Bay of Quinte Beaches and Shorewells Microbial Source Tracking Survey - 2013

Thomas A. Edge, Stephen Hill, and Eva Nowak

Water Science & Technology Directorate
National Water Research Institute
Environment Canada
Burlington, Ontario

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INTRODUCTION

The Bay of Quinte at the northeastern end of Lake Ontario is an important water supply supporting diverse recreational activities and serving as a source of drinking water for many communities. A number of beaches in the Bay area are impacted by fecal pollution at times. Beach closures are one of the Beneficial Use Impairments (BUIs) that must be addressed to delist the Bay of Quinte Area of Concern (AOC). Questions have also been raised about the extent of fecal pollution contaminating offshore waters in the Bay of Quinte that are used for recreation and as a source of drinking water, as well as shorewells that are also used as a source of drinking water in some communities. The potential for restrictions to consumption of drinking water is another Beneficial Use Impairment that needs to be addressed in the Bay of Quinte Area of Concern.

A field study was conducted from June to September 2013 to characterize the microbial water quality at four beaches in the Bay of Quinte area. Microbial water quality was also investigated at two stormwater outfalls near Deseronto and Frankford Beaches. In the fall 2013, several water samples were also collected from shorewells. This research provides a follow up and comparison to water quality research conducted in the Bay of Quinte area in 2009 (Hill et al. 2010), 2010 (Edge and Hill, 2011), and 2011 (Edge et al. 2012). The objective of the research was to understand the extent of fecal pollution at sampling locations, and to determine if there was evidence for human fecal contamination as a contributor to the fecal pollution. Human fecal pollution from sources like sewage treatment plant effluents, leaking septic systems, and sanitary sewer cross-connections to storm sewers are generally regarded to present the highest potential for human health risks from the occurrence of waterborne pathogens. These are the fecal pollution sources that are usually the first target for remediation efforts. The following study applied *E. coli* surveillance and a microbial source tracking approach to investigate the source of fecal pollution at Bay of Quinte study locations in 2013.

Microbial source tracking techniques compare the similarity of microorganisms from fecal pollution sources and water samples in order to make inferences about the source of water contamination (U.S. EPA, 2005; Edge and Schaefer, 2006). There are two general approaches to microbial source tracking: library-dependent methods and library-independent methods. Library-dependent methods select an indicator microorganism like *E. coli*, and collect hundreds to thousands of isolates from fecal sources and nearby water samples of interest. The similarity of fecal and water *E. coli* isolates is measured by techniques like DNA fingerprinting to infer the likely source of the water isolates. While these methods can provide very useful information for beach managers (Edge and Hill, 2007; Edge et al. 2007; Edge et al. 2010), they are relatively time consuming to perform, and are less useful over larger areas. For this reason, a library-independent method was selected for the microbial source tracking investigation in the Bay of Quinte area.

Library-independent source tracking methods are based on searching for host-specific microorganisms in water samples rather than building large libraries of fecal indicator bacteria. These host-specific microorganisms are adapted to specific gastrointestinal tracts, and have a restricted distribution, occurring only in the gut of their host such as

humans or ruminant animals. If the DNA sequence of such a microorganism is detected in a water sample, it is an indication of fecal contamination from that human or animal host. Some of the most promising library-independent methods are based on detecting host-specific strains of anaerobic bacteria in the *Bacteroidales*. This group of bacteria is generally found in much greater numbers in mammalian gastrointestinal tracts than *E. coli*. In addition, human-specific strains of *Bacteroidales* are increasingly under investigation as indicators of the presence of fecal contamination from sources like municipal sewage (Bernard and Field, 2000; Bower et al. 2005; Field and Samadpour, 2007; Gawler et al. 2007; Ahmed et al. 2009). The present study analyzed water samples for the occurrence of a DNA sequence unique to human strains of *Bacteroidales* in order to assess impacts from human sewage contamination at Bay of Quinte study locations.

METHODS

Study area

The four beach sampling locations are shown in Figure 1 (Frankford Beach, Kingsford Beach, Deseronto Beach, and Northport Beach) along with other previously studied tributary sampling locations. Sampling was continued at two stormwater outfalls near Deseronto and Frankford Beaches in 2013, and water samples were also collected from three shorewells (296 Old Orchard Rd, 52 Rowlands Ln, 1520 #35 CR).

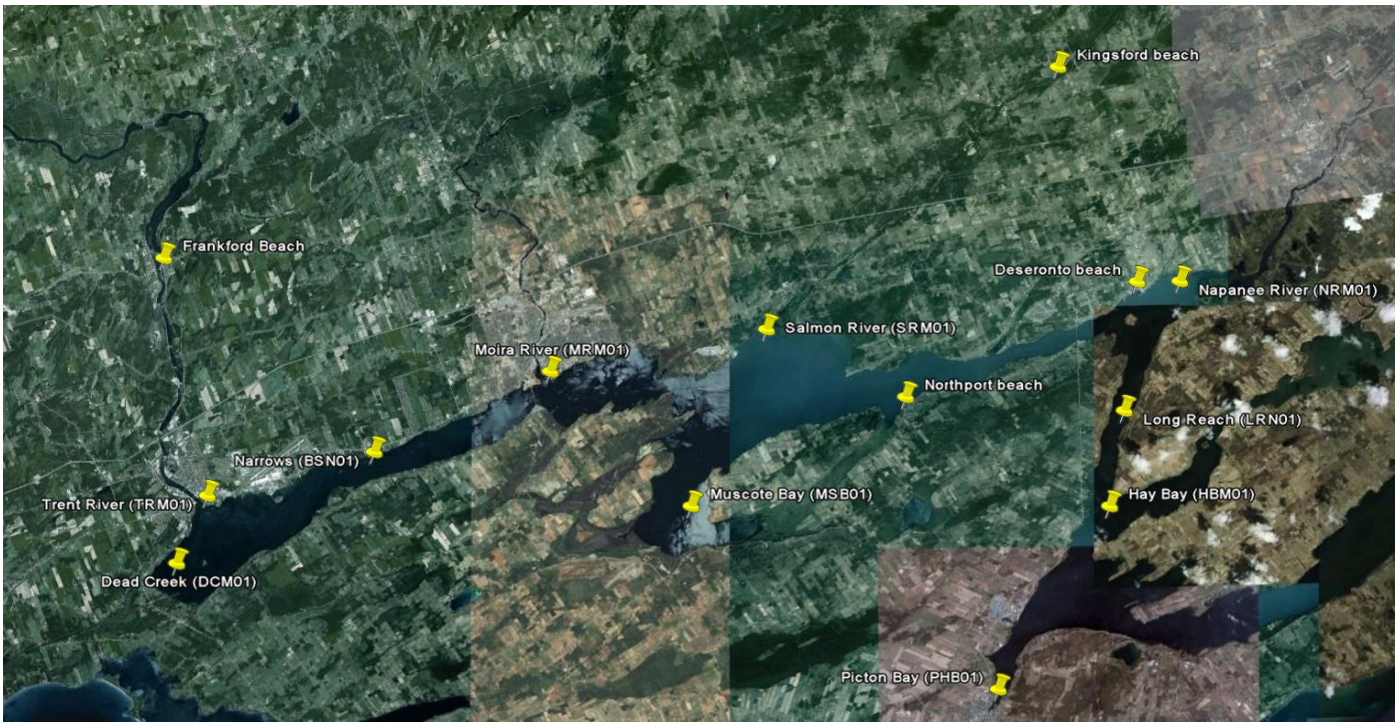

Figure 1. The Bay of Quinte study area showing the location of the four beach study sites (Deseronto, Frankford, Kingsford, and Northport).

Water and fecal reference sampling

Weekly water sampling was carried out at beaches as part of regular public health monitoring of beaches. Water sampling occurred from June 11 to September 23 at each beach. Three water samples were collected at each beach on each sampling day (corresponding to the outer and middle transects for the five water samples required by regular beach sampling protocols). Water samples were collected once at mid-day from three different shorewells on October 2, 2013. All water samples were collected in sterile polypropylene 500mL bottles, placed on ice in a cooler, and shipped overnight by courier to Environment Canada in Burlington for next day analysis.

Reference fecal samples from local municipal wastewater pollution sources were collected by Quinte Conservation from the Madoc sewage lagoon and the Belleville Sewage Treatment Plant in previous years. Additional reference samples from fecal pollution sources were previously collected from eastern Canada to test the host-specificity of the human *Bacteroidales* DNA marker assay. Municipal wastewater samples (raw untreated influent and final treated effluent) were collected from the Ashbridges Bay and Humber Sewage Treatment Plants, as well as final effluents from Sewage Treatment Plants in the Hamilton, Niagara and Atlantic regions. Fecal samples from dog and cat droppings were collected from Toronto, Ottawa and Atlantic Canada animal shelters. Fecal samples from bird droppings (e.g. gull, geese, duck) were collected from Toronto, Ottawa and Atlantic Canada. Livestock and poultry fecal samples were collected from farms in the Niagara Region, and Atlantic Canada. All wastewater effluent samples were placed on ice and returned to the National Water Research Institute, Environment Canada, in Burlington ON for analysis. Fecal samples were frozen at -20 C within several hours of collection.

E. coli enumeration

Water and wastewater samples were analyzed by membrane filtration and *E. coli* enumeration was expressed as colony forming units per 100 mL of water (CFU/100mL). Serial dilutions of water samples were performed and membrane filters were placed on the chromogenic differential coliform (DC) agar media supplemented with cefsulodin (Oxoid Inc.) for 18 hour incubation at 44.5°C. Sterile lab water samples were routinely filtered as negative controls to test potential for inadvertent sample contamination.

Bacteroidales DNA marker analysis

Water samples were assessed for the presence of strains of the anaerobic bacterium *Bacteroidales* that are associated with human fecal pollution (Bernhard and Field, 2000; Bower et al. 2005). This assay involved filtering as much water as the sample permitted, generally up to 300 mL for water samples. After filtration, the 0.45 µm membrane filters were frozen at -80C before subsequent DNA extraction steps. Each water sample was analyzed for the presence of human-specific strains of *Bacteroidales* bacteria (human *Bacteroidales* DNA marker HF183), as well as for the presence of a broader range of *Bacteroidales* bacteria (generic *Bacteroidales* DNA marker BAC32). Since the *Bacteroidales* group consists of a broad range of bacteria (beyond human-specific strains)

that can be commonly found in the environment, analysis for the generic *Bacteroidales* BAC32 marker serves as a form of positive reference to confirm the assay is capable of detecting and amplifying DNA targets in an environmental sample.

Membrane filters with total genomic DNA from water or wastewater samples were removed from -80C, and then homogenized in a Mini-Beadbeater (BioSpec Products Inc.) for 2 min. DNA was purified using a Powersoil DNA isolation kit (Mo BIO Laboratories, Inc.). A 1 µl extract was used as template in a polymerase chain reaction (PCR) assay using primer HF183F to amplify the human *Bacteroidales* DNA sequences and BAC32 to amplify generic *Bacteroidales* sequences if they were present in the sample. Primer BAC708R was the reverse primer for both reactions. For the PCR reaction, the following concentrations were used: 0.05 U/µl Hotmaster Taq and 1 x buffer (Intermedico), 0.8 mM dNTP mixture, 0.06% BSA, 1.56 pmol/ µl each primer and water to 25 µl. The PCR cycling conditions were: 2 min at 94°C followed by 35 cycles of 20 sec at 94°C, 10 sec anneal at 53°C for BAC32 or 63°C for HF183 primers, 50 sec at 65°C and a final single step at 65 °C for 7 min. A human fecal DNA extract was run as a positive control for each set of reactions, along with sterile water as a negative control. A 5 µl amount of dye DNA mix was loaded into wells of a 1.25% agarose gel, and run at 170 V for approximately 1 hr to resolve the bands which were visualized by staining with ethidium bromide and imaging under UV light.

RESULTS AND DISCUSSION

E. coli surveillance

The variability of *E. coli* concentrations at each beach transect is shown in Figure 2. All four beaches generally had good water quality in 2013, with Frankford Beach having the best water quality. The highest *E. coli* concentration measured in 2013 at Frankford Beach was only 69 CFU / 100 mL, indicating a clean beach. The other three beaches were generally clean, although *E. coli* concentrations could occasionally get higher at Kingsford Beach (820 CFU / 100 mL) Northport Beach (1620 CFU / 100 mL), and Deseronto Beach (1620 CFU / 100 mL). *E. coli* concentrations were very similar across the three transects at each beach on sampling days, and apparent differences in Figure 2 were largely due to missing data points for a transect (e.g. sample bottle broke in transit).

All four beaches would have been under the Blue Flag threshold value (maximum 20% of days posted) based on our data in 2013, although this did not include a full beach season of sampling starting in May. Frankford Beach did not have a sampling day > 100 *E. coli* CFU / 100 mL over our sampling period. Deseronto Beach had one sampling day > 100 *E. coli* CFU / 100 mL over our sampling period (1/16 = 6%). Northport Beach had one sampling day > 100 *E. coli* CFU / 100 mL over our sampling period (1/13 = 8%). Kingsford Beach had three sampling days > 100 *E. coli* CFU / 100 mL over our sampling period (3/16 = 19%). Restricting data analysis to just the beach season (i.e. June – August, and not including our sampling days on September 10, 17, and 23rd), would have further reduced the number of days exceeding 100 *E. coli* / 100 mL, as the one Deseronto

Beach exceedance, and two of the three Kingsford Beach exceedances were on these September sampling dates.

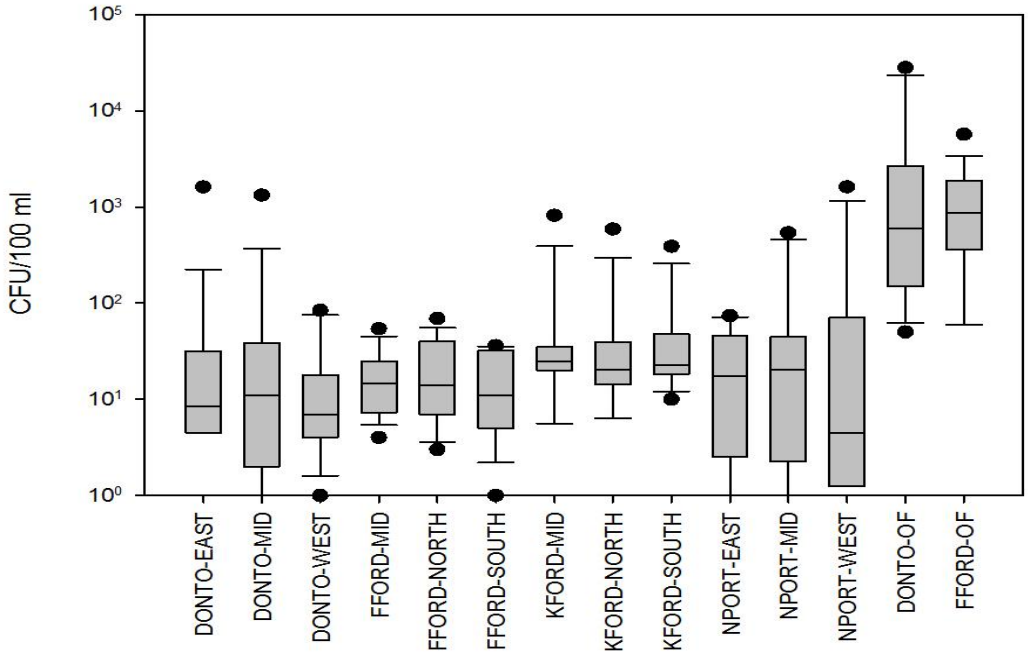

Figure 2. Box plots showing variation of *E. coli* concentrations at each Bay of Quinte beach transect, and two stormwater outfalls (OF) near Deseronto and Frankford Beaches over the sampling season in 2013 (June – September). The shaded boxes delimit the 25th and 75th percentile of data points, while the bar within each shaded box represents the median *E. coli* concentration. The sample size for each site was typically n=13 to 16.

The higher *E. coli* concentrations measured at Deseronto Beach in 2013 were likely related to the influence of a nearby stormwater outfall. The concentrations of *E. coli* in this outfall were normally above 100 CFU / 100 mL, and were measured as high as 28,100 CFU / 100 mL on September 10, 2013. *E. coli* concentrations were also normally above 100 CFU / 100 mL in the stormwater outfall near Frankford Beach. The highest concentration in the Frankford stormwater outfall in 2013 was measured at 5700 CFU / 100 mL. As described later, there were no human *Bacteroidales* DNA marker detections at these stormwater outfalls in 2013 suggesting no significant levels of human sewage contamination.

E. coli was not detected in the three shorewell water samples from October 2013.

The beach results from 2013 were generally consistent with the findings from previous sampling years (Table 1). Water quality conditions have been consistently good at Frankford Beach where *E. coli* concentrations were rarely above 100 CFU / 100 mL over the three sampling years. *E. coli* concentrations have also been relatively low at Northport Beach over these three sampling years. While water quality has not been as good at times at Kingsford Beach, the occasional spikes in *E. coli* concentrations at this beach are usually not much above 100 CFU / 100 mL and are not suggestive of high counts typically associated with significant human sewage contamination. Water quality at Deseronto Beach is often good, but as in past years, *E. coli* concentrations can spike above 1000 CFU / 100 mL at times at this beach. This is often associated with rain events, and the likely influence of a nearby stormwater outfall. The very high *E. coli* concentrations measured at Deseronto Beach in 2010 have not been seen since that year.

Table 1. *E. coli* concentrations (CFU / 100 mL) measured at three transects for each Bay of Quinte area beach over the 2010, 2011, and 2013 sampling seasons (June-September).

	2010			2011			2013		
Beach / transect	mean	min	max	mean	min	Max	mean	min	max
Deseronto Beach									
- east	1985	2	9000	9	0	68	105	0	1620
- mid	546	0	4700	57	0	590	100	0	1330
- west	418	1	3000	169	1	1090	18	1	84
Frankford Beach									
- north	40	12	99	50	7	87	21	3	69
- mid	117	4	1080	50	5	100	18	4	54
- south	29	4	96	44	10	85	17	1	36
Kingsford Beach									
- north	79	29	190	48	12	300	72	0	590
- mid	75	47	160	28	2	92	88	0	820
- south	91	31	170	33	14	95	63	10	390
Northport Beach									
- east	43	0	450	22	0	172	25	0	74
- mid	15	0	68	11	0	36	82	0	540
- west	20	0	150	6	0	35	154	0	1620

Bacteroidales DNA marker analyses

The host-specificity of the human *Bacteroidales* DNA marker has been examined using negative and positive control samples in our laboratory, as well as testing it against a variety of fecal samples collected from fecal pollution sources around the Bay of Quinte, southern Ontario, and Atlantic Canada. The human *Bacteroidales* DNA marker has been regularly amplified from human fecal samples run in our laboratory as positive control samples. It has not been detected in sterile water samples regularly run in our laboratory as negative control samples. The human *Bacteroidales* DNA marker has been detected in Madoc sewage lagoon samples from the Quinte area. The human *Bacteroidales* DNA marker has also been detected in 100 % of raw influent samples from the Niagara Region's Crystal Bay Sewage Treatment Plant (n=24), and 87 % of final effluent samples from Toronto's Ashbridge Bay Sewage Treatment Plant (n=52). Our results suggest the DNA marker is a conservative one, as it probably occurs below our level of detection in the final effluent from sewage treatment plants at times. A consequence is that our results from filtering 300 mL volumes represent more of a relative comparison of the occurrence of the human *Bacteroidales* DNA marker across water samples. Where the marker was not detected, the water sample could be truly negative for the DNA marker, or the DNA marker could be present, but only at a relatively low concentration below our limit of detection. In this sense, our % positive results for the human *Bacteroidales* DNA marker at a site are probably minimum values.

Host-specificity assessment of the human *Bacteroidales* DNA marker has indicated false positive results are rare. To date, the results of our host specificity testing of the human *Bacteroidales* DNA marker has not detected this marker in the following fresh fecal samples: dog (n=32), cat (n=24), gull (n=117), Canada geese (n=61), tern (n=24), cormorant (n=31), mallard duck (n=10), cow (n=75), pig (n=16), and chicken (n=21), raccoon (n=4), seal (n=3), rabbit (n=3), rat (n=1). In addition, several presumptive human *Bacteroidales* PCR amplicons obtained from Toronto water samples have been subjected to DNA sequencing for confirmatory analyses. The DNA sequences of these PCR amplicons were found to be most similar to *Bacteriodales* strains of *B. thetaiotaomicron* and *B. vulgatis* which have been associated with human fecal sources. However, host-specificity testing of the human DNA marker has found cross-amplification with two fresh fecal samples showing false positive results to date. One fecal sample was from a Toronto dog (1 of 33 samples tested) and one was from a Niagara chicken (1 of 22 samples tested). While the human *Bacteroidales* DNA marker is not expected to be a perfect host-specific marker for human fecal pollution (Kildare et al. 2007), our host-specificity testing to date, and results from other studies (Gawler et al. 2007; Ahmed et al. 2009) indicate there is little evidence for concern about significant effects from false positive results in our study around the Bay of Quinte.

Analyses for *Bacteroidales* DNA markers in water samples found that the generic BAC32 marker was detected in many of the water samples analyzed providing a measure to reduce concern about inhibition of PCR assays. The human *Bacteroidales* DNA marker was not detected in any of the beach water samples (n=181) collected in 2013, nor

in any stormwater samples from the outfalls near Deseronto (n=14) or Frankford Beaches (n=16) in 2013. The results provide no evidence of the presence of a significant level of human sewage contamination at these sampling locations over our 2013 study period.

These results are consistant with results from the 2010 and 2011 sampling periods for the human *Bacteroidales* DNA marker. Over three sampling years, the human *Bacteroidales* DNA marker has not been detected in about 500 water samples from the four Bay of Quinte beaches. This is consistent with generally low *E. coli* concentrations observed over much of the study period, and suggests the beaches are not impacted by any significant level of human sewage contamination. This is in contrast to some beaches in the Niagara or Toronto Areas of Concern where the same human *Bacteroidales* DNA marker can be detected in 40-50% of beach water samples.

Similarly, over two sampling years (2011 and 2013), the human *Bacteroidales* DNA marker has not been detected in 48 samples from the two stormwater outfalls near Deseronto and Frankford Beaches. This suggests that if there is any human sewage contamination in these stormwater systems, it is very low and below our level of detection. The levels of *E. coli* detected in these stormwater outfalls are typically below levels associated with sewage cross-connections, and are more likely from other animal sources such as pets or wildlife species. This is in contrast to stormwater outfalls around Toronto known to be contaminated by human sewage cross-connections where the same human *Bacteroidales* DNA marker can be detected in 80% of stormwater samples.

Overall, the microbial water quality at Bay of Quinte beaches was relatively clean over three sampling years. Our results have indicated that the beaches have typically not had beach postings exceeding the Blue Flag 20% posting criterion during the bathing season. There has been no evidence of any significant human sewage contamination at these beaches over the study period. Two nearby stormwater outfalls seemed to have typical levels of *E. coli*, and they did not show evidence of any significant level of human sewage cross-connections. Relatively higher *E. coli* concentrations could be detected at times at Deseronto Beach, and the nearby stormwater outfall likely contributes to *E. coli* contamination at this beach at times. *E. coli* spikes can also occur at other beaches associated with rain events. Steps should be taken to manage any potential risks associated with the stormwater outfall at Deseronto Beach, and the use of automatic rain rules could reduce bather exposure associated with rain events and degradation of water quality at Bay of Quinte beaches.

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