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Enumeration, variability, and transport of Escherichia coli in the foreshore reservoir and surface water at freshwater beaches

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Supervisor: Dr. Clare Robinson, The University of Western Ontario A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Civil and Environmental Engineering © Laura J. Vogel 2017

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Abstract

Beaches are of immense recreational, societal and economic value. This value, however, is considerably diminished by poor water quality. Fecal indicator bacteria (FIB) are measured at recreational beaches worldwide to assess the water quality. A beach closure or advisory is issued if FIB concentrations in surface water exceed recreational water quality standards. Due to the lengthy time required to enumerate FIB $(24 - 96$ hours), statistical and mechanistic models have been developed to predict water quality exceedances *a priori* and to better understand why and under what conditions water quality exceedances occur. These models as well as beach water quality management strategies are often based on limited mechanistic understanding of the fate and transport of FIB in the beach environment. For instance, FIB are known to accumulate at very high concentrations in foreshore sand and porewater at beaches (herein referred to as the foreshore reservoir). The dynamics of FIB accumulation in the foreshore reservoir and its subsequent release, including the impact on surface water quality exceedances, is unknown. It is also unclear how to best quantify the abundance of FIB in the reservoir including its partitioning between the sand and pore water. An increased understanding of the behavior of FIB at beaches is needed to improve the accuracy of predictive water quality models, develop effective measures to reduce water quality exceedances, improve water quality monitoring strategies, and ultimately to better protect human health at recreational beaches.

This thesis focuses on addressing key knowledge gaps regarding the behavior and quantification of FIB in the foreshore reservoir. In the first study, seasonal and daily variabilities in FIB concentrations in the foreshore reservoir and surface water are evaluated including determining the influence of environmental factors, such as temperature, waves, and rainfall. In this study, seasonal variability in FIB concentrations in the surface water and foreshore reservoir were found to depend on environmental factors, with some beaches showing a gradual increasing trend through the summer, then decreasing towards the beginning of fall. However, daily variation showed that FIB variability is much more complex and FIB may not simply accumulate over the summer months as previously thought. Further, this study showed for the first time that FIB may be able to replicate in

unseeded natural foreshore beach sand not subjected to external stimuli. The second study uses experimental and field data to evaluate the behavior of FIB in the beach environment during intensified wave conditions including the transfer of FIB from the foreshore reservoir to the surface water. This study showed that as wave height increased foreshore sand erosion resulted in elevated *E. coli* concentrations in surface water, as well as depletion of *E. coli* from the foreshore sand and pore water. *E. coli* initially attached to foreshore sand rather than initially residing in the pore water was found to be the main contributor to elevated surface water concentrations. Surface water *E. coli* concentrations were a function of not only wave height (and associated sand erosion) but also the time elapsed since a preceding period of high wave intensity. This finding is important for statistical regression models used to predict beach advisories. While calculations suggested that foreshore sand erosion may be the dominant mechanism for releasing *E. coli* to surface water during intensified wave conditions at a fine sand beach, comparative characterization of the *E. coli* distribution at a coarse sand-cobble beach suggested that interstitial pore water flow and discharge may be more important for coarser sand beaches. The third study compared the partitioning of FIB in the foreshore reservoir between the sand and pore water and evaluated different sampling methods for quantifying FIB in the foreshore reservoir at beaches with varying grain sizes. This study showed that the collection of the top 1 cm of unsaturated sand resulted in higher and more variable concentrations than the top 5 cm of sand. There were no statistical differences in *E. coli* concentrations when using different methods to sample the saturated sand. Overall, the unsaturated sand had the highest amount of *E. coli* when compared to saturated sand and pore water (considered on a bulk volumetric basis). Pore water sampled with a shovel resulted in the highest observed *E. coli* concentrations (only statistically significant at fine sand beaches) and lowest variability compared to other sampling methods. These findings presented will help determine the appropriate sampling strategy for characterizing FIB abundance in the foreshore reservoir as a means of predicting its potential impact on nearshore surface water quality and public health risk.

Overall, this thesis presents valuable information to health departments, beach managers, and scientists interested in improving water quality and water quality predictions at recreational beaches. Findings from this thesis increase understanding of FIB behavior, especially in the foreshore reservoir, and can be used to improve predictive water quality models, develop strategies to reduce FIB levels at beaches, and identify where and when a foreshore reservoir may be an important source of FIB to the surface water at a beach.

Keywords

Fecal indicator bacteria, *E. coli*, beaches, groundwater, sand, sand erosion, recreational water quality, sampling methods, accumulation, waves, replication, growth

Co-Authorship Statement

The thesis was written in accordance with the guidelines and regulations for an Integrated Article format stipulated by the School of Graduate and Postdoctoral Studies at the University of Western Ontario. The candidate designed, conducted and analyzed all of the experimental work in this thesis under the supervision of Dr. Clare Robinson. The themes and development of this work were performed in discussions with Dr. Clare Robinson. The candidate wrote three manuscripts that are included as chapters of this thesis. Full details for all chapters given below.

Chapter 2: Literature Review: Background

Contributions:

Laura Vogel: primary author/writer, developed outline, reviewed references, reviewed/revised chapter

Clare Robinson: reviewed/revised chapter

Chapter 3: Temporal Variations in the Abundance of Fecal Indicator Bacteria in Foreshore Sand and Porewater at Freshwater Beaches

Contributions:

Laura Vogel: primary author/writer, designed and developed field work protocol, performed field work, collected field samples, designed and conducted laboratory experiments, interpreted and analyzed the collected data.

Tom Edge: assisted with data interpretation, reviewed and revised chapter

Denis O'Carroll: assisted with data interpretation, reviewed and revised chapter

Clare Robinson: assisted with data interpretation, reviewed and revised chapter

Chapter 4: Release of Escherichia coli from Foreshore Sand and Pore Water during Intensified Wave Conditions at a Recreational Beach

Vogel, L., D. O'Carroll, T. Edge, C. Robinson. 2016. "Release of Escherichia coli from foreshore sand and pore water during intensified wave conditions at a recreational beach." Environmental Science and Technology. 50(11):5676-5684.

Contributions:

Laura Vogel: primary author/writer, designed and developed field work protocol, performed field work, collected field samples, designed and conducted laboratory experiments, interpreted and analyzed the collected data.

Denis O'Carroll: assisted with field sample collection and data interpretation, reviewed and revised chapter

Tom Edge: assisted with data interpretation, reviewed and revised chapter

Clare Robinson: assisted with field sample collection and data interpretation, reviewed and revised chapter

Chapter 5: Evaluation of Methods to Sample Fecal Indicator Bacteria in Foreshore Sand and Pore Water at Freshwater Beaches

Vogel, L., T. Edge, D. O'Carroll, H. Solo-Gabriele, C. Kushnir, C. Robinson. 2017. "Evaluation of methods to sample fecal indicator bacteria in foreshore sand and pore water at freshwater beaches." *Water Research*. 121:204-212.

Contributions:

Laura Vogel: primary author/writer, designed and developed field work protocol, performed field work, collected field samples, interpreted and analyzed the collected data

Tom Edge: assisted with data interpretation, reviewed and revised chapter

Denis O'Carroll: assisted with data interpretation, reviewed and revised chapter Helene Solo-Gabriele: assisted with data interpretation, reviewed and revised chapter Caitlin Kushnir: assisted with statistical analyses

Clare Robinson: assisted with data interpretation, reviewed and revised chapter

Dedication

This dissertation is dedicated to my rocks, my parents, without whom none of this was possible –

Ruthanne Deakin Vogel

And

Jeffrey David Vogel

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If I were to write down the names of everyone who helped me along the way, my acknowledgements section would be longer than the dissertation itself, which is apparently frowned upon. Therefore, I would like to acknowledge a few people who deserve a special shout out, but I would still like to thank everyone who played a part in my four years at Western.

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Chapter 1

1 Introduction

1.1 Background

Microbial contaminants in surface water at beaches can cause illnesses in swimmers, including the stomach flu, respiratory infection, ear infections, and skin infections. Most illnesses from swimming in contaminated waters last from a few days to weeks, however, in some cases long-term illness or even death can occur (Devine 2014). Contracting an illness from the beach is not exclusive to swimmers. Beachgoers can become ill without entering the water. For example, Heaney et al. (2009) found positive correlations between beachgoers who either dug in the sand or were buried in the sand at freshwater and marine beaches located within 7 miles of a sewage treatment plant and gastrointestinal illness.

Fecal indicator bacteria (FIB) are often monitored in the surface water at beaches to indicate the potential presence of harmful pathogens. The decision to post or close a beach is typically made based on grab samples from the surface water and by using standard FIB enumeration techniques. Using these methods it takes 24-96 hours to determine the concentrations of FIB in samples. This current procedure is not ideal due to the high temporal variability of FIB concentrations in the surface water (Boehm et al. 2002, Enns et al. 2012, Whitman et al. 2004). For instance, Boehm et al. (2002) showed that 70% of single-sample FIB exceedances at a marine beach lasted less than 1 hour while 40% lasted less than 10 minutes. Therefore, it is likely that any contamination event that caused an exceedance will have passed before a beach is closed or posted. In addition to the potential health risks associated with having a beach open during a contamination event, posting or closing a beach when there is no health threat can be detrimental to coastal and lakeside city economies. Rabinovici et al. (2004) estimated that closing Indiana Dunes State Park, a freshwater beach on Lake Michigan, may cause an economic loss of up to \$37,030 per day. This study also found that an unnecessary beach closures were issued on 12% of the sampling days over the bathing seasons (May – September) from 1998-2001. To reduce incorrect beach closures and postings and to be able to notify the public prior to an actual exceedance, statistical forecasting models of FIB concentrations in the surface water have been developed (e.g. Frick et al. 2008, Nevers and Whitman 2005, Olyphant and Whitman 2004). While these statistical models are proving to be valuable tools for beach managers, they are typically beach-specific and require large data sets of FIB concentrations, rainfall, wave height, solar radiation, wind speed/direction, temperature, and other parameters for a given beach. Further, these models provide limited mechanistic understanding of the underlying sources and fate of FIB in the beach environment.

FIB have been shown to accumulate in high numbers in the foreshore sand and pore water at beaches, herein referred to as the foreshore reservoir (Boehm et al. 2004, Kinzelman et al. 2004, Staley et al. 2015, Whitman and Nevers 2003). Considering concentrations on a volumetric basis, FIB concentrations in the foreshore reservoir have been found to be orders of magnitude higher than in adjacent surface water. In addition to serving as a potential direct health risk (Heaney et al. 2012, Heaney et al. 2009), the foreshore reservoir can serve as a source of FIB to the surface water, thereby causing a contamination event (Edge and Hill 2007, Gast et al. 2011, Phillips et al. 2014). It is currently not clear why FIB accumulate in high concentrations in the foreshore reservoir. The physical and environmental factors contributing to this accumulation, the pathways by which FIB are delivered to the sand and pore water, and the time-scale over which this accumulation occurs are not well understood. Once FIB accumulate in the foreshore reservoir, the mechanisms by which they are subsequently delivered to the surface water are also not clear. The overall variability of FIB concentrations in both the surface water and foreshore reservoir in response to different environmental forcing including periods of high wave intensity, as well as factors controlling this variability need to be determined. To understand the role and potential risk associated with FIB in the foreshore reservoir, there is a need for standard methods to quantify the amount and distribution of FIB in the reservoir. Currently, there are no widely accepted methods to collect samples from the foreshore sand and pore water for FIB enumeration. Therefore, different studies use different methods for collection and the reproducibility and comparability between these methods and thus studies are unknown. To improve the accuracy of statistical models in predicting FIB exceedances in the surface water, there is a need to develop a better understanding of why FIB accumulate in the foreshore reservoir and how we can measure this accumulation as well as the mechanisms of transport between the foreshore reservoir and surface water.

1.2 Research objectives

The overall goal of this study was to provide new knowledge of the behavior of FIB in the foreshore reservoir at freshwater beaches including the interconnectivity and exchange of FIB between the foreshore reservoir and surface water. The study is based on extensive field data collection at beaches on the Great Lakes combined with rigorous statistical analysis and a mass balance model to provide information needed to improve the current state of recreational water quality monitoring and modeling.

The first objective of this study was to identify short (daily) and long (seasonal) variability in *E. coli* concentrations in the foreshore reservoir and surface water at freshwater beaches including determining how different environmental forces (e.g. temperature, wave height, rainfall) influence this variability. This objective was met by collecting and analyzing seasonal *E. coli* concentrations and environmental data from three freshwater beaches in Southern Ontario together with daily sampling of the foreshore reservoir at one of the beaches over a 34-day period. The potential of replication of *E. coli* in unaltered natural foreshore beach sand was also evaluated to examine the potential for replication to contribute to FIB accumulation in the reservoir.

The second objective of this study was to determine for the first time how *E. coli* concentrations in the foreshore reservoir and surface water vary in response to varying wave conditions and to identify the pathway by which *E. coli* are transferred between the reservoir and surface water during intensified wave conditions. To address this objective, *E. coli* concentrations and environmental data were collected prior to, during, and after three wave events on a fine sand freshwater beach. In addition to statistical analyses, a mass balance model combined with laboratory experiments were used to determine the relative contribution of sand erosion and subsequent release of *E. coli* from sand to increases in surface water *E. coil* concentrations. Lastly, statistical analyses were performed to compare results from the field site to other beaches with varying sand types.

The third objective of this study was to compare different methods that have been used to sample *E. coli* in beach sand and pore water and also to improve understanding of the partitioning of *E. coli* between foreshore sand and pore water to ultimately improve beach monitoring programs. Three different sampling methods for saturated sand and pore water as well as two methods of sampling unsaturated sand were compared at six freshwater beaches with varying grain sizes. Results were compared to surface water samples taken at each field site to compare the partitioning of *E. coli* in the foreshore reservoir to concentrations in the surface water.

1.3 Thesis outline

The thesis is written in integrated article format. A brief description of each chapter is listed below.

Chapter 1 provides a brief overview of FIB at beaches and outlines the scope of the thesis.

Chapter 2 reviews the current literature on the occurrence, accumulation and transport of FIB in the foreshore reservoir at beaches as well as methods to sample FIB in the foreshore reservoir.

Chapter 3 investigates short-term (daily) and long-term (seasonal) variation of FIB in the foreshore reservoir and surface water at beaches and the factors (e.g. temperature, rainfall, waves, replication) affecting this variability.

Chapter 4 titled "Release of *Escherichia coli* from Foreshore Sand and Pore Water during Intensified Wave Conditions at a Recreational Beach" investigates the transport of *E. coli* from the foreshore reservoir to the surface water at beaches during intensified wave conditions.

Chapter 5 titled "Evaluation of Methods to Sample Fecal Indicator Bacteria in Foreshore Sand and Pore Water at Freshwater Beaches" evaluates the effect of sampling methods on the quantification of *E. coli* in sand and pore water and compares the partitioning of *E. coli* between different components of the reservoir (unsaturated sand, saturated sand, pore water) at beaches with varying sand grain sizes.

Chapter 6 summarizes the major conclusions of the thesis, discusses the implications of this study, and provides recommendations for further research.

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Chapter 2

2 Literature Review 2.1 Introduction

Immense social and economic benefits are derived from recreational swimming at beaches. It is estimated that approximately 928 million trips are made to the beach each year in the United States (National Oceanographic Atmospheric Administration 2005). Despite the benefits provided, up to 3.5 million people each year in the United States become ill from contact with raw sewage from sanitary sewer overflows, many instances of which occur at recreational beaches (Dorfman and Haren 2014). This number is likely higher than reported as many people who become ill after swimming in polluted waters are not aware of the cause of their illness and therefore do not report it to health officials. In 2005 there was an estimated 3000 days of beach closings and advisories in the Great Lakes. Research suggests that a 20% reduction in beach closures and advisories in the Great Lakes alone would lead to a net economic benefit of \$2 to \$3 billion dollars per year (Austin et al. 2007).

Exposure to microbial pathogens (e.g. *Salmonella, Campylobacter jejuni*, *Staphylococcus aureus*) from sewage and other sources poses a risk to swimmers in recreational waters through routes such as ingestion, inhalation, and skin contact (Boehm et al. 2009a, Enns et al. 2012). As FIB are present in high concentrations in sewage and runoff (Barthram and Rees 2000), epidemiology studies have shown a correlation between fecal indicator bacteria (FIB) levels and bather illness (e.g. gastrointestinal and respiratory illnesses, skin irritations) (Balarajan et al. 1991, Dewailly et al. 1986, Fleisher et al. 2010, Heaney et al. 2012, Hlavsa et al. 2015, Wade et al. 2008). Therefore, due to the challenges and high costs of quantifying harmful pathogens, FIB, such as enterococci in marine beaches and *Escherichia coli* (*E. coli*) in freshwater beaches, are used for recreational water quality monitoring as indicators of the human health risk. FIB water quality standards have been set for health departments to use to monitor recreational beaches (e.g. 100 colony forming units per 100 mL [CFU/100mL] based on a geometric mean for *E. coli* in Ontario, Canada (Ontario Ministry of the Environment and Energy 1999) and the United States (United States Environmental Protection Agency 2012), and 30 CFU/100mL for enterococci in the United States (United States Environmental Protection Agency 2012)). In most current practices, health departments take one

or a few grab samples from the surface water (from various depths, ankle- to waist-depth) and transport them back to the lab to analyze within 6 hours. There is approximately a 24-96 hour delay between when a water sample is taken and when the FIB concentration results are known due to the required incubation times. Therefore, if a sample is taken that exceeds water quality standards then it is possible that by the time the beach is closed or a sign is posted, the contamination event that caused the exceedance will have passed ((Boehm et al. 2002). Due to the lengthy time delay in obtaining water quality monitoring results, there is a need to be able to predict *a priori* when and where FIB concentrations in the surface water will be high. To achieve this there is an urgent need to clearly understand the behaviour and fate of FIB in the beach environment.

Health units in Canada and the United States are currently not required to sample sand or pore water as part of their beach monitoring programs (Health Canada 2012, United States Environmental Protection Agency 2012). However, current research shows that sand and pore water near the shoreline can harbor high amounts of FIB (Solo-Gabriele et al. 2015, Whitman and Nevers 2003). Herein, the pore water and sand in the foreshore area of a beach where FIB accumulates is referred to as the foreshore reservoir (Figure 2.1).

Figure 2.1: Components of the foreshore reservoir.

2.2 FIB in the surface water

Many studies have investigated FIB concentrations in the surface water at recreational beaches and how they vary spatially and temporally (Boehm et al. 2002, Edge et al. 2010, Edge and Hill 2007, Enns et al. 2012, Haack et al. 2003, Kleinheinz et al. 2006, Whitman and Nevers 2008). It is generally found that FIB concentrations decrease with increasing distance from shore. At a

Florida beach, knee-depth water samples had significantly higher enterococci concentrations than waist-depth water samples. While 43% of the samples taken at knee-depth exceeded the regulatory guideline (prior to 2012) of 104 CFU/100mL, only 5% of waist-depth samples exceeded this value. This is a concern as health departments take water samples at waist-depth for regulatory purposes, while most of the bathers spent their time between ankle- and knee-depth water (Enns et al. 2012). Whitman and Nevers (2003) observed the same pattern at a Lake Michigan Beach with *E. coli* concentrations substantially decreasing with increasing distance from the shore (Figure 2.2).

Figure 2.2: Average of *E. coli* **counts in sand (converted to CFU/100mL) and water (combined) by distance from shore. Error bars indicate ± 1 standard error of the mean. Figure reproduced from Whitman and Nevers (2003).**

In addition to spatial variations in FIB concentrations in surface water, temporal variations in concentrations are important in determining how and when to take a water sample. There are many physical factors that affect surface water FIB concentrations including rainfall (Ackerman and Weisberg 2003, Morrison et al. 2003, Olyphant and Whitman 2004), wind speed and direction (Olyphant and Whitman 2004, Smith et al. 1999), temperature (Ishii et al. 2007), wave activity (Gast et al. 2011, Phillips et al. 2014), and tides (Enns et al. 2012). Enns et al. (2012) observed that elevated solar radiation may contribute to decreases in surface water enterococci concentrations. Water samples taken in the morning were significantly lower than those taken in the evening (Figure 2.3), possibly due to increased solar radiation. Boehm et al. (2002) also concluded that FIB are very sensitive to sunlight and that the time of day that water samples are taken can significantly influence the outcome of the water quality tests. This study found that at least 70% of their single-sample exceedances lasted less than 1 hour and at least 40% lasted less

than 10 min. Therefore, the decision to close a beach should not solely be based on the concentration of FIB in a single grab sample that usually takes 24-96 hours to process. In addition to improving beach sampling protocols, understanding temporal and spatial variability in FIB surface water concentrations as well as controlling factors (e.g. rainfall, waves, temperature) is needed to improve predictive models that can close the time gap between when an exceedance occurs and when the public is notified.

Figure 2.3: Knee-depth water enterococci levels grouped by hour. Black squares indicate night samples (9 PM-5 AM), white squares indicate morning samples (6 AM-12 PM) and gray squares indicate afternoon samples (1 PM-8 PM). The dotted line indicates the percentage of samples each hour above the [water quality] advisory single sample guideline of 104 CFU/100 mL. Figure reproduced from Enns et al. (2012).

2.3 FIB in the foreshore reservoir

Recent studies have shown that pore water and sand in the foreshore area of a beach (within 1-2 m of the shoreline), at non-tidal beaches, e.g. the Great Lakes (Alm et al. 2006, Edge and Hill 2007, Ishii et al. 2007, Skalbeck et al. 2010, Whitman and Nevers 2003) and intertidal sand, at marine beaches (Wright et al. 2011), can act as a reservoir for FIB with concentrations of bacteria often much higher than in adjacent shallow waters. Davies et al. (1995) suggested that sand and pore water can provide a favorable, nonstarvation environment for FIB, where the die-off rate is lower than in surface water. Not only does the sand and pore water potentially provide a direct

route of exposure to humans and therefore represent a direct health risk (Bonilla et al. 2007, Heaney et al. 2009), it can also act as a non-point source whereby bacteria can be released into the surface waters by resuspension of sand grains or through interstitial pore water flow and groundwater discharge (Alm et al. 2006, Bai and Lung 2005, Boehm et al. 2004, Vogel et al. 2016, Whitman and Nevers 2003, 2008, Yamahara et al. 2007).

The build-up of FIB near the shoreline leads to the possibility of continuous exchange of FIB between the foreshore reservoir and the surface water. Ishii et al. (2007) found that *E. coli* concentrations in the upshore sand were patchy while concentrations in the foreshore sand were evenly distributed; suggesting a relationship between shallow surface water and the foreshore reservoir where wave action may homogenize *E. coli* in the foreshore area. A review by Halliday and Gast (2011) found that when concentrations of FIB in the sand were expressed in CFU/100g of dry sand, the ratio between the concentrations in shallow lake water (CFU/100mL) and sand ranged from 1:3-1:460 with concentrations of FIB in dry sand varying by 3 orders of magnitude. This variation may be explained by different climates and bacterial sources (e.g. point versus nonpoint sources) between the field studies included in the review, as well as by general spatial variation in FIB concentrations at beaches (Enns et al. 2012, Halliday and Gast 2011). In addition to large variations in concentrations of FIB in beach sand and pore water, there can also be significant differences in concentrations between different types of sand. Beach sand can range in grain size (fine, medium, and coarse grain) and their degree of uniformity (CU). Sources of contamination and the efficiency by which FIB attach to different sand types can account for the high range in concentrations found in the sand. Skalbeck et al. (2010) found that mean grain size and the degree of uniformity accounted for variation in FIB density with fine sand of uniform distribution found to have the highest concentrations. Piggot et al. (2012) found a unimodal relationship in the supratidal zone (just landward of the high tide mark) between sediment grain extracellular polymeric substances (EPS), the principal structural component of biofilms, and enterococci levels. They found maximum enterococci concentrations occurred at EPS levels of 7 μ g/g. They suggested that below 7 μ g/g, FIB gain protection from biofilms, however above this concentration of EPS, FIB may fall prey to competitive exclusion from the biofilm bacterial activity. This study also found higher levels of EPS and enterococci in supratidal sands over intertidal and subtidal sands (Piggot et al. 2012). The difference in the attachment and persistence of FIB in different types of sand grains adds additional uncertainty to determining sand

concentrations and the resulting risk of foreshore sands acting as a source of FIB to the surface water.

Based on DNA fingerprint analyses, multiplex PCR results, and surveying of culturable *E. coli*, Ishii et al. (2007) deduced that sand and sediment (offshore submerged sand) serve as both temporal sources and sinks of human and waterfowl-derived *E. coli*. When beach sand and sediment act as sources they can potentially contribute to high surface water concentrations and thus beach closures. In addition to sand, detrital material in the foreshore reservoir, such as decaying vegetation and algae, can harbour FIB and also be a source to surface waters (Grant et al. 2001, Haack et al. 2003, Whitman et al. 2003). For example, Whitman et al. (2003) measured *E. coli* concentrations in *Cladophora* over 6 log CFU/g.

2.3.1 Partitioning of FIB in the foreshore reservoir

Understanding how FIB are distributed and partition between the sand and pore water in the foreshore reservoir and the underlying physical and environmental factors is needed to determine the optimum approach for sampling FIB in the foreshore reservoir and quantifying their abundance. Whitman and Nevers (2003) found that *E. coli* concentrations were highest in the foreshore sand, followed by submerged knee-depth offshore sediment and surface water of increasing depth. Alm et al. (2003) found that *E. coli* concentrations at several Michigan beaches were highest in the first 5 cm below the sand surface in the foreshore area. This study also found that based on a unit weight basis, the mean summer concentrations of FIB were 3-38 times higher in the top 20 cm of wet foreshore sand than in the water column at the same Michigan beaches. FIB in unsaturated sand at moisture contents between 15% and 20% have been found to persist better than those in lower or higher moisture contents (Beversdorf et al. 2007). FIB in pore water have also been shown to have the highest concentrations around the water table and decrease with depth (Russell et al. 2012, Wu et al. 2017).

2.4 Fundamentals of FIB transport in porous media

The fate and transport of FIB in porous media is complex and controlled by interstitial pore water flow and the attachment and detachment of bacteria from sand grains (Solo-Gabriele et al. 2015). Bacteria are considered colloids which fall between 1-1000 nm in diameter (Levine 2009).

Colloids can move through the subsurface through advection with the interstitial pore water velocity, diffusion driven by concentration gradients in the pore water, and chemotaxis (Johnson et al. 1996). Colloid transport through the subsurface is often hindered by retention in the sediment. This hindrance is usually caused by attachment directly to the sediment grain surface or retention in the near surface zone. Transport from the pore water to the sediment surface or near surface zone is controlled by interception, diffusion, and sedimentation (Figure 2.4a). Colloid attachment to the surface or retention in the near surface zone is controlled by DLVO forces (e.g. van der Waals attraction, electrostatic attraction or repulsion) (Derjaguin and Landau 1993, Verwey and Overbeek 1955).

FIB movement in saturated porous media is typically described by Colloid Filtration Theory (CFT). The one-dimensional equation for bacterial transport in the aqueous phase, neglecting growth and decay, is given as:

$$
\frac{\partial C}{\partial t} = -v \frac{\partial C}{\partial x} + D \frac{\partial^2 C}{\partial x^2} - kC \tag{2.1}
$$

where C is the mass concentration of suspended bacteria in the aqueous phase (kg/m^3) , *v* is the pore water flow velocity (m/s) , *t* is time (s), *x* is distance traveled (m) , *D* is the dispersion coefficient (m^2/s), and *k* is the attachment rate coefficient (s^{-1}). CFT is generally used to predict *k*, the attachment rate coefficient, however, literature shows that CFT is not always appropriate in many environmental scenarios (Molnar et al. 2015). Classic CFT considers sedimentation, interception, and Brownian diffusion (Figure 2.4a), however, CFT does not take into account straining, geochemical heterogeneity, variable deposition rate coefficients, or preferential flow (Figure 2.4b) which can all affect the retention of FIB in the subsurface (Foppen 2007).

Figure 2.4: (a) Classical colloid filtration theory (CFT) mechanisms and (b) Relationship between CFT and other attachment mechanisms. Figure adapted from Foppen (2007).

Straining is not included in CFT. Sakthivadivel (1968) and Matthess et al. (1985) suggested that straining was only significant when considering particle: collector diameter (d_p/d_c) values above 5-18%. This would mean that only large colloids $(d_p \sim 10 \,\mu\text{m})$ in fine sediments would be strained. According to this theory, *E. coli*, with a length of about 2.0 µm and a diameter of 0.25-1.0 µm would not be influenced by straining. However, in the last 15 years more studies have reported that straining can occur for a much wider range of colloid and collector sizes and even for d_p/d_c ratios as low as 0.01% (Bradford et al. 2004, Bradford et al. 2003, Bradford et al. 2002, Xu et al. 2008). Bradford et al. (2006) conducted laboratory column experiments using *E. coli* 0157:H7 and

various sieve sizes of silica sand ($d_{50} = 710$, 360, 240, and 510 μ m) and found that straining tended to increase with decreasing sand size (increasingly smaller pores) and flow rate suggesting that bacteria, and other colloids, may travel shorter distances in finer grain sediment. In addition to increased straining with decreasing sand size, Bradford et al. (2002) showed that peak effluent colloid concentrations in column experiments using glass beads decrease with increasing colloid size (Figure 2.5). Even the effluent concentrations of colloids with a mean diameter of 1.0 μ m were reduced relative to the influent concentrations by approximately 40% in the column – this suggest that FIB transport through the beach aquifer may be influenced by straining. Due to the use of glass beads which are chemically homogenous, spherical, and smooth, the retention of the carboxyl colloids in the glass beads (which are both negatively charged) cannot be explained by attachment mechanisms and therefore must have been caused by straining.

Figure 2.5: Colloid concentration in the effluent relative to influent concentration as a function of pore volume for the indicated colloid sizes for a glass bead column. Figure reproduced from Bradford et al. (2002).

Transport of bacteria through the unsaturated zone brings in another important factor – the presence of air-water interfaces. Previous studies show that bacteria tend to accumulate at the airwater interface (Blanchard and Syzdek 1972, Powelson and Mills 1996). Using column experiments and a mechanistic model, Schäfer et al. (1998) found that the transport of bacteria through porous media was strongly reduced by decreasing the water saturation. The increased retention of bacteria in unsaturated porous media contributed to the accumulation at air-water interfaces. This suggests that unsaturated sands may have higher concentrations of FIB than saturated sands and that concentrations are expected to decrease with increasing sediment depth and moisture content.

Once colloids are associated with sediment particles, the shear stress associated with moving water can result in the release of colloids from the sediment. Once the moving water imposes a torque such that shear forces exceed the forces attaching the colloids to the sediment, the colloids are released (Ryan et al. 1998). Therefore, increasing pore water velocity will increase the shear forces and the subsequent colloid release (Kaplan et al. 1993, Shang et al. 2008). Saiers and Lenhart (2003) observed that increasing flow rates increased the number of colloids mobilized from silica sand. This study also found that at a given flow rate, a limited amount of colloids are released, however, when that flow rate is increased an additional amount of colloids are released. Through column experiments in unsaturated conditions, Shang et al. (2008) found that the peak colloid concentrations in the effluent occurred with the arrival of the infiltration front and that a larger flow rate led to a greater amount of colloids released from the column. The cumulative amount of colloids that were released was also proportional to the water content in the column once steady state flow was achieved.

2.5 Factors affecting abundance of FIB in the foreshore reservoir

Possible sources of FIB to the foreshore reservoir include point sources (e.g. raw sewage, sanitary sewer overflow or storm water discharge) and non-point sources (e.g. fecal droppings from birds or other animals, runoff from surrounding areas, and potentially septic systems) (Alm et al. 2017, Fujioka et al. 1988, Irvine and Pettibone 1993, Kim et al. 2004, Oshiro and Fujioka 1995). Surface water infiltration across the beach face can also be a source of FIB to the foreshore reservoir (Figure 2.6) (Byappanahalli et al. 2006, Gast et al. 2015, Ge et al. 2012, Ishii et al. 2007, Wu et al. 2017). Preliminary studies suggest that FIB accumulates in the foreshore reservoir due to favorable moisture content, high concentrations of nutrients, infiltration of possibly contaminated shallow surface water, and the reservoir's close proximity to surface sources (e.g. bird feces) than can transport FIB to the foreshore area via shallow unsaturated-saturated groundwater flow (Beversdorf et al. 2007, Lee et al. 2006).

2.5.1 Temporal variations of FIB in the foreshore reservoir

While several studies have investigated temporal variations of FIB in the surface water at beaches (Boehm et al. 2002, Enns et al. 2012, Whitman et al. 2004, Wright et al. 2011), few studies have examined these variations in foreshore sands and pore water. Understanding short- and long-term variability in FIB concentrations in the foreshore reservoir is needed to better understand the factors that affect FIB accumulation in the reservoir and the potential for the reservoir to impact surface water concentrations and cause a beach water quality advisory. Enns et al. (2012) conducted a 10-day intense sampling study, collecting hydrometereologic data, hydrodynamic data, bather densities, enterococci levels, and *S. aureus* levels in both water and sand. This study found that rainfall and tidal patterns considerably influenced enterococci concentrations in the water and sand on a short-term time-scale. However, this study mostly focused on the spatial and temporal changes in the surface water over the 10-day sampling period. Whitman and Nevers (2003) and Ishii et al. (2007) observed long-term increasing *E. coli* concentrations in foreshore surface sand over the bathing season (May – September). According to analysis of variance, correlation, cluster analysis, concentration gradients, temporal-spatial distribution, demographic patterns, and DNA fingerprinting, Whitman and Nevers (2003) concluded that *E. coli* may be able to survive and thrive during summer months in temperate beach sand without external inputs. These studies suggest that FIB may accumulate and persist in the foreshore reservoir over the bathing season leading to higher concentrations in the late summer months. No prior studies have evaluated short-term (i.e. daily) variability in FIB concentrations in the foreshore reservoir including the factors and processes controlling this temporal variability and how it is related to previously observed long-term trends. This information is needed to understand and potentially predict FIB concentrations in the foreshore reservoir as well as to understand how the variability may ultimately affect FIB concentrations in the surface water.

2.5.2 Groundwater as a source of FIB to the foreshore reservoir

The potential importance of groundwater in delivering FIB to the foreshore reservoir and subsequently to surface water will vary depending on the physical characteristics of the beach aquifer (e.g. grain size distribution, moisture content, biofilms, hydraulic conductivity) and groundwater flow conditions. Some studies have shown that land-derived groundwater may be an important pathway for transporting FIB from surficial aquifers to adjacent surface waters (Boehm et al. 2004, Foppen et al. 2007, Keswick et al. 1982). Boehm et al. (2004) found that enterococci suspended in saline groundwater was not significantly filtered by a sand packed column (10 cm in length) collected from a California beach, and therefore they suggested that enterococci may be

transported to surface water through the surficial aquifer. In addition to serving as a potential source and transport route for FIB, land-derived groundwater may deliver nutrients, such as inorganic nitrogen and orthophosphate, to the foreshore area which may contribute to enriched growth or persistence of FIB in the foreshore reservoir (Boehm et al. 2004). Conversely, some studies have concluded that land-derived groundwater is not a significant source of FIB to the beach environment as bacteria are typically not very mobile in the subsurface (Bitton and Harvey 1992, Brown and Boehm 2016, Harvey 1997). Harvey and Garabedlan (1991) showed from column experiments that 85% of nongrowing bacteria was removed by the sand within a 7 m travel distance. Foppen et al. (2007) suggested that due to the heterogeneity in most bacterial populations, consisting of both "slow" and "fast attachers", some bacteria may be filtered out of groundwater (approximately 5-20% in these experiments using 5 cm long columns), but some bacteria can travel high distances in the subsurface.

2.5.3 Surface water as a source of FIB to the foreshore reservoir

In addition to the foreshore reservoir serving as a potential source of FIB to surface water, surface water infiltration associated with tides or wave action may transport FIB from the surface water to foreshore reservoir (Ishii et al. 2007) (Figure 2.6). Gast et al. (2015) showed that enterococci from surface water were rapidly transported about 0.5-0.8 m vertically and 6 m horizontally into the beach subsurface by wave-driven surface water infiltration and associated pore water flow. Wu et al. (2017) presented field data showing that *E. coli* and enterococci can be transported 1 and 2 m, respectively, below the water table in the foreshore area. Wu et al. (2017) used this field data to validate a numerical model simulating the accumulation of FIB in a beach aquifer exposed to low energy (non-erosive) wave conditions and associated wave-induced surface water infiltration. Pore water FIB concentrations in the foreshore reservoir were found to rapidly approach steady state (i.e. after 0.5 days) as opposed to concentrations of FIB associated with the foreshore sand that continued to increase over time as *E. coli* continued to be delivered to the beach aquifer by surface water infiltration. This study also found that under certain beach conditions, FIB accumulation in the foreshore reservoir over 5-6 days due to wave-induced infiltration may be sufficient to trigger a beach advisory if the foreshore sand is eroded to the surface water.

Figure 2.6: Schematic of accumulation and transport of FIB in the foreshore reservoir under low energy wave conditions. Figure reproduced from Wu et al. (2017).

2.5.4 FIB replication in beach sand

The possibility of growth or replication of FIB in beach sand has been widely debated. It is important to know whether high concentrations of FIB in the foreshore reservoir are caused by FIB replicating in sand or whether FIB are coming from external sources. High concentrations of FIB due to replication does not indicate the same health risk as high concentrations from human sources. Experimental studies have shown an increase in FIB in sand after the addition of environmental stimuli (e.g. seawater, plankton, algae) or after alteration of the sand (e.g. autoclaving, inoculation) (Table 2.1). Byappanahalli et al. (2006) found a significant increase (approximately 2-logs) in *E. coli* concentrations when supplementing beach sand with plankton, while the control, not supplemented with plankton, did not exhibit any increase in concentrations (Figure 2.7). Yamahara et al. (2009) observed growth in enterococci concentrations in unseeded, unsterilized sand when subjected to intermittent wetting with seawater, similar to what would occur at the high tide line. Similar to Byappanahalli et al. (2006), there was no observed replication or growth in the control microcosms that were not subjected to wetting (Yamahara et al. 2009).

Figure 2.7: *In vitro* **growth of** *E. coli* **in beach sand amended and unamended with net-plankton from Lake Michigan. Error bars represent ± 1 standard error. Figure reproduced from Byappanahalli et al. (2006).**

Hartz et al. (2008) compared the change in FIB (enterococci and *E.* coli) concentrations in beach sand that was rinsed, dried, autoclaved, and inoculated with sand that was collected and used without sterilization. In the sterilized sand, enterococci increased by about 2-logs while the control sand did not exhibit the same growth. They suggested that FIB have the potential to survive and replicate in beach sand, but that increases in FIB concentrations at the magnitude they observed in their sterile sand experiment are unlikely to occur in the field. Similar to Hartz et al. (2008), Alm et al. (2006) also observed a significant increase in *E. coli* concentrations after inoculating sterilized beach sand with two *E. coli* isolates (Figure 2.8). Although several studies have shown that replication is possible, none have observed significant increases (replication) of FIB concentrations in unaltered, unseeded, natural beach sand not subjected to external stimuli. There is a need to understand whether FIB can replicate in the natural environmental conditions present in the foreshore reservoir to better evaluate the health risk associated with the reservoir.

Figure 2.8: Mean (± standard deviation) abundance of *E. coli* **in duplicate laboratory sand microcosms incubated at 19ºC. Squares indicate Experiment 1 and triangles indicate Experiment 2. Conditions were the same for both experiments. Figure reproduced from Alm et al. (2006).**

Paper	Site	Sterilized?	Seed	Amendments	Incubation Temperature	Findings
Byappanahalli 2006	Michigan City, Indiana	N _o	N/A	Plankton (microcrustacean s, rotifers, and filamentous algae)	23.5 C	Significant increase (about 2 logs) in E . <i>coli</i> numbers over 24 hours
Yamahara 2009	Lovers Point, California	N ₀	N/A	Intermittent "watering" with seawater	20 C	Increase at rates of 0.20 to 0.63 per day was observed during "watering" periods
Staley 2016	Burlington and Toronto, Ontario	No	Eight strains of E . coli taken from field site	Autoclaved lake water	15 C, 28 C	No significant increase in E . coli concentrations
Hartz 2008	Hollywood , Florida	Yes	Six isolates of $E.$ coli and enterococci	Sterile seawater, sea salts	20 C, 30 C, 40 C	Significant increase (about 2 log) within 2-3 days
Alm 2006	Port Huron, Michigan	Yes	Two E. coli isolates taken from field site	N/A	19 C	Significant increase (about 4 log) within 2 days
Yamahara 2012	Lovers Point, California	N _o	Primary treated sewage	Intermittent "watering" with seawater	22 C	Enterococci was significantly higher after "watering" periods
Standridge 1979	Madison, Wisconsin	Yes	Isolated fecal coliform organisms	N/A	20-22 C	Significant increase (over 1 log) in fecal coliform concentrations in 4 days

Table 2.1: Summary of replication studies focused on FIB in beach sand.

2.6 Release of FIB from the foreshore reservoir to surface water

Once contaminated, foreshore sands and pore water can act as a non-point source resulting in high FIB concentrations in shallow waters (Byappanahalli et al. 2003, Lee et al. 2006, Shibata et al. 2004, Whitman and Nevers 2003, Wright et al. 2011). However, the mechanisms by which FIB are transported from the foreshore reservoir to surface waters are unclear. FIB may be transported from the reservoir to surface waters via foreshore sand erosion and subsequent release of FIB from suspended sand grains, or alternatively via pore water flow and discharge. Russell et al. (2012) combined field and laboratory experiments to show that vertical infiltration of surface water through sand may deliver sand-associated FIB to saturated beach groundwater with FIB then transported to the adjacent surface water via pore water flow and discharge. Yamahara et al. (2007) found similar results with almost 100% of enterococci in a sand column mobilized and transported through the column when subjected to approximately four pore volumes of vertical flow. In contrast, Phillips et al. (2011) observed limited FIB mobility with 90% of enterococci initially in their column experiments remaining attached to the sand after being subjected to average pore water flows up to and sometimes over 40 cm/h. These contrasting findings are likely due to the different flow and sediment conditions and highlight the need to understand how specific beach conditions (i.e. sediment type) affect the relative contribution of different transport mechanisms in delivering *E. coli* from the foreshore reservoir to the surface water.

In addition to pore water flow and discharge, FIB can also be released from the sand/sediment to the surface water through erosion and sediment resuspension associated with wave action (Byappanahalli et al. 2003, Vogel et al. 2016, Whitman and Nevers 2003). Whitman and Nevers (2003) concluded that foreshore beach sand may be an important non-point source of *E. coli* to lake water rather than a net sink. A study on Lake Ontario using microbial source tracking (MST) techniques determined that *E. coli* recovered from ankle and knee-deep water samples collected up to 150 meters offshore, mostly came from beach sand (Edge and Hill 2007). Gast et al. (2011) showed that FIB in beach sands were redistributed during a period of high wave intensity, however, no simple redistribution pattern (e.g. net movement of sand-associated FIB from foreshore to offshore) was observed.

Mechanistic models often show a strong correlation between wave height and high FIB concentrations in surface waters (Feng et al. 2013, Ge et al. 2012). Coupling a microbehydrodynamic-morphological model with field measurements, Feng et al. (2013) recently concluded that foreshore sand and offshore sediment resuspension due to waves and tides were the main contributor of FIB to surface water at an embayed beach. However, this work assumed that the cross shore distribution of FIB associated with sand was stable over time – this is unlikely during intensified wave conditions as significant sediment redistribution typically occurs. Phillips et al. (2014) found that waves were only capable of releasing about 60% of the total bacteria in seeded foreshore sand in a laboratory wave flume experiment. Sand erosion, however, was limited in this laboratory study. The attachment and detachment of FIB to sand grains also affects its transport in the foreshore beach environment and the association of FIB with sand grains depends on the sand/sediment characteristics (e.g, fine vs. course grained sand). Haack et al. (2003) suggested that coarse sands compared to fine sands generally have low numbers of FIB and would therefore have little effect on the delivery of bacteria to surface water. While these studies indicate that the foreshore reservoir may be an important nonpoint source of FIB during periods of high wave intensity, the mechanisms by which FIB is transported from the foreshore reservoir to surface waters during these periods remains unclear.

2.7 Beach surface water quality models

Previous research has considered how environmental factors impact *E. coli* concentrations in the surface water (e.g. Boehm et al. 2002, Enns et al. 2012, Fujioka et al. 1981, Whitman et al. 2004). Understanding these relationships have been applied to develop statistical and mechanistic models for predicting beach water quality, and for improving conceptual understanding of FIB fate at beaches, respectively. Statistical models allow for decision makers to open or close a beach much earlier than obtaining water quality results, which in turn can help to better protect public health. Utilizing statistical models is also cost efficient, potentially eliminating the need for collecting frequent water quality samples. Nevers and Whitman (2005) developed statistical models based on environmental data such as wave height, turbidity, precipitation, and wind direction. Due to the influence of wind direction on the impact of a nearby river on the beach water quality, this study developed separate models for days with prevailing onshore and offshore winds. The models developed predicted *E. coli* concentrations with 64% and 32% of the variance explained by onshore and offshore winds, respectively. While *E. coli* concentrations were predicted fairly accurately during periods with onshore winds, when the source of bacteria (nearby river) was known, the models were not able to predict the water quality during periods of offshore winds, when the source of bacteria was not as clear.

Mechanistic models which consider fundamental physical and biological processes are considerably more complex have generally been developed to improve conceptual understanding of FIB concentrations and the fate of bacteria at beaches. These models have been able to evaluate the influence of different sources on water quality at numerous beach types (Feng et al. 2013, Ge et al. 2012, Zhu et al. 2011). As mentioned in Section 2.6, Feng et al. (2013) paired a mechanistic model with field data to show that foreshore and offshore sand was a leading source of FIB at their field site. The foreshore reservoir is not included in most statistical predictive surface water quality models. Safaie et al. (2016) used a combined modeling approach which uses insights derived from mechanistic models to improve statistical models and vice versa. Both statistical and mechanistic models require further development and will likely become a key tool for beach managers and health departments to protect human health at recreational beaches (Lušić et al. 2017).

2.8 Methods to sample the foreshore reservoir

As mentioned previously, recreational water quality guidelines worldwide do not currently require health units to sample sand or pore water as part of their monitoring programs (Health Canada 2012, United States Environmental Protection Agency 2012). Currently there is no single preferred method for the collection and analysis of sand/sediment or pore water samples (Solo-Gabriele et al. 2015). Various studies have quantified FIB in the foreshore reservoir by collecting unsaturated surface sand samples (Ferguson et al. 2005, Halliday et al. 2014, Whitman and Nevers 2003) or saturated sand samples (Byappanahalli et al. 2006, Desmarais et al. 2002, Hernandez et al. 2014), while other studies only sample the pore water (Boehm et al. 2004, Edge et al. 2010, Whitman et al. 2006). In addition to different components of the foreshore reservoir being sampled, there are also multiple methods being used to sample each component in the reservoir (i.e. unsaturated sand, saturated sand and pore water) (Table 2.2). Studies have sampled unsaturated surface sand by skimming the surface of the sand (Le Fevre and Lewis 2003, Lee et al. 2006, Staley et al. 2015), using a core (Gast et al. 2011, Kinzelman and McLellan 2009, Phillips et al. 2011), and taking

composite samples (Ishii et al. 2007, Shah et al. 2011, Yamahara et al. 2007). Saturated sand has been sampled using a core (Alm et al. 2003, Desmarais et al. 2002, Russell et al. 2012) or a shovel to reach the saturated sand (Byappanahalli et al. 2006, Staley et al. 2015, Whitman et al. 2006). Groundwater wells (Boehm et al. 2004), drive point samplers (Skalbeck et al. 2010, Vogel et al. 2016), and shovels (Edge et al. 2010, Whitman et al. 2006) have been used to access pore water for sampling. Within a given collection approach there are many other variables that can affect the quantification of *E. coli* in the reservoir including subsampling, amount of sample collected, size and type of sampling equipment used (i.e. length and diameter of core, sterile spoons), and sampling depth.

Table 2.2. Summary of sample collection methods used in select studies focused on FIB abundance in beach sand and pore water. Reproduced from Vogel et al. (2017).

*Drive Point samplers are well point systems that can be used to sample groundwater at depth while providing minimal disruption to the aquifer (Charette and Allen 2006)

 \mathbf{W}^T \mathbf{A} \mathbf{B} \mathbf{A} \mathbf{B} \mathbf{A} \mathbf{B} \mathbf{A} \mathbf{B} \mathbf{A} \mathbf{B} \mathbf{A} \mathbf{B} \mathbf{B} \mathbf{B} \mathbf{A} \mathbf{B} \mathbf{B} \mathbf{B} \mathbf{B} \mathbf{B} \mathbf{B} \mathbf{B} \mathbf{B} \math

Saturated Sand Sampling Methods

In addition to multiple techniques being used to sample sand for FIB, there are also several methods used to quantify that amount of FIB in a given sample. A study conducted by Boehm et al. (2009b) compared several different methods of extractions and different reagents. They compared 22 different methods of extraction and reported only slight differences between methods. They suggested that the easiest method with the highest FIB recovery consisted of 2 minutes of hand shaking within phosphate-buffered saline (PBS) or deionized water, a 30 second settling time, a one-rinse step, and a 10:1 eluant volume to sand weight ratio (100mL eluant: 10g sand) (Boehm et al. 2009b).

The accuracy of the different sampling methods in quantifying FIB in the foreshore reservoir, which is an important source of FIB to surface water as well as represents a potential direct human health risk (Heaney et al. 2012), is not understood. A standard method for quantifying the abundance of FIB in the foreshore reservoir (saturated and unsaturated foreshore sand with interstitial pore water) and the potential impact the reservoir may have on FIB concentrations in adjacent surface waters needs to be developed to evaluate the associated risk (Solo-Gabriele et al. 2015).

2.9 Conclusion

Although extensive research has been conducted to understand the abundance and transport of FIB in the beach environment including the role of foreshore sand and pore water as a potential reservoir and source of FIB to surface water, there are still major knowledge gaps. Currently, there are no standard methods for collection and analysis of sand and sediment samples. In order to properly compare FIB concentrations between studies and beaches, a standard method for the collection and analysis of beach sands needs to be adopted, like that of surface water sampling/analysis. According to the Guidelines for Canadian Recreational Water Quality (Health Canada 2012), there is not presently any conclusive evidence that there is a relationship between contact with beach sands and illness among beachgoers, therefore a guideline value cannot be established for the concentrations of FIB in beach sand. Further, it is thought that routine monitoring of sand samples for FIB is currently not practical and is therefore not recommended. If foreshore beach sands prove to be an important source of FIB for surrounding water, then a practical method of quantifying this risk needs to be developed to improve prediction of beach closures. When quantifying the potential risk to human health based on FIB concentrations, we need to take into account the possibility of accumulation and even replication of FIB in the foreshore reservoir at beaches. If FIB are able to thrive in the environment then they may not be the best indicator of sewage contamination or other health risks. Understanding short- and longterm variations in FIB concentrations and the environmental factors that affect this variation can also lead to better water quality prediction methods. Further, the transport processes that FIB undergo between the surface water and foreshore reservoir and the physical and environmental factors that affect these processes are currently not well understood. For example, the mechanisms controlling the release of FIB from the foreshore reservoir to the surface water are unclear. It is crucial to enhance understanding of the mechanisms that control the fate of FIB in beach sand and the transport of FIB between the surface water, pore water, and sand to improve the prediction of beach advisories. This is needed to improve water quality advisory models and thus help beach managers warn the public of contamination before the event, as opposed to 24-48 hours afterwards as is current practice.

2.10 References

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Chapter 3

3 Temporal variations in the abundance of fecal indicator bacteria in foreshore sand and porewater at freshwater beaches

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3.1 Introduction

High fecal indicator bacteria (FIB) in surface water leads to water quality advisories at recreational beaches, adversely impacting their recreational and economic value (Austin et al. 2007). In the United States and Canada, beach water quality advisories are issued based on concentrations of FIB (*E. coli* and enterococci at freshwater and marine beaches, respectively) in water samples taken between ankle- to chest-depth surface water (Enns et al. 2012, Health Canada 2012, United States Environmental Protection Agency 2012). Over the last decade it has been widely shown that FIB concentrations are often elevated in foreshore beach sand and pore water (herein referred to as the foreshore reservoir) on a bulk volumetric basis relative to adjacent surface water (e.g. Kinzelman et al. 2004, Russell et al. 2012, Staley et al. 2015, Whitman and Nevers 2003). Particularly at non-point source beaches, the foreshore reservoir can be an important source of FIB to nearshore surface waters thereby triggering a beach water quality advisory (Bai and Lung 2005, Edge and Hill 2007, Vogel et al. 2016, Yamahara et al. 2007). This reservoir may also represent a potential direct health risk to beachgoers (Heaney et al. 2009, Solo-Gabriele et al. 2015). While the influence of environmental factors (e.g., wave conditions, rainfall, temperature, UV, and currents) on surface water FIB concentrations has been well studied in order to improve prediction of beach water quality exceedances (e.g. Enns et al. 2012, Nevers and Whitman 2005, Olyphant and Whitman 2004, Vogel et al. 2016, Whitman et al. 2004), there is limited understanding of how FIB concentrations (sand and pore water) in the foreshore reservoir vary at long- (seasonal) and short-term (daily) time scales. Further, the environmental factors that affect this variability including the relationship between FIB concentrations in the surface water and foreshore reservoir are unclear (Russell et al. 2012, Whitman and Nevers 2003). Understanding short-term (daily) and long-term (seasonal) variability in foreshore sand and pore water FIB concentrations is needed to better understand environmental factors affecting FIB accumulation in the reservoir, when and if the reservoir will affect the surface water quality, and to improve management strategies for reducing microbial contamination at beaches.

The accumulation of FIB in the foreshore reservoir is complex due to the numerous sources which can contribute FIB to the reservoir, dynamic interactions and subsequent exchange of FIB between the foreshore reservoir and nearshore surface waters, and the various factors that affect the persistence of FIB in pore water and sand. Ishii et al. (2007) suggested that in addition to point sources (raw sewage, sanitary sewer and storm water discharge) that may contribute FIB to the foreshore reservoir, surface water exchange across the sediment-water interface in the foreshore area may deliver FIB from surface water to sand. Gast et al. (2015) and Wu et al. (2017) supported this theory with field experiments using microspheres and modelling, respectively. Gast et al. (2015) observed that microspheres, which they used as surrogates for bacteria, were able to be transported from their initial location (0.05 m below the sand surface just below the predicted high tide line) vertically to the groundwater below the sand by surface water infiltration. Wu et al. (2017) showed that FIB may accumulate in the foreshore reservoir under low energy wave conditions due to the continuous exchange of water across the sediment-water interface in the foreshore area. They found that the amount of FIB that can accumulate in the foreshore reservoir over a few days of low energy wave conditions may be sufficient to trigger a beach water quality advisory if the foreshore sand is subsequently eroded to the surface water if waves increase and become erosive. At marine beaches, Yamahara et al. (2009) found that in addition to tide-induced water exchange across the sediment-water interface delivering FIB to intertidal sands, periodic tidal wetting can stimulate growth of FIB in beach sands. Once delivered to the foreshore reservoir, the sand provides FIB protection from solar radiation which is known to increase die off rates in surface water (Enns et al. 2012, Whitman et al. 2004). Higher nutrient availability (Byappanahalli et al. 2006, Whitman et al. 2003) and favorable moisture conditions (15-19% (Beversdorf et al. 2007)) and temperature (Staley et al. 2016) in foreshore sands has also been shown to increase the persistence of FIB in the foreshore reservoir. During high energy (erosive) wave conditions FIB can be released from the foreshore sand and pore water to adjacent surface water through sand erosion as well as pore water flow (Gast et al. 2011, Vogel et al. 2016).

A few studies have examined seasonal variability in *E. coli* concentrations in the foreshore reservoir. Studies conducted at Great Lakes (freshwater) beaches have observed an increase in *E. coli* concentrations in the foreshore reservoir during the early summer months (Ishii et al. 2007, Whitman and Nevers 2003, Whitman et al. 1999). From sampling three times per week between April-September, Whitman and Nevers (2003) reported a gradual increase in *E. coli* concentrations in foreshore sand and surface water at a Lake Michigan beach throughout the sampling season with concentrations correlated to the air temperature (Whitman and Nevers 2003). Ishii et al. (2007) observed increasing *E. coli* concentrations in the foreshore reservoir as well as in the upshore sand and surface water from April through July with concentrations at all locations

declining after August. They observed relatively high *E. coli* concentrations in the fall compared to the early spring, even though the temperatures in the beach sediment were similar. Ishii et al. (2007) attributed this to *E. coli* persisting and continuing to accumulate in the reservoir through the summer months. However, this study was limited by only having one sampling event per month at one beach. Short term (daily) variability was not captured in these aforementioned studies and as such the influence environmental forcing on short term variability on FIB levels in the reservoir are unknown. Further, there is a need to evaluate the dynamics of FIB concentrations in the foreshore reservoir at different beach types (urban/rural, point source/non-point source, etc.) to more broadly understand and generalize factors controlling the temporal variability.

Temporal variability of FIB in the foreshore reservoir can be affected by varying sources of FIB (e.g. birds, stormwater inputs), environmental factors (e.g. rainfall, waves, temperature) as well as by changing bacterial persistence and potential growth. The possibility of growth or replication of FIB in the sand has been widely debated. Studies have investigated the possibility of *E. coli* (Alm et al. 2006, Byappanahalli et al. 2006, Craig et al. 2004, Hartz et al. 2008, Staley et al. 2016) and enterococci (Hartz et al. 2008, Yamahara et al. 2009) replication for different sand and experimental conditions (Table 3.1). Microcosm studies have shown an increase in FIB in beach sand after the addition of external stimuli (e.g. seawater, plankton, algae) or after alteration of the sand (e.g. autoclaving, inoculation). For example, Byappanahalli et al. (2006) observed significant growth of *E. coli* after supplementing foreshore sand with lake plankton and incubating at 23.5 °C. Hartz et al. (2008) showed a 2-log increase in enterococci concentrations after inoculating rinsed and autoclaved foreshore sand but did not observe the same replication in their control which was inoculated into unsterilized sand. This latter study suggests that although FIB have the potential to replicate in beach sand, it is unlikely to be significant in unsterile, natural sand due to competition effects and predation. In contrast, Yamahara et al. (2009) observed significant replication of enterococci in unseeded, unsterilized sand subjected to tidal wetting (intermittent wetting of the sand with filtered seawater). Similar to Hartz et al. (2008), their unaltered control microcosms that were not subjected to tidal wetting showed limited enterococci replication. Staley et al. (2016) conducted microcosm experiments at 15º and 28º C using unsterilized foreshore beach sand from a fine and coarse sand beach inundated with sterile beach water and inoculated with eight strains of *E. coli*. While this study observed some persistence of *E. coli* in the lower temperature microcosms, no significant increase in concentrations were observed. Although several studies

have shown that replication is possible, none have observed significant increases (replication) of FIB concentrations in unaltered, unseeded, natural beach sand not subjected to any external stimuli (e.g. added moisture or nutrients).

Paper	Site	Sterilized	Seed	Amendments	Incubation temperature	Findings
Alm 2006	Port Huron, Michigan	Yes	Two E. coli isolates from field site	N/A	19° C	Significant increase (~4 logs) within 2 days; persistence observed through experiment (36d)
Byappanahalli 2006	Michigan City, Indiana	N _o	N/A	Plankton (microcrustacea ns, rotifers, and filamentous algae)	23.5° C	Significant increase $(\sim 2 \text{ logs})$ in E . <i>coli</i> numbers over 24 hours; no increase in control; persistence observed in control for 5 days and through experiment $(7 d)$ for amended sand
Hartz 2008	Hollywoo d, Florida	Yes	Six isolates of E. coli and enterococ ci	Sterile seawater, sea salts	20° C, 30°C, 40°C	Significant increase $(\sim 2 \log)$ within 2-3 days and faster increased observed in highest temperature; no increase in nonseeded control; persistence observed through experiment (14 d)
Staley 2016	Burlington and Toronto, Ontario	N _o	Eight isolates of E. coli from field site	Autoclaved lake water	15° C, 28° C	No significant increase in E . coli concentrations; persistence observed through experiment (28 d); persistence was higher for lower temperature and finer grain sand
Standridge 1979	Madison, Wisconsin	Yes	Isolated fecal coliform organism S	N/A	20-22 C	Significant increase (over 1 log) in fecal coliform concentrations in 4 days; persistence observed through experiment (28 d)
Yamahara 2009	Lovers Point. California	N _o	N/A	Intermittent "watering" with filtered seawater	20° C	Significant increase $(-1 \log)$ in enterococci concentrations after "watering" period and no increase in control; persistence observed through experiment (45 d)
Yamahara 2012	Lovers Point, California	No	Primary treated sewage	Intermittent "watering" with filtered seawater	$22^{\circ}C$	Significant increase (~1 log) in enterococci concentrations after "watering" period and no increase in control; persistence observed through experiment (30 d); faster decay observed in control

Table 3.1: Summary of experimental studies that have investigated FIB replication in beach sand.

The objective of this study was to evaluate long (seasonal) and short (daily) variability in sand and pore water *E. coli* concentrations in the foreshore reservoir and surface water at freshwater beaches including evaluating the influence of different environmental forcing. Due to its potential control on the abundance of FIB in the reservoir, the study also evaluates if growth/replication of FIB is possible in unaltered natural foreshore beach sand. This study focuses on temporal variability in *E. coli* concentrations at three freshwater beaches on the Great Lakes that are impacted by different external sources of FIB, and have different sediment conditions. While many findings may be relevant for marine beaches, temporal variability at marine beaches are expected to differ due to tidal effects, salinity effects and in some occasions more constant (seasonal) surface water temperatures. The findings from this study are needed to improve understanding of the processes controlling the accumulation of FIB in the foreshore reservoir and its subsequent release to surface water, and thus to ultimately improve water quality predictions at recreational beaches.

3.2 Methods

3.2.1 Field site descriptions

Three exposed beaches (directly open to the lake) located in southern Ontario, Canada were selected for this study based on their different physical conditions and different external *E. coli* sources. Ipperwash Beach on Lake Huron is a fine sand non-urban beach $(d_{50} \text{ [median diameter]})$ $= 0.16$ mm; Coefficient of Uniformity [CU, calculated as d_{60}/d_{10} based on sieve size analysis] = 2.13) with frequent high wave conditions due to its north-west exposure. This beach has been studied extensively by Malott et al. (2016), Vogel et al. (2016), and Vogel et al. (2017). Approximately 23% (Strybos et al. 2011) of weekly waist-depth surface water samples collected from May-August 2005-2010 exceeded Ontario's recreational water quality standard (100 CFU/100mL) (Ontario Ministry of the Environment and Energy 1999). The foreshore beach slope (measured from the shoreline to approximately 6 m further landward) was 0.13 and the offshore beach slope (measured from ankle- to waist-depth surface water) was 0.022. The average groundwater hydraulic gradient at the beach site is around 0.014 (Malott et al. 2016). Ausable River enters Lake Huron approximately 6 km northeast of Ipperwash Beach and is a possible source of *E. coli* to the beach. Marie Curtis Beach is a coarse sand urban beach $(d_{50} = 1.37$ mm; $CU = 6.84$) on Lake Ontario with an average groundwater hydraulic gradient of 0.002. The

foreshore and offshore beach slopes were measured to be 0.12 and 0.091, respectively. Marie Curtis Beach was posted 61% of the time during summer months from 1995-2003 based on waistdepth surface water samples (Environmental Defence 2004). The major sources of *E. coli* at this beach are Canada geese, ducks, and other birds (Beach Guides 2015) as well as Etobicoke Creek which discharges to Lake Ontario at the southern extent of the beach ~ 200 m from the sampling location). Burlington Beach is a fine sand urban beach ($d_{50} = 0.20$ mm; CU = 1.49) on Lake Ontario. The foreshore and offshore beach slopes were measured to be 0.12 and 0.010, respectively. Water quality data from the summer months of 2009-2016 indicate that 23% (Lake Ontario Waterkeeper 2016) of waist-depth surface water samples exceeded Ontario's water quality standard of 100 CFU/100mL. Approximately 1.5 km from the field site is the Burlington Bay Canal which links Lake Ontario to Hamilton Harbor and may be a source of *E. coli* to the beach. Due to groundwater dewatering at a nearby construction site, groundwater was flowing landward at the beach (foreshore hydraulic gradient ranged from -0.005 to -0.01).

Climate data (temperature, rainfall, and wind), creek discharge, wave data (height and direction), and beach slope were collected for the three field sites if available. *E. coli* concentrations were compared to average daily temperature from the previous day, total rainfall and creek discharge added from the previous day and sampling day, and averaged wind and wave data from the previous 12 hours. These parameters have previously been found to correlate with *E. coli* concentrations in the surface water at beaches (e.g. Gast et al. 2011, Morrison et al. 2003, Olyphant and Whitman 2004, Phillips et al. 2014).

3.2.2 Field sample collection methods

Water and sand samples were collected biweekly at Burlington Beach and Marie Curtis Beach from May through November in 2013 and 2014 and at least once a week at Ipperwash Beach from May through November 2014. Daily sampling was also conducted at Ipperwash Beach from July 7, 2015 – August 10, 2015. The total number of samples collected at each beach is indicated in Table 3.2. For all sampling events at all beaches, pore water and sand samples were collected 1 m from the shoreline in three cross shore transects, approximately 10 m apart. Along each transect, pore water samples were collected by carefully digging a hole with a shovel, minimizing any sand collapsing into the hole, and collecting the pore water with a 250 mL bottle. Saturated sand was

collected by scraping a sterile spoon at the bottom of the hole and placing the sand in a sterile Whirlpak bag. The sand was collected after the pore water to avoid disturbing the sand and releasing sand-attached *E. coli* to the pore water. Unsaturated sand was collected by scraping a sterile spoon along the top 1 cm of undisturbed surface sand around the hole and placing the sand in a sterile Whirlpak bag. Approximately 100 g of sand was collected for each sample. These sampling techniques are discussed further by Vogel et al. (2017). Surface water samples were also collected along each transect at ankle- and waist-depth with 500 mL sterile propylene bottles.

3.2.3 Replication and die-off experiments

Approximately 10 kg of foreshore unsaturated sand was collected on September 1, 2015 at Ipperwash Beach and on June 27, 2016 from Burlington Beach and Marie Curtis Beach with the sand placed in a separate aseptic plastic container. The top 5 cm of surface sand was collected approximately 1 m from the shoreline. Within 4 hours of collection, the sand in each container was homogenized and a subsample (25 g) was taken for *E. coli* enumeration and moisture content. The containers were then placed in an environmental chamber (Thermo Scientific, Forma Environmental Chamber, Model: 3940) set to average summer conditions (20.2 °C and 74% humidity). The containers were covered with a lid but were not sealed. For each experiment, four sand samples (25 g) were collected from the container in the environmental chamber every 6-72 hours for approximately one month for *E. coli* enumeration (three sand samples) and moisture content measurements (one sand sample). Sand from Ipperwash beach was analyzed on 15 occasions, while sand from Marie Curtis Beach and Burlington Beach were analyzed on 12 occasions.

		Foreshore reservoir	Surface water			
	Unsaturated	Saturated	Pore water	Ankle-depth	Waist-depth	
	sand	sand	$(\log$	$(\log$	$(\log$	
	$(\log CFU/g)$	$(\log CFU/g)$	CFU/100mL	CFU/100mL)	CFU/100mL	
Ipperwash	0.83 ± 0.07	0.30 ± 0.05	2.33 ± 0.05	1.89 ± 0.04	1.51 ± 0.04	
n	227	245	254	199	208	
Burlington	1.20 ± 0.11	0.46 ± 0.11	2.42 ± 0.08	2.09 ± 0.09	1.19 ± 0.08	
n	76	72	77	78	78	
Marie	1.10 ± 0.10	0.62 ± 0.07	2.75 ± 0.07	2.23 ± 0.09	2.12 ± 0.09	
Curtis						
$\mathbf n$	69	69	72	71	71	

Table 3.2: Mean ± standard error *E. coli* **concentrations at the three field sites. The sample number (n) is provided for all sand and water samples.**

3.2.4 *E. coli* enumeration

Water and sand samples collected in the field were stored on ice, transported to the laboratory, and analyzed within 6 hours of sampling. Sand samples collected from the environmental chamber for the replication and die-off experiments were analyzed immediately upon subsampling. Water samples were filtered (0.45 μm pore size) using standard membrane filtration methods (American Public Health Association 1999) and placed on chromogenic differential coliform (DC) agar, supplemented with cefsulodin. The filter and agar were incubated at 44.5 °C for 20 hours and *E. coli* was then enumerated as colony forming units (CFU/100mL). To extract *E. coli* from the sand, 25 g from each homogenized sand sample was placed in a sterile polypropylene bottle, diluted with 250 mL of phosphate-buffered saline, hand shaken for 2 minutes, and allowed to settle for 2 minutes (Boehm et al. 2009b). The supernatant was then processed using the same method as the water samples. For the field samples, an additional 25 g from each sand sample was used to quantify the sand moisture content to enable expression of sand-associated *E. coli* as CFU/g of dry sand.

3.2.5 Statistics

Statistical analysis was performed on data using Microsoft Excel, Minitab (Minitab Inc., San Jose, CA), and Matlab (MathWorks, Natick, MA). *E. coli* concentrations were log-transformed prior to
analysis. ANOVA and Tukey's post hoc were used to analyze seasonal trends for Ipperwash Beach. Kruskal-Wallis was used to analyze seasonal trends for Marie Curtis Beach and Burlington Beach because of the smaller sample sizes. Pearson correlation analysis was performed to compare *E. coli* concentrations and environmental factors and test for linear relationships. A two-sample ttest was used when comparing *E. coli* concentrations between two components (pore water, saturated sand, unsaturated sand) of the foreshore reservoir. Results were considered significant with a p-value of less than 0.05.

3.3 Results and Discussion

3.3.1 Seasonal variations in the foreshore reservoir and surface water

Understanding seasonal variations in FIB concentrations in the foreshore sand and pore water at beaches is needed to improve beach water quality management programs including prediction of surface water quality exceedances, especially at nonpoint source beaches for which a relationship between foreshore reservoir and surface water concentrations has been shown (Alm et al. 2006, Vogel et al. 2017). *E. coli* concentrations in the foreshore reservoir (unsaturated sand, saturated sand and pore water) at the two urban beaches, Burlington Beach and Marie Curtis Beach, did not follow a distinct temporal trend over the sampling season (i.e., did not increase as air temperatures increased and then decrease with air temperature) (May – November; Figure 3.1b,d). For example, although the unsaturated sand concentrations increased from May-August and then decreased from August-November, the only statistically significant finding was that *E. coli* concentrations in the unsaturated sand were statistically lower in November than the rest of the season $(p<0.001$ for Marie Curtis (except for May), p=0.003 for Burlington). Similarly pore water *E. coli* concentrations at Marie Curtis Beach were statistically higher in June and August than in November (p=0.036), whereas at Burlington Beach pore water concentrations were statistically lower in September compared to August and October (p=0.005). While no statistical differences were observed for monthly saturated sand *E. coli* concentrations at Marie Curtis Beach (p=0.116), saturated sand concentrations were statistically lower in September than in June and August (p=0.025) at Burlington Beach.

E. coli concentrations in the surface water at Burlington Beach increased during the initial summer months with peak *E. coli* concentrations in the waist-depth water observed in August and in the ankle-depth water in October (Figure 3.1a). *E. coli* concentrations in the surface water during the peak months (October for ankle-depth and August for waist-depth) were statistically higher than concentrations in the months before (May, June, July) or after (November) this period $(p<0.001$ for ankle- and waist-depth). The high concentration of *E. coli* observed in the ankle-depth water towards the end of the monitoring season at Burlington Beach may be due to large amounts of algae observed in the shallow lake water during September and October. Unlike at Burlington Beach, Marie Curtis Beach did not follow a consistent trend with respect to *E. coli* concentrations in the surface water (Figure 3.1c). There was no statistical difference observed in *E. coli* concentrations in the surface water from June through November ($p=0.097$ for ankle-depth and p=0.299 for waist-depth samples). The month of May was not included in the statistical analysis since it only consisted of one sampling event.

Figure 3.1: Average monthly *E. coli* **concentrations in the surface water and foreshore reservoir at the three field sites. Air temperatures and concentrations at Ipperwash Beach were an average of 2014 and 2015, while temperatures and concentrations at Marie Curtis Beach and Burlington Beach were an average of 2013 and 2014. The number above each bar indicates the sample number for each month. Error bars indicate +/- one standard error from the mean. Ankle-depth, waist-depth, and pore water concentrations are reported in log CFU/100mL. Unsaturated sand and saturated sand concentrations are reported in log CFU/g.**

Pearson correlation analyses were performed to evaluate how the temporal variability in *E. coli* concentrations at a given beach may relate to different environmental conditions (temperature, waves, rainfall). *E. coli* concentrations and environmental data for individual sampling dates were used for this analysis and correlation plots are included in Appendix A. The distance between ankle- and waist-depth water was relatively small at Marie Curtis Beach \sim 10 m) due to a steep offshore beach slope. The smaller distance between ankle- and waist-depth led to a greater connectivity between the two locations, resulting in ankle- and waist-depth *E. coli* concentrations that were not significantly different from each other $(p=0.417)$ and similar results when comparing the concentrations to environmental conditions. *E. coli* concentrations in the unsaturated sand as well as ankle- and waist-depth surface water at Marie Curtis Beach were found to be positively correlated with mean daily temperature – as temperature increased higher *E. coli* concentrations were observed ($r=0.457$, $p=0.002$ for unsaturated sand; $r=0.570$, $p=0.007$ for ankle-depth; and r=0.457, p=0.042 for waist-depth). This suggests that *E. coli* may persist or replicate (as discussed in a later section) when the temperature is warmer, especially in the sand. Seasonal temperature variations do not seem to control *E. coli* concentrations in the unsaturated sand at Marie Curtis Beach, as seen with the lack of seasonal trends, but this result suggests that perhaps short-term temperature variations may be more important. *E. coli* concentrations in the unsaturated sand and ankle- and waist-depth water were also significantly correlated, indicating a link between the foreshore reservoir and surface water (r=0.611, p=0.002 for ankle-depth; and r=0.584, p=0.003 for waist-depth). Unsaturated sand *E. coli* concentrations were negatively correlated with wave height (r=-0.740, p=0.036) suggesting that *E. coli* may be released from the foreshore reservoir to surface water during period of high wave activity. This wash out from wave action is explored further in Chapter 4 (Gast et al. 2011, Vogel et al. 2016).

Ankle-depth water *E. coli* concentrations were also correlated with the flow rate in Etobicoke Creek (r=0.411, p=0.046), which discharges to the lake at the southern extent of the beach. Contributions from external inputs (Etobicoke Creek) may explain the absence of distinct seasonal trends in surface water *E. coli* concentrations at Marie Curtis Beach, in contrast to the other two beaches. There were no other correlations between *E. coli* concentrations and environmental conditions at Marie Curtis Beach.

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There were no correlations between mean daily environmental conditions (e.g. temperature, rainfall, wave height) and unsaturated sand or ankle-depth surface water *E. coli* concentrations at Burlington Beach. This may have been due to large amounts of algae covering the unsaturated sand and shallow surface water, possibly serving as a barrier from the external environment, toward the end of the 2014 season. *E. coli* is known to accumulate at high concentrations in algae, with average concentrations at ten Great Lakes beaches measured to be 5.3 log CFU/g (Whitman et al. 2003). Waist-depth surface water concentrations, however, were positively correlated with wave height ($r=0.530$, $p=0.020$), suggesting that there is a relationship between the shoreline/foreshore reservoir and nearshore surface waters. There was also a significant positive correlation between the saturated sand and temperature (r=0.496, p=0.019) at Burlington Beach. While concentrations in the unsaturated sand may also have been influenced by the temperature, this correlation may have been masked by the accumulation of algae. Further, a positive correlation was observed between rainfall and saturated sand (r=0.698, p<0.001) as well as pore water *E. coli* concentrations $(r=0.471, p=0.023)$ at Burlington Beach, but not between unsaturated sand $(r=0.275, p=0.228)$ or surface water concentrations $(r=0.106, p=0.640)$ and $r=-0.225, p=0.314$ for ankle- and waist-depth water, respectively). This finding suggests that rainfall may transport *E. coli* from unsaturated surface sand down to the saturated zone at Burlington Beach. This phenomena has been observed previously by Russell et al. (2012) and Gast et al. (2015).

In contrast to Burlington Beach and Marie Curtis Beach, *E. coli* concentrations in all components of the foreshore reservoir and surface water at Ipperwash Beach showed a distinct seasonal trend with concentrations increasing from May to August and decreasing from August to November (Figure 3.1e,f). The ankle-depth surface water, unsaturated sand, saturated sand, and pore water concentrations were all statistically higher in July, August, and September than in other months $(p<0.001$ for all), corresponding to the months with the highest average temperature. Additionally, *E. coli* concentrations in the unsaturated sand were statistically higher in August than in July or September $(p<0.001)$. This is consistent with the monthly trends in unsaturated sand concentrations shown by Ishii et al. (2007). Waist-depth surface water *E. coli* concentrations showed the same trend but were not significantly different during those months.

E. coli concentrations in the unsaturated and saturated sand as well as ankle-depth surface water were positively correlated with mean daily temperature $(r=0.675, p<0.001$ for unsaturated sand; $r=0.327$, $p=0.012$ for saturated sand, and $r=0.448$, $p<0.001$ for ankle-depth surface water). This positive correlation, especially in the unsaturated sand, may be attributed to increased persistence and possibly replication of *E. coli* in the sand with increasing temperature (Ishii et al. 2007). This is explored further in the Section 3.3.2. A positive correlation was observed between *E. coli* concentrations in the ankle-depth surface water and temperature $(r=0.448, p<0.001)$ as well as rainfall (r=0.218, p=0.004). The correlation between ankle-depth water concentrations and temperature may be due to the association between the ankle-depth concentrations and unsaturated sand concentrations (r=0.383, p=0.001). Increasing *E. coli* concentrations in the ankle-depth water with increasing rainfall may be related to increased discharge from Ausable Creek which is located 6 km north of the beach site. While some studies have shown that the foreshore reservoir can be a source of FIB to nearshore surface waters (Phillips et al. 2014, Vogel et al. 2016, Whitman and Nevers 2003) others have shown that surface waters may be a source of FIB to the foreshore reservoir (Byappanahalli et al. 2006, Ge et al. 2012, Wu et al. 2017). We expect it is likely a combination of both, with a continuous exchange of FIB, however, further work is needed to determine which typically occurs first (e.g. high FIB concentrations in nearshore surface water or in the foreshore reservoir). Vogel et al. (2016) and Vogel et al. (2017) discuss the strong connection between ankle-depth water and unsaturated sand, especially at Ipperwash Beach. Vogel et al. (2016) attributed an increase in surface water *E. coli* concentrations to increased wave conditions coupled with high *E. coli* concentrations in the unsaturated sand at Ipperwash Beach. However, a linear correlation was not observed between wave height and ankle-depth water concentrations during this study $(r<0.001, p=0.997)$. This may be due to the complexity and non-linearity of the relationship between *E. coli* concentrations in the surface water and wave height as observed in (Vogel et al. 2016). Even though there was not a linear correlation between the ankle-depth concentrations and wave height, they may still be related but dependent on other factors including *E. coli* concentrations in the foreshore reservoir and time since previous increased wave activity (Vogel et al. 2016). No significant correlations were observed between waist-depth water and rainfall, wave height or temperature. Due to the smaller offshore beach slope, there was a considerable amount of distance between ankle- and waist-depth at Ipperwash Beach (~30 m) compared to Marie Curtis Beach $(\sim 10 \text{ m})$. Therefore, the foreshore reservoir may not play as important of a role in waist-depth *E. coli* concentrations at Ipperwash Beach.

3.3.2 Growth/persistence experiments

To better understand temporal trends in foreshore *E. coli* concentrations, growth/persistence experiments were conducted on unaltered and unseeded sand from the three field sites. A large increase in *E. coli* concentrations was observed in the unaltered and unseeded unsaturated sand collected from Ipperwash Beach (Figure 3.2a). Within 46 hours, the concentration of *E. coli* in the sand increased from 3.50 log CFU/g $(3.3x10^2$ CFU/g) to 4.71 log CFU/g $(5.1x10^4$ CFU/g). *E. coli* concentrations remained above the initial concentration (C_0) for at least 15 days and were above the detection limit (>1 CFU/g or >0 log CFU/g) for over 30 days. This substantial increase in FIB concentrations in unaltered unseeded beach sand without external stimuli (e.g., addition of nutrients, intermittent rewetting) has not been observed previously.

In the experiment using Burlington Beach unsaturated sand, *E. coli* concentrations increased within the first 6 hours from 2.77 log CFU/g $(5.8x10^2 \text{ CFU/g})$ to 2.90 log CFU/g $(8.0x10^2 \text{ CFU/g})$ (Figure 3.2). Concentrations decreased below the initial concentration within 27 hours. There was no observed increase in *E. coli* concentrations in the unsaturated sand collected from Marie Curtis Beach (Figure 3.2). The greater replication observed in the unsaturated sand from Ipperwash Beach may have been due to its higher initial moisture content (20%) compared to the unsaturated sand collected at Burlington Beach (4%) and Marie Curtis Beach (5%). Beversdorf et al. (2007) showed that *E. coli* thrives at a moisture content of around 15-19%. The average moisture contents in the foreshore unsaturated sand over the two-year sampling period were 23%, 11%, and 17% at Ipperwash Beach, Marie Curtis Beach, and Burlington Beach, respectively. Due to the continuous movement of the shoreline, moisture content in the unsaturated sand can vary greatly (standard deviations for the moisture content in the unsaturated sand were 2%, 6%, and 6% for Ipperwash Beach, Burlington Beach, and Marie Curtis Beach, respectively over the field sampling period). Three days before sand was collected from Ipperwash Beach there was significant wave activity which may have increased the moisture content of foreshore sands, whereas Burlington Beach and Marie Curtis Beach had calm conditions (low wave height) for 11 days prior to sampling, resulting in drier foreshore sand. Moisture content decreased during the experiments because there was no external water source (Figure 3.2b). Overall the experimental results suggest that *E. coli* replication is possible and probable in unsaturated foreshore sand at Ipperwash Beach. In moister conditions, *E. coli* replication may also occur in the unsaturated foreshore sand at Burlington Beach and Marie

Curtis Beach. If FIB are able to replicate in sand at beaches then they may no longer indicate an increased presence of pathogens and would therefore no longer be a suitable indicator for fecal contamination.

Figure 3.2: Replication and die-off of *E. coli* **using unsaturated sand collected at the three field sites. (a)** *E. coli* **concentrations are normalized using the initial concentration (C/C0) Error bars indicate +/- one standard error from the mean. (b) Moisture content measured at each sampling time.**

3.3.3 High frequency variations in foreshore reservoir concentrations

Daily sampling was conducted at Ipperwash Beach for 34 days to evaluate high frequency temporal dynamics in foreshore reservoir *E. coli* concentrations. Samples were taken each day from the same location, measured from a permanent benchmark. The location of the shoreline was relatively consistent over the sampling period with 85% of the foreshore reservoir samples taken 1 m landward of the shoreline on that given day. Due to lakeward and landward movement of the shoreline, 15% of the foreshore reservoir samples were taken either further landward of the shoreline (up to 5 m landward) or from a location that was inundated. Statistical analyses showed no clear correlations between the various environmental factors (waves, rainfall, wind) and the *E. coli* concentrations. Most importantly, the sampling results indicate that *E. coli* concentrations in the unsaturated sand and ankle-depth water exhibit significant temporal variability (Figure 3.3). This conflicts with prior assumptions that have been made regarding FIB levels in the foreshore reservoir. For example, in interpreting their seasonal results, Ishii et al. (2007) suggested that *E. coli* persisted and accumulated in foreshore sand through the summer months, resulting in higher concentrations in the fall months than in the spring months. Although our long-term (seasonal) data agrees with Ishii et al. (2007) (Figure 3.1), the daily sampling data suggests that the temporal dynamics are considerably more complex. As seen in Figure 3.3, on Day 30 unsaturated sand concentrations decreased to 0.28 ± 0.07 log CFU/g and then four days later on Day 34, *E. coli* concentrations increased to 3.18 ± 0.12 log CFU/g. Similar large increases in *E. coli* concentrations were observed throughout the measurement period. The near depletion of *E. coli* in the unsaturated sand following by relatively large concentrations four days later is not consistent with a gradual seasonal accumulation. The low concentrations observed in the unsaturated sand on Day 30 were preceded by three days of high wave activity (*Hrms* > 0.55 m with onshore winds – set based on the upper quartile of observed H*rms* for 5-year wave data at the site) – erosion associated with this wave activity may have washed *E. coli* out of the foreshore reservoir. This pattern also occurred on Day 3 and Day 16 and was observed previously by Vogel et al. (2016). Between Days 30-34 the waves were smaller *(Hrms* < 0.55) with mostly offshore winds. As such *E. coli* concentrations may have rapidly increased over this period due to surface water infiltration and associated accumulation of *E. coli* in the foreshore sand (Wu et al. 2017) or replication. The results from the persistence/growth experiments presented above support the importance of replication as they showed that *E. coli* concentrations can increase over 2 logs within 46 hours in unsaturated sand from Ipperwash Beach. *E. coli* concentrations in the ankle-depth water followed a similar trend to the unsaturated sand concentrations, indicating a link between the surface water and sand, except after some extended periods of high waves when *E. coli* concentrations decreased or remained approximately the same in the sand while concentrations in the surface water increased (e.g. Days 8-9, 11-12, and 26-27). These trends were not statistically significant due to the low sample size. This is consistent with Vogel et al. (2016) who showed *E. coli* in the foreshore reservoir were transported to the surface water during periods of high wave activity.

Figure 3.3: Average daily *E. coli* **concentrations in the unsaturated sand and ankle-depth surface water at Ipperwash Beach from 7 July – 10 August 2015. Error bars indicate +/- one standard error from the mean. Black circles indicate wave height (H***rms***) when winds were coming onshore while grey circles indicate wave height when winds were offshore. Significant wave activity was defined as a period of at least 3 hours with wave height (***Hrms***) > 0.55 m and onshore winds. Blue shaded bars indicate a day where at least 2 mm of rainfall was recorded with the number on the bars indicating the daily rainfall amount.**

3.4 Conclusion

- *E. coli* concentrations in the surface water at three beach sites were found to depend on environmental conditions (e.g. temperature, rainfall, waves) and external sources (nearby creeks). Surface water concentrations at Ipperwash Beach and Burlington Beach were related to the foreshore reservoir concentrations and followed a seasonal trend with concentrations highest during the warmest months. The surface water *E. coli* concentrations at Marie Curtis Beach, which were related to the foreshore reservoir concentrations as well as flow discharge from Etobicoke Creek, did not follow any seasonal trend.
- The foreshore reservoir and ankle-depth surface water at Burlington Beach were covered with a layer of algae toward the end of the bathing season, resulting in little to no correlations between *E. coli* concentrations and environmental factors. This may be an issue when trying to use predictive models based on environmental data to predict water quality exceedances at beaches with algae or other debris that can serve as a barrier between the external environment and *E. coli* in the water/sand.
- A steeper offshore beach slope led to smaller distances between ankle- and waist-depth water at Marie Curtis Beach which resulted in similar *E. coli* concentrations at the two depths. Understanding variations in beach slope and the resulting *E. coli* patterns offshore is important, especially in some U.S. states where Health Departments currently sample at various depths (e.g. ankle-depth, knee-depth, waist depth) as part of their advisory program (Enns et al. 2012).
- A large increase in *E. coli* concentrations was observed in unaltered and unseeded unsaturated sand from Ipperwash Beach. This has not previously been observed. If FIB are able to thrive and even replicate in sand at beaches then they may no longer indicate an increased presence of pathogen contamination. It is critical to understand the potential for FIB to replicate in the beach environment if they are to be used as indicator bacteria for human health at recreational beaches. In addition, statistical and mechanistic models of FIB at beaches need to consider replication, however, more work is needed to parameterize these models as significant FIB growth was only observed at one of the three beaches included in this study.

• Short-term (daily) sampling showed significant temporal variability, indicating that prior studies which have suggested long-term (seasonal) accumulation of *E. coli* in the foreshore reservoir may not be correct and that short-term temporal dynamics are considerably more complex.

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Chapter 4

4 Release of Escherichia coli from foreshore sand and pore water during intensified wave conditions at a recreational beach

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4.1 Introduction

Elevated levels of fecal indicator bacteria (FIB) adversely impact the recreational and economic value of a beach (Austin et al. 2007). FIB such as enterococci at marine beaches, and *Escherichia coli* (*E. coli*) at freshwater beaches, are used for recreational water quality monitoring. The geometric mean (GM) standard for *E. coli* in Ontario for recreational waters is 100 Colony Forming Units per 100 mL (CFU/100mL) sampled at waist-depth (Ontario Ministry of the Environment and Energy 1999). If the indicator concentration is above this value, then a swimming advisory may be issued. It is well recognized that sand and pore water near the shoreline often act as a reservoir for FIB with sand and pore water FIB concentrations, considered on a bulk volumetric basis, much higher than concentrations in adjacent shallow waters (herein referred to as 'foreshore reservoir', Figure 4.2) (Alm et al. 2006, Boehm et al. 2004, Edge and Hill 2007, Ishii et al. 2007, Skalbeck et al. 2010, Whitman and Nevers 2003). Although FIB can freely reside in pore water, they have a high tendency to associate with sand due to a variety of mechanisms including Derjaguin-Landau-Verwey-Overbeek (DLVO) interactions and film straining (Bradford and Torkzaban 2008, Molnar et al. 2015). Therefore a large proportion of FIB are generally associated with sand (Whitman et al. 2014, Whitman and Nevers 2003).

Figure 4.1: Schematic of the foreshore reservoir and transport mechanisms.

The foreshore reservoir may contribute a significant amount of FIB to the surface water either by sand erosion or interstitial pore water flow and discharge (Figure 4.2) (Alm et al. 2006, Bai and Lung 2005, Boehm et al. 2004, Whitman and Nevers 2003, 2008, Yamahara et al. 2007). The potential importance of these two transport mechanisms remains uncertain. Russell et al. (2012) combined field and laboratory experiments to show that vertical infiltration of surface water through sand may deliver sand-associated FIB to beach groundwater with FIB subsequently released back to surface waters via pore water flow and discharge. In contrast, Phillips et al. (2011) observed limited FIB mobility with 90% of enterococci initially in their column experiments remaining attached to the sand. These contrasting findings are likely due to the different flow and sediment conditions and highlight the need to understand how specific beach conditions (i.e. sediment type) affect the relative importance of the different transport mechanisms in delivering *E. coli* from the foreshore reservoir to surface waters.

Statistical regression models often show a strong correlation between wave height and high FIB concentrations in surface waters (Feng et al. 2013, Ge et al. 2012). FIB may be released from the foreshore reservoir to surface waters during periods of high wave intensity (i.e., increased wave height and frequency) due to increased sand erosion, sediment resuspension, and interstitial pore water flow (Feng et al. 2013, Gast et al. 2011, Ge et al. 2012, Phillips et al. 2014). While prior studies have evaluated FIB variability in surface water in response to environmental variables (e.g., tides, solar radiation, rainfall) (Enns et al. 2012, Whitman and Nevers 2008), there is limited knowledge of FIB variability in both surface water and the foreshore reservoir over periods of high wave intensity, as well as factors controlling this variability.

Figure 4.2: Potential transport pathways for *E. coli* **transfer between the foreshore reservoir and adjacent surface water. Sampling locations (P1,P2, P3, ankle and waist) as well as initial (0 hours) sand elevation profile (black line) and water level (blue line) for Event3 are shown. The maximum wave run-up indicates the farthest location landward that the waves reached during Event3. Subset figure in top right hand corner shows the water levels at 0 hours (blue solid line, first sampling time) and 13 hours (blue dashed line, second sampling time), as well as the sand levels at 0 hours (black solid line) and 13 hours (black dotted line) for Event3. The cross-hatched area indicates the area of sand erosion between 0 hours and 13 hours. The locations of surface sand/subsurface sand/ offshore sediment samples (red squares) and pore water/water sample (black cross) for Event3 are shown.**

Previous marine studies have investigated the source of FIB to surface water during intensified wave conditions. Gast et al. (2011) showed that FIB in beach sands were redistributed during a period of high wave intensity, however, no simple redistribution pattern (e.g. net movement of sand-associated FIB from foreshore to offshore) was observed. Coupling a model with field measurements, Feng et al. (2013) recently concluded that foreshore sand and offshore sediment resuspension due to waves and tides were the main contributor of FIB to surface water at an embayed beach. Phillips et al. (2014) found that waves were only capable of releasing about 60% of the total bacteria in seeded foreshore sand in a laboratory wave flume experiment. Sand erosion however was limited in this laboratory study. While these studies indicate that the foreshore reservoir may be an important nonpoint source of FIB during periods of high wave intensity, the mechanisms by which FIB are transported from the foreshore reservoir to surface waters during these periods remains unclear.

This paper presents data from three field events at a freshwater beach that provide insight into the variability of *E. coli* concentrations in surface water and the foreshore reservoir in response to varying wave conditions. Periods of high wave intensity (defined by significant wave height [*Hsig*] being larger than a threshold wave height) occurred during each field event. A mass balance is conducted to determine the relative contribution of sand erosion to the release of *E. coli* from the foreshore reservoir to surface water for all field events. Finally, correlations between sand, pore water, and surface water concentrations are compared for the fine sand study beach and for a coarse sand-cobble beach to infer how the mechanisms by which *E. coli* are released from the foreshore reservoir to surface waters may differ for beaches of different sand type. It is important that the mechanisms by which *E. coli* is delivered to surface waters are understood so that water quality exceedances can be better predicted by statistical and process-based models.

4.2 Materials and methods

4.2.1 Field site descriptions

This study was conducted at Ipperwash Beach on Lake Huron, ON, Canada (Figure B.1). Ipperwash Beach is a dissipative beach extending over 10 km with homogeneous sand conditions (fine silica sand with little organic content; d_{50} [median diameter] = 0.16 mm; Coefficient of Uniformity [CU, calculated as d_{60}/d_{10} based on sieve size analysis (ASTM International 2009)] = 2.13). The beach frequently experiences periods of high wave intensity as well as calm periods with *Hsig* < 0.1 m. Approximately 23% of weekly surface water samples (waist-depth) from May to August 2005-2011 at Ipperwash Beach were found to exceed Ontario's water quality standard (100 CFU/100mL) (Strybos et al. 2011). Ausable River discharges into Lake Huron approximately 6 km northeast of the field site and may be the main source of *E. coli* to surface waters. Bird and other animal activity as well as storm water run-off are not observed to be significant sources of *E. coli*.

Additional sampling was conducted at Marie Curtis Beach to evaluate if our findings may be extrapolated to coarser sand beaches. Marie Curtis is a coarse sand-cobble $(d_{50} = 0.53$ mm, CU = 5.18) beach on Lake Ontario (Figure B.1). Data from the last 5 years show water quality exceedances at Marie Curtis Beach 37% of the time (City of Toronto 2016).

4.2.2 Water and sand sampling

Three 60-80 hour field events (Event1, Event2, Event3) were conducted at Ipperwash Beach on 17-20 June, 8-11 July, and 22-25 July 2014, respectively, to quantify the influence of variable wave conditions on *E. coli* concentrations in the surface water and foreshore reservoir. Periods of high wave intensity, defined by offshore *Hsig* being larger than a threshold wave height, assumed to be 0.55 m, for 3 hours, occurred during all field events. The criteria for high wave intensity was set based on the upper quartile of observed *Hsig* (0.55 m) for 5-year wave data at the site (Fisheries and Oceans Canada 2016). The sampling program was designed to capture high wave intensity periods with these periods predicted *a priori* using forecasted wind speed and direction (Government of Canada 2016). High wave intensity periods generally occur at Ipperwash Beach in response to winds greater than 15 km/hr from the north to north-west.

Surface water, pore water, and sand samples were collected at set locations along a cross-shore transect during the three field events (Figures 4.2 and A.2). Samples were taken 8-12 hours prior to *Hsig* increasing above 0.55 m, two or three times per day while *Hsig* remained high, and then daily once *Hsig* diminished. Sampling times and locations for all events are provided in Tables A.2- A.5. Water and sand samples were collected in sterile polypropylene bottles and Whirlpak® bags, respectively, with all samples collected in triplicate. While additional replicate samples would have been ideal to account for high spatial variability (Solo-Gabriele et al. 2015), this was not feasible as the time required for water and sand sample collection, transportation to the laboratory, and subsequent analysis was approximately 6 hours, while the sample interval was sometimes 4 hours. For all sampling times, surface water samples were collected in triplicate at ankle- and waist-depth. Pore water, surface sand and, subsurface sand samples were collected in triplicate at two locations (P1, P2) along the cross-shore transect (Figure 4.2). P1 was located 1 m landward of the initial (time $= 0$ hours) shoreline and P2 was located 1 m landward of the predicted maximum wave run-up limit. The maximum wave run-up limit was predicted based on our prior observations of the shoreline movement at the site. For Event3, an additional pore water/sand sampling location (P3) was added further onshore to account for the larger than predicted maximum wave run-up (Figures 4.2 and A.2).

Different methods were used to collect the pore water and sand samples during the field events. During Event3, triplicate sand samples were collected at P1, P2 and P3 at all sampling times using clear polyethylene cores (0.5 m length, 0.05 m diameter). The cores were hammered 0.5 m vertically into the sand, and then dug out from the side so as not to disturb the sample. The top 0.2 m and bottom 0.2 m of sand were removed from each core with sterile spoons and placed in Whirlpak® bags – these samples are referred to as surface sand and subsurface sand, respectively. Triplicate pore water samples at P1 during Event3 were collected using three drive-point samplers (Charette and Allen 2006) installed permanently over the event to enable pore water to be sampled when the location P1 was submerged. Triplicate pore water samples for other locations during Event3 (P2, P3) and for all locations during Event1 and Event2 were collected by digging three holes to the water table with a sterile shovel and collecting pore water that accumulated in the hole in sterile polypropylene bottles. For Event1 and Event2, surface sand was collected adjacent to the top of each pore water hole and subsurface sand was collected from the bottom of each hole. A similar pore water/sand sampling method was used by Edge et al. (2010). For Event3, triplicate offshore sediment samples were collected at ankle- and waist-depth by collecting the top 0.05 m of sediment from the lake bottom with a sterile spoon. Triplicate suspended sand samples in the water column at ankle-depth were also collected during Event₃ using a 0.34 x 0.34 m rigid frame covered with a fine mesh (0.1 mm aperture) (Kraus 1987). While using different sampling techniques may introduce some variability, testing suggests that the aforementioned methods are comparable (results not shown).

In addition to the three field events, weekly sampling was conducted at two locations on Ipperwash Beach over the 2014 bathing season (April - October). One sampling location was the site used for the field events and the other location was approximately 1 km south. For weekly sampling, triplicate pore water and sand (surface and subsurface) samples were collected from the foreshore reservoir (1 m landward of the shoreline) as well as triplicate surface water samples at ankle- and waist-depth. The same surface water, sand and pore water samples were collected biweekly at one location at Marie Curtis Beach over the 2014 bathing season. For weekly/biweekly sampling, foreshore sand and pore water samples were collected by digging three holes to the water table (sampling method described above). Inland groundwater samples (n=10) were also collected at Ipperwash Beach during the 2014 bathing season to evaluate if inland groundwater was a potential source of *E. coli* to the foreshore reservoir. These samples were collected 20-30 m landward of the shoreline using a drive point sampler (Charette and Allen 2006) installed up to 3 m below the sand surface.

E. coli in water and sand samples was enumerated using standard membrane filtration methods (U.S. Environmental Protection Agency 2002) with sand samples processed using methods recommended by Boehm et al. (2009b). Sand concentrations are reported as CFU per gram of dry weight. Statistics were performed on log10 transformed data using non-parametric tests (see Supporting Information for details on enumeration and statistical analysis methods).

4.2.3 Physical parameters

Wave height data during the field events were obtained from an offshore buoy located 37 km north of the site (Fisheries and Oceans Canada 2016). Sand levels along the cross-shore transect, from approximately 20 m landward of the shoreline to waist-depth water offshore, were surveyed at all sampling times during the field events using a total station. Surveyed sand levels were used to calculate erosion and accretion along the transect. Groundwater and surface water levels were also measured at all sampling times using groundwater wells and clear stilling wells, (Gibbes et al. 2007) respectively, installed along the transect at approximately 5 m intervals.

4.2.4 Mass balance calculations

Mass balance calculations were performed to evaluate the contribution of sand erosion to the increase in surface water *E. coli* concentrations observed during the field events. The calculations consider the numbers of *E. coli* associated with the sand that was eroded, and compare this with the increase in numbers of *E. coli* in the surface water between the first and second sampling times. Foreshore sand erosion and increases in surface water *E. coli* concentrations were greatest between these sampling times (Figures 4.2 and A.2).

The total number of *E. coli* released to surface water from the volume of sand eroded per unit width of shoreline (*N*) was calculated by:

$$
N = \sum_{i=1}^{n} (F\rho_s (1 - \phi)C_{sur_i} V_{sur_i} + F\rho_s (1 - \phi)C_{sub_i} V_{sub_i} + \phi C_{pw_i} V_{sub_i})
$$
(4.1)

where ρ_s is density of sand (2.65 g/cm³) (Terzaghi et al. 1996), and ϕ is the sand porosity (0.3) (Coduto et al. 2011). V_{sur_i} and V_{sub_i} [m³] are the volumes of eroded surface (unsaturated) sand and subsurface (saturated) sand, respectively, per unit width of shoreline, calculated for discrete 0.1 m intervals (*i*) in the cross-shore direction. These volumes were calculated based on sand elevation surveys at the first and second sampling times with the volume of eroded sand above (V_{sur_i}) and

below (V_{sub_i}) the water table determined using the measured groundwater levels at time = 0 hours. V_{subi} multiplied by ϕ was used to calculate the numbers of *E. coli* associated with the pore water. C_{sur_i} , C_{sub_i} and C_{pw_i} (CFU/g, CFU/100mL) are *E. coli* concentrations in the surface sand, subsurface sand and pore water, respectively, determined for each interval, *i*, by linearly interpolating between mean concentrations observed at sampling locations P1 and P2 (P3 also included for Event3) at time $= 0$ hours. *F* is the fraction of *E. coli* assumed to detach from the eroded sand as it is suspended. A shearing assay experiment was conducted to estimate the fraction (*F*) of *E. coli* associated with the sand that detaches and is released to surface water upon sand suspension. From the experiment it was found that 80%, 84% and 85% of *E. coli* was released from the sand after 100 s, 300 s, and 500 s of suspension, respectively. As such, $F = 0.8$ was used in (4.1) to provide a conservative estimate for the fraction of *E. coli* detached. Details of the shearing assay experiment and illustration of the sand mass balance calculation are provided in the Supporting Information. While our sampling was not able to fully account for the heterogeneous distribution of *E. coli* in sand and pore water (Solo-Gabriele et al. 2015), representing a limitation of these calculations, it is important to note that the sampling and mass balance calculations were performed for three separate field events and the general findings were consistent for all events.

To estimate the increase in numbers of *E. coli* in the surface water, the change in *E. coli* concentrations between the first and second sampling times were linearly interpolated between ankle- and waist-depth sampling locations at discrete spatial intervals, *i*. The surface water volume (assuming a 1 m width of shoreline) for each interval *i* was calculated using the bathymetry and lake water levels from the shoreline to waist-depth at the second sampling time. Alongshore processes and variability were neglected in the calculation. Offshore mixing further than waistdepth, and microbial decay were also assumed to be negligible. Although these factors affect the transport and fate of *E. coli* in shallow surface water, they are neglected here due to the short duration over which the mass balance calculation is performed (Russell et al. 2013).

4.3 Results and discussion

4.3.1 Physical conditions and observations at Ipperwash Beach

The log mean and standard error of ankle- and waist-depth surface water concentrations at Ipperwash Beach (both sampling sites) during the 2014 bathing season were 1.89±0.04 log CFU/100mL (n=196) and 1.53±0.04 log CFU/100mL (n=202), respectively. *E. coli* concentrations at this beach are similar to other non-urban Great Lake beaches (e.g. Ishii et al. 2007, Kleinheinz et al. 2006, Skalbeck et al. 2010, Alm et al. 2003, Whitman et al. 2004). Consistent with previous studies (Edge and Hill 2007, Enns et al. 2012, Whitman and Nevers 2003), ankle-depth *E. coli* concentrations were significantly higher than waist-depth concentrations (p<0.001). Ankle- and waist-depth concentrations were not significantly different between the two weekly sampling sites at Ipperwash Beach spaced 1 km apart ($p=0.81$ and $p=0.15$, respectively; Wilcoxon signed rank test). *E. coli* concentrations were below detection (< 1 CFU/100 mL) for all inland groundwater samples collected at the site used for the field events. This indicates that although the net groundwater flow is lakeward (average hydraulic gradient=-0.014; Figures 4.2 and A.2), inland groundwater is not expected to be a major source of *E. coli* to the foreshore reservoir at the site.

Wave height was variable over the three field events with maximum *Hsig* of 0.9 m, 1.2 m, and 2 m, respectively, recorded (Figure 4.3a,c,e). Two successive periods of high wave intensity (H_{sig} > 0.55 m), separated by less than 48 hours of *Hsig* < 0.55 m, were observed during the 60-80 hour field periods for Event1 and Event2 (Figure 4.3a,c). The wave period also varied during the field events with the peak wave period increasing from average values of 3.4, 2.9 and 3.7 sec for the 24 hours preceding each event to maximum values of 4.7, 5.2 and 4.3 sec during the high wave intensity periods for Event1, Event2 and Event3, respectively.

Figure 4.3: Mean log transformed *E. coli* **concentrations (± standard error) in the surface water and foreshore reservoir during each sampling event. (a), (c), and (e) show the ankle- and waist-depth** *E. coli* **concentrations for Event1, Event2, and Event3, respectively. (b), (d), and (f) show the surface sand** *E. coli* **concentrations at P1 for Event1, Event2, and Event3, respectively. (g) shows the** *E. coli* **concentrations in the pore water taken at P1 for Event3. (h) shows the** *E. coli* **concentrations in the suspended eroded sand collected at ankle-depth as well as the ankle- and waist-depth sediment collected from the lake bed for Event3. Offshore wave heights (** H_{sig} **) are indicated by the red dashed line in (a) – (h). The grey shading in (a) and (c) indicates periods of rainfall during Event1 and Event2, respectively.**

The total volume of sand eroded along the cross-shore monitoring transect (assuming 1 m shoreline width) was estimated to be 1.34 m^3 , 1.83 m^3 , and 1.27 m^3 for Event1, Event2 and Event3, respectively, with erosion of foreshore sand occurring mostly between the first and second sampling times (Figure B.2). The observed beach morphology change was compared with Deans parameter (or Gourlay parameter, Ω) (Wright and Short 1984) calculated using time-varying wave data (e.g., wave height, wave period) before and during the field events. Ω indicates the equilibrium beach profile shape expected for a given set of wave conditions with the profile being less reflective (steep foreshore gradient) and more dissipative (flat foreshore gradient) as Ω increases (Wright and Short 1984). Temporal changes in Ω indicate the tendency of a beach to erode/accrete as the morphology shifts towards the prevailing equilibrium profile (Wright and Short 1984). The average Deans parameter was 8, 20 and 17 for the 24 hours preceding each field event, respectively, and sharply increased to 39, 43 and 58, respectively, over the first 12 hours of each event. The increase in Ω is consistent with the observed beach profile change from a more reflective to a more dissipative shape (Figure B.2).

There was no rainfall in the 2 days prior to Event1 but 12 mm of rain fell from 26-30 hours after the initial sampling time (0 hours). For Event2 there was 26 mm of rainfall in the 24 hours before the initial sampling time and 12 mm fell over the period 2.5-3.5 hours after the initial sampling time. There was no rainfall in the 2 days prior to or during Event3. Rainfall at the site is not expected to have impacted the surface water concentrations during the field events with weekly sampling results showing a low correlation between rainfall in the previous 24 hours and ankleand waist-depth surface water *E. coli* concentrations (p=0.23 and 0.26, n=41, respectively). This low correlation despite Ausable River being the main source of fecal contamination to the site may be because summer rainfall events in the area are often localized. Therefore rainfall at the site does not necessarily correspond to rainfall in the Ausable River catchment.

4.3.2 Temporal variability in *E. coli* concentrations during field events

Surface water *E. coli* concentrations at ankle-depth $(2.11\pm0.06, 1.82\pm0.02$ and 2.50 ± 0.03 log CFU/100 mL) were statistically higher than waist-depth concentrations (below detection limit, 1.38±0.08 and 1.05±0.11 log CFU/100mL) at the initial sampling time (0 hours) for Event1, Event₂, and Event₃, respectively (Figure 4.3a,c,e; p=0.04, 0.04, and 0.04, respectively). Surface water *E. coli* concentrations showed similar temporal variability during all field events (Figure 4.3a,c,e). Concentrations in the ankle- and waist-depth water increased as *Hsig* increased with maximum concentrations observed near when the initial peak *Hsig* was recorded (12.5 hours for Event1, 9.7 hours for Event2, 12.5 hours for Event3). This also corresponded to the time when erosion of foreshore sand (between P1 and P2) was greatest (see Figures 4.2 and A.2). At the sampling time near to when the initial peak wave heights were recorded, for all field events the ankle- and waist-depth *E. coli* concentrations were not significantly different (p=0.19 for Event1, p=0.33 for Event2, p=0.50 for Event3). *E. coli* concentrations at both surface water locations decreased after this time despite a second period of high wave intensity (*Hsig* > 0.55 m) occurring during Event1 (~ 60 hours) and Event2 (~ 57 hours).

E. coli concentrations in the surface (unsaturated) sand 1 m landward of the initial shoreline (P1) were elevated and exhibited high variability between triplicate samples at the start (0 hours) compared to the end of each field event $(1.23\pm0.99$ compared with 0.07 ± 0.06 log CFU/g for Event1; 0.94 ± 0.32 compared with 0.54 ± 0.07 log CFU/g for Event2; 2.41 ± 0.31 compared with 1.19±0.08 log CFU/g for Event3; Figure 4.3b,d,f and Tables A.2, A.3 and A.5). *E. coli* in the pore water at P1 also decreased during Event3 (3.45±0.32 log CFU/100 mL at 0 hours compared with 2.65±0.31 log CFU/100 mL at 64 hours; Figure 4.3g). Note that pore water at P1 as well as other samples discussed below were only collected throughout the field event for Event3. *E. coli* in suspended eroded sand at ankle-depth water during Event3 was significantly higher near the peak wave height compared to later times (1.67 \pm 0.13 log CFU/g at 12.5 hours compared with 0.9 \pm 0.22 $log CFU/g$ at 24.5 hours; $p = 0.014$, Figure 4.3h). Suspended sand was only collected at three times during Event3 because there was negligible suspended sand at other sampling times. *E. coli* concentrations in the offshore sediment samples were statistically lower at the start of Event3 (0 hours) compared to the end (64 hours, p=0.04 for ankle-depth and p=0.04 for waist-depth, Figure 4.3h).The increase in offshore ankle-depth sediment concentrations followed by an increase in

offshore waist-depth sediment concentrations may be due to eroded foreshore sand settling as it was transported offshore. Maximum offshore sediment concentrations of 1.53±0.88 log CFU/g and 1.56±0.07 log CFU/g were observed at ankle- and waist-depth, respectively, at the final sampling time (64 hours). These mean concentrations were 13% and 15% of the initial foreshore (P1) surface sand concentration. Although conditions are different in the laboratory, this field result is consistent with the shearing assay experiment which found approximately 80% of *E. coli* is removed during sand suspension. The decrease in *E. coli* surface sand concentrations during all field events, as well as the decrease in pore water concentrations at P1 and suspended eroded sand concentrations during Event3 indicates that the amount of *E. coli* in the foreshore reservoir decreased during the events in response to the increase in wave height. The corresponding increase in *E. coli* concentrations in the surface water and offshore sediment between 0 hours and the time when the initial peak wave height was recorded suggests that *E. coli* may have being transferred from the foreshore reservoir to the surface water.

4.3.3 Depletion of the foreshore reservoir

A second period of high wave intensity ($H_{sig} > 0.55$ m) occurred during Event1 around 60 hours and Event2 around 57 hours (Figure 4.3a,c). Surface water *E. coli* concentrations did not increase during these periods as occurred for the initial high wave intensity periods. This may be because *E. coli* initially in the foreshore reservoir were depleted during the initial period of high wave intensity and, as indicated by low surface sand concentrations (Figure 4.3b,d, Tables A.2 and A.3), the original source of *E. coli* was no longer available. There was also limited foreshore sand erosion observed during the second periods of high wave intensity. A similar source wash-out phenomena is observed for *E. coli* in tributaries at the start of rainfall events (Jamieson et al. 2005). Our results suggest that for waves and associated sand erosion to considerably affect surface water *E. coli* concentrations there must be a preceding period of low wave conditions during which time *E. coli* is able to build-up in the foreshore reservoir. These periods may be characterized by lower than equilibrium Deans parameter values which would indicate accretionary conditions in the foreshore (Wright and Short 1984). Comparison of the initial foreshore surface sand concentrations at P1 for all field events with the amount of time elapsed since a period of high wave intensity at the site also supports our finding. The highest initial foreshore surface sand concentration (P1, 0 hours) was observed for Event3 (2.41 log CFU/g), followed by Event1 (1.23 log CFU/g) and Event2 (0.94

log CFU/g). Event3 and Event1 were preceded by 6 and 3 days, respectively, with *Hsig* < 0.55m, whereas *Hsig* reached 1.2 m in the 24 hours prior to the start of Event2. This finding suggests that statistical regression models used to predict *E. coli* concentrations in surface waters based on environmental variables (Feng et al. 2013, Ge et al. 2012) may be improved by considering, in addition to wave height, the time elapsed since a period of high wave intensity and potentially the temporal variability in Deans parameter (Wright and Short 1984).

4.3.4 Impact of magnitude of wave height

Data from the three events suggests that the magnitude of wave height may affect the time taken for *E. coli* concentrations in the ankle- and waist-depth water to decrease below the Ontario guideline value (2 log CFU/100 mL) after maximum concentrations are reached. For Event1, with maximum *Hsig* = 0.9 m, it took approximately 35 hours for ankle-depth *E. coli* concentrations to drop below 2 log CFU/100 mL following a concentration of 2.26 ± 0.03 log CFU/100 mL near the initial peak wave height (Figure 4.3a, 12.5-48 hours). For Event2, with a maximum $H_{sig} = 1.2$ m, this took approximately 24 hours (Figure 4.3c, 9.4-32.4 hours). Finally, during Event3, with maximum $H_{sig} = 2$ m, the ankle- and waist-depth *E. coli* concentrations were reduced from 2.26±0.03 log CFU/mL and 2.21±0.02 log CFU/mL, respectively, to less than 2 log CFU/100mL in only 8 hours following the peak wave height (Figure 4.3e, 12.5-20.5 hours). As samples were only taken at the times indicated in Figure 4.3, the actual time taken for concentrations to fall below 2 log CFU/100 mL may actually be less. The comparatively rapid decline in surface water *E. coli* concentrations during Event3 may be due to increased offshore mixing, in addition to sand being eroded more rapidly with more intense wave conditions resulting in faster depletion of the foreshore reservoir source (Thupaki et al. 2009).

4.3.5 Impact of wave run-up limit on pore water and sand concentrations

E. coli concentrations in the surface sand and pore water at P1, P2 and P3 as the maximum wave run-up propagated onshore and later receded during Event3 are shown in Figure 4.4. At the initial sampling time when the shoreline was approximately 1 m lakeward of P1, *E. coli* concentrations in the surface sand and pore water were highest at P1 and P2, and below detection at P3 (Figure 4.4, Table B.5). This gradient of decreasing sand and pore water concentrations onshore is

consistent with prior studies and generally attributed to the lake water interacting with the foreshore area only and increasing *E. coli* counts here (Alm et al. 2003, Kon et al. 2007). Pore water and sand *E. coli* concentrations at P1 and P2 decreased as the wave height increased and *E. coli* was released to surface waters. Once the maximum wave run-up reached P3 (20.5 hours), in contrast to the decrease in *E. coli* concentrations observed at P1 and P2 (Figure 4.4a,b), the pore water and surface sand concentrations at P3 increased from below detection at 0 hours to 3.37±0.27 log CFU/100mL and 1.36±0.33 log CFU/g, respectively, at 24.5 hours (Figure 4.4c). The observed increase in *E. coli* concentrations may be due to lake water infiltrating into the unsaturated sand in the wave run-up zone (Horn 2002, Li and Barry 2000) and therefore delivering *E. coli* from the surface water to the sand/pore water. Alternatively, the increase in measured *E. coli* concentrations may be due to the reviving of non-culturable bacteria through added moisture (Byappanahalli et al. 2006). Sand and pore water *E. coli* concentrations did not decrease significantly over the remainder of the event despite the maximum wave run-up receding lakeward. While more samples along the cross-shore transect as well as additional replicates at all sample locations would have been ideal to quantify spatial heterogeneity in pore water and sand concentrations, the number of samples collected at all sampling times was a trade-off with the high sampling frequency required to capture temporal variability. Additional sampling is recommended to confirm our findings with respect to the relationship between the run-up limit location and sand and pore water concentrations.

Figure 4.4: Mean log transformed *E. coli* **concentrations (± standard error) in the pore water and surface sand at (a) P1, (b) P2, and (c) P3 during Event3. Shaded areas in (a) and (b) indicate time when the maximum wave run-up was landward of the P1 and P2 sampling locations, respectively.**

4.3.6 Mass balance results

Total eroded volumes of sand over the cross-shore transect between the first and second sampling times (when the greatest amount of sand erosion occurred) were 0.68 m^3 , 0.45 m^3 and 0.41 m^3 of surface (unsaturated) sand (V_{sur}) and 0.66 m³, 0.26 m³ and 0.44 m³ of subsurface (saturated) sand (V_{sub}) per m of shoreline for Event1, Event2 and Event3, respectively (Table B.1). Using $F = 0.8$, the total *E. coli* associated with the eroded foreshore sand and thus potentially transported to the surface water between the first and second sampling times was calculated to be 7.71 log CFU, 7.00 log CFU, and 8.41 log CFU per m of shoreline for Event1, Event2 and Event3, respectively (Table B.1). These amounts can be compared with the estimated increase in total *E. coli* in the surface water over this period which were 7.27 log CFU, 7.61 log CFU, and 7.81 log CFU per m of

shoreline for Event1, Event2, and Event3, respectively (Table B.1). Comparison indicates that the *E. coli* released from erosion of foreshore sand alone was sufficient to account for the increase in total *E. coli* in the surface water for Event1 and Event3. In fact, *F* equal to only 29% and 17% would have achieved the increase in *E. coli* concentration observed in the surface water for Event1 and Event3, respectively. For Event2, calculations indicate that *E. coli* associated with the eroded sand was not sufficient to account for the observed increase in surface water concentrations. It is possible that contaminated foreshore sand may have been eroded, and subsequently accreted offshore, during the high wave intensity period that occurred in the 24 hours preceding Event2. Resuspension of this offshore sediment may have contributed to the increase in surface water *E. coli* concentrations during Event2. Prior erosion of foreshore sand is consistent with the initially lower *E. coli* concentrations in foreshore (P1) surface sand for Event2 (0.94 \pm 0.32 log CFU/g) compared with Event1 (1.23±0.99 log CFU/g) and Event3 (2.41±0.36 log CFU/g). Additional calculations were performed to test the sensitivity of the results to parameter values used for ϕ and *F*. The results were consistent regardless of the values adopted.

The percentage of *E. coli* associated with the different components of the foreshore reservoir (unsaturated surface sand, saturated subsurface sand and pore water) in the volume of eroded sand was calculated for each field event. Surface sand accounted for 99.6%, 84%, and 95% of *E. coli* potentially released via erosion from the foreshore reservoir between the first and second sampling times for Event1, Event2, and Event3, respectively. Based on the assumptions included in the mass balance, *E. coli* attached to sand is likely the main contributor of *E. coli* to surface water during high wave conditions rather than *E. coli* initially residing in pore water at this beach. Our finding that sand erosion may be a governing mechanism for transferring *E. coli* suggests that quantification of Ω over time, which provides indication of whether a beach is susceptible to erosion for given wave conditions, may be a useful approach to understand under what conditions the foreshore reservoir may be a potential source of *E. coli* to surface waters.

4.3.7 Comparison to coarse sand-cobble beach

While the mass balance calculations suggest that sand erosion alone was sufficient to account for the increase in surface water *E. coli* concentrations observed at Ipperwash Beach during high wave intensity periods preceded by calm periods, the mechanisms by which *E. coli* is released from the foreshore reservoir to adjacent surface waters may differ at beaches with different sand types. Here we compare the distribution of *E. coli* between the sand, pore water, and surface water for the fine sand study beach to the distribution for a coarse sand-cobble beach to infer potential release mechanisms. It has been found that *E. coli* have a higher tendency to attach to uniform fine-grain sand (Skalbeck et al. 2010). Other factors including organic matter content, biofilms, and moisture content also affect the tendency of *E. coli* to attach to sand (Boehm et al. 2009b, Piggot et al. 2012).

Weekly/biweekly sampling at Ipperwash Beach and Marie Curtis Beach over the 2014 bathing season found that pore water and ankle-depth surface water concentrations were strongly correlated at Marie Curtis Beach $(r_s=0.63, p<0.01, n=17)$ but not as strongly correlated at Ipperwash Beach (r_s =0.24, p=0.16, n=37; data provided in Table B.6). The higher correlation between pore water and ankle-depth concentrations at Marie Curtis Beach suggests greater connectivity between these two water entities. This may be due to higher saturated conductivity at Marie Curtis Beach (58 m/d; based on particle size analysis and Krumbein and Monk (1943)) compared with Ipperwash Beach (10 m/d) leading to higher water exchange across the sedimentwater interface in the foreshore area. While *E. coli* concentrations in the pore water were significantly higher at Marie Curtis Beach $(2.73\pm0.07 \log CFU/100mL, n=69)$ than at Ipperwash Beach (2.34±0.05 log CFU/100mL, n=253; p<0.01, Mann Whitney U Test), *E. coli* concentrations in the surface sand were not significantly different between the two beaches (1.19 \pm 0.09 log CFU/g, n=66, 0.94 ± 0.06 log CFU/g, n=214; p=0.65, Mann Whitney U Test). This suggests that there may be less attachment of *E. coli* to sand at Marie Curtis Beach compared to Ipperwash Beach. Consequently, sand erosion may not deliver as much *E. coli* to surface waters during a dry-weather high wave intensity period at a coarse sand-cobble beach, like Marie Curtis Beach, compared with a fine sand beach. Through-beach pore water transport due to the higher water exchange may be a more important mechanism for delivering *E. coli* from the foreshore reservoir to surface water at coarser beaches than sand erosion (Beversdorf et al. 2007, Wright et al. 2011).

4.4 Environmental implications

This work provides important insights into the transfer of *E. coli* from the foreshore reservoir (sand and pore water) to adjacent surface waters during periods of high wave intensity. The findings are important for improving statistical and process-based models used to predict water quality

exceedances. The work indicates that sand erosion may be the main mechanism by which *E. coli* is transferred from the foreshore reservoir to surface waters during high wave intensity periods at fine sand beaches. However, this may not be the case for coarser sand beaches where interstitial pore water flow and discharge, as opposed to sand erosion, may be more important. This work suggests that sand size and size distribution are key to understanding the mechanisms governing the release of *E. coli* to surface water, however, additional work is needed to better understand this. Future work is also needed to determine if erosion is also important for mobilizing different bacterial, protozoan, and viral pathogens in the foreshore reservoir.

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Chapter 5

5 Evaluation of methods to sample fecal indicator bacteria in foreshore sand and pore water at freshwater beaches

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5.1 Introduction

Microbial pathogens at beaches can lead to bather illness (Dufour 1984, Marion et al. 2010). Due to the difficulties and cost of quantifying harmful pathogens, fecal indicator bacteria (FIB), such as enterococci in marine beaches and *Escherichia coli* (*E. coli*) in freshwater beaches, are used for recreational water quality monitoring as indicators of the human health risk. In the United States and Canada, health departments determine the health risks at a beach based on water samples taken between ankle- to chest-depth in the surface water (Enns et al. 2012). FIB are often orders of magnitude higher in sand and pore water near the shoreline (herein referred to as the foreshore reservoir) than in adjacent shallow surface waters, upshore sand, and offshore sediment at freshwater beaches (Kinzelman et al. 2004, Staley et al. 2015, Vogel et al. 2016, Whitman and Nevers 2003) and at marine beaches (Yamahara et al. 2007). While further research is required, some studies have shown that the foreshore reservoir can act as a potential direct health risk to beachgoers (Heaney et al. 2009, Solo-Gabriele et al. 2015). The foreshore reservoir consists of unsaturated sand (sand above the water table with variable moisture content), saturated sand (sand below the water table), and pore water (water in the interstitial spaces of the sand). An example of higher *E. coli* concentrations in the foreshore reservoir than surface water is a study by Whitman and Nevers (2003) which reported pore water concentrations several orders of magnitude higher than those in the adjacent shallow water at a Chicago beach. For sand, there are currently no healthbased guideline levels for acceptable *E. coli* levels. In lieu of sand guideline levels, the water quality guideline can be used as a benchmark recognizing that the benchmark may correspond to a different risk level. Given the benchmark and considering concentrations on a bulk volumetric basis, Whitman and Nevers (2003) found sand samples collected at the Chicago beach had *E. coli* concentrations that exceeded the U.S. EPA guideline value of 235 CFU/100mL 95% of the time for foreshore sand and 76% of the time for offshore sand. Due to the high FIB levels in the foreshore reservoir, it can act as a non-point source of contamination to adjacent surface waters through routes such as sand erosion, and bacterial detachment from sand combined with groundwater flow and discharge (Alm et al. 2003, Brown and Boehm 2016, Byappanahalli et al. 2006, Edge and Hill 2007, Vogel et al. 2016, Whitman and Nevers 2003, Yamahara et al. 2007).

Health units do not currently sample the sand or pore water, nor are they required to do so (Health Canada 2012, United States Environmental Protection Agency 2012). In addition, unlike surface

water, there is no widely accepted method to collect samples from the foreshore reservoir for FIB enumeration (Health Canada 2012). Previous studies have quantified FIB presence in this reservoir by sampling the pore water, unsaturated sand, and saturated sand (Table 5.1). Methods that have been used to sample the unsaturated sand include skimming the surface sand, using a sterile core sample, and taking composite samples. For saturated sand sampling, methods include using a sterilized core or a shovel to reach the saturated sand (Table 5.1). Groundwater wells, drive point samplers (Charette and Allen 2006), and shovels have been used to access pore water in the foreshore area to collect samples (Table 5.1). For a given collection approach, the type and size of equipment used (i.e. length and diameter of sterile core), as well as amount of sample collected can also vary. It is important to understand how *E. coli* concentrations vary based on sampling technique so health departments, beach managers, and researchers can select the sampling method that best suits their needs as well as better interpret sampling results given a specific method used.

Table 5.1: Summary of sample collection methods used in select studies focused on FIB abundance in beach sand and pore water.

Pore Water Sampling Methods

*Drive Point samplers are well point systems that can be used to sample groundwater at depth while providing minimal disruption to the aquifer (Charette and Allen 2006)

Unsaturated Sand Sampling Methods

Saturated Sand Sampling Methods

A recent review paper by Solo-Gabriele et al. (2015) suggests that beach managers may need a better conceptual understanding of the foreshore reservoir at their beaches in order to understand and predict surface water quality exceedances. Past research indicates the complexity of the partitioning and accumulation of FIB in the different components of the foreshore reservoir. For example, FIB that are unable to persist in surface water (e.g. those sensitive to solar radiation or limited nutrients) may find sand a more favorable habitat and may proliferate in the foreshore reservoir (LaLiberte and Grimes 1982, Obiri-Danso and Jones 2000). Russell et al. (2012) observed that FIB concentrations in foreshore pore water are highest close to the water table and then rapidly decrease with depth. Beversdorf et al. (2007) found that *E. coli* levels in the sand were greatest when the moisture content was between 15% and 19%, indicating that unsaturated sand may contain higher concentrations of FIB than saturated sand, which usually has a moisture content above 20%. The partitioning of FIB between the components of the foreshore reservoir and the relationships between the components need to be better understood to determine the optimum way of sampling as well as quantifying the abundance of FIB in the reservoir.

The physical characteristics of a freshwater beach (location, sand type, wave exposure) may affect the distribution of FIB in the foreshore reservoir and in turn affect the results obtained when using different methods to sample the reservoir. In the foreshore reservoir, FIB can either attach to sand grains through a variety of mechanisms (e.g., attachment to biofilms and sand grains, straining at grain to grain contacts), reside freely in the pore water, or accumulate at the air/water interface. The efficiency by which FIB attach to different sand types or exist freely in the pore water depends on sand characteristics such as grain size, uniformity, moisture content and mineralogy, as well as the water chemistry, including ionic strength. As a result these factors influence the high variability in FIB sand concentrations between beaches (Alm et al. 2003, Hernandez et al. 2014, Piggot et al. 2012, Skalbeck et al. 2010). Skalbeck et al. (2010) found that *E. coli* sand concentrations increase with decreasing grain diameter and increasing uniformity. Their results suggest well sorted, fine grain sands may be a more favorable habitat for FIB due to the larger surface area of grain per unit volume of sand. Lee et al. (2006) showed that FIB concentrations were higher in the foreshore sand at sheltered beaches rather than wave exposed beaches. The variability in the distribution of FIB between different components of the foreshore reservoir as influenced by differences in physical characteristics adds uncertainty to characterizing the abundance of FIB in the reservoir.

Studies at freshwater and marine beaches have quantified the abundance of FIB in the foreshore reservoir by collecting sand samples (Shibata and Solo-Gabriele 2012, Vogel et al. 2016, Whitman and Nevers 2003, Wright et al. 2011), while other studies sample the pore water (Boehm et al. 2004, Skalbeck et al. 2010, Staley et al. 2015). In addition to sampling different components of the foreshore reservoir, various methods have been used to sample these components (Table 5.1). The ability of different sampling strategies to adequately express the abundance of FIB in the foreshore reservoir is unclear. Solo-Gabriele et al. (2015) indicated that sampling for FIB in sand should be considered for inclusion in regulatory programs that aim to protect recreational beach users from infectious diseases. If sampling of the foreshore reservoir is to be included in regulatory sampling, we must first develop robust scientific understanding of the components and methods used to express *E. coli* in the reservoir and how they may vary based on different beach characteristics. The objectives of this study were as follows: (1) determine the effect of sampling methods on the quantification of *E. coli* in the foreshore reservoir for freshwater beaches, (2) compare the partitioning of *E. coli* between different components of the reservoir (i.e. unsaturated sand, saturated sand, and pore water), and (3) determine how the sampling method or partitioning of *E. coli* within each component of the reservoir varies between freshwater beaches with different grain size. While this paper focuses on sampling methods for freshwater beaches, many of the findings are relevant for marine beaches. Sampling at marine beaches, however, may be more complicated due to tide-induced water level fluctuations, varying unsaturated zone depth, and salinity effects.

5.2 Methods

5.2.1 Field site descriptions

Six beaches along the Great Lakes, in southern Ontario were selected for sampling based on their physical parameters and high frequency of surface water quality exceedances. Beach sands were defined in terms of their grain size (fraction that is 50% finer, d_{50}) (Wentworth 1922) and their coefficient of uniformity (CU, calculated as d_{60}/d_{10} based on sieve size analysis) (ASTM 2009). Two fine grain $(0.125 \times d_{50} \times 0.250 \text{ mm})$, two medium grain $(0.251 \times d_{50} \times 0.500 \text{ mm})$, and two coarse to very coarse grain $(0.501 \leq d_{50} \leq 2.00 \text{ mm})$ sand beaches were selected. Field sites were also designated as "bird impacted" or "not bird impacted", and "sheltered" or "wave exposed". If there

were confirmatory microbial source tracking results, or at least 20 birds sighted at a field site on at least one of the sampling trips, the beach was designated as bird impacted. Sheltered beaches were characterized as beaches that are partially or fully protected from wave action by land or manmade physical barriers (root mean square wave height (H_{rms}) typically ranging from $0 - 0.5$ m), whereas exposed beaches are directly open to the lake (*Hrms* typically ranging from 0.5 – 2 m) (Feng et al. 2016). Since some interdependency may exist between the physical characteristics of the field sites, statistical analysis focused on examining only the effect of sand grain size. *E. coli* concentrations and their partitioning between different components of the foreshore reservoir, however, may also be impacted by the degree of wave shelter and bird presence. With only six beaches included in our study, we recommend similar studies be conducted at other beaches including marine beaches to further test our study findings. Details on our field sites are provided in Table 5.2.

Table 5.2: Summary of beach characteristics at individual Ontario beaches.

Ipperwash Beach (located on Lake Huron) and Burlington Beach (located on Lake Ontario) are characterized as fine grain sand beaches. Both beaches are exposed and can experience high wave activity. A recent study on Ipperwash Beach showed that release of sand-associated *E. coli* from the foreshore reservoir by sand erosion caused by wave heights (H_{rms}) between $0.5 - 2$ m significantly increased *E. coli* surface water concentrations (Vogel et al. 2016). The two medium sand beaches were Sunnyside Beach (described by Edge et al. (2010) and Staley and Edge (2016)) and Bronte Beach. These beaches are located on Lake Ontario and sheltered from wave activity. Sunnyside Beach is protected by several breakwater structures parallel to the shoreline. Bronte Beach is protected by a breakwater structure that runs along the northeast quadrant of the beach and delineates the outlet of Bronte Harbour. Bayfront Beach (located on Hamilton Harbour) and Marie Curtis Beach (located on Lake Ontario) were selected as the two coarse to very coarse sand/cobble beaches. Bayfront Beach (described by Edge and Hill (2007)) is sheltered by land that extends past the beach on either side and reduces water circulation. Bayfront Beach has the highest percentage of historical water quality exceedances compared to the other beaches (Table 5.2), potentially due to high gull and Canada geese numbers at this beach (Edge and Hill 2007). Marie Curtis beach is exposed to Lake Ontario with Canada geese, ducks, and other birds frequently observed along the shoreline (Beach Guides 2015).

5.2.2 Sample collection methods

Three to four sampling events were conducted at all six beaches during the 2014 and 2015 bathing seasons. To evaluate how measured *E. coli* concentrations in the foreshore reservoir depend on the specific sample collection method used, three pore water (PW) and saturated sand (SAT) sampling methods were tested (shovel method, careful excavation method, and drive point/core method), as well as two unsaturated sand (UNSAT) sampling methods (1 cm depth, 5 cm depth). The unsaturated and saturated sand samples where comprised of both the sand-associated *E. coli* and *E. coli freely residing in the pore water. The moisture content, and thus pore water volume, is* lower in the unsaturated sand. All sand and pore water samples were collected in the foreshore area (approximately one meter landward from the shoreline) with replicate samples (4-5) collected for each sampling method on all sampling events. For all sampling events, 4-5 replicate surface water samples (500 mL) were also collected at ankle-depth.

5.2.2.1 Shovel method (*PW-Shovel*, *SAT-Shovel*)

The shovel method consisted of digging a hole with a sterilized 1.5 m long digging shovel to the water table, while limiting the amount of surface sand collapsing in the hole. If a hole started collapsing, the hole was abandoned and a new hole was dug beside it. The shovel was sterilized using isopropyl alcohol and rinsing with sterile DI water. Pore water was collected by placing a sterile 250 mL polypropylene bottle at the bottom of the hole and allowing the pore water seeping into the hole to fill the bottle (PW-Shovel). Once the pore water was collected, approximately 100 g of saturated sand was collected by using a sterile tablespoon to scoop the bottom 1 cm of sand from the hole and place it into a Whirlpak® bag (SAT-Shovel). These methods for pore water and saturated sand collection were used by Edge et al. (2010), Staley et al. (2015), and Vogel et al. (2016). The shovel method may not be suitable for collecting pore water and saturated sand at a beach with a deep water table or at macrotidal marine beaches when sampling is conducted near the high tide mark. For these conditions, the sides of the hole may collapse during sampling.

5.2.2.2 Careful excavation method (*PW-Careful*, *SAT-Careful*)

When collecting samples with the shovel method, the samples can be contaminated by unsaturated surface sand falling into the hole. The careful excavation method (Careful) aimed to avoid any contamination of the samples by minimizing disturbance during sampling. For this method, a sheet of sterilized polymethyl methacrylate (0.25 x 0.30 m), or Plexiglas, was used to scrape away the sand surface and carefully excavate a hole to the water table. During excavation, no surface sand was permitted to fall into the hole. Once sufficient pore water seeped into the hole, it was collected using a 60 mL plastic, sterile syringe (PW-Careful). After the pore water was collected, a sterile spoon was used to collect the saturated sand in a similar manner as for the shovel method (SAT-Careful).

5.2.2.3 Drive point/core method (*PW-Drive*, *SAT-Core*)

The following sampling methods (PW-Drive and SAT-Core) were used as methods of collecting pore water and saturated sand that result in the least amount of sample disturbance. Limiting disturbance of a sample during collection enables concentrations of *E. coli* in the pore water and sand to be better quantified without sand-associated *E. coli* being released to the pore water. To collect pore water, a drive point sampler was driven vertically downwards until the screen (AMS Stainless Steel piezometer drive point, 5 cm length screen; (Charette and Allen 2006)) was located at the water table. A peristaltic pump was used to collect water from the tubing attached to the drive point sampler (flow rate ≈ 2 mL/s). One volume of tubing was discarded to flush the line and prevent cross contamination between samples, and the sample was stored in a sterile polypropylene bottle (PW-Drive). This method has been used by Skalbeck et al. (2010) and Vogel et al. (2016). To collect saturated sand, clear polyethylene cores (0.25 m length, 0.05 m diameter) were hammered vertically into the sand and then dug out from the side as to not disturb the sample. Six cm of sand at the water table was taken from the core and stored in a sterile Whirlpak® bag (SAT-Core). Using a core to collect saturated sand has been used in numerous studies (e.g. Gast et al. (2011), Edge and Hill (2007), Russell et al. (2012)).

5.2.2.4 Unsaturated sand methods (*UNSAT-1cm*, *UNSAT-5cm*)

Two methods were evaluated for the collection of unsaturated surface sand from the foreshore reservoir. For the first method a sterile spoon was used to collect the top 1 cm of sand (UNSAT-1cm). This method of skimming the surface has been used by Lee et al. (2006), Staley et al. (2015), and Wright et al. (2011). For the second method, a sterile polyethylene core (0.05 m diameter) was used to collect approximately the top 5 cm depth of sand (UNSAT-5cm). Desmarais et al. (2002), Alm et al. (2003), and Edge and Hill (2007) have used this second method to collect unsaturated sand. Approximately 100 g of sand was collected and stored in Whirlpak® bags for each method.

5.2.3. *E. coli* enumeration

After collection, water and sand samples were stored on ice, transported to the laboratory, and analyzed within 6 hours. Water samples were filtered (0.45 μm pore size) using standard membrane filtration methods (American Public Health Association 1999) and placed on chromogenic differential coliform (DC) agar, supplemented with cefsulodin. The filter and agar were incubated at 44.5 °C for 20 hours and *E. coli* was then enumerated as colony forming units (CFU/100mL). To extract *E. coli* from the sand, 25 g from each homogenized sand sample was placed in a sterile polypropylene bottle, diluted with 250 mL of phosphate-buffered saline, hand shaken for 2 minutes, and allowed to settle for 2 minutes (Boehm et al. 2009b). The supernatant was then processed using the same method as the water samples. An additional 25 g

from each sand sample was used to quantify the sand moisture content and enable expression of sand-associated *E. coli* as CFU/g of dry sand. To compare the amount of *E. coli* in the sand and water, concentrations were converted to a bulk volumetric basis ($CFU/cm³$). For this conversion, sand concentrations were multiplied by the density of sand ($\rho = 2.65$ g/cm³ (Terzaghi et al. 1996)) and the proportion of the bulk volume taken up by sand grains $(1-\phi)$, where ϕ is porosity (0.3) (Coduto et al. 2011)). Pore water concentrations were converted from CFU/100mL to a bulk volumetric concentration (CFU/cm³) by multiplying by the porosity (ϕ).

5.2.4 Statistics and data analysis

All *E. coli* concentrations were log transformed and the transformed values were determined to be normally distributed prior to analysis. Statistical analyses were performed using Minitab (Minitab Inc., State College, PA) and SPSS (IBM Corp., Armonk, NY). Generalized estimating equations (GEE) were used to compare *E. coli* concentrations between different methods. This method was used to account for potential clustering in the data due to grouping data from different beaches and sampling days. Variability between datasets was evaluated using Levene's test, which analyzes the variance of the datasets. Since variance is the square of the standard deviation, standard deviation is also used throughout the paper as a measure of variability. Pearson correlation analysis was performed to compare *E. coli* concentrations between the components of the foreshore reservoir and surface water. These correlations were also run using GEE to obtain a p-value that accounted for potential data clustering. Results were considered significant with a p-value of less than 0.05.

All statistical analyses were first run on data obtained from individual beaches. Beaches were then grouped by grain size (fine, medium, coarse) and data were evaluated for relationships between grain size and *E. coli* concentrations as determined using a specific sampling method or partitioning of *E. coli* between the foreshore reservoir components. If a relationship was observed, then the preferred sampling method may vary for beaches with different grain sizes. Lastly, data from all beaches were combined to determine if there was an overall pattern between different sampling methods or *E. coli* partitioning independent of beach type.

5.3 Results and discussion

5.3.1 Comparison of methods used to characterize the foreshore bacteria reservoir

5.3.1.1 Comparison of pore water sampling methods

When the data from individual beaches were analyzed, no statistical differences were observed between pore water sampling methods. When the data were grouped by grain size, the PW-Shovel method (2.66 log CFU/100mL) resulted in statistically higher *E. coli* concentrations than the PW-Drive method $(2.18 \text{ log } CFU/100mL; p<0.001)$ and the PW-Careful method $(2.43 \text{ log }$ CFU/100mL; p=0.011) at fine sand beaches. There was no significant difference observed between *E. coli* concentrations in the pore water when using the PW-Shovel, PW-Careful, and PW-Drive methods at medium and coarse sand beaches. Our results suggest that selecting a method to sample pore water may be more important at fine sand beaches as opposed to medium and coarse sand beaches where the methods produce similar results. We note that these results may be also be due to other beach characteristics (e.g. exposed versus sheltered) in addition to grain size.

The data from all beaches were combined to determine if there was an overall pattern in measured *E. coli* concentrations based on sampling method. Averaged results for the different sampling methods are provided in Table 5.3. After combining the data from all beaches, the PW-Shovel method (3.47 log CFU/100mL) resulted in statistically higher *E. coli* concentrations in the pore water than the PW-Drive method (2.95 log CFU/100mL; $p<0.001$) and the PW-Careful method (3.33 log CFU/100mL; p<0.001). The PW-Careful method also had statistically higher concentrations than the PW-Drive method $(p<0.001)$ (see Table 5.3). This is mostly consistent with the findings when only the data for the fine sand beaches were considered. The higher pore water concentrations found when the PW-Shovel method was used may be due to contamination of the pore water sample by sand falling into the hole or by *E. coli* being released from sand, biofilms, or the air/water interface as it is disturbed by the shoveling. The PW-Drive method is the least disruptive sampling method and therefore it is thought that this method may provide a more representative sample of *E. coli* freely residing in the pore water. It is possible that the tendency for a greater amount of *E. coli* to attach to finer grain sand may be the reason that a significant difference was observed between PW sampling methods for fine sand beaches but not for medium and coarse sand beaches. If more *E. coli* is attached to the sand rather than freely residing in the pore water, then the *E. coli* detachment as the sand is disturbed would be greater, increasing the amount of *E. coli* measured in the pore water. Therefore, there would be a larger difference observed between sampling methods at finer sand beaches, as seen in our study. The potential for *E. coli* to detach from sand once it is disturbed is supported by laboratory experiments by Vogel et al. (2016) which showed up to 85% detachment from sand suspension alone. This theory requires further investigation through experimental work. The data suggest that if the objective of a sampling program is to obtain a "worst case scenario" of pore water concentrations or to obtain a preliminary estimate of the total amount of *E. coli* in the saturated portion of the foreshore reservoir then the PW-Shovel method may be suitable. The PW-Shovel method is also the easiest sampling method to use and the least variable (discussed below; Table 5.3). However, if the objective is to obtain an estimation of the amount of *E. coli* freely residing in the pore water (not including *E. coli* attached to the sand) then the PW-Drive method may be more suitable.

Table 5.3: *E. coli* **concentrations and statistical test results for the different sampling methods examined with the data from all beaches combined. Groupings refer to statistically significant differences in concentration.**

	$\mathbf n$	Mean	Standard Deviation	Grouping				
Pore Water (log CFU/100 mL)								
PW-Shovel	78	3.47	1.11	\mathbf{A}				
PW-Careful	78	3.33	1.30	B				
PW-Drive	75	2.95	1.27	\mathcal{C}				
Saturated Sand (log CFU/g)								
SAT-Shovel	75	1.31	1.05	\mathbf{A}				
SAT-Careful	75	1.40	1.36	\mathbf{A}				
SAT-Core	76	1.70	1.35	A				
Unsaturated Sand (log CFU/g)								
UNSAT-1cm	78	2.23	1.30	A				
UNSAT-5cm	17	1.63	0.84	B				

FIB concentrations in pore water exhibit high spatial heterogeneity and this can cause high variability between multiple samples collected at a beach on a given day (Ishii et al. 2007, Kleinheinz et al. 2006). It is important to understand how the magnitude of this variability may vary depending on the sampling method used. For example, beach managers may prefer a sampling method with lower variability between samples, so fewer samples are required at a given time to obtain higher confidence around the mean. No statistical differences were observed when the variability for the different methods was analyzed for individual beaches or by grouping data based on grain size. When the data for all beaches were combined, although the variability in *E. coli* concentrations between the methods also did not differ significantly $(p=0.354)$, the PW-Shovel method had a lower standard deviation (standard deviation=1.11 log CFU/100mL) than the PW-Careful method (1.30 log CFU/100mL) and the PW-Drive method (1.27 log CFU/100mL). When collecting pore water using the PW-Shovel method, a larger volume of pore water is mixed due to the larger diameter of the hole (compared to the other sampling methods) – this may result in less variability between samples compared to the PW-Careful and PW-Drive methods. In this way, PW-Shovel may be considered a composite sampling method for pore water. Beach managers may prefer using the PW-Shovel method as it can be more representative of the overall foreshore reservoir at the beach and less biased by horizontally isolated zones of higher or lower *E. coli* concentrations. However, this method may be more biased due to vertical heterogeneity in the subsurface (due to sand from different layers falling into the hole and releasing *E. coli*). Alternatively, multiple PW-Careful or PW-Drive samples could be collected and composited to ensure a more accurate representation that captures the spatial heterogeneity at the beach.

5.3.1.2 Comparison of unsaturated sand sampling methods

No significant differences were observed between the unsaturated sand sampling methods when data from the individual beaches were analyzed separately or when data were grouped based on grain size. However, when the data from all the beaches were combined, the UNSAT-1cm method $(2.23 \log CFU/g)$ had statistically higher concentrations than the UNSAT-5cm method $(1.63 \log 10^{-10})$ CFU/g ; p<0.001; Table 5.3). This is consistent with previous studies that have shown the top layer of surface sand has higher *E. coli* concentrations than deeper layers (Alm et al. 2003, Desmarais et al. 2002). There was no significant difference observed between the moisture content of the sand collected using the two methods ($p=0.937$). The UNSAT-1cm method (standard deviation=1.30 log CFU/g) also had statistically more variable concentrations than the UNSAT-5cm method (standard deviation=0.84 log CFU/g; $p=0.032$). The higher mean and variability observed in the top 1 cm of unsaturated sand may be due to the deposition of fecal sources to the surface of the sand (e.g. bird droppings, trash, and run-off). A range of *E. coli* retention mechanisms may also cause higher concentrations of *E. coli* in the top layer of sand (e.g., film straining, retention on biofilms or on sand surfaces, retention at the air/water interface) (Bradford et al. 2006, DeNovio et al. 2004). The results suggest that if the objective of the sampling program is to assess the highest possible amount of *E. coli* in the unsaturated sand then the UNSAT-1cm method may be suitable, however, due to the larger variability (or *E. coli* "patchiness"), a greater number of samples may have to be taken to obtain an accurate representation of the mean.

5.3.1.3 Comparison of saturated sand sampling methods

Similar to the unsaturated sand results, no consistent trends were observed between sampling methods for saturated sand when the data from each beach were analyzed separately or when data were grouped together based on grain size. Further, after combining the data collected at all the beaches there was no statistical difference observed in the mean values between the saturated sand sampling methods (SAT-Shovel, SAT-Careful, SAT-Core; p=0.280; Table 5.3). This is likely due to the large standard deviations for all the sampling methods compared to the low mean *E. coli* concentrations observed (e.g. standard deviation of all saturated sand sampling methods=1.25 log CFU/g and mean concentration of all saturated sand sampling methods=1.47 log CFU/g). When comparing the standard deviations, saturated sand collected using the SAT-Shovel method (standard deviation=1.05 log CFU/g) resulted in lower variability in *E. coli* concentrations than saturated sand collected using the other methods (standard deviation=1.36 and 1.35 log CFU/g for the SAT-Careful and SAT-Core methods, respectively). This result is consistent with the PW-Shovel method having the smallest variability for the pore water sampling methods, and similarly may be attributed to the larger sampling area when the shovel method is used. As there was no significant difference between the means of the *E. coli* concentrations observed when using the different saturated sand sampling methods, SAT-Shovel may be the preferred method for sampling the saturated foreshore sand since this method is the simplest to implement in the field and has the smallest variation, resulting in fewer samples required to obtain an accurate representation of the mean *E. coli* concentration.

5.3.2 Comparison of *E. coli* in the different components of the reservoir

5.3.2.1 Comparison of components using all methods

Understanding how *E. coli* distributes between the different components of the foreshore reservoir (unsaturated sand, saturated sand, and pore water) is important for regulators to determine how to best sample and thus manage their beach. For example, if the amount of *E. coli* in the pore water and sand were related, then sampling pore water only (which requires less work for analysis than sand) may provide a suitable indication of the amount of *E. coli* in the foreshore reservoir. For comparison purposes, bulk volumetric units are used here. Also, *E. coli* concentrations measured using the different sampling methods are combined to compare the amount of *E. coli* in the different components of the reservoir.

Firstly, no statistical differences were observed between the different components of the reservoir when the data from each beach were analyzed separately (data provided in Table 5.4). When data were grouped to evaluate whether *E. coli* concentrations in the components of the foreshore reservoir are related for beaches with a certain sand grain size, it was found that as the grain size increased, the correlation between *E. coli* concentrations in the unsaturated sand and pore water increased (r=0.29, p=0.29 for fine sand; r=0.52, p<0.001 for medium sand; r=0.80, p<0.001 for coarse sand). This result is consistent with Bradford et al. (2006) as well as many colloid (microbial) studies that predict increased colloid retention in media with smaller grain sizes (Molnar et al. 2015). Retention of *E. coli* by these mechanisms in the unsaturated sand would increase concentrations in the unsaturated sand while pore water concentrations remain the same, resulting in little to no correlation between the two components. Although the concentration of *E. coli* in the unsaturated sand was not the highest at the fine sand beaches (1.40 log CFU/g for fine, 2.88 log CFU/g for medium, and 2.56 log CFU/g for coarse), the ratio of *E. coli* in the unsaturated sand to the saturated sand (based on log transformed concentrations) was highest for the fine sand beaches (1.9), in comparison to the medium (1.3), and coarse sand beaches (1.1). This may be due to increased retention of *E. coli* in the surficial unsaturated sand for finer sand beaches resulting in less downward transport of *E. coli* to the saturated zone. By contrast, at a coarse sand beach proportionally less *E. coli* is attached to the surficial unsaturated sand resulting in increased downward *E. coli* transport and thus increased pore water concentrations.

	$\mathbf n$	Pore Water (log CFU/100mL)	Saturated Sand $(\log CFU/g)$	Unsaturated Sand $(\log CFU/g)$	Ankle-Depth (log CFU/100mL)
Burlington	13	2.31 ± 0.57	0.50 ± 0.71	1.91 ± 0.84	2.38 ± 0.36
Ipperwash	16	2.52 ± 0.81	0.65 ± 0.86	0.90 ± 1.12	1.59 ± 0.66
Bronte	12	4.03 ± 0.80	2.16 ± 0.88	2.52 ± 0.77	2.44 ± 0.42
Sunnyside	12	4.55 ± 0.65	2.76 ± 0.76	3.24 ± 0.46	2.41 ± 0.49
Bayfront Park	13	4.18 ± 1.01	2.12 ± 1.40	3.79 ± 0.75	3.45 ± 0.28
Marie Curtis	12	2.19 ± 0.78	$0.57 + 0.71$	1.48 ± 0.80	2.28 ± 0.12

Table 5.4: *E. coli* **concentrations at beach study sites with mean values ± standard deviations determined by combining the data for all sampling methods for a given component of the reservoir. n corresponds to the number of samples for each method (n was taken over 3-4 sampling events at each site).**

In addition to grain size having an effect on the distribution of *E. coli* in the foreshore reservoir, the degree of beach exposure and bird presence may also have an effect, although we note that these differences may be caused by interdependencies between the beach characteristics. Consistent with Lee et al. (2006) and Yamahara et al. (2007), FIB concentrations were higher at sheltered beaches than at wave exposed beaches in the pore water $(4.36\pm0.87 \log CFU/100mL$ and 2.73±1.03 log CFU/100mL at sheltered and exposed beaches, respectively), unsaturated sand $(3.19\pm0.84 \text{ log CFU/g and } 1.38\pm1.05 \text{ log CFU/g})$, saturated sand $(2.34\pm1.10 \text{ log CFU/g and } 1.38\pm1.05 \text{ log CFU/g})$ 0.58 ± 0.77 log CFU/g), and in the ankle-depth water $(2.85\pm0.67 \log CFU/100mL$ and 2.13 ± 0.57 log CFU/100mL). Similar to Bonilla et al. (2007), *E. coli* concentrations were also higher at bird impacted beaches than at non-bird impacted beaches in the pore water $(3.74\pm1.22 \log CFU/100mL)$ and 2.43±0.72 log CFU/100mL at bird and non-bird impacted beaches, respectively), unsaturated sand $(2.76\pm1.11 \log CFU/g$ and $1.35\pm1.12 \log CFU/g$, saturated sand $(1.91\pm1.27 \log CFU/g$ and 0.58 ± 0.80 log CFU/g), and in the ankle-depth water $(2.60\pm0.57 \log CFU/100mL$ and 1.96 ± 0.67 log CFU/100mL).

The amount of *E. coli* in the different components of the reservoir was analyzed with the data from the six beaches combined. Unsaturated sand statistically had the highest *E. coli* concentrations $(3.93 \log CFU/cm^3)$ followed by saturated sand $(2.73 \log CFU/cm^3)$, and then pore water $(0.98 \log 10^{-3})$

 $CFU/cm³$ (p<0.001). Higher *E. coli* concentrations in the unsaturated sand over the saturated sand are consistent with Yamahara et al. (2007) and Beversdorf et al. (2007). In addition, pore water (standard deviation= $0.37 \log CFU/cm^3$) was statistically less variable than unsaturated (standard deviation=2.32 log CFU/cm³) and saturated sand (standard deviation=2.35 log CFU/cm³; p<0.001). The results suggest that sampling the unsaturated sand may provide the "worst case scenario" for the amount of *E. coli* in the foreshore reservoir although multiple samples are required to determine the true mean concentration of *E. coli* due to variability caused by high spatial heterogeneity in the unsaturated sand.

When comparing the different components of the reservoir at a given sampling location with data for all the beaches combined, a very strong significant correlation was observed between the saturated sand and the pore water $(r=0.953, p<0.001$; Table 5.5). This correlation is most likely a result of the saturated sand samples being a composite of sand and pore water, indicating that sampling either the saturated sand or pore water provides a good indication of the amount of *E. coli* in the saturated subsurface (not including unsaturated sand). The unsaturated sand was also correlated with the pore water, but not as strongly ($r=0.682$, $p<0.001$). While the unsaturated and saturated sand were correlated ($r=0.695$, $p=0.004$), the correlation was higher for pore water and saturated sand.

	Pore Water	Saturated Sand	Unsaturated Sand	Ankle-Depth Water
Pore Water		$0.953 \approx 0.001$	$0.682 \, \textcolor{blue}{<} 0.001)$	0.262(0.001)
Saturated Sand		1	0.695(0.004)	0.300(0.005)
Unsaturated Sand			1	$0.579 \, (\leq 0.001)$
Ankle-Depth Water				

Table 5.5: Correlations between different components of the foreshore reservoir and surface water for all beaches combined. Results are displayed as r(p).

E. coli concentrations in the foreshore reservoir components were compared to ankle-depth water concentrations to test for significant correlations. The component that was most representative of the ankle-depth water was the unsaturated sand $(r=0.579, p<0.001;$ Table 5.5). This correlation was likely dominated by the contribution from the exposed beaches where a strong relationship between the ankle-depth water and unsaturated sand *E. coli* concentrations has been observed in previous studies at exposed beaches (Alm et al. 2003, Skalbeck et al. 2010) but not necessarily at sheltered beaches (Edge and Hill 2007). The relationship between the ankle-depth water concentrations and unsaturated sand concentrations at exposed beaches may be due to continuous exchange of *E. coli* between the surface water and unsaturated sand due to wave-induced infiltration-exfiltration processes (Alm et al. 2003, Wu et al. 2017, Yamahara et al. 2007). Understanding the relationship between the surface water concentrations and foreshore sand concentrations provides insight into the extent of the exchange of *E. coli* between the foreshore reservoir and surface water as well as whether collecting surface water samples provides any indication of the abundance of FIB in the foreshore reservoir. This information can be beneficial from a regulatory compliance perspective. Correlations were also observed between the surface water and the pore water (r=0.262, p=0.001) and the saturated sand (r=0.300, p=0.005).

5.3.2.2 Comparison of components using individual methods

After no statistical differences were observed when data from each beach were analyzed separately and after data were grouped together based on grain size, data from all beaches were combined and analyzed to determine if the sampling method used affected assessment of the distribution of *E. coli* in the saturated foreshore reservoir – i.e. the partitioning of *E. coli* between the saturated sand versus pore water. At all sampling locations and times, the percentage of *E. coli* in the pore water relative to the total *E. coli* in the saturated reservoir (considering bulk volume concentrations) was calculated for each of the three sampling methods used (shovel, careful, and drive/core). Statistically, the shovel sampling method results in the highest percentage of *E. coli* in the pore water and in turn, the lowest percentage of *E. coli* attached to the saturated sand, followed by the careful excavation method, which was followed by the drive point/core method (Figure 5.1) ($p=0.001$). As discussed in Section 3.1.1, the disturbance caused by digging with a shovel may cause *E. coli* to detach from the sand resulting in higher pore water concentrations and lower saturated sand concentrations. The least disruptive method was the drive point method,

which caused the least amount of *E. coli* detachment from the sand. Understanding the tendency of *E. coli* to remain attached to the sand or alternatively detach to the pore water, based on the sampling method used, is important in deciding which sampling method is the most appropriate for a given purpose.

Figure 5.1: Percentage of *E. coli* **in the saturated sand and in the pore water relative to the total** *E. coli* **in the saturated reservoir (considering bulk volumetric concentrations) considering the data from all beaches. Percentages for each method are statistically different from one another (p=0.001). Error bars indicate ± one standard deviation from the mean of the percentage.**

5.4 Conclusion

Improved understanding of the partitioning of FIB between the different components of the foreshore reservoir (pore water, unsaturated sand, saturated sand) as well as how different sampling methods affect the measured FIB concentrations in these components is essential to develop better monitoring programs to protect public health at recreational beaches. Findings from this study have the following implications for sampling programs designed to assess FIB contamination:

• Selection of an appropriate method for sampling pore water is most significant at fine sand beaches $(0.125 \leq d_{50} \leq 0.250$ mm). At these beaches the PW-Shovel method resulted in statistically higher measured *E. coli* pore water concentrations compared to PW-Careful and PW-Drive methods. While the PW-Shovel method also resulted in higher *E. coli* concentrations at most medium and coarse sand study beaches, the differences were not statistically significant. At medium and coarse sand beaches using the PW-Shovel method may be appropriate as it is the easiest method and also provides the least variability in *E. coli* concentrations meaning less samples may need to be collected.

- The depth over which an unsaturated (surface) sand sample is collected significantly affects the sampling results. The top 1-cm of unsaturated sand (UNSAT-1cm) has higher and more variable *E. coli* concentrations than the top 5-cm of unsaturated sand (UNSAT-5 cm). Choosing the appropriate sampling depth depends on whether the objective of a sampling program is to identify the "worst case scenario" of *E. coli* concentrations or determine a representative amount of *E. coli* associated with the upper unsaturated sand layer.
- For saturated sand, *E. coli* concentrations were highly variable relative to their mean concentrations and so the mean values were not statistically different for each of the sampling methods.
- The highest *E. coli* concentrations in all reservoir components (pore water, unsaturated sand, saturated sand) were found at sheltered beaches and those impacted by birds.
- As sand grain size increased, the correlation between *E. coli* concentrations in the unsaturated sand and pore water increased which may be due to finer sand retaining a higher amount of *E. coli* in the unsaturated surface sand rather than allowing *E. coli* to more consistently distribute within the reservoir.
- If foreshore sand or pore water is added into the sampling regime for public health monitoring, the decision about the number of samples to be taken should reflect the large variability observed between replicate samples collected for the different components of the foreshore reservoir.

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Chapter 6

6 Conclusions and recommendations

6.1 Summary

This thesis addresses key knowledge gaps pertaining to fecal indicator bacteria (FIB) in foreshore beach sand and pore water (i.e. foreshore reservoir) at beaches. First, factors affecting the abundance and accumulation of FIB in the foreshore reservoir and how the reservoir affects surface water FIB concentrations were explored. Second, the pathways by which FIB that have accumulated in the foreshore reservoir may be transport to adjacent surface waters under high wave conditions were investigated. Finally, methods to sample FIB in the foreshore sand and pore water were compared to provide recommendations on how to appropriately determine the abundance of FIB in the foreshore reservoir. Ultimately this research provides new knowledge needed to better predict water quality exceedances at beaches and to improve beach water quality monitoring programs and modeling.

Sampling of *E. coli* concentrations in the foreshore reservoir at three Great Lake beaches over the bathing season (May – October) combined with high frequency daily sampling at one beach indicate complex temporal dynamics in foreshore reservoir *E. coli* concentrations. Seasonal variability in *E. coli* concentrations in the surface water at the three beaches were found to depend on environmental factors (e.g. temperature, rainfall, waves) as well as external *E. coli* sources (e.g. nearby tributaries). Surface water *E. coli* concentrations at beaches without external inputs from a tributary were found to be related to *E. coli* concentrations in the foreshore reservoir with the seasonal trend following a similar trend to that of the air temperature. Surface water *E. coli* concentrations at a beach adjacent to a creek did not follow any seasonal patterns with surface water concentrations related to the creek flow rates as well as to the foreshore reservoir concentrations. Daily sampling of the foreshore reservoir at one beach showed significant variability on a daily-time scale. Data indicate that *E. coli* does not simply accumulate in the foreshore reservoir over the bathing season as previously thought (Ishii et al. 2007, Whitman and Nevers 2003). This study further showed for the first time that *E. coli* may replicate in unseeded natural foreshore beach sand, not subjected to any external stimuli. Replication may in part explain the large temporal variations observed in FIB concentrations in unsaturated sand.

Based on intensive field sampling during periods of high wave intensity, it was found that sand erosion rather than pore water flow and discharge may be the dominant mechanism for the transfer of *E. coli* from the foreshore reservoir to surface water at fine sand beaches. A mass balance showed that the amount of *E. coli* measured in eroded sand prior to the wave event was sufficient to account for the measured increase in *E. coli* concentrations in the surface water. Field data indicated that in order for waves and associated sand erosion to significantly affect surface water *E. coli* concentrations, there must be a preceding period of calm non-erosive wave conditions during which *E. coli* can build-up in the foreshore reservoir. The magnitude of the average wave height also affected the time for *E. coli* concentrations in the surface water to decrease after the maximum concentrations have occurred. This is likely due to increased offshore mixing associated with higher wave activity. In addition, *E. coli* concentrations in the upgradient beach area (i.e. landward of the initial foreshore zone) were found to increase as the shoreline moved landward in response to larger wave activity. This may be due to lake water infiltrating into the upgradient unsaturated sand and delivering *E. coli* to the sand/pore water or due to the reviving of nonculturable bacteria through added moisture.

Beaches that are sheltered from waves and those with large bird populations were found to have higher *E. coli* concentrations in the surface water, as well as in the foreshore pore water, unsaturated sand, and saturated sand. After comparing methods for sampling FIB in pore water at six beaches with different sand characteristics, it was found that the sampling method chosen significantly affected the observed porewater FIB concentrations at fine sand beaches, but not at medium or coarse grain sand beaches. Data indicate that collecting pore water microbial samples using a shovel (PW-Shovel method) may be the most appropriate method at medium and coarse sand beaches as it is logistically the easiest method and provides little variability between samples compared to other methods. The method used for sampling at a fine sand beach should be determined based on the purpose of sampling. The study also found that the depth over which unsaturated sand samples are collected affects the FIB concentration with the highest FIB concentrations observed in the top 1 cm below the sand surface. This top layer of unsaturated surface sand was found to retain a larger proportion of *E. coli* at fine sand beaches compared to

coarse grain sands which showed a more even distribution of FIB over the top 5 cm below the sediment surface. Due to the highly variable concentrations observed in saturated sand relative to the mean concentrations, the saturated sand concentrations were not statistically different when different sampling methods were used. Finally, the high variability in FIB concentrations observed in all of the components of the foreshore reservoir (i.e. pore water, unsaturated sand, saturated sand) highlights the need for replicate samples, especially if foreshore sand and pore water are to be added to the sampling regime for public health monitoring.

6.2 Implications

The research presented in this thesis has important implications for beach water quality monitoring practices and modeling. The current practice for informing the public of a potential health hazard at a beach is inadequate. The lengthy time associated with FIB enumeration leads to beach advisories and closures that occur well after a water quality exceedance event has passed. New knowledge from this thesis can be applied to improve the accuracy of statistical and process-based models developed to predict beach water quality exceedances. This is critical for protecting human health at recreational beaches.

The techniques used in this thesis to evaluate how environmental factors, such as temperature and proximity to rivers/creeks, affect FIB concentrations in the foreshore reservoir on both a daily and seasonal scale can be applied to individual beaches to better predict when a water quality exceedance will occur. This information can then be combined with the results from Chapter 4 to determine how FIB in the foreshore reservoir can be transported to the surface water and cause a water quality exceedance. This thesis also highlighted the importance of sand grain size at beaches and how this may affect different management and monitoring approaches at individual beaches. For example, sand erosion may be the dominant mechanism that transfers FIB from the foreshore reservoir to surface water at fine sand beaches, however, interstitial pore water flow and discharge may be a more important mechanism for the transport of FIB from the foreshore reservoir to surface water at coarse sand beaches. Chapter 5 also concluded that sand grain size affects the distribution of FIB in the foreshore reservoir as well as the concentrations observed when using different methods to sample foreshore sand and pore water. The variations associated with the different components of the reservoir and different sampling methods needs to be considered in determining the optimum approach to assess risk. Significant growth of *E. coli* was also observed in unaltered and unseeded beach sand, not subjected to any external stimuli. Therefore, the ability of *E. coli* in representing a contamination event where there is an increased presence of pathogens may not be suitable if *E. coli* are able to thrive and even replicate in the beach environment.

6.3 Recommendations and future work

Although this thesis addressed key knowledge gaps related to recreational water quality monitoring and modeling, there are some limitations. Future work is required to address remaining uncertainties. For example, Chapter 3 explored variability of FIB in the foreshore reservoir, but was limited by the number of field sites. Environmental data was also limited to do the remoteness of some of the field sites. Further, short-term (daily) sampling was only conducted at one field site, limiting the applicability of the results to other beaches. The following are recommendations for future work aimed at improving understanding of FIB accumulation and variability in the foreshore reservoir.

- ❖ Evaluate the dynamics of FIB concentrations in the foreshore reservoir at different beach types (urban/rural, point source/non-point source, etc.) to more broadly understand and generalize factors controlling the temporal variability.
- ❖ Compare the relative occurrence and accumulation of pathogens relative to FIB in the foreshore reservoir.
- ❖ Conduct epidemiological studies at different types of beaches (urban/rural, point source/non-point source, fine sand/coarse sand etc.) to determine the health risk associated with high FIB levels in the foreshore reservoir. Also, conduct epidemiological studies at specifically non-point source beaches that have the potential for FIB growth in the sand to determine the health risk associated with the increased levels of FIB.
- ❖ Conduct combined field and laboratory studies to determine why FIB may replicate in the foreshore reservoir at some beaches but not at others and what parameters control this.
- ❖ While there is most likely a continuous exchange between the foreshore reservoir and surface water, detailed field studies need to be conducted to explicitly evaluate if high FIB presence in the foreshore reservoir usually comes first and leads to high concentrations in the surface water, or if the reverse is true.
- ❖ Investigate the importance of biofilm and organic matter build-up at the shoreline in the accumulation of FIB in the foreshore reservoir.
- ❖ Use microbial source tracking techniques to determine sources of FIB in the foreshore reservoir including markers for human sewage as well as birds and other wildlife.
- \div Investigate beach restoration and design options that may reduce the accumulation and potential replication of FIB in the foreshore reservoir (e.g. beach grooming, wildlife deterrents).

Chapter 4 focused on the influence of high wave conditions on the transport of FIB from the foreshore reservoir to the surface water. The majority of the data presented in this chapter was from one field site, limiting the applicability of the results to other beaches, especially marine beaches. This study exclusively focused on the movement of *E. coli*, and therefore the applicability of these results for the transport of other bacterial, protozoan, or viral pathogens remains unclear.The following are recommendations to further enhance and generalize our understanding of FIB transport from the foreshore reservoir to surface waters.

- ❖ Determine if erosion and interstitial pore water flow is also important for mobilizing bacterial, protozoan, and viral pathogens from the foreshore reservoir to surface water.
- ❖ Conduct rigorous field studies to examine FIB transport at beaches with different grain sizes (medium, coarse) to determine if sand erosion is the dominant transport mechanism from the foreshore reservoir to surface water at non fine sand beaches.
- ❖ Conduct field or modeling studies to determine the length of time FIB are generally in the foreshore reservoir before they are flushed out by the surface water or die.
- ❖ Determine if the mechanisms by which FIB is releases from the foreshore reservoir to surface water varies for beaches with engineering structures (e.g. breakwater structure) or at sheltered embayed beaches.
- ❖ Use field data to develop coupled groundwater-surface water mechanistic models to evaluate the release of FIB from the foreshore reservoir and the subsequent fate of FIB in the surface water (i.e. due to offshore mixing).

Chapter 5 compared results obtained when different methods were used to sample the foreshore reservoir and evaluated how FIB partitions between the components of the reservoir (i.e.
unsaturated sand, saturated sand, pore water). This study was limited by the number and types of field sites and would benefit from additional results from other beaches. The limited number of field sites in addition to the numerous variables compared (e.g. grain size, exposure to waves, impact of birds) made it difficult to differentiate results based on individual variables. The following are recommendations for work that is required to finalize a standard sampling method for foreshore sand and pore water to enumerate FIB as may be required in the future for beach monitoring programs.

- ❖ Compare sand and pore water sampling methods at more beaches, especially marine beaches, to determine if results are consistent to the beaches examined in this study.
- ❖ Compare the methods and results in this study to sampling other bacteria and pathogens to determine if the results are consistent.
- ❖ Communicate with government stakeholders and health departments to evaluate the feasibility of adding foreshore reservoir sampling to current water quality sampling protocols.
- ❖ Explore other sampling methods such as composite samples and longer core samples that may be used to sample sand and pore water at beaches.

6.4 References

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Appendix A: Supplementary Material for "Temporal variations in the abundance of fecal indicator bacteria in foreshore sand and porewater at freshwater beaches"

A.1 Correlation plots for Burlington Beach

Figure A.1: *E. coli* **concentrations in the unsaturated sand at Burlington Beach compared to temperature.**

Figure A.2: *E. coli* **concentrations in the saturated sand at Burlington Beach compared to temperature.**

Figure A.3: *E. coli* **concentrations in the pore water at Burlington Beach compared to temperature.**

Figure A.4: *E. coli* **concentrations in the ankle-depth water at Burlington Beach compared to temperature.**

Figure A.5: *E. coli* **concentrations in the waist-depth water at Burlington Beach compared to temperature.**

Figure A.6: *E. coli* **concentrations in the unsaturated sand at Burlington Beach compared to wave height.**

Figure A.7: *E. coli* **concentrations in the saturated sand at Burlington Beach compared to wave height.**

Figure A.8: *E. coli* **concentrations in the pore water at Burlington Beach compared to wave height.**

Figure A.9: *E. coli* **concentrations in the ankle-depth water at Burlington Beach compared to wave height.**

Figure A.10: *E. coli* **concentrations in the waist-depth water at Burlington Beach compared to wave height.**

Figure A.11: *E. coli* **concentrations in the unsaturated sand at Burlington Beach compared to rainfall.**

Figure A.12: *E. coli* **concentrations in the saturated sand at Burlington Beach compared to rainfall.**

Figure A.13: *E. coli* **concentrations in the pore water at Burlington Beach compared to rainfall.**

Figure A.14: *E. coli* **concentrations in the ankle-depth water at Burlington Beach compared to rainfall.**

Figure A.15: *E. coli* **concentrations in the waist-depth water at Burlington Beach compared to rainfall.**

Figure A.16: *E. coli* **concentrations in the unsaturated sand at Marie Curtis Beach compared to temperature.**

Figure A.17: *E. coli* **concentrations in the saturated sand at Marie Curtis Beach compared to temperature.**

Figure A.18: *E. coli* **concentrations in the pore water at Marie Curtis Beach compared to temperature.**

Figure A.19: *E. coli* **concentrations in the ankle-depth water at Marie Curtis Beach compared to temperature.**

Figure A.20: *E. coli* **concentrations in the waist-depth water at Marie Curtis Beach compared to temperature.**

Figure A.21: *E. coli* **concentrations in the unsaturated sand at Marie Curtis Beach compared to wave height.**

Figure A.22: *E. coli* **concentrations in the saturated sand at Marie Curtis Beach compared to wave height.**

Figure A.23: *E. coli* **concentrations in the pore water at Marie Curtis Beach compared to wave height.**

Figure A.24: *E. coli* **concentrations in the ankle-depth water at Marie Curtis Beach compared to wave height.**

Figure A.25: *E. coli* **concentrations in the waist-depth water at Marie Curtis Beach compared to wave height.**

Figure A.26: *E. coli* **concentrations in the unsaturated sand at Marie Curtis Beach compared to rainfall.**

Figure A.27: *E. coli* **concentrations in the saturated sand at Marie Curtis Beach compared to rainfall.**

Figure A.28: *E. coli* **concentrations in the pore water at Marie Curtis Beach compared to rainfall.**

Figure A.29: *E. coli* **concentrations in the ankle-depth water at Marie Curtis Beach compared to rainfall.**

Figure A.30: *E. coli* **concentrations in the waist-depth water at Marie Curtis Beach compared to rainfall.**

Figure A.31: *E. coli* **concentrations in the unsaturated sand at Marie Curtis Beach compared to flow rate in Etobicoke Creek.**

Figure A.32: *E. coli* **concentrations in the saturated sand at Marie Curtis Beach compared to flow rate in Etobicoke Creek.**

Figure A.33: *E. coli* **concentrations in the pore water at Marie Curtis Beach compared to flow rate in Etobicoke Creek.**

Figure A.34: *E. coli* **concentrations in the ankle-depth water at Marie Curtis Beach compared to flow rate in Etobicoke Creek.**

Figure A.35: *E. coli* **concentrations in the waist-depth water at Marie Curtis Beach compared to flow rate in Etobicoke Creek.**

A.3 Correlation plots for Ipperwash Beach

Figure A.36: *E. coli* **concentrations in the unsaturated sand at Ipperwash Beach compared to temperature.**

Figure A.37: *E. coli* **concentrations in the saturated sand at Ipperwash Beach compared to temperature.**

Figure A.38: *E. coli* **concentrations in the pore water at Ipperwash Beach compared to temperature.**

Figure A.39: *E. coli* **concentrations in the ankle-depth water at Ipperwash Beach compared to temperature.**

Figure A.40: *E. coli* **concentrations in the waist-depth water at Ipperwash Beach compared to temperature.**

Figure A.41: *E. coli* **concentrations in the unsaturated sand at Ipperwash Beach compared to wave height.**

Figure A.42: *E. coli* **concentrations in the saturated sand at Ipperwash Beach compared to wave height.**

Figure A.43: *E. coli* **concentrations in the pore water at Ipperwash Beach compared to wave height.**

Figure A.44: *E. coli* **concentrations in the ankle-depth water at Ipperwash Beach compared to wave height.**

Figure A.45: *E. coli* **concentrations in the waist-depth water at Ipperwash Beach compared to wave height.**

Figure A.46: *E. coli* **concentrations in the unsaturated sand at Ipperwash Beach compared to rainfall.**

Figure A.47: *E. coli* **concentrations in the saturated sand at Ipperwash Beach compared to rainfall.**

Figure A.48: *E. coli* **concentrations in the pore water at Ipperwash Beach compared to rainfall.**

Figure A.49: *E. coli* **concentrations in the ankle-depth water at Ipperwash Beach compared to rainfall.**

Figure A.50: *E. coli* **concentrations in the waist-depth water at Ipperwash Beach compared to rainfall.**

Appendix B: Supplementary Material for "Release of Escherichia coli from foreshore sand and pore water during intensified wave conditions at a recreational beach"

B.1 Location of field sites

Figure B.1: Location of field sites (Ipperwash Beach and Marie Curtis Beach).

B.2 Water levels, sand levels, and sample locations for field events

Figure B.2: Measured sand levels, water levels and sampling locations for (a) Event1, (b) Event2, and (c) Event3.

B.3 *E. coli* enumeration methods

After collection, water and sand samples were stored in a cooler with ice packs, transported to the laboratory, and analyzed within 6 hours. Water samples were filtered (0.45 μm pore size) using standard membrane filtration methods (U.S. Environmental Protection Agency 2002) then placed on chromogenic differential coliform (DC) agar, supplemented with cefsulodin, incubated at 44.5 °C for 20 hours, then enumerated as colony forming units (CFU/100mL). To extract *E. coli* from the sand, 25 g from each sand sample was placed in a sterile polypropylene bottle, diluted with 250 mL of phosphate-buffered saline, hand shaken for 2 minutes, and allowed to settle for 2 minutes (Boehm et al. 2009b). The supernatant was then processed using the same method as the water samples. An additional 25 g from each sand sample was used to quantify the sand moisture content gravimetrically by weighing the sand samples before and after being placed in an oven at 110 °C for 24 hours. Moisture contents were used to express sand-associated *E. coli* as CFU/g of dry weight.

B.4 Statistical analyses

Statistical analyses were performed on log10 transformed data using SigmaPlot (Systat Software Inc., San Jose, CA) and Minitab (Minitab Inc., State College, PA). Due to the large variability and small sample sizes non-parametric tests were used. The Mann-Whitney U Test was used to compare *E. coli* concentrations during the three field events (Event1, Event2, Event3). This test was also used to compare *E. coli* concentrations in the sand and pore water between Ipperwash Beach and Marie Curtis Beach. The Spearman rank-order correlation test was used to compare *E. coli* concentrations in the foreshore reservoir and ankle-depth water at Ipperwash Beach and Marie Curtis Beach. The Wilcoxon signed rank test was used to compare ankle- and waist-depth concentrations along one transect and between two transects 1 km apart at Ipperwash Beach. Water samples below the detection limit were recorded as 1 CFU/100mL for data analysis. Sand samples that were below the detection limit were recorded as 1 CFU and then divided by the dry weight. Results were considered significant with a p-value of less than 0.05.

Figure B.3: Conceptual diagram illustrating how the mass balance calculation was performed to quantify the total amount of E. coli associated with the volume of sand that eroded between the first and second sampling times. Vsur and Vsub are the volume of eroded sand above and below the water table, respectively. C_{sub} and C_{pw} [CFU/g, CFU/100mL] are E. coli concentrations in the subsurface sand and pore water, respectively. ϕ **is porosity which was estimated to be 0.3 (Coduto et al. 2011).**

Table B.1: Input values and results for mass balance calculations for all field events. Total *E. coli* **is reported as log total CFU (assuming one meter width of shoreline). "BDL" denotes below detection limit and "--" indicates no samples were collected.**

B.6 Shearing assay experiment

To estimate the percent detachment of *E. coli* from the sand upon suspension, a shearing assay experiment was conducted with sand from Ipperwash Beach. Surface (unsaturated) sand was collected 1 m landward of the shoreline using sterile spoons. Similar to Phillips et al. (2014), a gyratory shaker set to 300 revolutions per minute (RPM) was used to agitate a sand-water mixture for a set amount of time (100 s, 300 s, 500 s). The gyrating speed was set to 300 RPM because this was found to be the lowest speed for which at least 50% of the sand was suspended. For each set agitation time (100 s, 200 s, 300 s), 25 g of sand was placed in four beakers and 120 mL of sterile distilled water was gently poured on top with care taken to minimize sand disturbance. Two beakers were control beakers and were set on the bench for the set time. The other two beakers were placed in the gyratory shaker and agitated for the set time. Once the set time was reached, the supernatant from each beaker was poured into a separate beaker and *E. coli* in the supernatant of each beaker was enumerated using methods described above. The 25 g of sand was also enumerated using sand enumeration methods described above. The percent of *E. coli* released from the sand after the set agitation times was calculated as the *E. coli* in the supernatant divided by the sum of the *E. coli* in the supernatant and the *E. coli* associated with the sand, converted to volumetric units, after being in the gyrator. It was found that 80%, 84% and 85% of *E. coli* was released from the sand after 100 s, 300 s, and 500 s of suspension, respectively. In comparison 44%, 32%, and 38% of *E. coli* was released after 100 s, 300 s, and 500 s, respectively, for the control beakers. It is thought that the *E. coli* in the supernatant in the control beakers was a combination of *E. coli* that was initially freely-residing in the pore water, and *E. coli* that was detached from the sand as water was poured into the beaker. These control experiment results are consistent with those of Phillips et al. 2014 who reported 43% enterococci release in control

experiments. $F = 0.8$ was used in the sand mass balance calculation (4.1) to provide a conservative estimate for the fraction of *E. coli* detached upon sand erosion and suspension.

B.7 Sampling results for field events

Table B.2: Log transformed mean ± standard error *E. coli* **concentrations during Event1. All samples were collected in triplicate. "BDL" denotes** below detection limit and "-" indicates no samples were collected. The detection limit for water/pore water was 0 log CFU/100 mL and the detection **limit for sand samples was approximately -1 log CFU/g.**

Table B.3: Log transformed mean ± standard error *E. coli* **concentrations during Event2. All samples were collected in triplicate. "-" indicates no samples were collected.**

Table B.4: Log transformed mean ± standard error *E. coli* **concentrations in samples collected at ankle- and waist-depth during Event3. All samples were collected in triplicate. "-" indicates no samples were collected.**

B.8 Weekly/biweekly sampling at Ipperwash Beach and Marie Curtis Beach

	Ipperwash Beach		Marie Curtis Beach	
	$Mean + Std Error$	$\mathbf n$	Mean $+$ Std Error	n
Ankle-Depth (log CFU/100mL)	1.89 ± 0.04	196	2.22 ± 0.09	67
Waist-Depth (log CFU/100mL)	1.53 ± 0.04	202	2.09 ± 0.09	68
Pore Water (log CFU/100mL)	2.34 ± 0.05	253	2.73 ± 0.07	69
Surface Sand ($log CFU/g$)	0.94 ± 0.06	214	1.19 ± 0.09	66

Table B.6: Weekly/biweeky sampling results for Ipperwash Beach and Marie Curtis Beach.

B.9 References

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Appendix C: Supplementary Material for "Evaluation of methods to sample fecal indicator bacteria in foreshore sand and pore water at freshwater beaches"

C.1 Sampling methods

Figure C.1: Photos of the three sampling methods used.
Appendix D: Reproduction Licenses

D.1 Chapter 4

ACS Publications **Title:** Release of Escherichia coli from Foreshore Sand and Pore Water during Intensified Wave Conditions at a Recreational Beach **Author:** Laura J. Vogel, Denis M. O'Carroll, Thomas A. Edge et al **Publication:** Environmental Science & Technology **Publisher:** American Chemical Society **Date:** Jun 1, 2016 Copyright © 2014, American Chemical Society

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D.2 Chapter 5

Curriculum Vitae

Publications:

- **Vogel, L.**, T. Edge, D. O'Carroll, H. Solo-Gabriele, C. Robinson. 2017. "Evaluation of methods to sample fecal indicator bacteria in foreshore sand and pore water at freshwater beaches." *Water Research*. 121: 2014-212.
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