* has not been fully supported (Haack *et* al., 2003; Kleinheinz *et al*., 2009). *E. coli* levels were 16.49 times more likely to be >100 CFU/100 mL when turbidity levels were greater than 5.0 NTU. This relationship is well documented, with a positive correlation being reported in the literature (Francy *et al*., 2013; Marion *et al*., 2015). Furthermore, heavy rainfall will increase turbidity within the water column mainly through sediment runoff (Lawler *et al*., 2006; Göransson *et al*., 2013). Increased turbidity within the water column represents an influx

of sediment particles in the water column which in turn provides increased protection and nutrients for suspended *E. coli*. As a result, the addition of 7 day precipitation and turbidity, and their interaction, into the final predictor model is to be expected.

The predictor model indicates that pH showed significant interactions with water temperature and turbidity but is not significant individually. Furthermore, removing pH from the predictor equation decreased the goodness-of-fit of the model. The research surrounding the relationship between pH and *E. coli* levels is sparse, as highlighted by Brauwere and others (2014). However, Hipsey *et al*. (2008) report that FC appear to have higher mortality rates outside of the pH 6-8 range. Measured pH levels within the beaches did not surpass this range and remained consistently at approximately 7. Therefore, the observed pH would have little to no effect on *E. coli* levels within the models. However, pH has a weak negative correlation with water temperature and turbidity (Shibata *et al*., 2004; Ortega *et al*., 2009) such that as these factors increase the pH will move outside of the neutral range. The pH variable was left in the interactions of the final model as it likely increased the adjusted R^2 value but did not have any effect on *E. coli* levels measured within our data.

The above predictor model is a simplified version of what would occur in a real water system. However, it does highlight how *E. coli* levels within the water are influenced by weather and WQP. Turbidity and previous day precipitation are common variables in *E. coli* predictor models within the literature (Francy *et al*., 2013; Brauwere *et al*., 2014). Rainfall specifically has been shown to correlate quite heavily with beach closures (Kleinheinz *et* al., 2009; Bush *et al*., 2014). Furthermore, Health Canada recommends that beaches remain closed after period of heavy rainfall in order to ensure public safety (Health Canada, 2012b). Further research should therefore be completed to assess the ability of precipitation to assess public risk and determine beach closures.

Chapter 6 – Conclusion

6.1 – Project Summary

Within this study, water samples were collected from four local freshwater beaches before, during, and after the beach season. Throughout most of the sampling season *E. coli* levels remained below the maximum allowed concentration. Furthermore, *E. coli* levels during, and after the beach season were greater than those observed before the beach season, although there was no significant difference between during and after the beach season. There was no significant difference in *E. coli* levels between the tested beaches. *E. coli* levels obtained in this study therefore indicate that the beaches may not be heavily contaminated by feces.

E. coli O157:H7 was only detected in one water sample while *L. monocytogenes* and *Salmonella* showed moderate prevalences of 25% and 29.1% respectively. Additionally, the triplex PCR only detected *Campylobacter* in one sample, corresponding to *C. jejuni*. Humans may act as the largest source of fecal contamination at these beaches as it was detected in 13.6% of water samples. Dogs showed a similar prevalence of 10.7% although contamination was detected only by the BacCan marker, a result likely brought about by a difference in specificity between the two markers and the difference in target abundance in dog feces. Avian species did not represent a large source of fecal contamination at the tested beaches as the GFD markers was only detected once throughout the entire sampling season. Overall, the test beaches do not appear to be heavily contaminated by fecal matter and should generally be safe for public use.

Within this study, *E. coli* functioned as was intended and could represent an adequate indicator of fecal contamination in the four test beaches. Low levels of contamination were observed within the test beaches and *E. coli* remained below the maximum allowed concentration throughout most of the sampling season. Although levels remained below 100 CFU/g for the most part, *E. coli* was still observed in tested sediment samples. Additionally, *E. coli* levels were noted to significantly fluctuate on a day-to-day basis, highlighting the retrospective nature of using *E. coli* as a fecal indicator. In this study, *E. coli* was unable to predict the presence of fecal contamination markers and enteric pathogens, although the low prevalence of these markers could have influenced this finding. It is therefore important that future studies further test the validity of *E. coli* for use an indicator of fecal contamination.

E. coli also appeared to be influenced by WQP and weather parameters. Logistic regression indicated that *E. coli* were less likely to be increased with increasing water temperature while increased within increasing turgidity and previous day precipitation. The influence of WQP on *E. coli* levels was further highlighted by the resulting stepwise regression prediction model: *E. coli* (100 CFU/100 mL) = Water temperature + Turbidity + DO + 7-day precipitation + Water Temperature:DO + Water Temperature:pH + Turbidity:pH + Turbidity:7-day precipitation. This model highlights the complex interaction within and between WQP and *E. coli* levels. The influence of turbidity and previous day precipitation could be used by the HRM to help develop a simpler and more reliable method of assessing and managing risk within the test beaches. The prediction model should be expanded further to include more variables in the hope to create solid WQP guidelines for use in conjunction with *E. coli* in recreational waters.

6.2 - Recommendations for Further Research

1. Extend sampling period year-round to fully explore *E. coli*, coliform, pathogen, and fecal marker prevalence patterns within the test beaches.

2. Include water samples from the input, output, and middle of each lake to get a better understanding of fecal coliform, pathogen, and fecal marker distribution.

3. Include more sediment sampling trips and test for the presence of fecal contamination markers and pathogens to further explore the relationship between sediments and water columns at the beaches.

4. Identify genotypes of *E. coli* isolates taken from sediment samples and relate types found within column to allow for the assessment of water column *E. coli* source.

5. Quantify the levels of pathogens and fecal contamination markers in order to better assess public health risk associated with each beach.

6. Include spontaneous sampling runs to capture storm events to compare how *E. coli*, fecal markers, and pathogen prevalence changes between baseline and storm events – allowing for a more in depth analysis of distribution of *E. coli* within test lakes.

7. Further expand predictor model by adding more variables and testing the resulting model's ability to assess risk in Nova Scotia recreational waters.

Bibliography

Acheson, D., Hohmann, E.L. (2001). Nontyphoidal Salmonellosis, *Clinical Infectious Diseases, 32*(2), 263-269.

Ackerman, D., Weisberg, S.B. (2003). Relationships between rainfall and beach bacterial concentrations on Santa Moniva bay beaches. *Journal of Water and Health*, *1*(2), 85-89.

Adesiyun, A.A., Campbell, M., Kaminjolo, J.S. (1997). Prevalence of bacterial enteropathogens in pet dogs in Trinidad. *Journal of Veterinary Medicine*, *44*, 19-27.

Ahmed, W., Neller, R., Katouli, M. (2005). Host species-specific metabolic fingerprint database for *Enterococci* and *Escherichia coli* and its application to identify sources of fecal contamination in surface waters. *Applied and Environmental Microbiology, 71*(8), 4461-4468.

Ahmed, W., Stewart, J., Gardner, T., Powell, D., Brooks, P., Sullivan, D., Tindale, N. (2007). Sourcing faecal pollution: a combination of library-dependent and library-independent methods to identify human faecal pollution in non-sewered catchments. *Water Research*, *41*(16), 3771-3779.

Alderisio, K.A., DeLuca, N. (1999). Seasonal enumeration of fecal coliform bacteria from the feces of Ring-Billed Gulls (*Larus delawarensis*) and Canada Geese (*Branta Canadensis*). *Applied and Environmental Microbiology, 65*(12), 5628-5630.

Alm, E.W., Burke, J., Hagan, E. (2006). Persistence and potential growth of the fecal indicator bacteria, *Escherichia coli,* in shoreline sand at Lake Huron. *Journal of Great Lakes Research, 32,* 401-405.

Amorim, E., Ramos, S., Bordalo, A.A. (2014). Relevance of temporal and spatial variability for monitoring the microbiological water quality in an urban bathing area. *Ocean and Coast Management, 91*, 41-49.

Anderson, K.L., Whitlock, J.E., Harwood, V.J. (2005). Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. *Applied and Environmental Microbiology, 71*(6), 3041-3048.

Angulo, F.J., Tippen, S., Sharp, D.J., Payne, B.J., Collier, C., Hill, J.E., Barrett, T.J., Clark, R.M., Geldreich, E.E., Donnell, H., Swerdlow, D.L. (1997). A community waterborne outbreak of salmonellosis and the effectiveness of a boil water order. *American Journal of Public Health, 87*(4), 580-584.

Armbruster, D.A., Pry, T. (2008). Limit of blank, limit of detection and limit of quantification. *Clinical Biochemist Reviews*, *1*, 49-52.

Badgley, B.D., Nayak, B.S., Harwood, V.J. (2010). The importance of sediment and submerged aquatic vegetation as potential habitats for persistent strains of *enterococci* in a subtropical watershed. *Water Research, 44*, 5857-5866.

Bagcigil, A.F., Ikiz, S., Dokuzeylu, B., Basaran, B., Or, E., Ozgur, N.Y. (2007). Fecal shedding of *Salmonella* spp. in dogs. *Journal of Veterinary Medical Science*, *69*, 775-777.

Baker-Austin, C., Rangdale, R., Lowther, J., Lees, D.N. (2010). Application of mitochondrial DNA analysis for microbial source tracking purposes in shellfish harvesting waters. *Water Science and Technology*, *61*(1), 1-7.

Baldursson, S., Karanis, P. (2011). Waterborne transmission of protozoan parasites: review of worldwide outbreaks - An update 2004-2010. *Water Research, 45*, 6603-6614.

Ballesté, E., Bonjoch, X., Belanche, L.A., Blanch, A.R. (2010). Molecular indicators used in the development of predictive models for microbial source tracking. *Applied and Environmental Microbiology*, *76*(6), 1789-1795.

Barcina, I., Arana, I., Iriberri, J., Egea, L. (1986). Factors affecting the survival of *E. coli* in a river. *Hydrobiologia, 141*, 249-253.

Bernhard, A.E., Field, K.G. (2000a). Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16s ribosomal DNA genetic markers from fecal Anaerobes. *Applied and Environmental Microbiology, 66*(4), 1587-1594.

Bernhard, A.E., Field, K.G. (2000b). A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides-Prevotella* genes encoding 16S rRNA. *Applied and Environmental Microbiology*, *66*(10), 4571-4574.

Beutin, L. (1999). *Escherichia coli* as a pathogen in dogs and cats. *Veterinary Research*, *30*, 258-298.

Beversdorf, L.J., Bornstein-Forst, S.M., McLellan, S.L. (2006). The potential for beach sand to serve as a reservoir for *Escherichia coli* and the physical influences on cell die-off. *Journal of Applied Microbiology*, *102*(5), 1372-1381.

Bielaszewska, M., Janda, J., Blahova, K., Minarikova, H., Jikova, E., Karmali, M.A., Laubova, J., Sikulova, J., Preston, M.A., Khakhria, R., Karch, H., Klazarova, H., Nyc, O. (1997). Human *Escherichia coli* O157:H7 infection associated with the consumption of unpasteurized goat's milk. *Epidemiology & Infection, 119,* 299-305.

Black, R.E., Levine, M.M., Clements, L., Hughes, T.P., Blaser, M.J. (1988). Experimental *Campylobacter jejuni* infections in humans. *The Journal of Infectious Diseases, 157*, 472-479.

Blaser, M.J., Newman, L.S. (1982). A review of human Salmonellosis: I. Infective dose. *Reviews of Infectious Diseases*, *4*(6), 1096-1106.

Boehm, A.B. (2003). Tiered approach for identification of a human fecal pollution source at a recreational beach: Case Study at Avalon Bay, Catalina Island, California. *Environmental Science and Technology, 37*(4), 673-680.

Boehm, A.B., Griffith, J., McGee, Edge, T.A., Solo-Gabriele, H.M., Whitman, R., Cao, Y., Getrich, Jay, J.A., Ferguson, D., Goodwin, K.D., Lee, C.M., Madison, M., Weisberg, S.B. (2009). Faecal indicator bacteria enumeration in beach sand: a comparison study of extraction methods in medium to coarse sands. *Journal of Applied Microbiology*, *107*, 1740-1750.

Boehm, A.B., Van De Werfhorst, L.C., Griffith, J.F., Holden, P.A., Jay, J.A., Shanks, O.C., Wang, D., Weisberg, S.B. (2013). Performance of forty-one microbial source tracking methods: A twenty-seven lab evaluation study. *Water Research, 47*, 6812-6828.

Boehm, A.B., Soller, J.A., Shanks, O.C. (2015). Human-associated fecal quantitative polymerase chain reaction measurements and simulated risk of gastrointestinal illness in recreational waters contaminated with raw sewage. *Environmental Science and Technology Letters*, *2*(10), 270-275.

Bogenhagen, D., Clayton, D.A. (1974). The number of mitochondrial deoxyribonucleic acid genomes in mouse L and human HeLa cells. *The Journal of Biological Chemistry, 249*(24), 7991-7995.

Bolton, F.J., Surman, S.B., Martin, K., Wareing, D.R., Humphrey, T.J. (1999). Presence of *Campylobacter* and *Salmonella* in sand from bathing beaches. *Epidemiology and Infection*, *122*(1), 7-13.

Bonjoch, X., Ballesté, E., Blanch, A.R. (2004). Multiplex PCR with 16s rRNA gene-target primers of *Bifidobacterium* spp. to identify sources of fecal contamination. *Applied and Environmental Microbiology,* 3171-3175.

Bonilla, T.D., Nowosielski, K., Cuvelier, M., Hartz, A., Green, M., Esiobu, N., McCorquodale, D.S., Fleisher, J.M., Rogerson, A. (2007). Prevalence and distribution of fecal indicator organisms in South Florida beach sand and preliminary assessment of health effects associated with beach sand exposure. *Marine Pollution Bulletin, 54*, 1472-1482.

Bortolussi, R. (2008). Listeriosis: a primer. *Canadian Medical Association Journal*, *179*(8), 795-797.

Bouzid, M., Halai, K., Jeffreys, D., Hunter, P.R. (2015). The prevalence of *Giardia* infection in dogs and cats, a systematic review and meta-analysis of prevalence studies from stool samples. *Veterinary Parasitology, 207*, 181-202.

Brauwere, A., Ouattara, N.K., Servais, P. (2014). Modeling fecal indicator bacteria concentrations in natural surface waters: a review. *Critical Reviews in Environmental Science and Technology*, *44*, 2380-2453.

Budzińska, K., Wroński, G., Szejniuk, B. (2012). Survival time of bacteria *Listeria monocytogenes* in water environment and sewage. *Polish Journal of Environmental Studies, 21*(1), 31-37.

Bush, K.F., Fossani, C.L., Li, S., Mukherjee, B., Gronlund, C.J., O'Neill, M.S. (2014). Extreme precipitation and beach closures in the Great Lakes region: evaluating risk among the elderly. *International Journal of Environmental Research and Public Health*, *11*(2), 2014-2032.

Byappanahalli, M., Fujioka, R. (2004). Indigenous soil bacteria and low moisture may limit but allow faecal bacteria to multiply and become a minor population in tropical soils. *Water Science and Technology, 50*(1), 27-32.

Byappanahalli, M.N., Roll, B.M., Fujioka, R.S. (2012). Evidence for occurrence, persistence, and growth potential of *Escherichia coli* and *Enterococci* in Hawaii's soil environment. *Microbes and Environments, 27*(2), 164-170.

Cabral, J.P.S. (2010). Water microbiology. Bacterial pathogens and water. *International Journal of Environmental Research and Public Health*, *7*(10), 3657-3703.

Caldwell, J.M., Levine, J.F. (2009). Domestic wastewater influent profiling using mitochondrial real-time PCR for source tracking animal contamination. *Journal of Microbiological Methods, 77,* 17-22.

Casarez, E.A., Pillai, S.D., Giovanni, G.D. (2007). Genotype diversity of *Escherichia coli* isolates in natural waters determine by PFGE and ERIC-PCR. *Water Research*, *41*(16), 3643-3648.

Centre for Disease Control and Prevention, 2014a. *Morbidity and mortality weekly report: recreational water-associated disease outbreaks – United States, 2009-2010*. [online] Available at: < http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6301a2.htm> [Accessed 3 January 2016].

Centre for Disease Control and Prevention. 2014b. *Salmonella*: *What is Salmonellosis*?. [online]. Available at: <http://www.cdc.gov/salmonella/general/> [Accessed 30 December 2014].

Chen, H.J., Chang, H. (2014). Response of discharge, TSS, and *E. coli* to rainfall events in urban, suburban, and rural watersheds. *Environmental Science: Processes & Impacts*, *16*(10), 2313-2324.

Cheng, C., Lin, W., Thien, K., Phan, L., Tran, N.N., Farmer, D. (2008). Rapid detection of Salmonella in foods using Real-Time PCR. *Journal of Food Protection, 71*(12), 2436-2441.

Cherry, W.B., Hanks, J.B., Thomason, B.M., Murlin, A.M., Biddle, J.W., Croom, J.M. (1972). *Salmonellae* as an index of pollution of surface waters. *Applied Microbiology, 24*(3), 334-340.

Ciebin, B.W., Brodsky, M.H., Eddington, R. Horsnell, G., Choney, A., Palmateer, G., Ley, A., Joshi, R., Shears, G. (1995). Comparative evaluation of modified m-FC and m-TEC media for membrane filter enumeration of *Escherichia coli* in water.

Clark, C.G., Price, L., Ahmed, R., Woodward, D.L., Melito, P.L., Rodgers, F.G., Jamieson, F., Ciebin, B., Li, A., Ellis, A. (2003). Characterization of waterborne outbreak-associated *Campylobacter jejuni,* Walkerton, Ontario. *Emerging Infectious Disease Journal, 9*(10), 1232-1241.

Converse, R.R., Kinzelman, J.L., Sams, E.A… Wade, T.J. (2012). Dramatic improvements in beach water quality following gull removal. *Environmental Science & Technology, 46*, 10206-10213.

Cooley, M.B., Quinones, B., Oryang, D., Mandrell, R.E., Gorski, L. (2014). Prevalence of shiga toxin producing *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes* at public access watershed sites in a California central coast agricultural regions. *Frontiers in Cellular and Infection Microbiology*, *4*(30), 1-13.

Coupe, S., Delabre, K., Pouillot, R., Houdart, S., Santillana-hayat, M., Derouin, F. (2006). Detection of *Cryptosporidium*, *Giardia* and *Enterocytozoon bieneusi* in surface water, indicating recreational areas: a one-year perspective study. *FEMS Immunology and Medical Microbiology, 47*, 351-359.

Curtis, T.P., Mara, D.D., Silva, S.A. (1992). Influence of pH, oxygen, and humic substances on ability of sunlight to damage fecal coliforms in waster stabilization pond water. *Applied and Environmental Microbiology, 58*(4), 1335-1343.

Daly, E.R., Roy, S.J., Blaney, D.D., Manning, J.S., Hill, V.R., Xiao, L., Stull, J.W. (2010). Outbreak of Giardiasis associated with a community drinking-water source. *Epidemiology and infection, 138*, 491-500.

D'Aoust, J.Y. (1985). Infective dose of *Salmonella typhimurium* in cheddar cheese. *American Journal of Epidemiology, 122*, 717-720.

Desai, A.M., Rifai, H. (2013). *Escherichia coli* concentrations in urban watersheds exhibit diurnal sag: implications for water-quality monitoring and assessment. *Journal of the American Water Resources Association, 49*(4), 766-779.

Devane, M.L., Nicol, C., Ball, A., Klena, J.D., Scholes, P., Hudson, J.A., Baker, M.G., Gilpin, B.J., Garrett, N., Savill, M.G. (2005). The occurrence of *Campylobacter* subtypes in environmental reservoirs and potential transmission routes. *Journal of Applied Microbiology, 96*, 980-990.

Dick, L.K., Bernhard, A.E., Brodeur, T.J., Domingo, J.W.S., Simpson, J.M., Walters, S.P., Field, K.G. (2005). Host distributions of uncultivated fecal *Bacteroidales* bacteria reveal genetic markers for fecal source identification. *Applied and Environmental Microbiology, 71*(6), 3184-3191.

Dick, L.K., Stelzer, E.A., Bertke, E.E., Fong, D.L., Stoeckel, D.M. (2010). Relative decay of *Bacteroidales* microbial source tracking markers and cultivated *Escherichia coli* in freshwater microcosms. *Applied and Environmental Microbiology, 76*(10), 3255-3262.

Dombek, P.E., Johnson, L.K., Zimmerley, S.T., Sadowsky, M.J. (2000). Use of repetitive DNA sequences to differentiate *Escherichia coli* isolates from human and animal sources. *Applied and Environmental Microbiology, 66*(6), 2572-2577.

Dorevitch, S., Dworkin, M.S., DeFlorio, S.A., Janda, W.M., Wuellner, J., Hershow, R.C. (2012). Enteric pathogens in stool samples of Chicago-area water recreators with new-onset gastrointestinal symptoms. *Water Research*, *46*, 4961-4972.

Dwight, R.H., Baker, D.B., Semenza, J.C., Olson, B.H. (2004). Health effects associated with recreational coastal water use: urban versus rural California. *American Journal of Public Health,* 94(4), 565-567.

Edge, T. A., Hill, S. (2007). Multiple lines of evidence to identify the sources of fecal pollution at a freshwater beach in Hamilton Harbour, Lake Ontario. *Water Research, 41,* 3585-35-94.

Edge, T.A., Khan, I.U.H., Bouchard, R., Guo, J., Hill, S., Locas, A., Moore, L., Neumann, N., Nowak, E., Payment, P., Yang, R., Yerubandi, R., Watson, S. (2013). Occurrence of waterborne pathogens and *Escherichia coli* at offshore drinking water intakes in Lake Ontario. *Applied and Environmental Microbiology, 79*(19), 5799-5813.

Environmental Protection Agency, (1999). *Importance of turbidity*. [pdf]. Available at: <http://www.epa.gov/ogwdw/mdbp/pdf/turbidity/chap_07.pdf> [Accessed 18 January 2015].

Environmental Protection Agency (2012a). *Water: monitoring & assessment – turbidity*. [online] Available at: <http://water.epa.gov/type/rsl/monitoring/vms55.cfm> [Accessed 25 November 2015].

Environmental Protection Agency (2012b). *Water: monitoring & assessment – total solids*. [online] Available at: <http://water.epa.gov/type/rsl/monitoring/vms58.cfm> [Accessed 25 November 2015].

Ehsan, A., Casaert, S., Levecke, B., Rooy, L., Pelicaen, J., Smis, A., Backer, J., Vervaeke, B., Smedt, S., Schoonbaert, F., Lammens, S., Warmoes, T., Geurden, T., Claerebout, E. (2015). *Cryptosporidium* and *Giardia* in recreational water in Belgium. *Journal of Water and Health, 13*, 870-878.

Ezzat, R.F., Hussein, H.A., Baban, T.S., Rashid, A.T., Abdullah, K,M. (2010). Typhoid ulcer causing life-threatining bleeding from Dieilafoy's lesion of the ileum in a seven-year-old child: a case study. *Journal of Medical Case Reports, 4*, 171.

Fallacara, D.M., Monahan, C.M., Morishita, T.Y., Bremer, C.A., Wack, R.F. (2004). Survey of parasites and bacterial pathogens from free-living waterfowl in zoological settings. *Avian Sources, 48*(4), 759-767.

Ferens, W.A., Hovde, C.J. (2011). *Escherichia coli* O157:H7: animal reservoir and sources of human infection. *Foodborne Pathogens and Disease*, *8*(4), 465-487.

Ferguson, A.S., Layton, A.C., Mailloux, B.J… Geen, A.V. (2012). Comparison of fecal indicators with pathogenic bacteria and rotavirus in groundwater. *Science of the Total Environment, 432*, 324-322.

Field, K.G., Samadpour, M. (2007). Fecal source tracking, the indicator paradigm, and managing water quality. *Water Research, 41*, 3517-3538.

Finley, R., Ribble, C., Aramini, J., Vandermeer, M., Popa, M., Litman, M., Reid-Smith, R. (2007). The risk of *Salmonellae* shedding by dogs fed *Salmonella* contaminated commercial raw food diets. *The Canadian Veterinary Journal*, *48*(1), 69-75.

Flint, K.P. (1987). The long-term survival of *Escherichia coli* in river water. *Journal of Applied Bacteriology*, *63*, 261-270.

Fondriest Environmental. 2013. *Dissolved Oxygen: Fundamentals of environmental measurements*. [online] Available at: <http://www.fondriest.com/environmental-measurements/parameters/water-quality/dissolved-oxygen/ > [Accessed 30 January 2016].

Fondriest Environmental. 2014. *Turbidity, total suspended solids and water quality*. [online] Available at: <http://www.fondriest.com/environmental-measurements/parameters/water-quality/turbidity-total-suspended-solids-water-clarity/> [Accessed 30 January 2016].

Francy, D.S., Stelzer, E.A., Duris, J.W., Brady, A.M.G., Harrison, J.H., Johnson, H.E., Ware, M.W. (2013). Predictive models for *Escherichia coli* concentrations at inland lake beaches and relationship of model variables to pathogen detection. *Applied and Environmental Microbiology*, *79*(5), 1676-1688.

Fremaux, B., Gritzfeld, T., Boa, T., Yost, C.K. (2009). Evaluation of host-specific *Bacteroidales* 15S rRNA gene markers as a complementary tool for detecting fecal pollution in a prairie watershed. *Water Research, 43*, 4838-4849.

Galván, A.L., Magnet, A., Izquierdo, F., Vadillo, C.F., Peralta, R.H., Angulo, S., Fenoy, S., Aguila, C. (2014). A year-long study of *Cryptosporidium* species and subtypes in recreational, drinking and wastewater from the central area of Spain. *Science of the Total Environment, 469*, 368375.

Gardner, T.B., Hill, D.R. (2001). Treatment of Giardiasis. *Clinical Microbiology Reviews*, *14*(1), 114-128.

Gauthier, F., Archibald, F. (2001). 'The ecology of "faecal indicator" bacteria commonly found in pulp and paper mill water systems. Water Research, *35*, 2207–2218.

Ge, Z., Whitman, R.L., Nevers, M.B., Phanikumar, M.S. (2012). Water-induced mass transport affects daily *Escherichia coli* fluctuations in nearshore water. *Environmental Science and Technology, 46*, 2204-2211.

Gillespie, I.A., O'Brien, S.J., Frost, J.A., Adak, G.K., Horby, P., Swan A.V., Painter, M.J., Neal, K.R. (2002). A case-case comparison of *Campylobacter coli* and *Campylobacter jejuni* infection: A tool for generating hypotheses. *Emerging Infectious Diseases, 8*(9), 937-942.

Göransson, G., Larson, M., Bendz, D. (2013). Variation in turbidity with precipitation and flow in a regulated river system. *Hydrology and Earth System Sciences*, *17*, 2529-2542.

Goto, D.K., Yan, T. (2011). Genotypic diversity of *Escherichia coli* in the water and soil of tropical watersheds in Hawaii. *Applied and Environmental Microbiology, 77*(12), 3988-3997.

Gourmelon, M., Caprais, M.P., Segura, R., Mennec, C., Lozach, S., Piriou, J.Y., Rince, A. (2007). Evaluation of two library-independent microbial source tracking methods to identify sources of fecal contamination in French estuaries. *Applied and Environmental Microbiology, 73*(15), 4857-4866.

Graczyk, T.K., Fayer, R., Trout, J.T., Lewis, E.J., Farley, C.A., Sulaiman, I., Lal, A.A. (1998). *Giardia sp.* cysts and infectious *Cryptosporidium parvum* oocysts in the feces of migratory Canada Geese (*Branta canadensis*). *Applied and Environmental Microbiology, 64*(7), 2736-2738.

Grant, M.A. (1997). A new membrane filtration medium for simultaneous detection and enumeration of *Escherichia coli* and total coliforms. *Applied and Environmental Microbiology, 63*(9), 3526-3530.

Graziani, C., Mughini-Gras, L., Owczarek, S., Dionisi, A.M., Luzzi, I., Busani, L. (2013). Distribution of *Salmonella enterica* isolates from human cases in Italy, 1980 to 2011. *Eurosurveillance*, *18*(27), 19-27.

Green, H.C., Dick, L.K., Samadpour, M., Field, K.G. (2012). Genetic markers for rapid PCR-based identification of gull, Canada goose, duck, and chicken fecal contamination in water. *Applied and Environmental Microbiology, 78*(2), 503-510.

Green, H.C., Haugland, R.A., Varma, M… Shanks, O.C. (2014). Improved HF183 quantitative real-time PCR assay for characterization of human fecal pollution in ambient surface water samples. *Applied and Environmental Microbiology*, *80*(10), 3086-3094.

Guber, A.K., Fry, J., Ives, R.L., Rose, J.B. (2014). *Escherichia coli* survival in, and release from, White-Tailed Deer feces. *Applied and Environmental Microbiology*, *81*(3), 1168-1176.

Guy, R.A., Payment, P., Krull, U.J., Horgen, P. A. (2003) Real-time PCR for quantification of *Giardia* and *Cryptosporidium* in environmental water samples and sewage. *Applied and Environmental Microbiology*, *69*(9), 5178-5185.

Haack, S.K., Fogarty, L.S., Wright, C. (2003). *Escherichia coli* and *Enterococci* at beaches in the Grand Traverse Bay, Lake Michigan: sources, characteristics, and environmental pathways. *Environmental Science and Technology, 37*, 3275-3282.

Haley, B.J., Cole, D.J., Lipp, E.K. (2009). Distribution, diversity, and seasonality of waterborne salmonella in a rural watershed. *Applied and Environmental Microbiology, 75*(5), 1248-1455.

Hamilton, M.J., Yan, T., Sadowsky, M.J. (2006). Development of Goose- and Duck-specific DNA markers to determine sources of *Escherichia coli* in waterways. *Applied and Environmental Microbiology,* 4012-4019.

Hancock, D.D., Besser, T.E., Kinsel, M.L., Tarr, P.I, Rice, D.H., Paros, M.G. (1994). The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington State. *Epidemiology & Infection, 113,* 199-207.

Hansen, D.L., Ishii, S., Sadowsky, M.J., Hicks, R.E. (2009). *Escherichia coli* populations in Great Lakes waterfowl exhibit spatial stability and temporal shifting. *Applied and Environmental Microbiology, 75*, 1546-1551.

Hara-Kudo, Y., Takatori, K. (2011). Contamination level and ingestion dose of foodborne pathogens associated with infections. *Epidemiology & Infection, 139*, 1505-1510.

Hartel, P.G., Summer, J.D., Hill, J.L., Collins, J.V., Entry, J.A., Segars, W.I. (2002). Geographic variability of *Escherichia coli* ribotypes from animals in Idaho and Georgia. *Journal of Environmental Quality, 31*(4), 1273-1278.

Harwood, V.J., Whitlock, J., Withington, V. (2000). Classification of antibiotic resistance patterns of indicator bacteria by discriminant analysis: use in predicting the source of fecal contamination in subtropical waters. *Applied and Environmental Microbiology, 66*(9), 3698-3704.

Harwood, V.J., Levine, A.D., Scott, T.M., Chivukula V., Lukasik, J., Farrah, S.R., Rose, J.B. (2005). Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. *Applied and Environmental Microbiology, 71*(6), 3163-3170.

Haugland, R.A., Siefring, S.C., Wymer, L.J., Brenner, K.P., Dufour, A.P. (2004) Comparison of *Enterococcus* measurements in freshwater at two recreational beaches by quantitative polymerase chain reaction and membrane filter culture analysis. *Water Research, 39*, 559-568.

Haugland, R.A., Varma, M., Sivaganesan, M., Kelty, C., Preed, L., Shanks, O.C. (2010). Evaluation of genetic markers from the 16S rRNA gene V2 region for use in quantitative detection of selected *Bacteroidales* species and human fecal waste by qPCR. *Systematic and Applied Microbiology, 33,* 348-357.

Health Canada, 2012a. *Guidelines for Canadian recreational water quality.* [online] Available at: <http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/guide_water-2012-guide_eau/index-eng.php> [Accessed 20 October 2015].

Health Canada, 2012b. *Environmental and Workplace Health: Recreational Water*. [online]. Available at: <http://www.hc-sc.gc.ca/ewh-semt/water-eau/recreat/index-eng.php> [Accessed 17 March 2016].

Heany, C.D., Sams, E., Wing, S., Marshall, S., Brenner, K., Dufour, A.P., Wade, T.J. (2009). Contact with beach sand among beachgoers and risk of illness. *Journal of Epidemiology, 170,* 164-172.

Helmi, K., Skraber, S., Burnet, J., Leblanc, L., Hoffman, L., Cauchie, H. (2011). Two-year monitoring of *Cryptosporidium parvum* and *Giardia lamblia* occurrence in a recreational and drinking reservoir using standard microscopic and molecular biology techniques. *Environmental Monitoring and Assessment*, *179*, 163-175.

Hipsey, M.R., Antenucci, J.P., Brookes, J.D. (2008). A generic, process-based model of microbial pollution in aquatic systems. *Water Resources Research*, *44*, 1-26.

Hof, H. (2003). History and epidemiology of Listeriosis. *FEMS Immunology & Medical Microbiology*, *35*(3), 199-202.

Hörman, A., Rimhanen-Finne, R., Maunula, L., Bonsdorff, C., Torvela, N., Heikinheimo, A., Hänninen, M. (2004). *Campylobacter* spp., *Giardia* spp., *Cryptosporidium* spp., Noroviruses, and indicator organisms in surface water in Southwestern Finland, 2000-2001. *Applied and Environmental Microbiology, 70*(1), 87-95.

Horrocks, S.M., Anderson, R.C., Nisbet, D.J., Ricke, S.C. (2009). Incidence and ecology of *Campyobacter jejuni* and *coli* in animals. *Anarobe, 15*, 18-25.

Hrudey, S.E., Huck, P.M., Payment, P., Gillham, R.W., Hrudey, E.J. (2002). Walkerton: lessons learned in comparison with waterborne outbreaks in the development world. *Journal of Environmental Engineering & Science, 1*(6), 397-407.

Huey, G., Meyer, M.L. (2010). Turbidity as an indicator of water quality in diverse watersheds of the Upper Pecos River Basin. *Water, 2*, 273-284.

Hunter, P.R., Thompson, R.C.A. (2005). The zoonotic transmission of *Giardia* and *Cryptosporidium*. *International Journal for Parasitology, 35*, 1181-1190.

Ibekwe, A.M., Watt, P.M., Grieve, C.M., Sharma, V.K., Lyons, S.R. (2002). Multiplex fluorogenic real-time PCR for detection and quantification of *Escherichia coli* O157:H7 in dairy wastewater wetlands. *Applied and Environmental Microbiology, 68*(10), 4853-4862.

Ishii, S., Hansen, D.L., Hicks, R.E., Sadowsky, M.J. (2007). Beach sand and sediment are temporal sinks and sources of *Escherichia coli* in Lake Superior. *Environmental Science and Technology, 41*, 2203-2209.

Jackson, K.A., Iwamoto, M.I., Swerdlow, D. (2010). Pregnancy-associated Listeroisis. *Epidemiology and Infection*, *138*(10), 1503-1509.

Jeng, H.A. (2005). Impact of urban stormwater runoff on estuarine environmental quality. *Estuarine, Coastal, and Shelf Science*, *63*(4), 513-526.

Jenson, P., Aalbaek, B., Aslam, R., Dalsgaard, A. (2001). Specificity for field enumeration of *Escherichia coli* in tropical surface waters. *Journal of Microbiological Methods, 45,* 135-141.

Jiménez, L., Muñiz, I., Toranzos, G.A., Hazen, T.C. (1989). Survival and activity of *Salmonella typhimurium* and *Escherichia coli* in tropical freshwater. *Journal of Applied Bacteriology*, *67*(1), 61-69.

Jokinen, C.J. Schreier, H., Mauro, W., Taboada, E., Issac-Renton, J.L., Topp, E., Edge, T. Thomas, J.E., Gannon, V.P.J. (2010). The occurrence and sources of *Campylobacter* spp.., *Salmonella enterica*, and *Escherichia coli* O157:H7 in Salmon River, British Columbia, Canada. *Journal of Water and Health*, *8*, 374-386.

Jorge, J.F., Costa, A.B., Rodrigues, J.L., Girão, E.S., Luiz, R.S., Sousa, A.Q., Moore, S.R., Menezes, S.R., Leitão, T.M. (2014). *Salmonella typhi* liver abscess overlying a metastatic melanoma. *The American Journal of Tropical Medicine and hygiene, 90*(4), 716-718.

Jothikumar, N., da Silva, A.J., Moura, I., Qvarnstrom, Y., Hill, V.R. (2008). Detection and differentiation of *Cryptosporidium hominis* and *Cryptosporidium parvum* by dual TaqMan assays. *Journal of Medical Microbiology, 57*, 1099-1105.

Kaakoush, N.O., Castaño-Rodriguez, N., Mitchell, H.M., Man, S.M. (2015). Global epidemiology of *Campylobacter* infection. *Clinical Microbiology Reviews*, *28*(3), 687-720.

Kamra, A., Kessie, G., Chen, J… Nair, P.P. (2005). Exfoliated colonic epithelial cells: surrogate targets for evaluation of bioactive food components in cancer prevention. *The Journal of Nutrition, 135*(11), 2719-2722.

Karch, H., Tarr, P.I., Bielaszewska, M. (2005). Enterohaemorrhagic *Escherichia coli* in human medicine. *International Journal of Medical Microbiology, 295*(6), 405-418.

Kemp, R., Leatherbarrow, A.J.H., Williams, N.J., Hart, C.A., H.E., Clough, Turner, J., Wright, E.J., French, N.P. (2005). Prevalence and genetic diversity of *Campylobacter* spp. in environmental water samples from a 100-sqaure-kilometer predominantly dairy farming area. *Applied and Environmental Microbiology, 71*(4), 1876-1882.

Khan, I., Edge, T.A. (2007). Development of a novel triplex PCR assay for the detection and differentiation of thermophilic species of *Campylobacter* using 16S-23S rDNA internal transcribe spacer (ITS) region. *Journal of Applied Microbiology, 103*, 2561-2569.

Khan, I., Edge, T.A. (2013). Investigation of the prevalence of thermophilic *Campylobacter* species at Lake Simcoe recreational beaches. *Inland Waters, 3*(1), 93-104.

Kildare, B.J., Leutenegger, C.M., McSwain, B.S., Bambic, D.G., Rajal, V.B., Wuertz, S. (2007). 16s rRNA-based assays for quantitative detection of universal, human-, cow-, and dog-specific fecal *Bacteroidales*: a Bayesian approach. *Water Research, 41,* 3701-3715.

King, G., Zeng, L. (2001). Logistic regression in rare events data. *Political Analysis*, *9*, 137-163.

Kinzelman, J., McLellan, S.L., Amick, A., Preedit, J., Scopel, C.O., Olapade, O., Sngh, A., Sedmak, G. (2008). Identification of human enteric pathogens in gull feces at Southwestern Lake Michigan bathing beaches. *Canadian Journal of Microbiology*, *54*(12), 1006-1015.

Kirs, M., Harwood, V.J., Fidler, A.E., Gillespie, P.A., Fyfe, W.R., Blackwood, A.D., Cornelisen, C.D. (2011). Source tracking faecal contamination in an urbanised and rural waterway in the Nelson-Tasman region, New Zealand. *New Zealand Journal of Marine and Freshwater Research, 45*(1), 43-58.

Kleinheinz, G.T., McDermott, C.M., Hughes, S., Brown, A. (2009). Effects of rainfall on *E. coli* concentration at Door County, Wisconsin Beaches. *International Journal of Microbiology, 2009*.

Korajkic, A., Brownell, M.J., Harwood, V.J. (2010). Investigation of human sewage pollution and pathogen analysis at Florida Gulf coast Beaches. *Journal of Applied Microbiology*, *100*, 174-183.

Korhonen, L.K., Martikaien, P.J. (1991). Survival of *Escherichia coli* and *Campylobacter jejuni* in untreated and filtered lake water. *Journal of Applied Microbiology, 71*, 379-382.

Kortbaoui, R., Locas, A., Imbeau, M., Payment, P., Villemur, R. (2009). Universal mitochondrial PCR combined with species-specific dot-blot assay as a source-tracking method of human, bovine, chicken, ovine, and procine in fecal-contaminated source-water. *Water Research*, *43*(7), 2002-2010.

Kudva, I.T., Hatfield, P.G., Hovde, C.J. (1996). *Escherichia coli* O157:H7 in microbial flora of sheep. *Journal of Clinical Microbiology, 34,* 431-433.

Kuhn, R.C., Rock, C.M., Oshima, K.H. (2002). Occurrence of *Cryptosporidium* and *Giardia* in wild ducks along the Rio Grande River Valley in Southern New Mexico. *Applied and Environmental Microbiology*, *68*(1), 161-165.

Kühn, I., Allestam, G., Engdahl, M., Stenström, T.A. (1998). Biochemical fingerprinting of coliform bacterial populations – comparisons between polluted river water and factory effluents. *Environmental and Applied Microbiology, 57*(11), 3171.

Kumar, R.R., Park, B.J., Cho, J.Y. (2013). Application and environmental risks of livestock manure. *Journal of the Korean Society for Applied Biological Chemistry*, *56*, 497-503.

Kuusi, M., Nuorti, J.P., Hanninen, M., Koskela, M., Jussila, V., Kela, E., Miettinen, I., Ruutum P. (2005). A large outbreak of Campylobacteriosis associated with a municipal water supply in Finland. *Epidemiology and Infection, 133*, 593-601.

Layton, A., McKay, L., Williams, D., Garrett, V., Gentry, R., Sayler, G. (2006). Development of *Bacteroides* 16S rRNA Gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water. *Applied and Envrionmental Microbiology, 72*(6), 4214-4224.

Layton, B.A., Cao, Y., Ebentier, D.L.,… Griffith, J.F. (2013). Performance of human fecal anaerobe-associated PCR-based assays in a multi-laboratory method evaluation study. *Water Research, 47*, 6897-6908.

Lauber, C.L., Hamady, M., Knight, R., Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology, 75*(15), 5111-5120.

Lawler, D.M., Petts, G.E., Foster, I.D.L., Harper, S. (2006). Turbidity dynamics during spring storm events in an urban headwater river systems: The Upper Tame, West Midlands, UK. *Science of the Total Environment*, *360*, 109-126.

Lee, D.Y., Weir, S.C., Lee, H., Trevors, J.T. (2010). Quantitative identification of fecal water pollution sources by TaqMan real-time PCR assays using *Bacteroidales* 16s rRNA genetic markers. *Applied Microbiology and Biotechnology, 88*, 1373-1383.

Lee, C., Agidl, S., Marion, J.W., Lee, J. (2012). *Arcobacter* in Lake Erie beach waters: an emerging gastrointestinal pathogen linked with human-associated fecal contamination. *Applied and Environmental Microbiology, 78*(16), 5511-5519.]

Levantesi, C., Bonadonna, L., Briancesco, R., Grohmann, E., Toze, S., Tandoi, V. (2012). *Salmonella* in surface and drinking water: occurrence and water-mediated transmission. *Food Research International, 45*, 587-602.

Lévesque, B., Brousseau, P., Simard, P., Dewailly, E., Meisels, M., Ramsay, D., Joly, J. (1993). Impact of the Ring-Billed Gull (*Larus delawarensis*) on the microbiological quality of recreational water. *Applied and Environmental Microbiology, 59*(4), 1228-1230.

Linke, K., Ruckerl, I., Brugger, K., Karpiskova, R., Walland, J., Muri-Klinger, S., Tichy, A., Wagner, M., Stessl, B. (2014). Reservoirs of *Listeria* in three environmental ecosystems. *Applied and Environmental Microbiology*, *80*(18),5583-5592.

Lopez-Torres, A.J., Hazan, T.C., Toranzos, G.A. (1987). Distribution and in situ survival and activity of *Klebsiella pneumonia* and *Escherichia coli* in a tropical rain forest watershed. *Current Microbiology, 15*, 213-218.

Lowden, P., Wallis, C., Gee, N., Hilton, A. (2015). Investigation the prevalence of *Salmonella* in dogs within the midlands region of the United Kingdom. *BMC Veterinary Research*, *11*(239).

Lund, M., Nordentoft, S., Pedersen, K., Madsen, M. (2004). Detection of *Campylobacter* spp. in chicken fecal samples by real-time PCR. *Journal of Clinical Microbiology*, *42*, 5125-5132.

Lyautey, E., Lapen, D.R., Wilkes, G., McCleary, K., Pagotto, F., Tyler, K., Hartmann, A., Piveteau, P., Rieu, A., Robertson, W.J., Medeiros, D.T., Edge, T.A., Gannon, V., Topp, E. (2007). Distribution and characteristics of *Listeria monocytogenes* isolates from surface waters of South Nation River Watershed, Ontario, Canada. *Applied and Environmental Microbiology, 73*(17), 5401-5410.

MacKenzie, W.R., Hoxie, N.J., Proctor, M.E… Davis, J.P. (1994). A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *The New England Journal of Medicine, 331,* 161-167.

Marion, J.W., Lee, J., Lemeshow, S., Buckley, T.J. (2010). Association of gastrointestinal illness and recreational water exposure at an inland U.S. beach. *Water Research*, *44*, 4796-4804.

Marion, J.W., Burrowes, V., Lee, C.S., Lee, J. (2015). Changes in microbial water quality associated with an extreme recreational water event in Ohio, United States. *Water Quality*, *Exposure and Health*, *7*, 491-501.

Matsuki, T., Watanabe, K., Fujimoto, J., Takada, T., Tanaka, R. (2004). Use of 16s rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in human feces. *Applied and Environmental Microbiology, 70*(12), 7720-7728.

McEgan, R., Mootian, G., Goodridge, L.D., Schaffner, D.W., Danyluk, M.D. (2013). Predicting *Salmonella* populations from biological, chemical, and physical indicators in Florida surface waters. *Applied and Environmental Microbiology, 79*(13), 4094-4105.

McLain, J.E.T., Williams, C.F. (2008). Seasonal variation in accurate identification of *Escherichia coli* within a constructed wetland receiving tertiary-treated municipal effluent. *Water Research, 42*(15), 4041-4048.

McQuaig, S., Griffith, J., Harwood, V.J. (2012). Association of fecal indicator bacteria with human viruses and microbial source tracking markers at coastal beaches impacted by nonpoint source pollution. *Applied and Environmental Microbiology, 78*(18).

Meerburg, B.G., Koene, M.G.J., Kleijn, D. (2011). *Escherichia coli* concentrations in feces of geese, coots, and gulls residing on recreational water in the Netherlands. *Vector Borne Zoonotic Diseases, 6*, 601-603.

Melby, K., Gondrosen, B., Gregusson, S., Ribe, H., Dahl, O.P. (1991). Waterborne *Campylobacteriosis* in northern Norway. *Internation Journal of Food Microbiology, 12*(2), 151-156.

Moriñigo, M.A., Córnax, R., Muñoz, M.A., Romero, P. (1990). Relationships between *Salmonella* spp and indicator microorganisms in polluted natural waters. *Water Research, 1*, 117-120.

Morris, R.D., Naumova, E.N., Levin, R., Munasinghe, R.L. (1996). Temporal variation in drinking water turbidity and diagnosed gastroenteritis in Milwaukee. *American Journal of Public Health, 86*(2), 237-239.

Moss, J.A., Gordy, J., Synder, R.A. (2014). Effective concentration and detection of *Cryptosporidium*, *Giardia*, and the *Microsporidia* from environmental matrices. *Journal of Pathogens*, 1-10.

Müller, T., Ulrich, A., Ott, E.M., Müller, M. (2001). Identification of plant-associated *enterococci*. *Journal of Applied Microbiology, 91*(2), 268-278.

Murphy, J., devane, M.L., Robson, B., Gilpin, B.J. (2005). Genotypic characterization of bacteria cultured from duck faeces. *Journal of Applied Microbiology*, *99*(2), 301-309.

Murphy, C., Carroll, C., Jordan, K.N. (2006). Environmental survival mechanisms of the foodborne pathogen *Campylobacter jejuni*. *Journal of Applied Microbiology, 100*(4), 623-632.

Nakabachi, A., Ishikawa, H., Kudo, T. (2003). Extraordinary proliferation of microorganisms in aposymbiotic pea aphids, *Acyrthosiphon pisum*. *Journal of Invertebrate Pathology, 82*(3), 152-161.

Nevers, M.B., Whitman, R.L. (2005). Nowcast modeling of *Escherichia coli* concentrations at multiple urban beaches of southern Lake Michigan. *Water Research*, *39*, 5250-5260.

Niewolak, S. (1998). Total viable count and concentration of enteric bacteria in bottom sediments from the Czarna Hancza River, Northeast Poland. *Polish Journal of Environmental Studies, 7*(5), 295-306.

Nshimyimana, J.P., Ekklesia, E., Shanahan, P., Chua, L.H.C., Thompson, J.R. (2014). Distribution and abundance of human-specific *Bacteroides* and relation to traditional indicators in an urban tropical catchment. *Journal of Applied Microbiology, 116*, 1369-1383.

Obiri-Danso, K., Jones, K., (2000). Intertidal sediments as a reservoir for hippurate negative *Campylobacters, Salmonellae* and faecal indicators in three WU recognised bathing waters in North West England. *Water Research*, *34*(2), 519-527.

Obiri-Danso, K., Paul, N., Jones, K. (2001). The effects of UVB and temperature on the survival of natural populations and pure cultures of *Campylobacter jejuni, Camp. coli, Camp. lari* and urease-positive thermophilic campylobacters (UPTC) in surface waters. *Journal of Applied Microbiology, 90*, 256-267.

Ohkuma, M., Noda, S., Hongoh, Y., Kudo, T. (2002). Diverse bacteria related to the *Bacteroides* subgroup of the CFB phylum within the gut symbiotic communities of various Termites. *Bioscience Biotechnology Biochemistry, 66*(1), 78-84.

Olsen, S.J., Bishop, R., Brenner, F.W., Roels, T.H., Bean, N., Tauxe, R.V., Slutsker, L. (2001). The changing epidemiology of *Salmonella*: trends in serotypes isolated from humans in the United States, 1987-1997.

Olson, M.E., Leonard, N.J., Strout, J. (2010). Prevalence and diagnosis of *Giardia* infection in dogs and cats using a fecal antigen test and fecal smear. *The Canadian Veterinary Journal*, *51*(6), 640-642.

Ortega, C., Solo-Gabriele, H.M., Abdelzahar, A., Wright, M., Deng, Y. (2009). Correlations between microbial indicators, pathogens, and environmental factors in a subtropical Estuary. *Marine Pollution Bulletin, 58*, 1374-1381.

Payan, A., Ebdon, J., Taylor, H., Gantzer, C., Ottoson, J., Papageorgiou, G.T., Blanch, A.R., Lucena, F., Jofre, J., Muniesa, M. (2005). Method of isolation of *Bacteriophage* host strains suitable for tracking sources of fecal pollution in water. *Applied and Environmental Microbiology, 71*(9), 5659-5662.

Phillips, M.C., Solo-Gabriele, H.M., Piggot, A.M., Klaus, J.S., Zhang, Y. (2011). Relationships between sand and water quality at recreational beaches. *Water Research, 45*, 6763-6769.

Phillips, M.C., Feng, Z., Vogel, L.J., Reniers, A.J.H.M., Haus, B.K., Enns, A.A., Zhang, Y., Hernandez, D.B., Solo-Gabriele, H.M. (2014). Microbial release from seeded beach sediments during wave conditions. *Marine Pollution Bulletin, 79*, 114-122.

Piorkowski, G., Jamieson, R., Bezanson, G., Truelstrup Hansen, L., Yost, C. (2014). Reach specificity in sediment *E. coli* population turnover and interaction with waterborne populations. *Science of the Total Environment*, *496*, 402-413.

Polo, F., Figueras, M.J., Inza, I., Sala, J., Fleisher, J.M., Guarro, J. (1999). Prevalence of *Salmonella* serotypes in environmental waters and their relationships with indicator organisms. *Antonie van Leeuwenhoek*, *75*(4), 285-292.

Prüss, A. (1998). Review of epidemiological studies on health effects from exposure to recreational water. *International Journal of Epidemiology, 27,* 1-9.

Ramalho, J., Correia, J. (2013). Non-typhoidal *Salmonella* severe sepsis in an immunocompetent young woman. *BMJ Case Reports, 32*(2), 263-269.

Rautelin, H., Koota, K., Essen, R.V., Jahkola, M., Siitonen, A., Kosunen, T.U. (1990). Waterborne *Campylobacter jejuni* epidemic in a Finnish hospital for rheumatic diseases. *Scandinavian Journal of Infectious Diseases, 22*(3), 321-326.

Reischer, G.H., Kasper, D.C., Steinborn, R., Farnleitner, A.H., Mach, R.L. (2007). A quantitative real-time PCR assay for the highly sensitive and specific detection of human faecal

influence in spring water from a large alpine catchment area. *Letters in Applied Microbiology, 44* (4).

Reischer, G.H., Ebdon, J.E., Bauer, J.M… Farnleitner, A.H. (2013). Performance characteristics of qPCR assays targeting human- and ruminant-associated *Bacteroidetes* for microbial source tracking across sixteen countries on six continents. *Environmental Science and Technology*, *47*(15), 8548-8556.

Rhodes, M.W., Kator, H. (1988). Survival of *Escherichia coli* and *Salmonella* spp. in estuarine environments. *Applied and Environmental Microbiology*, *54*(12), 2902-2907.

Rodrigues, C.G., Melo, R.T., Fonseca, B.B., Martins, P.D., Ferreira, F.A., Araujo, B.J., Rossi, D.A. (2015). Occurrence and characterization of *Campylobacter* spp. isolates in dogs, cats and children. *Pesquisa Veterinaria Brasileira*, *35*(4), 365-370.

Rodriguez-Lazaro, D., Hernandaz, M., Scortti, M., Esteve, T., Vazquez-Boland, J.A., Pla, M. (2004). Quantitative detection of *Listeria monocytogenes* and *Listeria innocua* by Real-Time PCR: assessment of hly, iap, and lin02483 targets and AmpliFluor technology. *Applied and Environmental Microbiology, 70*(3), 1366-1377.

Roslev, P., Bukh, A.S. (2011). State of the art molecular markers for fecal pollutions source tracking in water. *Applied and Microbial Biotechnology*, *89*, 1341-1355.

Ryu, H., Griffith, J.F., Khan, I., Hill, S., Edge, T.A., Toledo-Hernandez, C., Gonzalez-Nieves, J., Domingo, J.S. (2012). Comparison of gull feces-specific assays targeting the 16s rRNA genes of *Catellicoccus marimammalium* and *Streptococcus* spp.. *Applied and Environmental Microbiology, 78(6),* 1909.

Sakulwira, K., vanapongtipagorn, P., Theamboonlers, A., Oraveerakul, K., Poovorawan, Y. (2003). Prevalence of canine coronavirus and parvovirus infections in dogs with gastroenteritis in Thailand. *Veterinarni Medicina*, *48*(6), 163-167.

Sampson, R.W., Swiatnicki, S.A., Osinga, V.L., Supita, J.L., McDermott, C.M., Kleinheinz, G.T. (2006). Effects of temperature and sand on *E. coli* survival in a northern lake water microcosm. *Journal of Water Health*, *4*(3), 389-393.

Santos, R.L., Zhang, S., Tsolis, R.M., Kingsley, R.A., Adams, L.G., Bäumler, A.J. (2001). Animal models of *Salmonella* infections: enteritis versus typhoid fever. *Microbes and Infection*, *3*, 1335-1344.

Sauders, B.D., Overdevest, J., Fortes, E., Windham, K., Schukken, Y., Lembo, A., Wiedmann, M. (2012). Diversity of *Listeria* species in urban and natural environments. *Applied and Environments*, *78*(12), 4420-4433.

Sauer, E.P., VandeWalle, J.L., Bootsma, M.J., McLellan, S.L. (2011). Detection of the human specific *Bacteroides* genetic marker provides evidence of widespread sewage contamination of stormwater in the urban environment. *Water Research*, *45*, 4081-4091.

Savage, D.C. (2001). Microbial biota of the human intestine: a tribute to some pioneering scientists. *Current Issues Intestinal Microbiology, 2*(1), 1-15.

Savichtcheva, O., Okayama, N., Okabe, S. (2007). Relationships between *Bacteroides* 16s rRNA genetic markers and presence of bacterial enteric pathogens and conventional fecal indicators. *Water Research, 41*(16), 3615-3628.

Schets, F.M., Wijnen, J.H., Schijven, J.F., Schoon, H., Husman, A.M. (2008). Monitoring of waterborne pathogens in surface waters in Amsterdam, the Netherlands, and the potential health risk associated with exposure to *Cryptosporidium* and *Giardia* in these waters. *Applied* and *Environmental Microbiology, 74*(7), 2069-2078.

Schill, W.B., Mathes, M.V. (2008). Real-Time PCR detection and quantification of nine potential sources of fecal contamination by analysis of mitochondrial cytochrome b targets. *Environmental Science and Technology*

Schriewer, A., Goodwin, K.D., Sinigalliano, C.D… Wuertz, S. (2013). Performance evaluation of canine-associated *Bacteroidales* assays in a multi-laboratory comparison study. *Water Research, 47*, 6909-6920.

Schwartz, J., Levin, R., Hodge, K. (1997). Drinking water turbidity and pediatric hospital use for gastrointestinal illness in Philadelphia. *Epidemiology, 8*(6), 615-620.

Scott, T.M., Rose, J.B., Jenkins, T.C., Farrah, S.R., Lukasik, J. (2002). Microbial source tracking: current methodology and future directions. *Applied and Environmental Microbiology*, *68*(12), 5796-5803.

Sercu, B., Werfhorst, L.C., Murray, J.L., Holdon, P.A. (2011). Terrestrial sources homogenize bacterial water quality during rainfall in two urbanized watersheds in Santa Barbara, CA. *Microbial Ecology*, *62*(3), 574-583.

Seurinck, S., Defoirdt, T., Verstraete, W., Siciliano, S.D. (2005). Detection and quantification of the human-specific HF183 *Bacteroides* 16S rRNA genetic marker with real-time PCR for assessment of human faecal pollution in freshwater. *Environmental Microbiology, 7*(2), 249-258.

Shelton, D.R., Higgins, J.A., Van Kessel, J.S., Peachepsky, Y.A., Belt, K., Karns, J.S. (2004). Estimation of viable *Escherichia coli* O157:H7 in surface waters using enrichment in conjunction with immunological detection. *Journal of Microbiological Methods, 58*, 223-231.

Shibata, T., Solo-Gabriele, H.M., Fleming, L.E., Elmir, S. (2004). Monitoring marine recreational water quality using multiple microbial indicators in an urban tropical environment. *Water Resources, 38*(13), 3119-3131.

Shukla, R., Giraldo, P., Kraliz, A., Finnigan, M., Sanchez, A.L. (2006). *Cryptosporidium* spp. and other zoonotic enteric parasites in a sample of domestic dogs and cats in the Niagara region of Ontario. *The Canadian Veterinary Journal*, *47*(12), 1179-1184.

Silk, B.J., Date, K.A., Jackson, K.A…Mahon, B.E. (2012). Invasive Listeroisis in the foodborne disease active surveillance network. 2004-2009: further targeted prevention needed for higher-risk groups. *Clinical Infectious Diseases*, *54,* 5396-5404.

Silkie, S.S., Nelson, K.L. (2009). Concentrations of host-specific and generic fecal markers measured by quantitative PCR in raw sewage and fresh animal feces. *Water Research, 43*, 4860-4871.

Simpson, J.M., Domingo, J.W.S., Reasoner, D.J. (2002). Microbial source tracking: state of the science. *Environmental Science and Technology*, *36*(24), 5279-5288.

Sinigalliano, C.D., Fleisher, J.M, Gidley, M.L… Fleming, L.E. (2010). Traditional and molecular analyses for fecal indicator bacteria in non-point source subtropical recreational marine waters. *Water Research, 44,* 3763-3772.

Somarelli, J.A., Makareqicz, J.C., Sia, R., Simon, R. (2007). Wildlife identified as a major source of *Escherichia coli* in agriculturally dominated watersheds by BOX A1R-derived genetic fingerprints. *Journal of Environmental Management, 82*, 60-65.

Staley, Z.R., Vogel, L., Robinson, C., Edge, T.A. (2015). Differential occurrence of *Escherichia coli* and human *Bacteroidales* at two Great Lakes beaches. *Journal of Great Lakes Research*, *41*, 530-535.

Staley, Z.R., Robinson, C., Edge, T.A. (2016). Comparison of the occurrence and survival of fecal indicator bacteria in recreational sand between urban beach, playground and sandbox settings in Toronto, Ontario. *Science of the Total Environment, 541*, 520-527.

Stea, E.C., Truelstrup Hansen, L., Jamieson, R.C., Yost, C.K. (2015a). Fecal contamination in the surface waters of a rural- and an urban-source watershed. *Journal of Environmental Quality, 44*, 1556-1567.

Stea, E.C., Purdue, L.M., Jamieson, R.C., Yost, C.K., Truelstrup Hansen, L. (2015b). Comparison of the prevalences and diversities of *Listeria monocytogenes* in an urban and a rural agricultural watershed. *Applied and Environmental Microbiology, 81*(11), 3812-3822.

Stewart, J.R., Ellender, D., Gooch, J.A., Jiang, S., Myoda, S.P., Weisberg, S.B. (2003). Recommendations for microbial source tracking: lessons from a methods comparison study. *Journal of Water and Health*, *1*(4), 225-231.

Stoeckel, D.M., Harwood, V.J. (2007). Performance, design, and analysis in microbial source tracking studies. *Applied and Environmental Microbiology*, *73*(8), 2405-2415.

St-Pierre, K., Lévesque, S., Frost, E., Carrier, N., Arbeit, R.D., Michaud, S. (2009). Thermotolerant coliforms are not a good surrogate for *Campylobacter* spp. in environmental waters. *Applied and Environmental Microbiology, 75*(21), 6736-6744.

Strawn, L.K., Fortes, E.D., Bihn, E.A., Nightingale, K.K., Gröhn, Y.T., Worobo, R.W., Wiedmann, M., Bergholz, P.W. (2012). Landscape and meteorological factors affecting

prevalence of three foodborne pathogens in fruit and vegetable farms. *Applied and Environmental Microbiology*, *249*, 1-12.

Tambalo, D.D., Boa, T., Liljebjelke K., Yost, C.K. (2012). Evaluation of two quantitative PCR assays using *Bacteroidales* and mitochondrial DNA for tracking dog fecal contamination in waterbodies. *Journal of Microbiological Methods, 91*, 459-467.

Thomas, C., Hill, D.J., Mabey, M. (1999). Evaluation of the effect of temperature and nutrients on the survival of *Campylobacter* spp. in water microcosms. *Journal of Applied Microbiology*, *86*(6), 1024-1032.

Till, D., McBride, G., Ball, A., Taylor, K., Pyle, E. (2008). Large-scale freshwater microbiological study: rationale, results and risks. *Journal of Water and Health, 6*, 443-460.

Tuttle, J., Gomez, T., Doyle, M.P., Wells, J.G., Zhao, T., Tauxe, R.V., Griffen, P.M. (1999). Lessons from a large outbreak of *Escherichia coli* O157:H7 infections: insights into the infectious dose and method of widespread contamination of hamburger patties. *Epidemiology & Infection, 128*, 185-192.

Van Dyke, M.I., Morton, V.K., McLellan, N.L., Huck, P.M. (2010). The occurrence of *Campylobacter* in river water and waterfowl within a watershed in southern Ontario, Canada. *Journal of Applied Microbiology*, *109*(3), 1053-1066.

Verweij, J.J., Schinkel, J., Laeijendecker, D., Rooyen, M.A.A., Lieshout, L., Polderman, A.M. (2003). Real-time PCR for the detection of *Giardia lamblia*. *Molecular and Cellular Probes, 17*(5), 223-225.

Vierheilig, J., Farnleitner, A.H., Kollanur, D., Blöschl, G., Reischer, G.H. (2012). High abundance of genetic *Bacteroidetes* markers for total fecal pollution in pristine alpine soils suggests lack in specificity for feces.

Wade, T.J., Calderon, R.L., Sams, E., Beach, M., Brenner, K.P., Williams, A.H., Dufour, A.P. (2006). Rapidly measured indicators of recreational water quality are predictive of swimming-associated gastrointestinal illness. *Environmental Health Prospective, 114*(1), 24-28.

Wade, T.J., Calderon, R.L., Brenner, K.P., Sams, E., Beach, M., Haugland, R., Wymer, L., Defourm A.P. (2008). High sensitivity to swimming-associated gastrointestinal illness: results using a rapid assay of recreational water column. *Epidemiology, 19*(3), 375-383.

Wallis, P.M., Buchanan-Mappin, J.M., Faubert, G.M., Belosevic, M. (1984). Reservoirs of *Giardia* spp. in southwestern Alberta. *Journal of Wildlife Diseases*, *20*(4), 279-283.

Wali, G.M. (1991). Bickerstaff's brainstem encephalitis associated with typhoid fever. *BMJ Postgraduate Medical Journal*, *67*(793), 1011-1012.

Walker, J.W., Duivenboden R., Neale, M.W. (2015). A tiered approach for the identification of faecal pollution sources on an Auckland urban beach. *New Zealand Journal of Marine and Freshwater Research*, *49*(3), 333-345.

Walters, S.P., Gannon, V.P.J., Field, K.G. (2007). Detection of *Bacteroidales* fecal indicators and the zoonotic pathogens *E. coli* O157:H7, *Salmonella,* and *Campylobacter* in river water. *Environmental Science & Technology, 41,* 1856-1862.

Walters, S.P., Thebo, A.L., Boehm, A.B. (2011). Impact of urbanization and agriculture on the occurrence of bacterial pathogens and stx genes in coastal waterbodies of central California. *Water Research, 45*, 1752-1762.

Wang, J.D., Solo-Gabriele, H.M., Abdelzaher, A.M., Fleming, L.E. (2010). Estimation of enterococci input from bathers and animals on recreational beach using camera images. *Marine Pollution Bulletin*, *60*, 1270-1278.

Weiskel, P.K., Howes, B.I., Heufelder, G.R. (1996). Coliform contamination of a coastal embayment: sources and transport pathways. *Environmental Science & Technology, 30,* 1872-1881.

Whitlock, J.E., Jones, D.T., Harwood, V.J. (2002). Identification of the sources of fecal coliforms in an urban watershed using antibiotic resistance analysis. *Water Research*, *36,* 4273-4282.

Whitman, R.L., Nevers, M.B. (2003). Foreshore sand as a source of *Escherichia coli* in nearshore water of a Lake Michigan beach. *Applied and Environmental Microbiology*, *69*(9), 5555-5562.

Whitman, R.L., Shivley, D.A., Pawlik, H., Nevers, M.B., Byappanahalli, M.N. (2003). Occurrence of *Escherichia coli* and *Enterococci* in *Cladophora* (*Chlorophyta*) in nearshore water and beach sand of Lake Michigan. *Applied and Environmental Microbiology, 69*(8), 4714-4719.

Whitman, R.L., Nevers, M.B., Korinek, G.C., Byappanahalli, M.N. (2004). Solar and temporal effects on *Escherichia coli* concentration at a Lake Michigan swimming beach. *Applied and Environmental Microbiology, 70*(7), 4276-4286.

Whitman, R.L., Nevers, M.B., Byappanahalli, M.N. (2006). Examination of the watershed-wide distribution of *Escherichia coli* along Southern Lake Michigan: an integrated approach. *Applied and Environmental Microbiology*, *72*(11), 7301-7310.

Whitman, R.L., Przybyla-Kelly, K., Shivley, D.A., Nevers, M.B., Byappanahalli, M.N. (2009). Hand-mouth transfer and potential for exposure to *E. coli* and F^{+}-coliphage in beach sand, Chicago, Illinois. *Journal of Water and Health, 07,* 623-629.

Wilkes, G., Edge, T., Gannon, V., Jokinen, C., Lyautey, E., Mederios, D., Neumann, N., Ruecker, N., Topp, E., Lapen, D.R. (2009). Seasonal relationships among indicator bacteria, pathogenic bacteria, *Cryptospordium* oocysts, *Giardia* cysts, and hydrological indices for surface waters within an agricultural landscape. *Water Research, 43*, 2209-2223.

Wilson, D.J., Gabriel, E., Leatherbarrow, A.J.H., Cheesbrough, J., Gee, S., Bolton, E., Fox, A., Fearnhead, P., Hart, C.A., Diggle, P.J. (2008). Tracing the Source of Campylobacteriosis. *PLoS Genetics*, *4*(9).

Winfield, M.D., Groisman, E.A. (2003). Role of Nonhost Environments in the Lifestyles of *Salmonella* and *Escherichia coli*. *Applied and Environmental Microbiology, 69*(7), 3687-3694.

Wong, M., Kumar, L., Jenkins, T.M., Xagoraraki, I., Phanikumar, M.S., Rose, J.B. (2009). Evaluation of public health risks at recreational beaches in Lake Michigan via detection of enteric viruses and a human-specific bacteriological marker. *Water Research*, *43*, 1137-1149.

Workman, S.N., Mathison, G.E., Lavoie, M.C. (2005). Pet dogs and chicken meat as reservoirs of *Campylobacter* spp. in Barbados. *Journal of Clinical Microbiology*, *43*(6), 2642-2650.

World Health Organization. 2011a. *Enterohaemorrhagic Escherichia coli (EHEC) Fact Sheet N°125*. [online] Available at: <http://www.who.int/mediacentre/factsheets/fs125/en/> [Accessed 29 December 2014].

World Health Organization. 2011b. *Campylobacter fact sheet N°225*. [online] Available at: <http://www.who.int/mediacentre/factsheets/fs255/en/> [Accessed 4 January 2015].

World Health Organization. 2015. *Water Sanitation Health: Burden of disease and cost-effective estimates*. [online] Available at: <http://www.who.inr/water_sanitation_health/disease/ burden/en> [Accessed 31 January 2015].

Wright, M.E., Solo-Gabriele, H.M., Elmir, S., Fleming, L.E. (2009). Microbial load from animal feces at a recreational beach. *Marine Pollution Bulletin, 58*(11), 1649-1656.

Wu, J., Long, S.C., Das, D., Dormer, S.M. (2011). Are microbial indicators and pathogens correlated? A statistical analysis of 40 years of research. *Journal of Water and Health, 9*(2), 265-278.

Yamahara, K.M., Sassoubre, L.M., Goodwin, K.D., Boehm, A.B. (2012). Occurrence and persistence of bacterial pathogens and indicator organisms in beach sand along the California coast. *Applied and Environmental Microbiology, 78*(6), 1733-1745.

Zhu, X., Wang, J.D., Solo-Gabriele, H.M., Fleming, L.E. (2011). A water quality modeling study of non-point sources at recreational marine beaches. *Water Research*, *45*, 2985-2995.

Zmirou, D., Pena, L., Ledrans, M., Letertre, A. (2003). Risks associated with the microbiological quality of bodies of fresh and marine water used for recreational purposes: summary estimates based on published epidemiological studies. *Archives of Environmental Health, 58*(11), 703-711.

Appendices

Appendix A- Equations for Calculations Used in this Study:

1) Sensitivity and Specificity (Page 13)

$$Sensitivity = \frac{True\ Positives}{(True\ Positives + False\ Negatives)}$$

$$Sensitivity = \frac{True\ Negatives}{(True\ Negatives + False\ Positives)}$$

2) Total Suspended Solids (Page 26)

$$TSS = \frac{Initial\ weight\ of\ filter}{weight\ of\ dried\ filter}$$

3) Turbidity (Page 26)

$$Turbidity = Blank - (Average\ turbidity\ reading)$$

4) Colony Forming Units (Page 29)

$$CFU = Number\ of\ colonies * \frac{1}{Dilution} * \frac{1}{Volume}$$

5) 95% CI of Geometric Means (Page 48)

$$Geometric\ 95\%\ CI = 10^{(Log\ of\ mean\ 95\%\ CI)}$$

Appendix B – Media Used for the Enrichment of Selected Pathogens Within this Study

Table B.1: Media used for the enrichment of *E. coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, and *Campylobacter* spp..

Target Microbe	Enrichment media	Additives
E. coli and *Salmonella*	Buffered peptone water	None
E. coli O157:H7	Tryptic soy broth	Novobiocin at 20 µg / mL
Salmonella spp.	Rappaport-Vassiliadis Salmonella enrichment broth	None
Campylobacter spp.	Bolton Broth	/500 mL: 1 vial Oxoid Bolton broth selective supplement and 25 mL laked horse blood
Listeria monocytogenes	Listeria Enrichment Broth (UVM formulation)	/500 mL: 1 vial Oxoid *Listeria* primary selective enrichment supplement (UVM-1)
	Fraser Broth	/500 mL: 1 vial Oxoid Fraser selective supplement

Appendix C - Assay Controls

Table C.1: Controls utilized for all pathogenic and DNA assay controls

Control	Pathogens	Control Protocol
Pathogenic Enrichment and Filtering Controls:		
Day 1 Negative media (-M1)	*Listeria*, *E. coli/ Salmonella, Campylobacter*,	**Enrichment:** Corresponding media is run through appropriate incubation protocol (Table 3.3). **Sediments:** 90 mL of sterile PS is filtered, filter is incubated with MCB for 24 hours at 37° C.
Day 2 Negative media (-M2)	*Listeria, Salmonella, E. coli*	***Listeria****: 1* mL of day 1 negative media control is placed into 9 mL of corresponding day 2 media and incubated according to Table 3.3. ***Salmonella*/*E. coli***: Day 1 media is run through *E. coli* and *Salmonella* beads separately and then put into corresponding day 2 media.
Negative Beads (-D)	*Salmonella* and *E. coli* – immunomagnetic separation	Sterilized distilled water (dH_2O) is run through both the *Salmonella* and *E. coli* immunomagnetic separation protocols and then placed into appropriate day 2 media.
Negative Filter (-F)	*Salmonella, E. coli, Listeria, Giardia, Cryptosporidium, Campylobacter,* MCB/Sediments	**Bacteria**: For each pathogen, 500 mL of sterilized dH_2O is filtered and the filter is placed in the appropriate enrichment media. **Protozoans**: 500 mL of dH_2O is filtered and the filter is placed in 10 mL of sterile dH_2O. **MCB**: 100 mL of sterile dH_2O is filtered, filter is incubated with MCB for 24 hours at 37°C.
DNA Extraction Controls		
Negative Media (-M)	*Listeria*, *E. coli/ Salmonella, Campylobacter*,	2 mL aliquots of BPW, TSB, RVS, FB, and BB –M controls are combined and then centrifuged for 10 minutes at 3200x *g*. Supernatant is poured off, pellet is resuspended, and 250 µL of re-suspension is run through MoBio PowerSoil DNA extraction kit.
Negative Filter (-F)	*Listeria*, *E. coli/ Salmonella, Campylobacter*,	**Bacteria**: 2 mL aliquots of BPW, TSB, RVS, FB, and BB –M controls are combined and then centrifuged for 10 minutes at 3200x *g*. Supernatant is poured off, pellet is resuspended, and 250 µL of re-suspension is run through MoBio PowerSoil DNA extraction kit **Protozoans:** Negative filter control (for *Giardia* and *Cryptosporidium*) is vortexed for 3 minutes at

		max speed. The filter is aseptically removed and remaining water is centrifuged at 3200x *g* for 10 minutes. Supernatant is discarded and 150 µL of re-suspension is run through Zymo DNA extraction kit.
Negative Bead (-D)	*Salmonella* and *E. coli*	2 mL aliquots of TSB and RVS -D controls are combined, vortexed briefly, and centrifuged for 10 minutes at 3200x *g*. Supernatant is discarded, pellet is resuspended, and 250 µL of re-suspension is run through MoBio PowerSoil DNA extraction kit.
Negative Extraction (-X)	All DNA extractions	For each extraction, sterile dH_2O is run through MoBio PowerSoil DNA extraction kit (for enriched pathogens) or Zymo DNA extraction kit (For *Giardia* and *cryptosporidium)*.

Appendix D – Statistical Information Regarding Day-to-day Deviation in *E. coli* levels

Table D.1: Geometric means and associated 95% CI of *E. coli* levels observed on consecutive sampling days.

Sampling Site	Sampling Dates	Geometric Mean	95% CI
Springfield	August 30th	0.793	0.108 – 5.79
	August 31st	6.25	3.86 – 10.1
	September 7th	12.63	9.27 – 17/1
	September 8th	0.629	0.233 – 1.70
	September 9th	1.00	1
	September 22nd	11.5	0.0120 – 1.17E4
	September 23rd	55.1	26.9 – 113
	September 24th	21.5	0.919 – 504
Kinsmen	August 30th	2.62	0.277 – 24.7
	August 31st	6.86	3.83 – 12.2
	September 7th	109	37.8 – 314
	September 8th	1.58	0.114 – 22.0
	September 9th	0.793	0.293 – 2.14
	September 22nd	67.3	2.70 – 1.67E3
	September 23rd	1.06E3	125 – 9.12E3
	September 24th	123	34.5 – 438
Sandy	August 30th	1.81	0.457 – 7.22
	August 31st	2.51	0.932 – 6.800
	September 7th	5.73	1.37 – 23.9
	September 8th	1.14	0.121 – 10.7
	September 9th	1.58	0.587 – 4.28
	September 22nd	300	300
	September 23rd	181	112 – 291
	September 24th	186	48.4 – 717
Birch Cove	August 30th	0.629	0.233 – 1.70
	August 31st	3.41	1.04 – 11.2
	September 7th	2.88	1.21 – 6.85
	September 8th	1.44	0.139 – 14.8
	September 9th	0.629	0.233 – 1.70
	September 22nd	80.1	20.3 – 314
	September 23rd	115	51.1 – 263
	September 24th	7.93	2.93 – 21.4