INFORMATION REPORT

TO: Mayor and Members Board of Health	**WARD(S) AFFECTED:** CITY WIDE
COMMITTEE DATE: November 28, 2011	
SUBJECT/REPORT NO: Public Health Services 2011 Public Beach Sampling Summary Report BOH11042 (City Wide)	
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Council Direction:

N/A

Information:

Executive Summary

This Report is an update to the Board of Health regarding the water quality at Hamilton's public beaches and what Public Health Services does to monitor the water quality at these beaches.

Public Works, Public Health Services, Hamilton Harbour Remedial Action Plan (RAP), National Water Research Institute, and the Bay Area Restoration Council (BARC) have been working together to improve the water quality at Hamilton Harbour Beaches. Measures including keeping birds off the beach and beach maintenance that have been in place at Pier 4 Beach since August 2005 have improved the water quality significantly at that beach (see Graph 2 – page 4).

There is an opportunity to improve the water quality at Bayfront Park Beach as well. As part of the Waterfront Masterplan, Public Works plans to bring forward a 2012 Capital Budget request to begin pre-design work for shoreline improvements to improve the water quality at Bayfront Beach. The request would be approved through the 2012 capital budget process. The City of Hamilton's Strategic Plan (Focus Area 6) aims to

remove Hamilton Harbour (Harbour) from Great Lakes area of concern list by 2015. Getting there includes a decreasing in the number of days when beaches are closed (6.8).

Background

Hamilton Public Health Services monitors recreational water quality at three public beaches along Lake Ontario, two beaches in Hamilton Harbour, and at three Conservation Areas according to the Ministry of Health and Long Term Care Beach Management Protocol under the Mandatory Public Health Standards and Protocols (2008). Beach water is tested for Escherichia coli. E. coli are bacteria normally and naturally found in the intestines of humans and warm-blooded animals E. coli in the water at public beaches indicates the presence of fecal contamination and the potential presence of other harmful micro organisms in the water.

Public warnings are issued when E. coli reaches or exceeds 100 E.coli bacteria cells per 100 ml of water (100 CFU's per 100 ml). Beach water quality with E. coli concentrations at or above 100 CFU's per 100 ml of water represent an increased risk of human infection. When E. coli concentrations are at or above 100 CFU's per 100 ml warning signs are posted at the beach advising potential bathers that the water may pose a health risk due to unacceptable levels of bacteria. At the same time the Public Health Services' Safewater website (www.hamilton.ca/beaches) and the Safe Water Information Line (905-546-2189) outgoing phone message are updated to reflect the changed beach water quality status.

Public Health Units in Ontario sample and test the recreational beach waters at public beaches according to the Ontario Public Health Standards Beach Management Protocol. The Beach Management Protocol requires that public beaches be sampled and tested for E.coli at least once per week during the swimming season. The frequency of sampling may be altered based on a variety of factors, with the most important factors being historic water quality or a sudden deterioration in water quality.

2011 Beach Sampling Results

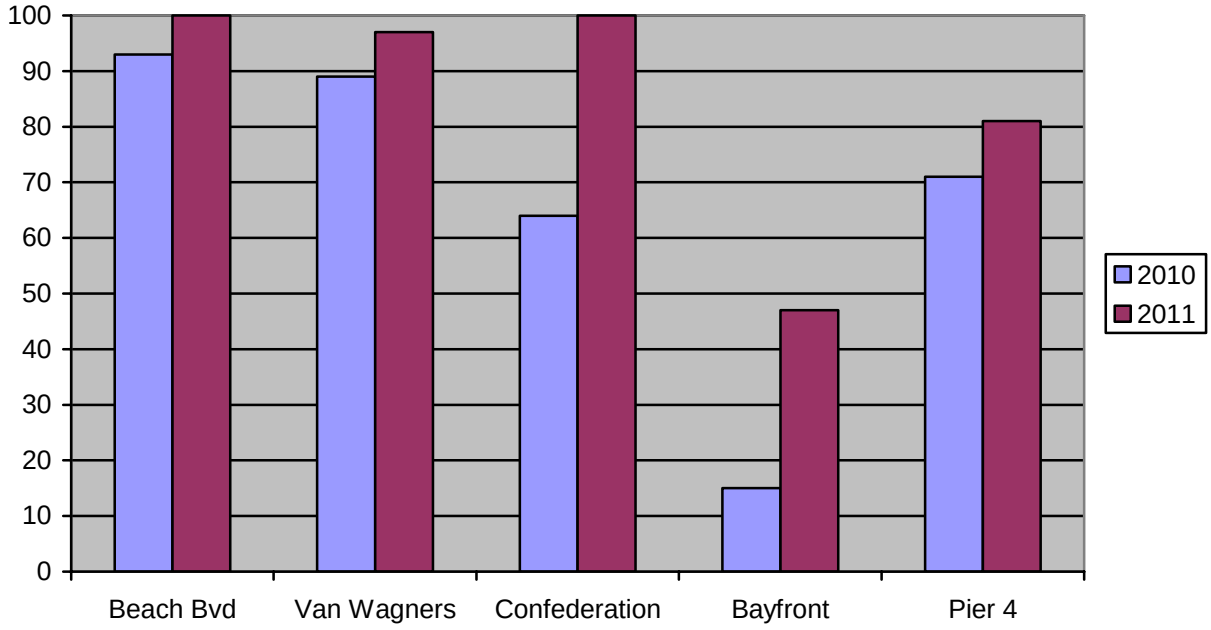
In 2011 the public beach sampling program occurred over a 15 week period starting the week of May 24, 2011 and ending September 2, 2011. Table 1 on the next page summarises the data from the 2011 beach season.

Table 1: 2011 Bathing Beach Monitoring Summary

Waterbody	Name of Beach	# of sampling sites	# weeks sampled	# days in bathing season	# days closed	% days Beach open
Lake Ontario	Beach Boulevard	14	15	108	0	100
	Van Wagners	6	15	108	3	97
	Confederation Park	6	15	108	0	100
Hamilton Harbour	Bayfront Park Beach	5	15	108	57	47
	Pier 4 Beach	5	15	108	21	81
Christie Reservoir	Christie Conservation	5	15	108	3	97
Lake Niapenco	Binbrook Conservation	5	15	108	3	97
Valens Reservoir	Valens Conservation	5	15	108	7	93

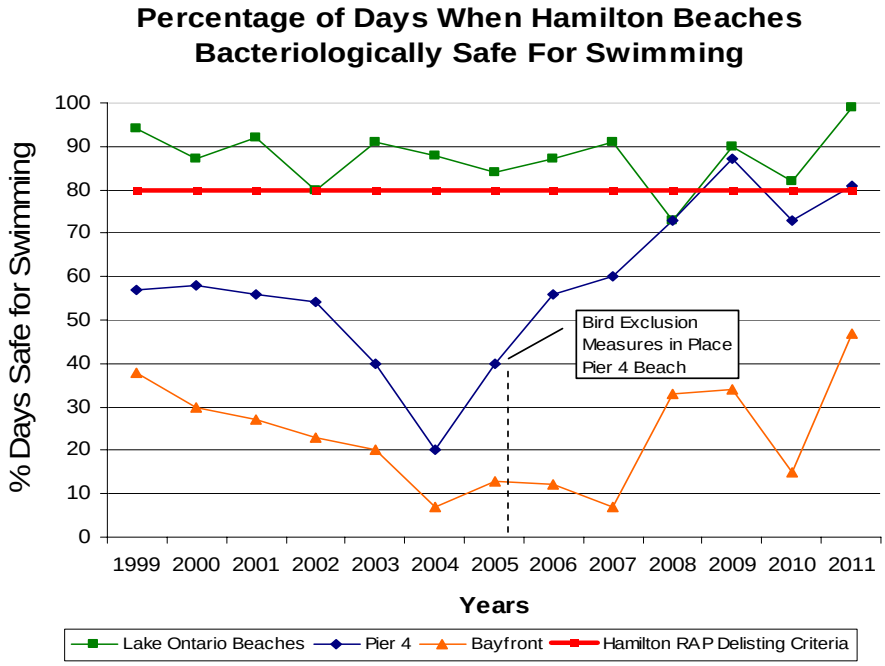
All Lake Ontario and Hamilton Harbour beaches had fewer days closed during the 2011 bathing season compared to 2010 as shown in **Graph 1** (next page). The geometric mean concentration of E. coli bacteria in the water samples collected weekly over 15 weeks at Beach Blvd Beach and Confederation Park Beach was below 100 CFU's/100 ml. This is good news. This may be attributed to lower than average rainfall in June and July of 2011. Heavy rain increases surface water run-off that can disturb sediments and make water cloudy. Storm events are also associated with increased wave action which also disturbs sediments. Combined June-July rainfall for Hamilton was 101.8 mm compared to June-July normal precipitation of 170.1 (source: Environment Canada). More beach closures were seen towards the end of the season when rainfall levels increased and water temperatures began to rise.

Graph 1: Hamilton Beaches Days Open 2011 vs 2010 (percentage)



Graph 2 below shows that the two Hamilton Harbour beaches (Pier 4 Beach and Bayfront Park Beach) have historically not met the RAP criteria due to high E. coli concentrations.

Graph 2:



Pier 4 Beach

Pier 4 Beach has made steady and significant improvement since implementation in 2005 of experimental bird exclusion measures and beach maintenance (removal of bird droppings, etc) by the Public Works Department, Parks and Cemeteries Section. The bird exclusion project at Pier 4 Beach involved several main activities:

- Installation of a buoy line to deter geese from swimming into the beach area when they cannot fly during moulting season.
- Installation of a fence around the perimeter of the beach to deter birds from walking into beach areas. An accidental finding is that the buoy line might inhibit the landing and take-off of Canada geese due to the short distance between the shoreline and the buoy line.
- Removal of visible fecal matter from the beach.
- Planting of shrubs around the perimeter of the beach and use of trained dogs to harass the geese.

Since 2005 the water quality at Pier 4 Beach results has improved steadily with the beach exceeding 80% open for the first time in 2009, and again in 2011.

Continuing and improving the bird exclusion measures and diverting rainfall drainage and other beach improvements at Pier 4 should continue to improve the water quality and recreational access.

Bayfront Beach

Excessive E. coli concentrations are a constant problem at Bayfront Beach. Research indicates that waterfowl are the likely source of the E.coli that is adversely affecting the water quality at Bayfront Beach (see Appendices A, B, C to Report BOH11042). The "Toward Safe Harbours 2008" report by BARC (Bay Area Restoration Council) cites the aforementioned research and supports the belief that that Canada geese, ring-billed gulls and other waterfowl are a source of *E. coli* at Harbour beaches. This report can be accessed at www.hamiltonharbour.ca/whysave-harbourreports.htm. There are a large number of Canada geese and seagulls in the area. They deposit fecal matter on the beach, in the swimming area waters, and on the grassy slopes and paved surfaces that are washed onto the beach and ultimately the water after heavy rains.

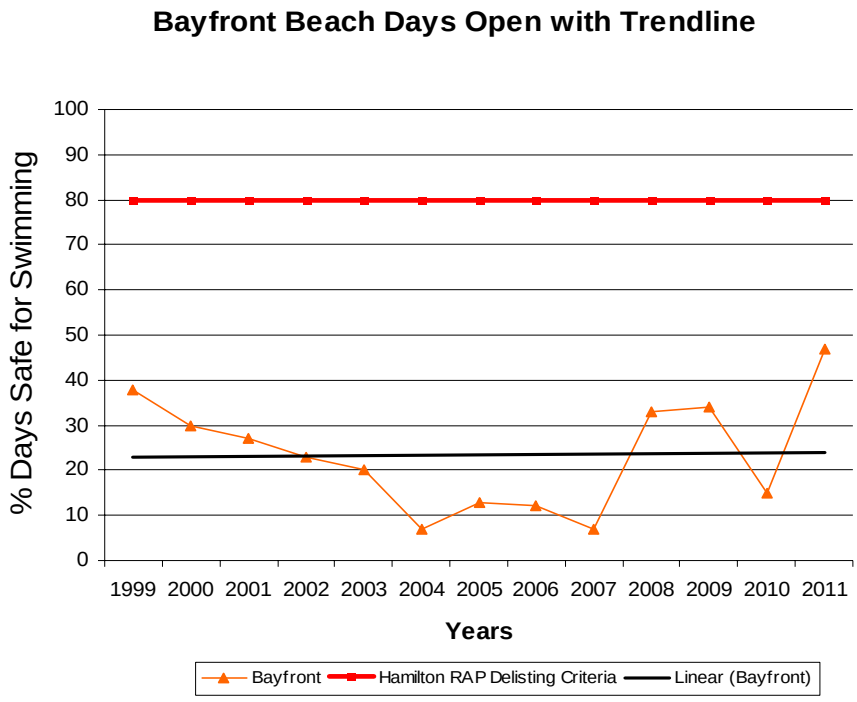
The water quality has improved at Pier 4 Beach since measures were taken to keep Canada geese away. This indicates that waterfowl are likely causing the recreational beach closures in Hamilton Harbour. Excluding waterfowl and their fecal droppings as much as possible from the Harbour beaches and swimming areas should improve the recreational water quality and access.

In August 2010 a buoy system similar to Pier 4 Beach was installed at Bayfront Beach in attempt to deter Canada geese from swimming into the Bayfront swimming area during the 6 week period when the geese cannot fly due to feather moulting. In April 2011 shrubs were planted around the edges of Bayfront Beach as a deterrent to the geese. Increased harassment from trained dogs occurred from April-October 2011, and beach grooming remained the same as prior seasons.

Bayfront Park beach water quality appears to be weakly improving over the past 4 years, but it will not likely meet the delisting criteria without additional bird exclusion measures and physical changes to the beach, nearby shoreline, and nearby landscape. Although the number of "days safe for swimming" improved for Bayfront Park in 2011 it also did at other area beaches. The long term trend at Bayfront Park show that water quality has not improved in the past decade (see Graph 3 – next page) and suggests that it is not likely to in the future without active measures to improve water quality at this beach.

The West Harbour Recreational Master Plan recommended park improvements to the swimming area at Bayfront Beach that included the introduction of a physical separation of the swimming area from the harbour to provide the opportunity for treatment of the beach water. In light of the recent improvements observed at Pier 4 beach the City of Hamilton Public Works Department is intending to bring forward a 2012 Capital Budget request to commence pre-design for park shoreline improvements to improve the water quality at Bayfront Beach. Public Health Services supports these measures and will play a consultative role in this project.

Graph 3



Cyanobacteria (Blue Green Algae)

Warning signs were posted at Hamilton Harbour beaches and other recreational water access points on September 1, 2011 due the presence of toxin producing cyanobacteria, commonly referred to as blue-green algae. The concentrations of microcystin toxin in water samples collected from the Bayfront Boat Launch and the Macassa Bay Yacht Club were 67 and 39 micrograms per litre of water (ug/L) respectively. The Health Canada Guideline for microcystins in recreational water is 20 ug/L. On October 4, 2011 a sample collected from the Hamilton Harbour West Marina boat launch had over 300 ug/L microcystin. Blue-green algae like surface blooms have been observed and reported at numerous recreational access points. Warnings were issued to all recreational water users (yacht clubs, etc) and the Public Works Department's Operations and Waste Management Division. As of November 1, 2011 blue green algae-like blooms continue to affect recreational water access points in western Hamilton Harbour.

Appendices Attached:

Appendix A
Appendix B
Appendix C

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Multiple lines of evidence to identify the sources of fecal pollution at a freshwater beach in Hamilton Harbour, Lake Ontario

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ABSTRACT

Multiple microbial source-tracking methods were investigated to determine the source of elevated *Escherichia coli* levels at Bayfront Park Beach in Hamilton Harbour, Lake Ontario. *E. coli* concentrations were highest in wet foreshore sand (114,000 CFU/g dry sand) and ankle-depth water (177,000 CFU/100 mL), declining rapidly in deeper waters. Many gull and geese droppings were enumerated each week on the foreshore sand within 2 m of the waterline. Both antimicrobial resistance analysis and rep-PCR DNA fingerprinting of *E. coli* collected at the beach and nearby fecal pollution sources indicated that *E. coli* in sand and water samples were predominantly from bird droppings rather than from pet droppings or municipal wastewater. Both methods indicated a trend of decreasing bird contamination, and increasing wastewater contamination, moving offshore from the beach. When foreshore sand was treated as a reservoir and secondary source of *E. coli*, waterborne *E. coli* were found to be more similar to sand isolates than bird or wastewater isolates out to 150 m offshore. Multiple lines of evidence indicated the importance of bird droppings and foreshore sand as primary and secondary sources of *E. coli* contamination in beach water at Bayfront Park.

1. Introduction

Fecal contamination of beaches can present significant public health risks, loss of recreational opportunities, and costly impacts for local economies. Around the Great Lakes, almost one-third of the beaches in Canada and the United States had swimming advisories, postings, or closures in 2003 (Environment Canada and US Environmental Protection Agency, 2006). Diverse fecal contamination sources contribute to these beach advisories, including point sources such as municipal wastewater effluents, and non-point sources such as agricultural run-off and wildlife droppings. It is important to identify the source of fecal contamination at beaches in order to better understand public health risks and correctly target fecal pollution prevention actions.

Municipal wastewater is a familiar source of fecal contamination at beaches (Dorfman et al., 2004; Bower et al., 2006). While improvements continue to be made to control sources such as sewage treatment plant effluents and combined sewer overflows, beach closures persist in many communities around the Great Lakes. There is growing recognition that, in addition to point sources, a better understanding is needed of the significance of non-point sources of fecal contamination (Kinzelman et al., 2004). For example, fecal droppings from

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birds (Levesque et al., 1993), impervious surface runoff (Scopel et al., 2006), mats of *Cladophora* green alga (Whitman et al., 2003), and foreshore sand (Whitman and Nevers, 2003) can serve as non-point sources of fecal indicator bacteria adversely impacting recreational waters.

Beaches in Hamilton Harbour, Lake Ontario, have been frequently closed in recent years despite investments in municipal wastewater infrastructure and storage tanks to control combined sewer overflows (Hall et al., 2006). It had been assumed that beach closures were probably the result of municipal wastewater contamination. However, recent investigations have suggested that bird droppings might be a contributor to the elevated numbers of *Escherichia coli* in beach waters (Charlton and Milne, 2004; Edge and Hill, 2004, 2005). The following study applied multiple lines of evidence to determine the source of *E. coli* contaminating Bayfront Park Beach in Hamilton Harbour. The field of microbial source tracking has developed in recent years to provide a toolbox of methods that are available for identifying the source of fecal contamination in aquatic ecosystems (Simpson et al., 2002). However, the field is still evolving, and there is recognition that multiple lines of evidence are generally needed to resolve fecal contamination problems (USEPA, 2005a; Edge and Schaefer, 2006; Rochelle and De Leon, 2006). For this reason, antimicrobial resistance analysis (Whitlock et al., 2002) and rep-PCR DNA fingerprinting (Johnson et al., 2004) methods were applied in parallel, along with *E. coli* monitoring and beach observations, to determine the source of *E. coli* at Bayfront Park Beach.

2. Materials and methods

2.1. Study site and field observations

Hamilton Harbour is a 2150 ha embayment at the western end of Lake Ontario. It is situated in an urban setting surrounded by the cities of Hamilton and Burlington (population of 640,000 in 2001). Four municipal wastewater treatment plants discharge into the harbor area, and combined sewer overflow storage tanks occasionally overflow during storm events. There are large populations of ring-billed gulls (*Larus domesticus*) and Canada geese (*Branta canadensis*) around the harbor, and they are increasingly common in beach areas. Hamilton Harbour is listed as a Great Lakes Area of Concern, and beach closures are identified as one of the beneficial use impairments that are being addressed through a Remedial Action Plan (Hall et al., 2006). The harbor supports an active recreational environment for windsurfers and boaters, although beaches have often been closed in recent years as a result of high *E. coli* levels (O'Connor, 2003). Bayfront Park Beach is a 160 m crescent-shaped beach that is situated at the end of a promontory and set in a protective bay that reduces water circulation from the rest of the harbor. Over the 2004 bathing season, weekly observations were made of the number of animals and their fecal droppings around Bayfront Park Beach. Animals were enumerated on the beach and adjacent grassy areas, and fresh fecal droppings were counted along the beach within 2 m of the waterline.

2.2. Water, sand, and fecal sampling

Water and sand samples were collected at Bayfront Park Beach each Monday morning over the 2004 bathing season. Water samples were collected at the middle of the beach by wading out from the shoreline for ankle- and knee-depth samples. Additional surface water samples were collected by boat at about 150 m directly offshore of the beach at the mouth of the bay (6 m depth) and further offshore in the middle of the harbor (24 m depth). All water samples were collected in sterile bottles and returned on ice to the laboratory for analysis within several hours of collection. Two water samples were collected at each sampling location, and *E. coli* counts were expressed as the mean of the two replicates.

Sand samples were obtained from the wet foreshore sand within a meter of the waterline, and to a depth of about 15 cm, using a sterile plastic core (diameter = 2.5 cm). About 20 g of wet sand was recovered from the cores, placed in Whirlpak bags, and returned to the laboratory on ice for analysis within several hours of collection. Two adjacent sand cores were collected and *E. coli* counts were expressed as the mean of the two replicates.

Fecal samples were collected simultaneously with water and sand sampling. Municipal wastewater samples were obtained from combined sewer overflow storage tanks and three municipal wastewater treatment plant effluents (Hamilton Woodward, Dundas, and Waterdown Plants). Samples of feces from gulls, Canada geese, and mallard ducks (*Anas platyrhyncos*) were obtained from fresh fecal droppings on the beach in numbers approximating their representation on the beach. Additional fecal samples were collected from Canada geese droppings adjacent to the beach, and occasional dog droppings elsewhere in the Park. Fecal samples were also obtained from fresh droppings of stray dogs and cats at the City of Hamilton animal shelter. Fecal dropping samples were obtained using sterile culturette cotton swabs (BD Inc.). The swabs were stored on ice and returned to the laboratory for analysis within several hours of collection.

2.3. E. coli enumeration and isolation

Water and municipal wastewater effluent samples were analyzed by membrane filtration and *E. coli* enumeration was expressed as CFU/100 mL. Water samples were diluted and membrane filters were placed on chromogenic differential coliform (DC) agar media supplemented with cefsulodin (Oxoid Inc.) for 18 h incubation at 44.5 °C. Sterile water samples were filtered as negative controls. Sand samples were analyzed by a blender-based method and *E. coli* counts were expressed as CFU/gram of dry sand. Wet sand was weighed to 10 g and placed into 150 mL of phosphate buffer in a Waring blender. The sand was blended for 1 min and then left standing for another minute. The supernatant was then filtered following the membrane filtration procedure. Ten grams of wet sand was also dried overnight to get a dry weight conversion factor. Fecal swabs were streaked onto mFC agar (Difco Inc.) and incubated at 44.5 °C for 18 h. Isolates showing a typical blue color on mFC agar were selected for further *E. coli* identification confirmation tests. *E. coli* isolates

obtained from mFC agar or DC agar typically showed normal responses when grown on the other agar (data not shown).

E. coli were isolated from the weekly water, sand, and fecal samples to provide *E. coli* isolates representative of the beach area over the bathing season. Up to 12 *E. coli* isolates were randomly selected from DC agar plates for each water or sand sample. Between three and five *E. coli* isolates were randomly selected from mFC agar plates for each fecal swab. The isolates were picked with a sterile toothpick and streaked onto MacConkey agar (Difco Inc.) for overnight growth at 37 °C. Putative *E. coli* isolates on MacConkey plates were then tested for glucuronidase activity by growth and fluorescence in EC-MUG (Difco Inc.), and for indole production by growth in 1% (w/v) tryptone (Difco Inc.) and reaction with Kovac's reagent (Oxoid Inc.). Isolates positive for both tests were stored in 96-well Matrix plates (Matrix Technologies Corp., Hudson, NH) at −80 °C in tryptic soy broth and 15% (v/v) glycerol. *E. coli* ATCC 29194 and *Klebsiella* ATCC 33495 were used as positive and negative controls, respectively, during confirmation tests.

2.4. Antimicrobial resistance analysis

E. coli from 96-well Matrix plates were thawed and incubated overnight in a microplate containing 200 μL per well of EC-MUG broth at 44.5 °C. A 96-floating pin replicator (V&P Scientific, San Diego, CA) was used to transfer *E. coli* isolates to the surface of rectangular tryptic soy agar plates. The 12 antimicrobials (and three concentrations of each) used were as follows: ampicillin (5, 16, 32 μg/mL), cephalothin (5, 16, 32 μg/mL), chlorotetracycline (20, 40, 80 μg/mL), cloramphenicol (5, 16, 32 μg/mL), erythromycin (25, 50, 100 μg/mL), irgasan (= triclosan) (0.01, 0.1, 0.5 μg/mL), kanamycin (1, 5, 16 μg/mL), oxytetracycline (1, 5, 16 μg/mL), penicillin G (25, 50, 100 U), streptomycin (1, 5, 16 μg/mL), sulfamethoxazole (50, 200, 512 μg/mL), and tetracycline (1, 5, 16 μg/mL). Agar plates were incubated for 18 h at 37 °C and growth of *E. coli* isolates on plates with antimicrobials was compared to their growth on control plates without antimicrobials. To quantify their relative growth, plates were scanned on a standard optical scanner as TIF files, and optical density readings of colonies were obtained with the BMNIA filter of Bionumerics ver. 4.0 (Applied Maths, Austin, TX) after rolling ball background subtraction. *E. coli* antimicrobial resistance was measured as a continuous variable (ratio of its optical density on the antimicrobial plate relative to the control plate) and as a binary variable (an isolate was considered resistant to an antimicrobial if its growth was >0.73 of its growth on a control plate without the antimicrobial). The value of 0.73 was derived as a practical threshold after examining several thousand *E. coli* isolates and determining the optimal optical density for discriminating between susceptible and resistant responses across different antimicrobials. When data were recorded as binary, *E. coli* isolates were occasionally found to be resistant at a high concentration of an antimicrobial, while also susceptible at a lower concentration. In these cases, the data were corrected and scored as resistant at the lower concentration. Negative control wells (blank wells) and positive control wells (wells with other *E. coli* strains with known profiles) were included on antimicrobial resistance plates. The reproducibility of the method for ratio data was assessed by repeatedly testing (six times) the profiles of 88 different *E. coli* isolates. The isolates were clustered, and it was found that the average similarity of an isolate to one of its replicates was 86%.

Prior to statistical analysis of antimicrobial resistance data, *E. coli* isolates with identical antimicrobial resistance binary profiles from the same fecal dropping or wastewater sample (or sand sample) were removed to reduce library bias. The resulting library of *E. coli* antimicrobial resistance profiles was analyzed by discriminant analysis (SAS, 1999—PROC DISCRIM procedure) using a non-parametric nearest-neighbor ($k = 5$) approach (Ritter et al., 2003). A two-way analysis of the library was performed to discriminate between bird and wastewater *E. coli* classes. Three-way analyses of the library were also performed to discriminate between bird, wastewater, and pet *E. coli* classes, and between bird, wastewater, and sand *E. coli* classes.

The performance of the library was evaluated by internal and external accuracy measures. The internal accuracy of the library was evaluated by calculating average rates of correct classification (ARCC) using resubstitution and the less-biased jack-knife method. A crossvalidation evaluation was also performed by selecting fecal samples from each source class, such that 30% of the *E. coli* isolates from each class were removed from the library. The removed isolates were then presented as "unknowns" for assignment to a source class. In addition, a mock database was constructed in which isolates were randomly assigned to each source group (bird or wastewater) to test whether, inadvertently, analysis of the randomized database would provide artifactual correct classifications. The external accuracy of the library was evaluated by its ability to predict the correct class for *E. coli* proficiency isolates collected independently from the library from duck droppings at LaSalle Park across the harbor ($n = 457$), water samples likely contaminated by wastewater from nearby Redhill and Stoney Creeks ($n = 55$), and sand samples from Beachway Park Beach on Lake Ontario outside the harbor ($n = 113$).

When the library was applied to assign water and sand *E. coli* isolates, an isolate was classified as "unknown source" when it could not be assigned to either bird or wastewater source classes with a probability of greater than 0.67. An *E. coli* isolate was classified as "unknown source" in three-way analyses when it could not be assigned to one of the three classes with a probability of greater than 0.5. These probability thresholds were chosen as a practical approach to minimizing incorrect classifications. A minimum detection percentage (Whitlock et al., 2002; Wiggins et al., 2003) was calculated based on misclassification rates to consider a conservative minimum limit for considering that a particular fecal source was present in water or sand samples.

2.5. Rep-PCR DNA fingerprinting analysis

Rep-PCR fingerprinting was performed using a BOX-PCR primer approach. A 96-pin replicator was used to transfer *E. coli* isolates to 96-well microplates containing 200 μL of tryptic soy broth in each well. Isolates were incubated at 37 °C for 16–18 h. In addition to the test isolates, four positive

controls with known BOX-PCR fingerprints and a negative control were added to each plate. Plates were centrifuged for 10 min at 3050 *g* to form a cell pellet. The cells were washed by removing the supernatant and resuspending the cells in 200 μL of sterile water. A PCR plate was filled with 5 μL of Lyse-N-Go reagent (Fisher Scientific, Nepean, Ont.) to which 5 μL of the cell suspension was added. Heating and cooling the suspension in a thermocycler as per the manufacturer's instructions lysed the cells, making the DNA available in a PCR stable solution. Fifteen microliters of master mix was created and added to achieve the following concentrations in the final 25 μL solution: 1 × Eppendorf HotMaster Taq buffer, 0.25 mM each dNTP, 5% (vol/vol) DMSO, 400 nM BOX primer (sequence 5′-CTACggCAAggCgACgCTgACg-3′), and 0.1 U/μL HotMaster Taq (Eppendorf, Mississauga, Ont.) and ultrapure water. The amplification cycling conditions were as follows: initial denaturation of 2 min at 94 °C, followed by 35 cycles of 20 s at 94 °C, 20 s at 60 °C, and 5 min at 65 °C, with a final extension of 5 min at 65 °C. Electrophoresis of the PCR products was done in a 1.25% agarose gel in TAE buffer with three rows of 50 wells. Three microliters of sample combined with loading dye was loaded into the wells. Three microliters of a $\frac{1}{2}$ dilution of Promega 1 kb ladder was used as a standard in four wells per row. A voltage of 170 V was applied until the bottom dye marker reached the end of the gel (approximately 3.5 h). The gel was stained in ethidium bromide for 30 min and destained in water for 20 min. Following staining, DNA bands were visualized by exposure to UV light and the image was captured at an exposure just below the saturation level of the brightest bands in the ladder.

Gel images were imported into Bionumerics ver. 4.00. Automatic lane and band calling were used; however, since most analyses were conducted using lane curves rather than band matchings, manual alterations were not made. DNA fingerprint comparisons were based on using a Pearson coefficient (0.28% optimization) and UPGMA clustering. Isolates that did not have at least one band with a volume of 2000 were removed to exclude failed amplifications. The reproducibility of the controls was found to be approximately 90%, which was the value used to remove *E. coli* isolates (clones) from the same fecal dropping or wastewater sample (or sand sample) to reduce library bias. Similar to antimicrobial resistance analysis, the *E. coli* rep-PCR DNA fingerprinting library was analyzed by two-way and three-way cluster analyses for birds, municipal wastewater, pets, and sand source classes. Performance of the DNA fingerprint library was evaluated in BioNumerics by simulating jack-knife-based ARCC using a maximum similarity measure and nearest-neighbor approach. Libraries were classified against themselves using $K = 7$, with nearest-neighbor source matches needing to be greater than 4 ($K = 7$ was used rather than $K = 6$ because one match would be the unknown isolate against itself, so there must be at least three other matches to a source before the isolate could be classified as such). ARCCs were expressed as a percentage of those isolates that could be identified after "unknown" source isolates were removed. When the DNA fingerprint library was applied to assign unknown water and sand isolates, they were compared to the fecal isolates using maximum similarity and a $K = 6$ nearest-neighbor approach. When a water or sand isolate had a tie with the number of nearest-neighbor matches for two fecal source classes, it was classified as "unknown source." Minimum detection percentages were calculated as they were for antimicrobial resistance analyses.

3. Results

Weekly monitoring results for cumulative numbers of bird droppings on foreshore sand and *E. coli* concentrations in ankle-depth water at Bayfront Park Beach are presented in Fig. 1. The highest concentrations of *E. coli* were found in ankle-depth water, dropping off rapidly at knee depth, and again at sites further offshore. The concentration of *E. coli* reached 177,000 CFU/100 mL in ankle-depth water on August 3. *E. coli* concentrations also peaked on this day at knee depth (8750 CFU/100 mL) and at the offshore bay (425 CFU/100 mL) and mid-harbor (162 CFU/100 mL) sites. *E. coli* numbers were otherwise less than 100 CFU/100 mL at the two offshore sites over the sampling period. High concentrations of *E. coli* were found in wet foreshore sand ranging from 248 to 114,000 CFU/g dry sand. The sand concentrations generally increased over the sampling period and exceeded 100,000 CFU/g dry sand on July 26 and August 3.

Birds were the only significant animal fecal source observed in the beach area over the sampling period. Ring-billed gulls were observed at every sampling time, with up to about 160 gulls observed on the beach on some days. Canada geese were also common, with numbers increasing noticeably in early June. Up to about 175 geese could be observed on the beach and surrounding grass areas on some days. Small numbers of mallard ducks were occasionally observed on the beach. While dogs were walked in Bayfront Park, they were very rarely seen on the beach and their fecal droppings were only occasionally observed elsewhere in the Park area. Large numbers of gull and geese droppings were deposited close to the waterline, and at times, droppings were observed directly in the water, and on the sand subject to waves washing up onto the beach. Up to 808 gull droppings were counted along the beach on sampling days in the early spring, while up to 707 Canada geese droppings were counted on the beach in late July. Weekly counts of gull or Canada geese droppings were not significantly correlated with ankle-depth *E. coli* concentrations at Bayfront Park Beach.

A total of 1966 *E. coli* isolates were collected from Bayfront Park area fecal sources (Table 1). Simultaneously, 1615 isolates were collected from water and sand samples at Bayfront Park Beach. *E. coli* isolates from municipal wastewater sources showed a higher frequency of antimicrobial resistance than *E. coli* from bird or pet droppings. The frequency of antimicrobial resistance was lowest in *E. coli* from beach sand and water samples. An evaluation of the two-way and three-way fecal source discriminatory analyses is provided in Table 2. Two-way antimicrobial resistance and rep-PCR analyses resulted in jack-knife ARCCs of 84% and 82%, respectively. Two-way analyses of antimicrobial resistance data found that using the ratio data provided a higher ARCC than binary data (72%), so ratio data were used in subsequent two-way analyses. Two-way analysis of the randomly assigned bird and wastewater *E. coli* isolates had a low jack-knife ARCC of

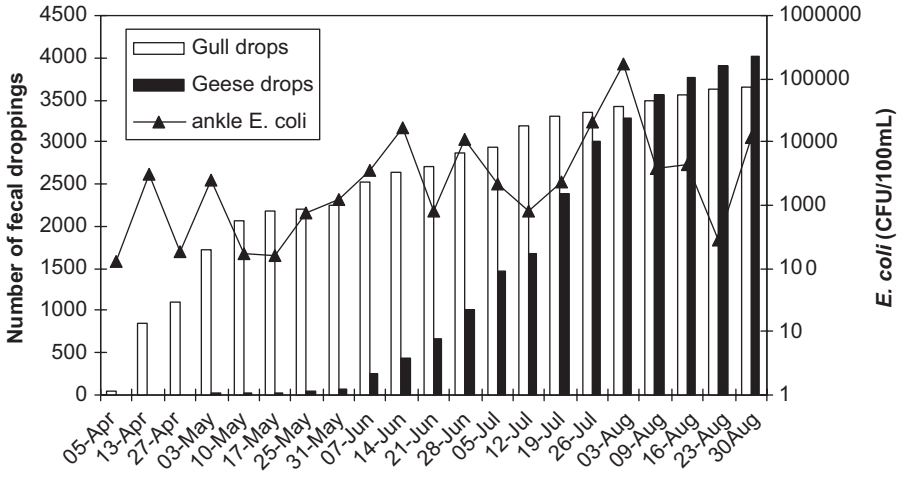

Fig. 1 – Cumulative numbers of bird fecal droppings and concentration of *E. coli* in ankle depth water at Bayfront Park Beach in 2004.

Table 1 – Sources of *Escherichia coli* isolates for antimicrobial resistance and rep-PCR DNA fingerprinting analyses

Source	No. of *E. coli* isolates					
	Antimicrobial resistance analysis			Rep-PCR analysis		
	No. of samples	Total	Decloned	No. of samples	Total	Decloned
Gulls	166	390	348	69	165	119
Canada geese	183	454	409	81	200	152
Ducks	27	99	82	8	23	18
Total birds	376	943	839	158	388	289
Dogs	38	186	143	38	186	96
Cats	46	203	165	46	199	87
Total pets	84	389	308	84	385	183
STP effluent	58	373	317	53	194	173
CSO tank	22	261	211	19	196	143
Total wastewater	80	634	528	72	390	316
Bayfront sand	35	370	295	27	196	138
Total	575	2336	1970	341	1359	926

49.5%, similar to the result expected by chance in a two-way analysis (50%). The crossvalidation test of the two-way antimicrobial resistance analysis found that 80% of the removed isolates were correctly assigned to their source class. Evaluation of the external accuracy of the two-way antimicrobial resistance analysis found that 64% of duck isolates and 61% of suspected wastewater isolates were correctly assigned to their source class. Some three-way analyses (e.g. antimicrobial resistance) had lower ARCC values than two-way analyses, but were still much better than expected by chance for each class (33%). The cross-validation test of the sand three-way antimicrobial resistance analysis found that 62% of the removed isolates were correctly assigned to their source class. Evaluation of the external accuracy of this three-way antimicrobial resistance analysis found that 50% of duck isolates, 54% of suspected wastewater isolates, and interestingly, 88% of Beachway sand isolates were correctly assigned to their source class.

When *E. coli* from water and sand samples were classified in the two-way analysis, both antimicrobial resistance and rep-PCR methods clearly indicated that most *E. coli* in sand and shallow ankle- and knee-depth water were more similar to *E. coli* from birds rather than wastewater sources (Fig. 2). Birds were the only fecal source that consistently exceeded minimum detection percentages for both antimicrobial resistance and DNA fingerprinting analyses. The rep-PCR method suggested a trend toward increasing presence of *E. coli* from wastewater sources at offshore sites, although the DNA fingerprinting results were not above the minimum detection percentage.

In the pet three-way analysis of *E. coli* from water and sand, both methods still indicated the prominence of *E. coli* from

Table 2 – Evaluation of the *Escherichia coli* library by antimicrobial resistance and rep-PCR DNA fingerprinting analyses

Discrimination analyses	N[a]	ARCC-1[b]	ARCC-2[c]	MDP[d]
Bird-wastewater (2-way)				
Antimicrobial resistance analysis	1367	90	84	19
Rep-PCR DNA fingerprinting	605	ND[e]	82	36
Bird-wastewater-pet (3-way)				
Antimicrobial resistance analysis	1675	87	80	24
Rep-PCR DNA fingerprinting	788	ND	83	34
Bird-wastewater-sand (3-way)				
Antimicrobial resistance analysis	1662	83	72	25
Rep-PCR DNA fingerprinting	743	ND	84	31

[a] Number of *E. coli* fecal isolates.
[b] Average rate of correct classification using resubstitution method.
[c] Average rate of correct classification using jack-knife method.
[d] Minimum detection percentage derived as described in Materials and methods.
[e] Not determined.

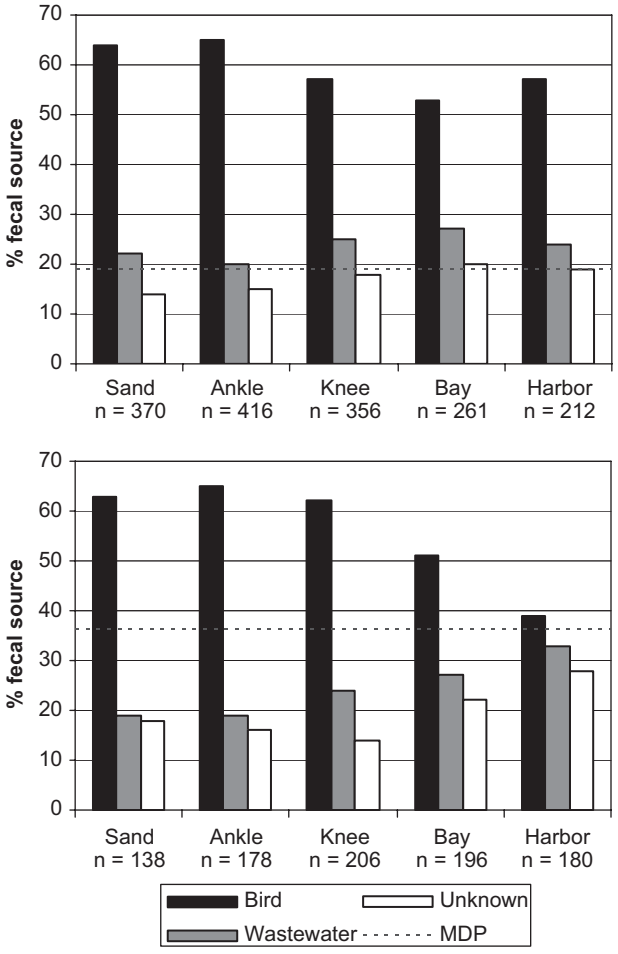

Fig. 2 – Two-way assignment of *Escherichia coli* isolates in Bayfront Park Beach sand and water samples to bird or wastewater fecal sources by antimicrobial resistance (top) and rep-PCR DNA fingerprinting (bottom) analyses. MDP = minimum detection percentage.

birds rather than from wastewater or pets in sand and shallow water (Fig. 3). However, unlike rep-PCR results, antimicrobial resistance analysis indicated *E. coli* from pets in ankle-depth water, and a greater prominence of *E. coli* from wastewater at offshore sites. When sand was treated as a reservoir and secondary source of *E. coli* in the three-way analysis, both methods indicated *E. coli* from ankle- and knee-depth water were mostly similar to *E. coli* from sand samples, rather than bird droppings or wastewater sources (Fig. 4). The prominence of *E. coli* from sand seemed to extend out to the mouth of the bay sampling site about 150 m offshore. Both methods also indicated that a transition occurred between knee depth and the mouth of the bay where *E. coli* from wastewater became more prominent than *E. coli* from birds.

4. Discussion

The highest concentrations of *E. coli* in water at Bayfront Park Beach were found in ankle-depth water, dropping rapidly as one moved offshore. Water samples from ankle-depth water exceeded Ontario provincial recreational water quality guidelines (geometric mean of 100 *E. coli* CFU/100 mL) at every sampling time. The *E. coli* concentrations in ankle-depth water reached as high as 177,000 CFU/100 mL, and were probably related to the protected nature of Bayfront Park Beach providing less water circulation and increased residence time of nearshore waters. The finding of such *E. coli* concentration gradients in beach waters has also been reported at other Great Lakes beaches (Whitman and Nevers, 2003; Sampson et al., 2005; US EPA, 2005b; Kleinheinz et al., 2006). At present, it is uncertain if high *E. coli* levels in shallow water present an increased public health risk for children who commonly play there. Epidemiology studies conducted to date at beaches have typically measured indicator bacteria densities in waters of swimming depth, and have addressed risks to adult swimmers rather than to infants and toddlers (US EPA, 2005b).

High concentrations of *E. coli* were found in the wet foreshore sand at Bayfront Park Beach, reaching over 100,000 CFU/g dry sand on two sampling occasions. *E. coli* concentrations in foreshore sand have been reported at other Great Lakes beaches, ranging from around 10 CFU/g dry sand (Alm et al., 2003) to 1.1×10^4 CFU/100 mL (Whitman and Nevers, 2003), and 20,000 CFU/g dry sand (Kinzelman et al., 2004). Whitman and Nevers noted that proper expression of *E. coli* counts in wet sand is unresolved. As there are no standard methods to measure *E. coli* in sand, it is difficult to compare results from Bayfront Park Beach with other studies.

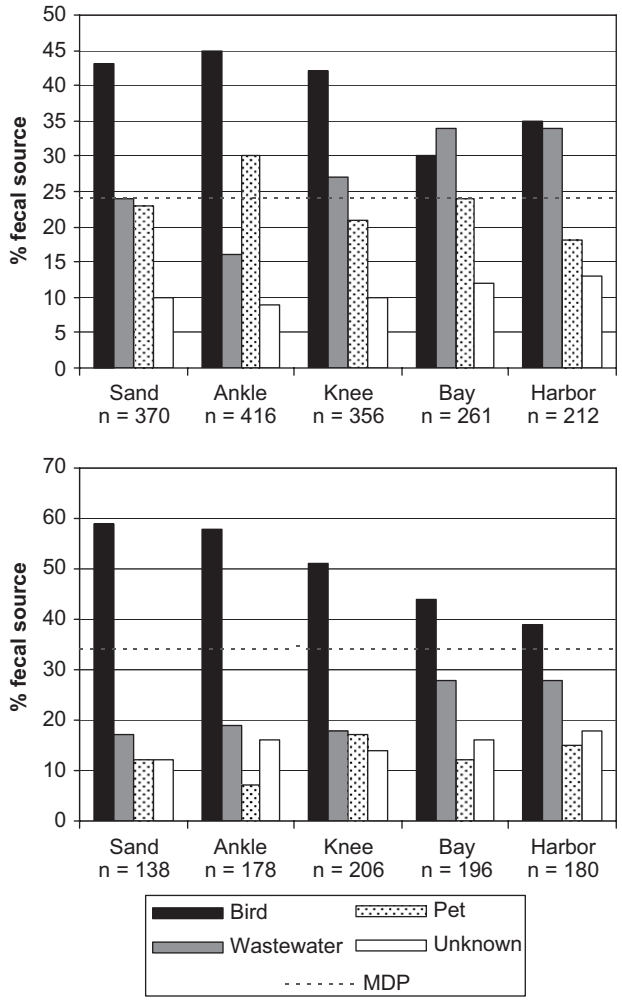

Fig. 3 – Three-way assignment of *Escherichia coli* isolates in Bayfront Park Beach sand and water samples to bird, wastewater, or pet fecal sources by antimicrobial resistance (top) and rep-PCR DNA fingerprinting (bottom) analyses. MDP = minimum detection percentage.

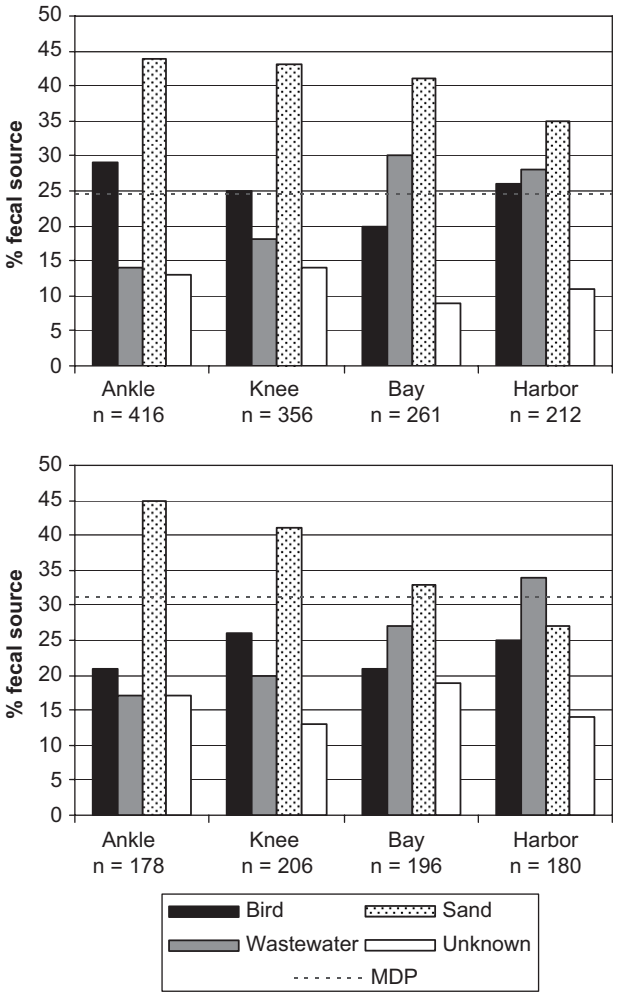

Fig. 4 – Three-way assignment of *Escherichia coli* isolates in Bayfront Park Beach water samples to bird, wastewater, or sand sources by antimicrobial resistance (top) and rep-PCR DNA fingerprinting (bottom) analyses. MDP = minimum detection percentage.

In our source-tracking study, we chose to use a blender-based extraction method in a rigorous attempt to recover a representative sample of *E. coli* cells including those that might be in biofilms or more closely adhering to sand particles. Irrespective of measurement method, high numbers of *E. coli* in sand relative to adjacent beach water suggests that foreshore sand can serve as a potential reservoir and non-point source of *E. coli* (Whitman and Nevers, 2003; Alm et al., 2003; Kinzelman et al., 2004).

Since Bayfront Park Beach was posted for most of the bathing season, people were rarely seen on the beach, and ring-billed gulls and Canada geese were the only animals regularly observed. Gulls were regularly observed standing at the water's edge, and their fecal droppings were observed directly in the water or on the wet sand subject to wave action. Canada geese, and their droppings, became more numerous on the beach at the beginning of June. These gull and geese droppings would have been a significant source of *E. coli*. Gould and Fletcher (1978) studied caged gulls and found that individual gulls could produce between 34 and 62 fecal droppings in 24 h. Alderisio and DeLuca (1999) found that gull feces had 3.68×10^8 fecal coliforms per gram of feces, while the geese had 1.53×10^4 fecal coliforms per gram of feces. Fogarty et al. (2003) reported *E. coli* numbers in gull feces from a Chicago beach as high as 1.9×10^9 CFU/g of feces. The gull and Canada geese droppings on Bayfront Park Beach would have provided a continuous loading of *E. coli* into foreshore sand over the bathing season.

Both antimicrobial resistance and rep-PCR DNA fingerprinting methods indicated the importance of *E. coli* contamination from bird droppings rather than from pet droppings or municipal wastewater sources at Bayfront Park Beach. We chose to interpret the microbial source-tracking results in a more qualitative sense, since the basis for drawing accurate quantitative conclusions has not been well established (Griffith et al., 2003; Stoeckel et al., 2004; Moore et al., 2005; US EPA, 2005a; Stoeckel and Harwood, 2007). The results from antimicrobial resistance analyses were similar to those from

other studies in finding higher frequencies of resistance in *E. coli* from municipal wastewater than from wildlife fecal sources (Guan et al., 2002; Edge and Hill, 2005; Salmore et al., 2006). They were also consistent with DNA microarray analyses, which found antimicrobial resistance genes more common in *E. coli* from the middle of Hamilton Harbour than in ankle-depth water at Bayfront Park Beach (Hamelin et al., 2006). The results from both microbial source-tracking methods, as well as enumeration of *E. coli* in sand and water samples, and observations of numerous bird fecal droppings provide multiple lines of evidence to indicate that birds were a more prominent source of *E. coli* contaminating Bayfront Park Beach than wastewater or pet sources over the 2004 bathing season.

Bird droppings have been reported to contribute to impairment of water quality at other beaches around the Great Lakes area (Standridge et al., 1979; Levesque et al., 1993; Whitman and Nevers, 2003; McLellan and Salmore, 2003). It was notable that the bird droppings could contribute to concentrations of *E. coli* as high as 177,000 CFU/100 mL in ankle-depth water at Bayfront Park Beach. Abulreesh et al. (2004) reported levels of *E. coli* up to 300,000 CFU/100 mL in British amenity ponds impacted by ducks and geese. Kirschner et al. (2004) reported levels of *E. coli* reaching 13,000 CFU/100 mL in shallow saline pools, whose fecal inputs were exclusively from birds such as gulls, geese and ducks. Such high *E. coli* concentrations are more typical of those measured at sources like stormwater or combined sewer overflow outfalls (Salmore et al., 2006; Bower et al., 2006; Scopel et al., 2006). While these high levels of *E. coli* are suggestive of human health risks, the risks associated with shallow beach water contaminated by high levels of *E. coli* from bird sources remain uncertain. While health risks might be lower than if the *E. coli* were from municipal wastewater sources, bird droppings can also be a source of pathogens (Levesque et al., 2000; Jones, 2005).

Both microbial source-tracking methods suggested that the frequency of *E. coli* from municipal wastewater sources seemed to be higher at sites further offshore in Hamilton Harbour. Four municipal wastewater treatment plants discharge into the harbor, and combined sewer overflow storage tanks occasionally overflow during storm events. It is likely that these sources of municipal wastewater contributed to *E. coli* contamination in the offshore waters. Hamelin et al. (2006) found that *E. coli* from the middle of Hamilton Harbour more commonly possessed virulence and antimicrobial resistance genes than *E. coli* isolates collected from ankle-depth water at Bayfront Park Beach. The possibility of sporadic municipal wastewater contamination from storm events, and continuous bird dropping contamination from beach sand, presents water-sampling challenges for microbial source-tracking studies. Our weekly water-sampling regime did not specifically capture wet weather events, and thus represents an integration of weekly *E. coli* contamination at Bayfront Park Beach waters over a whole bathing season. Microbial source-tracking water-sampling designs will need to be applied at the appropriate scale to the problem they are addressing (e.g. determining the predominant source of fecal contamination for a specific event, or for a whole bathing season).

The persistence of *E. coli* in foreshore sand is a poorly understood complication for applying microbial-source tracking methods at beaches. Gordon et al. (2002), Topp et al. (2003), and McLellan (2004) identified the differential survival of *E. coli* strains in secondary habitats outside the gut as a problem for microbial-source tracking studies. If there is significant differential survival of *E. coli* strains in beach sand, then the *E. coli* strain composition in the sand may no longer closely reflect the *E. coli* strain composition in the original fecal source (e.g. goose dropping). In addition, foreshore sand may serve as a reservoir for fecal indicator bacteria allowing them to persist for long periods of time and be resuspended in beach water through wave actions (LeFevre and Lewis, 2003; Whitman and Nevers, 2003; Kinzelman et al., 2004). In this case, resuspended *E. coli* may not be a reliable reflection of recent sources of fecal contamination. McLellan (2004) suggested that this might have accounted for the surprisingly low diversity of *E. coli* rep-PCR DNA fingerprints in beach water, and their unexpectedly low frequency of resemblance to *E. coli* from nearby gulls at Lake Michigan beaches.

When foreshore sand was treated as a reservoir and secondary source of *E. coli* at Bayfront Park Beach, both microbial source-tracking methods found that *E. coli* in the adjacent beach water were more similar to *E. coli* from the sand than from bird droppings or wastewater sources. It is possible the sand *E. coli* isolates may have originated largely from birds, but represent a unique subset of bird isolates with different survival characteristics, better enabling them to persist in sand and be mobilized into adjacent beach water. The similarity between *E. coli* in sand and water samples seemed to extend to the mouth of the bay about 150 m offshore, suggesting that beach sand was a continuous active source of *E. coli* loading into adjacent water over the beach season rather than a passive sink. These results are consistent with Whitman and Nevers (2003), who argued that while there is a continuous bidirectional flux of *E. coli* between sand and water, there was a net movement of *E. coli* from the sand lakeward at a Lake Michigan beach. The complexity of *E. coli* fluxes at the sand–water interface raises questions for microbial source-tracking studies, the appropriate grooming and management practices for reducing *E. coli* concentrations in sand, and for understanding the reliability of *E. coli* as an indicator of health risks in wet foreshore sand and shallow beach water where children play.

The library-dependent microbial source-tracking methods applied in this study provided results consistent with other lines of evidence to indicate that bird fecal droppings and foreshore sand were more prominent sources of *E. coli* contamination at Bayfront Park Beach than pet droppings or municipal wastewater. Similar results have been reported elsewhere in the Great Lakes, where more localized non-point sources of fecal contamination have unexpectedly been prominent causes of elevated *E. coli* levels at beaches rather than familiar point sources like municipal wastewater outfalls (McLellan and Salmore, 2003; Scopel et al., 2006). While *E. coli* library-dependent methods have disadvantages in terms of the costs and complexities of library building, they have advantages when validated library-independent methods for key fecal sources (e.g. birds) do not yet exist, and when results need to be communicated to end users who make decisions

using *E. coli* as a water quality indicator. Though more research is required to evaluate *E. coli* as a fecal source identifier, antimicrobial resistance and rep-PCR DNA fingerprinting analyses in this study provided useful results for identifying the most prominent source of fecal contamination over the temporal and spatial boundaries of a bathing season at Bayfront Park Beach on Lake Ontario.

5. Conclusions

1. *E. coli* library-based microbial source-tracking methods using antimicrobial resistance analysis and rep-PCR DNA fingerprinting identified the relative prominence of sources of fecal pollution over a bathing season at a freshwater beach on Lake Ontario, Canada.
2. Bird fecal droppings can be an important source of *E. coli* contamination in foreshore sand of temperate freshwater beaches.
3. Foreshore sand can serve as a significant reservoir of *E. coli*, and an important secondary source of *E. coli* contamination into adjacent beach waters.
4. A better understanding is needed of the survival and ecology of *E. coli* at the sand–water interface of beaches to inform sand-grooming practices and beach-management decisions to protect public health.

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NOTE / NOTE

Occurrence of antibiotic resistance in *Escherichia coli* from surface waters and fecal pollution sources near Hamilton, Ontario

Thomas A. Edge and Stephen Hill

Abstract: Antibiotic resistance was examined in 462 *Escherichia coli* isolates from surface waters and fecal pollution sources around Hamilton, Ontario. *Escherichia coli* were resistant to the highest concentrations of each of the 14 antibiotics studied, although the prevalence of high resistance was mostly low. Two of 12 *E. coli* isolates from sewage in a CSO tank had multiple resistance to ampicillin, ciprofloxacin, gentamicin, and tetracycline above their clinical breakpoints. Antibiotic resistance was less prevalent in *E. coli* from bird feces than from municipal wastewater sources. A discriminant function calculated from antibiotic resistance data provided an average rate of correct classification of 68% for discriminating *E. coli* from bird and wastewater fecal pollution sources. The preliminary microbial source tracking results suggest that, at times, bird feces might be a more prominent contributor of *E. coli* to Bayfront Park beach waters than municipal wastewater sources.

Key words: antibiotic resistance, *Escherichia coli*, surface water, fecal pollution.

Résumé : La résistance aux antibiotiques a été examinée chez 462 isolats de *Escherichia coli* issus d'eaux de surface et de sources de pollution fécale aux environs d'Hamilton, Ontario. Des *E. coli* furent résistants aux plus hautes concentrations de chacun des 14 antibiotiques étudiés, bien que la prévalence de la résistance élevée était plutôt basse. Deux des 12 isolats de *E. coli* provenant d'eau d'égouts d'un déversoir d'eau excédentaire avaient une résistance multiple à l'ampicilline, la ciprofloxacine, la gentamycine et la tétracycline au delà de leur seuil clinique. La résistance aux antibiotiques était moins prévalente chez les *E. coli* de fèces d'oiseaux que de sources d'eaux usées municipales. Une fonction de discrimination calculée à partir des données de résistance aux antibiotiques a fourni un taux de classification exacte de 68 % pour discriminer *E. coli* de sources de pollution fécale d'oiseaux versus d'eaux usées. Les résultats préliminaires de dépistage des sources microbiennes indiquent que les fèces d'oiseaux pourraient parfois contribuer davantage au *E. coli* des eaux de la plage de Bayfront Park que les sources d'eaux usées municipales.

Mots clés : résistance aux antibiotiques, *Escherichia coli*, eaux de surface, pollution fécale.

[Traduit par la Rédaction]

The spread of enteric bacteria with antibiotic resistance is a growing public health concern. Whereas hospital settings and the retail food supply are increasingly recognized as important sources of these bacteria (Karlowsky et al. 2003; Gorbach 2001), the significance of waterborne sources is less understood. Large quantities of enteric bacteria from human and animal fecal wastes can be released into rivers and lakes that serve as sources of water for drinking, recreation, or irrigation. A better understanding is needed about the prevalence of antibiotic resistance in these enteric bacteria and the significance of their occurrence in aquatic ecosystems. The potential of antibiotic-resistance analyses for microbial source tracking of fecal pollution also needs further investigation.

Escherichia coli is a useful enteric bacterium in the study of waterborne transfer of antibiotic resistance. It is adapted to human and other warm-blooded animal gastrointestinal tracts, and is readily exposed to a variety of medical and veterinary antibiotic treatments. *E. coli* can be a prominent carrier of antibiotic resistance among the commensal Enterobacteriaceae in the gut (Osterblad et al. 2000), and it is capable of transferring antibiotic resistance genes to pathogens in fecal flora such as *E. coli* O157 and *Salmonella* spp. (Blake et al. 2003). Since *E. coli* is more of a transient inhabitant of aquatic ecosystems in temperate climates, it is less likely to reflect naturally occurring sources of antibiotic resistance in microbial communities of aquatic ecosystems.

To date, there has been little investigation of the prevalence of antibiotic resistance in *E. coli* occurring in many ar-

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eas around the Great Lakes, which serve as significant sources of drinking and recreational waters. The following study investigated the occurrence of antibiotic resistance in *E. coli* from surface waters and fecal pollution sources near Hamilton, Ontario. Hamilton Harbour is situated at the western end of Lake Ontario, and is surrounded by the cities of Hamilton and Burlington (population of 640 000 in 2001). Drinking water is obtained from pipes extending offshore into Lake Ontario. The Harbour supports public beaches and an active recreational environment for boaters and windsurfers, although the beaches (e.g., Bayfront Park) have often been closed in recent years as a result of high *E. coli* levels (O'Connor 2003). Four municipal wastewater treatment plants discharge into the Harbour area, and combined sewer overflow (CSO) storage tanks occasionally overflow. The three major tributaries that drain into the Harbour are Spencer Creek, Red Hill Creek, and Grindstone Creek. There is little livestock or agricultural activity in the urban area surrounding the Harbour, although birds such as Canada geese, gulls, and ducks are common in beach areas.

Water, wastewater, and fecal samples were obtained between August 26 and October 23, 2002, within an area of about a 10-km radius from the centre of the Harbour. An additional preliminary beach water sample was collected on March 18, 2002. Water samples were collected in sterile bottles from the following locations: *i*) the shoreline at Bayfront Park Beach in Hamilton Harbour; *ii*) the lower lift of raw water obtained from offshore Lake Ontario for Hamilton drinking water; and *iii*) surface waters in Red Hill Creek (at Mount Albion), Ancaster Creek (tributary of Red Hill Creek), and Spencer Creek (at Dundas, HWY 5, and Westover). Samples of treated wastewater effluent were obtained from Hamilton, Dundas, and Waterdown sewage treatment plants, and a sample of untreated municipal wastewater was obtained from the Main and King CSO tank in Hamilton. Sterile culturette transport swabs (BD Inc., Oakville, Ont.) were used to collect samples of fresh feces from Canada geese (n = 32), gulls (n = 46), and mallard ducks (n = 16) that were deposited on Bayfront Park beach and the ground in the surrounding area. All samples were placed on ice packs and returned to the lab for *E. coli* isolation the same day.

Escherichia coli from Bayfront Park beach water samples were isolated after 0.45 μm membrane filtration and overnight incubation on mFC agar (BD Inc.) at 44.5 °C. mFC agar plates were swabbed with Culturette tips, and up to 3 *E. coli* isolates were selected on a random basis from each plate after overnight incubation at 44.5 °C. *E. coli* from the lower lift, creek surface waters, and municipal wastewaters were isolated on DC agar (Oxoid Inc., Nepean, Ont.) by the City of Hamilton's Environmental Laboratory (Hamilton, Ont.). Up to twelve isolates were selected on a random basis from these agar plates. Confirmatory identification of all *E. coli* isolates included testing for growth on MacConkey agar (Difco Inc.) at 37 °C, positive EC-MUG fluorescence (Difco Inc.) at 44.5 °C, and production of indole from tryptophan (Difco Inc.) at 37 °C using Kovac's reagent (Oxoid Inc.). Positive (*E. coli* ATCC 29194) and negative (*Klebsiella* ATCC 33495) control strains were used during *E. coli* confirmation testing. *E. coli* isolates were stored at –80 °C in 50% glycerol.

Antibiotic resistance profiles of *E. coli* isolates were obtained by agar dilution. The following antibiotics (and concentrations) were added to Tryptic soy agar (Difco Inc.): amoxicillin (50, 75, 100, and 125 $\mu g \cdot mL^{-1}$); ampicillin (10, 20, 30, and 50 $\mu g \cdot mL^{-1}$); cephalothin (10, 15, 20, and 25 $\mu g \cdot mL^{-1}$); chlorotetracycline (20, 40, 60, and 80 $\mu g \cdot mL^{-1}$); ciprofloxacin (5, 10, 15, and 20 $\mu g \cdot mL^{-1}$); erythromycin (30, 50, 70, and 90 $\mu g \cdot mL^{-1}$); gentamicin (5, 10, 15, and 20 $\mu g \cdot mL^{-1}$); kanamycin (10, 20, 30, and 40 $\mu g \cdot mL^{-1}$); neomycin (10, 20, 30, and 50 $\mu g \cdot mL^{-1}$); oxytetracycline (10, 20, 40, and 60 $\mu g \cdot mL^{-1}$); penicillin G (25, 50, 75, and 100 $U \cdot mL^{-1}$); streptomycin (10, 20, 40, and 60 $\mu g \cdot mL^{-1}$); sulfamethoxazole (5, 10, 30, and 50 $\mu g \cdot mL^{-1}$), and tetracycline (5, 10, 15, and 30 $\mu g \cdot mL^{-1}$). *Escherichia coli* isolates in 96-well microplates were transferred to agar plates using a 96 pin replicator, and the agar plates were incubated for 24 h at 37 °C. An *E. coli* isolate was classified as resistant if its growth was not markedly different from that on an agar control plate without antibiotics. This classification was made more rigorous by using an Alpha Imager (Alpha Innotech Corp., San Leendro, Calif.) to transilluminate agar plates and measure optical density of colony growth. An optical density reading of >0.15 was found to be useful for classifying *E. coli* isolates as resistant to an antibiotic. A multiple antibiotic resistance (MAR) index was calculated for *E. coli* from each water site and fecal source following Guan et al. (2002). The following ten antibiotics (and concentrations) were used for the MAR calculations: ampicillin (10 $\mu g \cdot mL^{-1}$); cephalothin (15 $\mu g \cdot mL^{-1}$); chlorotetracycline (20 $\mu g \cdot mL^{-1}$); gentamicin (10 $\mu g \cdot mL^{-1}$); kanamycin (20 $\mu g \cdot mL^{-1}$); neomycin (50 $\mu g \cdot mL^{-1}$); oxytetracycline (20 $\mu g \cdot mL^{-1}$); penicillin G (75 $U \cdot mL^{-1}$); streptomycin (20 $\mu g \cdot mL^{-1}$); and tetracycline (20 $\mu g \cdot mL^{-1}$).

A total of 462 *E. coli* isolates were screened for antibiotic resistance from the Hamilton area (Table 1). *E. coli* were resistant to the highest concentrations of each of the 14 antibiotics studied, although the prevalence of high resistance was usually low.

Antibiotic resistance was most prevalent in *E. coli* from untreated sewage in the CSO tank, although only 12 isolates were studied from this source. The CSO tank was the only location where *E. coli* had any resistance to ciprofloxacin and gentamicin. The taxonomic identification of the *E. coli* isolates that were resistant to ciprofloxacin and gentamicin was confirmed by an additional API test. The CSO tank also had a higher prevalence of *E. coli* resistance to amoxicillin (125 $\mu g \cdot mL^{-1}$), ampicillin (50 $\mu g \cdot mL^{-1}$), and penicillin (100 $U \cdot mL^{-1}$), and a higher MAR index than other locations.

Resistance to antibiotics like ciprofloxacin in the Enterobacteriaceae is a growing concern in clinical settings (Karlowsky et al. 2003). Whereas they were more prevalent in the CSO tank, ciprofloxacin- and gentamicin-resistant *E coli* represented <1% of the 462 *E. coli* studied from the Hamilton area. These results were comparable with previous environmental studies that have found prevalence of *E. coli* resistance to ciprofloxacin and gentamicin at <2%–3% in wastewater sources (Guan et al. 2002; Reinthaler et al. 2003), and <1% in surface waters (Ash et al. 2002; Roe et al. 2003). Livermore et al. (2001) did not find any ciprofloxacin resistance among 177 *E. coli* isolates from magpies and 61 isolates from rabbits in the United Kingdom. Fallacara et al.

Table 1. Percentage of *E. coli* resistant to each antibiotic (and calculated MAR index) for each sampling location.

Location	*n*	amx	amp	cep	chlo	cip	ery	gen	kan	neo	oxy	pen	strp	sulf	tet	MAR
Water																
BayFront beach (March)	17	0	0	0	0	0	6	0	0	0	0	0	0	100	0	0.035
BayFront beach (Sep-Oct)	40	3	3	18	5	0	18	0	0	0	8	3	3	90	8	0.080
Total BayFront beach	57	2	2	12	4	0	14	0	0	0	5	2	2	93	5	0.067
Lower lift intake	8	0	0	0	0	0	63	0	0	0	0	0	0	100	0	0.013
Ancaster Creek	11	0	0	0	0	0	73	0	0	0	0	0	0	100	0	0.009
Red Hill Creek	22	0	0	0	0	0	0	0	0	0	0	0	0	95	0	0.018
Stoney Creek	12	0	0	8	0	0	8	0	0	0	0	0	0	92	0	0.025
Spencer Creek	39	5	5	3	5	0	3	0	5	5	5	5	8	97	5	0.105
Total surface waters	149	2	2	6	3	0	15	0	1	1	3	2	3	95	3	0.059
Waste Water																
CSO tank	12	25	25	25	17	17	42	17	0	0	17	25	17	100	17	0.200
Dundas STP effluent	27	0	4	0	4	0	7	0	7	7	4	7	4	85	4	0.063
Hamilton STP effluent	12	8	8	17	8	0	25	0	0	0	8	8	8	92	8	0.117
Waterdown STP effluent	30	3	7	3	0	0	13	0	0	0	3	7	0	73	3	0.027
Total wastewaters	81	6	9	7	5	3	17	3	3	3	6	10	5	84	6	0.080
Animal origin																
Canada goose	86	0	0	0	0	0	13	0	0	0	5	0	0	56	2	0.040
Mallard duck	38	2	5	13	3	0	24	0	0	0	8	5	5	92	3	0.092
Gull	108	1	1	13	3	0	9	0	1	1	3	3	1	94	4	0.077
Total birds	232	1	1	8	2	0	13	0	<1	<1	4	2	1	79	3	0.07

Note: amx, amoxicillin 125 $\mu g \cdot mL^{-1}$; amp, ampicillin 50 $\mu g \cdot mL^{-1}$; cep, cephalothin 25 $\mu g \cdot mL^{-1}$; chlo, chlorotetracycline 80 $\mu g \cdot mL^{-1}$; cip, ciprofloxacin 20 $\mu g \cdot mL^{-1}$; ery, erythromycin 90 $\mu g \cdot mL^{-1}$; gen, gentamicin 20 $\mu g \cdot mL^{-1}$; kan, kanamycin 40 $\mu g \cdot mL^{-1}$; neo, neomycin 50 $\mu g \cdot mL^{-1}$; oxy, oxytetracycline 60 $\mu g \cdot mL^{-1}$; pen, penicillin 100 $U \cdot mL^{-1}$; strp, streptomycin 60 $\mu g \cdot mL^{-1}$; sulf, sulfamethoxazole 50 $\mu g \cdot mL^{-1}$; tet, tetracycline 30 $\mu g \cdot mL^{-1}$; and MAR, multiple antibiotic resistance index.

(2001) found no ciprofloxacin resistance for 190 *E. coli* from waterfowl in urban Ohio parks, although 2 isolates were resistant to gentamicin at 10 $\mu g \cdot mL^{-1}$.

The prevalence of antibiotic resistance in *E. coli* found in surface waters sampled around Hamilton was generally low and comparable to results from Appalachia Bay, Florida (Parveen et al. 1997), Baltimore Harbour and nearby river waters (Kaspar et al. 1990), and West Virginia groundwaters (McKeon et al. 1995). However, it can be difficult to compare results across studies. This is likely to be a continuing challenge since there are no standard antibiotics (or concentrations) used across fields such as microbial source tracking, clinical and veterinary medicine, and water quality monitoring. Prevalence of erythromycin resistance in *E. coli* around Hamilton was highest in 2 surface water locations (lower lift and Ancaster Creek) presumed to be relatively uncontaminated by fecal pollution sources. Whereas erythromycin (and its derivatives) have been found at detectable levels in surface waters more commonly than other antibiotics, these levels have been suggested to be below those that would select for resistant bacteria (Summers 2002). One of 40 *E. coli* isolates from Bayfront Park beach water had multiple resistance to ampicillin and tetracycline above their breakpoints, indicating MAR isolates can occur in recreational waters.

Different patterns of antibiotic resistance for *E. coli* from human, domestic animals, and wildlife have been explored for discriminating among sources of fecal pollution (Kaspar et al. 1990; Parveen et al. 1997; Guan et al. 2002; Simpson et al. 2002; Whitlock et al. 2002; Harwood et al. 2003). Antibiotic resistance data were analyzed for a preliminary investigation of the source of fecal pollution contaminating Bayfront Park beach water. A discriminant function was calculated to distinguish the *E. coli* from the 2 prominent nearby fecal contamination sources, bird feces and municipal wastewater. The complete antibiotic resistance data set was first screened by the PROC STEPDISC procedure (stepwise method) (SAS Institute Inc. 1999, version 8.0; Cary, N.C.) to identify a smaller set of antibiotics for discrimination purposes (and allowing for inclusion of a maximum of 1 concentration for each antibiotic). The discriminant function was calculated using the nonparametric nearest neighbour (k = 5) method in the PROC DISCRIM procedure (SAS Institute Inc. 1999, v8.0). The average rate of correct classification (ARCC) was calculated using the crossvalidation method in PROC DISCRIM (rather than the more biased resubstitution method). Where waterborne *E. coli* isolates could not be classified by the discriminant function as either "bird" or "wastewater" with a probability of greater than 0.67, their source was classified as "unknown."

The discriminant function was calculated using the best 4 discriminators identified as significant ($p < 0.05$) by the PROC STEPDISC procedure: ampicillin (10 $\mu g \cdot mL^{-1}$), cephalothin (10 $\mu g \cdot mL^{-1}$), penicillin (25 $U \cdot mL^{-1}$), and streptomycin (10 $\mu g \cdot mL^{-1}$). This discriminant function was statistically significant ($p < 0.0001$), and had an ARCC of 68% for discriminating *E. coli* from bird feces and wastewater sources. When the discriminant function was calculated with *E. coli* randomly assigned to bird and wastewater sources, it was not statistically significant ($p > 0.05$), and had an ARCC of 37%. When the 57 Bayfront Park beach water *E. coli* were classified by the discriminant function, 36 (63%) were classified as from bird feces, 2 (4%) were classified as from wastewater, and 19 (33%) *E. coli* were classified as from unknown sources.

These preliminary microbial source tracking results suggest that, at times, bird feces may be a more prominent contributor of *E. coli* to Bayfront beach waters than municipal wastewater sources. This is consistent with frequent observations of many gulls, Canada geese, and ducks (and their droppings) on the beach. However, the results need to be interpreted with caution since the number of *E. coli* isolates studied was low for microbial source tracking studies. Library-dependent methods based upon small numbers of isolates can produce artifacts of source-independent groupings (Whitlock et al. 2002; Harwood et al. 2003). Whereas randomizing the current library did not indicate this occurred, further work is needed to better understand the sources of fecal contamination at Bayfront Park beach.

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Escherichia coli in water and ground water at beaches in Lake Huron, Lake Ontario, and Hamilton Harbour

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ABSTRACT*:* Escherichia coli (E. coli) *have been used as an indicator organism for human faecal material and possible pathogen contamination of beaches for many years. In the public health sense there is not a requirement to understand in all cases the sources of the* E. coli *that may cause beach closures or postings. We began to survey* E. coli *in Hamilton Harbour in 1998 because beaches were posted despite control of combined sewer overflows which were thought to be the source of contamination. Our surveys led to more detailed work that indicated bacteria came from the beach. We repeated this work on beaches of Lake Huron and Lake Ontario with similar results. In Hamilton Harbour the beach is heavily littered with goose droppings which support the premise that birds are the cause of the beach postings. At other beaches several sources may be present but delineation is complicated by the storage and possible growth of* E. coli *in the ground water of beach sand. We suggest that the usefulness of* E .coli *as an indicator of human pathogens be re-investigated.*

INDEX WORDS: E. coli*, ground water, beaches, Lake Huron, Lake Ontario, Hamilton Harbour*

INTRODUCTION

Health advisory postings hereafter referred to as beach postings and closures, have been common throughout the Great Lakes during the summer months on beaches in high use areas. Great Lakes postings or closures are potentially caused by *Escherichia coli* (*E. coli*) from sewage treatment plants (STPs) (Remedial Action Plan for Hamilton Harbour 1992), faulty or old septic systems (Whitman and Nevers 2003), agriculture (Palmateer *et al*. 1989), gull faeces (Whitman and Nevers 2003), and *Cladophora* (Whitman *et al* 2003). *E.coli* is used as indicator for the presence of potential human pathogens. A disconcerting observation is that beaches without obvious human *E. coli* sources exhibit levels higher than the Provincial Water Quality Standard (PWQS) of 100 colony forming units (cfu)/100 mL. For example, beaches established in the west end of Hamilton Harbour in 1992 were posted for June, July and August each year even after the diversion of combined sewage overflows (CSOs) to CSO holding tanks which came on-line between 1994 and 2003 (Hamilton Harbour RAP Stakeholders 2003). From five spatial surveys of Hamilton Harbour during June, July and August 1998, we were surprised to find *E. coli* concentrations generally low - below 100 cfu/100 mL and suitable for swimming. However, with the implementation of the Hamilton Harbour Remedial Action Plan (HHRAP), beach closures remained a problem as there was little understanding of the source of *E. coli* contamination. This study of *E. coli* includes results gathered from groundwater and lake water at beaches in Hamilton Harbour, Lake Ontario and Lake Huron. Kerry's Creek, Pine River, Clark Creek, Royal Oak Creek, Nine Mile Creek, and the Maitland River between Kincardine and Goderich Ontario (Lake Huron) were also sampled for *E. coli.*

METHODS

Study Site

The focus of this study is the southeast shore of Lake Huron between Goderich, Ontario and Kincardine, Ontario; two beaches on western Lake Ontario between Hamilton and Burlington, Ontario (Fig. 1), and Bayfront Beach in Hamilton Harbour. Kincardine and Goderich areas include cottages, homes, beaches, marinas, and agriculture. Hamilton Harbour area includes homes, beaches, marinas, heavy industry and four sewage treatment plants two of which discharge into Hamilton Harbour directly and two that discharge into Hamilton Harbour via Grindstone Creek and Cootes Paradise. The beaches of Burlington and Hamilton are exposed to Lake Ontario. These areas experience beach posting or closures from *E. coli* greater than 100 cfu/100 mL on a regular basis throughout the summer months but do not have any obvious human *E. coli* sources.

Field Methods

Samples taken by boat were collected from stations along transects from near shore to approximately 1 to 2 km offshore by boat, hereafter known as “boat transects”. Stations were approximately 200 m apart. Global Positioning coordinates for each transect are in Table 1**.** Three transects were sampled for *E. coli* at Kincardine, Ontario on July 28, 2003. Transect 1 is located approximately 200 m north of the Penetangore River. Transect 2 is located off the Penetangore River and Transect 3 is located off Station Beach. One transect was sampled off Poplar Beach on July 28, 2003. One transect was sampled off Bruce Beach July 28 and August 20, 2003. One transect was sampled on July 30, 2003 off Lurgan Beach, Point Clark Beach, Amberley Beach, Rotary Park Beach and

Main Beach. Boat transects at Bayfront Beach, Burlington Beach, and Hamilton Beach consist of five stations sampled weekly between May and September 2001 to 2003. The four near shore stations were approximately 50 m apart and the fifth offshore station was approximately 1 km from shore. Data from July 28, 2003 will be used to illustrate typical high *E. coli* numbers in the near shore. Samples were collected using a 250 mL sterile bottle attached to a pole and submerged up to 1 m below the surface of the water. Depth of water ranged between 0.5 m near shore to 15 m offshore.

Samples of beach ground water were collected by excavating a hole using a sterilized shovel. The holes were approximately 0.5 m deep and 2 m from water (A) and 1 m from water (B). Each hole was then left to fill with groundwater. Water was then collected by submerging a sterilized plastic bottle into the groundwater. Near shore lake water was also collected along the same transect by wading approximately 1 m (C) from shore and wading approximately 5 m from shore to 1m depth (D) (Fig. 2). Hereafter, this transect will be known as “beach transects”. Beach transect coordinates were positioned with Global Positioning System Zone 17 WGS 84 (Table 2). Four beach transects were completed at Station Beach on Lake Huron on July 28, 2003. Transects were approximately 60 m apart. Three beach transects approximately 30 m apart were completed at Bruce Beach on October 15, 2003. Four beach transects approximately 100 m apart were completed at Point Clark Beach on July 31, 2003. One ground water sample only was collected Kintail Beach on July 30, 2003. Three beach transects approximately 50 m apart were completed at Rotary Park Beach on October 14, 2003. Two beach transects, approximately 30 m apart, were completed at Main Beach on October 14, 2003.

Three beach transects approximately 50 m apart were completed at Bayfront Beach in Hamilton Harbour on October 30, 2003 and February 8, 2005. Three beach transects approximately 200 m apart were completed at Burlington Beach on Lake Ontario on October 30, 2003. Four beach transects approximately 400 m apart were completed at Hamilton Beach on Lake Ontario.

Tributary water samples were collected at Penetangore River July 28 and August 19, 2003, Royal Oak Creek and, Clark Creek on July 31, 2003; Pine River on July 30 and August 19, 2003; Kerry's Creek, Nine Mile River, and the Maitland River on July 30, 2003. The samples were collected in 250 mL sterilized plastic bottles by wading with exception of the Penetangore River. Tributary coordinates were positioned with Global Positioning System Zone 17 WGS 84 (Table 3)

Laboratory Analysis

All samples were kept on ice until plated within 7 hours of collection on Coliplates™ (Environmental Bio-Detection Products, Brampton, Ontario). Distilled water was incubated as a blank with each batch of samples to test whether contamination was occurring in our plating methods. The plates consist of premade agar solution. Plates were counted using a UV light after an incubation period of 24 hours at 35 deg C. The Most Probable Number (MPN) was derived as per procedures from Environmental Bio-Detection Products (1996). Hereafter, 100 MPN/100 mL will be used as the swimming guideline.

RESULTS

Kincardine to Goderich

Table 4 shows *E. coli* results of samples collected by boat. Generally, most samples collected by boat on the southeast shore of Lake Huron were below the PWQS (100 MPN/100 mL) whereas most ground water *E.coli* numbers in beaches of the southeast shore of Lake Huron were above the PWQS (100 MPN/100 mL (Table 5).

With the Kincardine STP outflow approximately 1 km south of Station Beach ($44^{o}10'02"N$ $81^{o}39'17"W$), *E. coli* results were still well under 100 MPN/100 mL at transects A, B, and C from offshore to nearshore (<3 to 28 MPN/100 mL) including the Penetangore River (Table 4). However, three beach transects on Station Beach (Table 5) had very high *E. coli* in groundwater of beach sand (>2424 MPN/100 mL) but decreased to an average of 101 MPN/100 mL 1 m into the lake water. The fourth transect samples were all below 100 MPN/100 mL. This shows that there can be considerable variation in *E. coli* numbers in groundwater of the beach sand.

Boat transects off Poplar Beach, Bruce Beach and Lurgan Beach (D, E, F, and G) had very low *E. coli* concentrations (<3 MPN/100 mL to 36 MPN/100 mL) (Table 4) however, at Lurgan Beach (G) there was a slight increase near shore, although still below 100 MPN/100 mL. Two samples of Poplar Beach groundwater had concentrations of >2424 MPN/100 mL and 161 MPN/100 mL (Table 5). At Bruce Beach 1 sample of groundwater in beach transect 1 was slightly above 100 MPN/100 mL (146 MPN/100ml) (Table 5). Royal Oak Creek, a tributary of the Pine River, flows into Lake Huron at the

north end of Lurgan Beach. Results from Royal Oak Creek and Pine River on July 30, 2003 were well above 100 MPN/100 mL (1696 and >2424 MPN/100 mL, respectively) (Table 4).

Point Clark boat transect (H) results were similar to Lurgan Beach (Table 4). Low *E. coli* numbers were evident in the offshore water (<3 MPN/100 mL), but showed a slight increase to 16 MPN/100 mL in the nearshore water. Clark Creek which flows into Lake Huron at Point Clark, had *E. coli* results well above 100 MPN/100 mL (>2424 MPN/100 mL) (Table 4). Beach transects at Point Clark show that the highest *E. coli* numbers were evident in groundwater samples 2 m from the surf zone (A) (average of 167 MPN/100 mL) (Table 5). *E. coli* groundwater numbers, however, were an order of magnitude less than at Station beach. Groundwater samples at 1 m above the surf zone (B) and 5 m in the water (C) were below 100 MPN/100 mL (average of 49 MPN/100 mL and 32 MPN/100 mL at B and C respectively) except transect 4 (161 MPN/100 mL and 166 MPN/100 mL at B and C respectively) although still higher than the offshore transect (Table 5). Again, there was considerable variability along the beach.

A boat transect off Amberley Beach (I) had low *E. coli* concentrations offshore (Table 4) however, these increased to above 100 MPN/100 mL near shore (119 MPN/100 mL).

One groundwater sample was taken at Kintail Beach (Table 5). The result was 938 MPN/100 mL. Kerry's Creek flows into Lake Huron at the south end of Kintail Beach. *E. coli* was well above 100 MPN/100 mL at >2424 MPN/100 mL (Table 4). Nine Mile

River located approximately 5 km south of Kerry's Creek, was also sampled and had 83 MPN/100 mL of *E. coli* (Table 4).

Two boat transects were completed in Goderich (J and K) (Table 4). Transect J was located off the STP outflow (43°44'26"N 81°43'59"W) approximately 0.5 km south of Rotary Park Beach and Main Beach. Results showed *E. coli* numbers <3 MPN/100 mL. A boat transect between Rotary Park Beach and Main Beach show an increase in *E. coli* from offshore to nearshore (339 MPN/100 mL) (Table 4). The mouth of the Maitland River, which is approximately 0.75 km north of Main Beach and Rotary Park Beach in Goderich, was also sampled with results showing well above 100 MPN/100 mL (>2424 MPN/100 mL) (Table 6). The usual direction of near shore flow is to the north in this area (Sheng and Rao, 2006); thus, it seems difficult to clearly associate the extreme nearshore number at Rotary Park Beach with either the STP or the Maitland River. Beach transects sampled at Rotary Park Beach on October 14, 2003 show groundwater *E. coli* well above 100MPN/100 mL (average of 798 MPN/100 mL) (Table 5), and remained above 100MPN/100 mL 1 m into the lake water (C) (average of 305 MPN/100 mL) except transect 1C was below 100MPN/100 mL (83 MPN/100 mL). Results then rapidly decreased to close to or at 100MPN/100 mL at D. Two beach transects were sampled at Main Beach (Table 5). Ground water samples were well above 100MPN/100 mL at 1696 and >2424 MPN/100 ml. Results 1 m into the water were still above 100MPN/100 mL at 119 and 858 MPN/100 ml, then decreased to below 100MPN/100 mL 10 m offshore (98 MPN/ 100mL 36 MPN/ 100 mL).

Bayfront Park in Hamilton Harbour

Generally, *E. coli* numbers are at or below the PWQS spatially in Hamilton Harbour (Fig. 3). *E. coli* numbers generally increase towards the beach to above the PWQS (Fig. 4) and *E. coli* routinely exceeds the PWQS in the groundwater of beach sand (Table 5).

E. coli concentrations from spatial surveys in Hamilton Harbour generally are not high enough to support the notion that the beaches are contaminated by the open waters, although, evidence can be found of the largest wastewater treatment plant in the southeast corner of Hamilton Harbour and the second largest in the northeast area of Hamilton Harbour (Fig. 1). For example, spatial results of *E. coli* numbers in 2001 in Hamilton Harbour were below 100 MPN/100 mL during the Skyway and Woodward STP chlorination period (geometric mean of 60 MPN/ 100L) and after chlorination terminated (geometric mean of 98 MPN/ 100 mL) (Fig. 3). Chlorination of sewage begins May 15 and terminates October 15 every year. Figure 4 shows a boat transect of *E. coli* concentrations at Bayfront Beach. *E. coli* increased from 16 MPN/100 mL offshore to well above 100 MPN/100 mL (559 MPN/100 mL) at the beach (Table 4). All *E. coli* concentrations between 2001 and 2003 with averages are shown in Figure 5. The inshore station in 2001 had the highest *E. coli* count for 54% of the total weeks sampled. The inshore station in 2002 had the highest *E. coli* count for 78% of the total weeks sampled and in 2003 the inshore station had the highest *E. coli* count for 85% of the total weeks sampled.

E. coli numbers in groundwater samples at Bayfront Beach were highest 1 m from surf zone (B) (>2424 MPN/100 mL) then steadily decrease at 1m into the lake water (C) (average of 483 MPN/100 mL) to below 100 MPN/100 mL at 10 m into lake water (D) (average of 37 MPN/100 mL) at all three transects (Table 5) during the ice free season. In February 2005, groundwater samples of *E. coli* numbers were highest 1 m and 10 m into the lake water (C and D) (average of 1880 MPN/100mL and 1631 respectively). Groundwater *E. coli* at A and B were lower than C and D however, remained above the PWQO at 176 MPN/100 mL (A) and 985 MPN/100 mL (B)(Table 5).

Burlington Beach and Hamilton Beach

Figure 6 shows a boat transect of *E. coli* concentrations at Burlington Beach and Hamilton Beach. Generally, *E. coli* numbers at Burlington and Hamilton Beach remained below the PWQS (100 MPN/mL) for most samples collected, whereas ground water samples on Burlington Beach and Hamilton Beach routinely exceeded the PWQS (100 MPN/100 mL) (Table 5).

The nearshore station at Burlington Beach in 2001 had higher *E. coli* count for 63% of the total weeks sampled compared to the offshore station. The inshore station in 2002, had the highest *E. coli* count for 73% of the total weeks sampled compared to the offshore station and the near shore station in 2003, had the highest *E. coli* count for 30% of the total weeks sampled compared to the offshore station. In 2003 the counts were lower (below 100 MPN/100 mL) than previous years therefore no trend from near shore

to offshore was noticeable. The near shore station at Hamilton Beach in 2001 had the highest *E. coli* count for 41% of the total weeks sampled compared to the offshore station. The nearshore station in 2002, had the highest *E. coli* count for 40% of the total weeks sampled compared to the offshore station and the nearshore station in 2003 had the highest *E. coli* count for 36% of the total weeks sampled compared to the offshore station. Most values were at or below 100 MPN/100 mL therefore no trend from near shore to offshore was noticeable. Figure 7 illustrates all data between 2001 and 2003 with averages. All inshore average numbers were higher than offshore except for Burlington Beach in 2003 where the offshore station average was 52 MPN/100 mL. Hamilton Beach in 2003 showed a slight increase to offshore, however, the difference between the inshore and offshore numbers was an average of only 9 MPN/100 mL and results were below 100 MPN/100 mL.

Three beach transects were sampled on Burlington Beach (Table 5). Transect 2 had high *E. coli* in groundwater (>2424 and 559 MPN/100ml), but decreased to 33 and 3 MPN/100 mL in the lake water. Transect 1 had low *E. coli* in groundwater, but increased to >2424 MPN/100 mL at 1 m from the surf zone, then rapidly decreasd to 3 MPN/100 mL at 10 m from the surf zone. Transect 3 had low *E. coli* below 100 MPN/100 mL for groundwater and lake water.

Four beach transects were sampled on Hamilton Beach (Table 5). Transect 2 showed high *E. coli* results in groundwater 2 m from the surf zone (>2424 MPN/100 mL) then rapidly decreased at 1 m from the surf zone (510 MPN/100 mLl) to 5 then 3 MPN/100 mL 10 m

in lake water. Transect 3 showed high *E. coli* results in groundwater 2 m above the surf zone (>2424 MPN/100 mL) then rapidly decreased at 1 m above surf zone (69 MPN/100 mL) then 3 MPN/100 mL 1 m and 10 m in lake water. Transects 1 and 4 results remained below 100 MPN/100 mL in groundwater and lake water. Again, there was a great deal of variability along a beach at stations sampled within 50 m.

DISCUSSION

During this study three important factors were noted:

1) Even with the influence of sewage outflows and heavy agriculture in the case of Kincardine to Goderich, *E. coli* numbers in the offshore water were usually at or below the Provincial Water Quality Standard (PWQS) of 100 MPN/100 mL; however, beaches are often posted. Observations were similar in Hamilton Harbour. With implementation of the Hamilton Harbour Remedial Action Plan recommendations, and recently installed combined sewer overflow collectors, offshore samples were at or below the PWQS and Bayfront Beach was still posted. Burlington Beach and Hamilton Beach have no direct influence from human *E. coli* sources yet beach samples numbers were elevated.

2) *E. coli* numbers tended to increase from offshore to nearshore and were highest in beach ground water. Whitman and Nevers (2003) and MOE (1979) found similar results. This illustrates the phenomenon that materials introduced at lake shores do not readily mix offshore and that the bacteria in the water may come from the beaches themselves.

3) During this study, creeks sampled were well above 100 MPN/100 mL. Unfortunately, near shore samples gathered at the creek mouths were not collected on the same day. Creeks tended to have elevated *E. coli* numbers, but nearshore results approximately 50 m from creek mouths were at or below 100 MPN/100 mL. Weather during the time of creek sampling was clear and sunny. Results may have been different during a rain event but, in our sampling, high *E. coli* numbers in a stream were not predictive of high numbers in the water of adjacent beaches.

With this evidence it appears that groundwater in sand may act as a storage facility for bacteria. Sediment particles provide more surface area for bacteria to colonize than is available to free floating bacteria in water (Whitman and Nevers 2003 and Doyle *et al.* 1992). *E. coli* and other pathogens can survive in freshwater sediments for months (Burton *et al.* 1987) as opposed to a water environment where the half-life of *E. coli* is about one day (Winfield and Groisman 2003). However, because of a continuous load of *E. coli* from human and animals, a constant population occurs (Winfield and Groisman 2003). High bacteria levels in the upper layer of sand may be released into the water through agitation of sediment from wave action and people/animals (Burton *et al.* 1987 and Whitman and Nevers 2003). Cities and counties of Ontario follow the Ontario Ministry of Health and Long Term Care Beach Management Protocol (1992) for sampling beach water to determine beach postings. Samples are obtained about 15 to 30 cm below the water surface at 1 and 1.5 m depth. If these samples are taken during or after a turbulent event, *E. coli* numbers may be elevated simply due to the liberation of *E.*

coli stored in the beach. These results may be misconstrued as offshore pathogens coming inshore from, for example, sewage.

High levels of bacteria in sand may originate from various sources. These include:

1) Faulty and outdated septic systems may be a factor on Lake Huron. There are many seasonal and full time residents lining the beaches – especially Amberley Beach. However, there are no active septic systems on Burlington Beach and Hamilton Beach and *E. coli* numbers are still elevated. From a sand replacement study, Whitman and Nevers, (2003) found that *E. coli* increased to pre-sand replacement numbers after only two weeks. Therefore, since septic systems at most Lake Huron beaches are located at least 20 m from the beach sand, contamination from groundwater, which travels approximately 20 to 25 m/year in sand is not likely a factor (Dr. Allan Crowe, Environment Canada, National Water Research Institute, Aquatic Ecosystem Management Research Branch, Groundwater Remediation, 2003 personal communication).

2) Agriculture runoff may be a contributing factor during high water events – high rains and spring runoff. The shoreline between Kincardine and Goderich is in a region with the second highest number of livestock animals in Canada (Statistics Canada 2001). The elevated *E. coli* numbers close to shore illustrate that materials introduced to the nearshore do not readily leave. Thus, *E. coli* from streams could remain in nearshore water after flow events. At the time of our sampling, however, in Lake Huron, stream velocities from our own observations were very low. In addition, the presence of high

numbers of *E. coli* at the water's edge seems more consistent with the notion that the beaches themselves are the ongoing source. Studies have shown that *E. coli* can live 3 to 19 days in summer and remain viable in winter for up to 68 days (Davis *et al*). Therefore effects from spring runoff cannot be precluded.

3) Gull (*Larus argentatus, Larus delawarensis*) faeces contain large numbers of *E. coli* and may be considered as a significant source if large flocks are evident (Whitman and Nevers 2003). In this study, the greatest number of birds observed was at Rotary Park Beach in Goderich and at Bayfront Beach in Hamilton Harbour. The nearshore water of the beach transects illustrated increased *E. coli* numbers. Whether there is a relationship between the elevated *E. coli* numbers and numbers of birds is unknown. In the past there did not seem to be an *E. coli* gradient at Burlington Beach (Sherry 1986) but gulls and geese are a more common feature at all beaches as populations have increased enormously in the last 30 years (Canadian Wildlife Service, 2006). Indeed, the beach at Bayfront Park is often intensely littered with geese and gull droppings; here bird faeces are a source of *E. coli* to the beach groundwater and lake water. The Regional Municipality of Halton found elevated *E. coli* counts at Kelso Beach located north of Burlington Ontario. Large numbers of birds, mainly geese and gulls, tended to inhabit the beach daily. A large net-like structure was engineered in place over the beach area to prevent birds from accumulating on the sand. The *E. coli* counts decreased dramatically to 0 colony forming units (cfu)/100 mL on a regular basis thereafter leaving little doubt that birds were the source of *E. coli* (T. Colaco Regional Municipality of Halton, personal

communication). Further studies are underway on genotyping the bacteria as a way of identifying sources.

4) *Cladophora* may harbour bacteria (Whitman *et al.* 2003). The southeast shore of Lake Huron has had significant algae problems in the past, predominantly *Cladophora* (Neil and Owen, 1964). Floating *Cladophora* mats wash up on the beaches leaving malodorous piles. During this study, there were no "algal events" observed. Whitman *et al.* (2003) concluded that *Cladophora* mats can harbour large numbers of *E. coli* which can survive longer than six months.

5) Elevated *E. coli* numbers may come from bathers (Obiri-Danso and Jones 1999). During the time of this study there were very few bathers.

6) Infiltration of rain through bird and animal faeces, dead birds and fish and other debris on the surface of the sand could potentially cause an increase in *E. coli* numbers in ground water.

In summary, three areas were used for this study. Each area is unique. Lake Huron beaches and surrounding area consist mostly of cottages, small marinas and agriculture. Hamilton Harbour consists of heavy industry and four sewage treatment plants two of which discharge into Hamilton Harbour directly and two that discharge into Hamilton Harbour via Grindstone Creek and Cootes Paradise. Burlington Beach and Hamilton Beach are located on the west end of Lake Ontario exposed to the full fetch. From this

study, low bacteria levels were evident in offshore areas. Beach transects showed a dramatic increase in *E. coli* numbers from offshore water to beach ground water. *Perhaps the most important finding for beach management is that beach groundwater can be a long term reservoir for* E. coli. Thus, resuspension events can produce elevated numbers near shore but, apparently, notable resuspension events are not always required. The original *E. coli* contamination may come from any or all of the sources but duration of storage and the possibility of multiple low level contamination events or ongoing wildlife contamination may lead to confusion as to where the root problems originate. Whereas, formerly, sewage contamination was the subject of testing for *E .coli* as an indicator of potential human pathogens, this study and others show the situation is much more complex. Intensive studies are needed of all sources and a determination of whether they are associated with important pathogens would be worthwhile.

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Table 1. Global positioning coordinates for boat transects

Transects sampled by boat	Date Sampled	Latitude	Longitude
Kincardine N of Penetangore R.	28 Jul 03	44° 12’18”	81 °37’37”
Station Beach Transect #1	28 Jul 03	44° 11’13”	81 °39’15”
Station Beach Transect #2	28 Jul 03	44° 10’42”	81 °39’08”
Poplar Beach	28 Jul 03	44° 09’42”	81 °41’58”
Bruce Beach	28 Jul 03 30 Aug 03	44° 07’30”	81 °44’06”
Lurgan Beach	30 Jul 03	44° 05’57”	81 °45’48”
Point Clark	30 Jul 03	44° 04’38”	81 °46’09”
Amberley Beach	30 Jul 03	44° 02’54”	81 °45’43”
Main Beach	30 Jul 03	43° 44’05”	81 °43’41”
Rotary Park Beach	30 Jul 03	43° 43’48”	81 °44’17”
Bayfront Beach Station 1 nearshore	May to Sept 01 to 03	43°16’18”	79°52’29”
Bayfront Beach Station 2	May to Sept 01 to 03	43°16’18”	79°52’30”
Bayfront Beach Station 3	May to Sept 01 to 03	43°16’19”	79°52’31”
Bayfront Beach Station 4	May to Sept 01 to 03	43°16’20”	79°52’33”
Bayfront Beach Station 5 offshore	May to Sept 01 to 03	43°16’24”	79°52’38”
Burlington Beach Station 1 near shore	May to Sept 01 to 03	43°18’31”	79°47’56”
Burlington Beach Station 2	May to Sept 01 to 03	43°18’31”	79°47’55”
Burlington Beach Station 3	May to Sept 01 to 03	43°18’32”	79°47’54”
Burlington Beach Station 4	May to Sept 01 to 03	43°18’32”	79°47’52”
Burlington Beach Station 5 offshore	May to Sept 01 to 03	43°18’33”	79°47’46”
Hamilton Beach Station 1 near shore	May to Sept 01 to 03	43°16’22”	79°46’37”
Hamilton Beach Station 2	May to Sept 01 to 03	43°16’22”	79°46’36”
Hamilton Beach Station 3	May to Sept 01 to 03	43°16’23”	79°46’35”
Hamilton Beach Station 4	May to Sept 01 to 03	43°16’25”	79°46’31”
Hamilton Beach Station 5 offshore	May to Sept 01 to 03	43°16’27”	79°46’27”

Table 2. Global positioning coordinates for beach transects.

Beach Transect	Date Sampled	Latitude	Longitude
Lake Huron			
Station Beach #1	28 Jul 03	44° 10’ 35”	81° 38’ 36”
Station Beach #2	28 Jul 03	44° 10’ 37”	81° 38’ 38”
Station Beach #3	28 Jul 03	44° 10’ 40”	81° 38’ 41”
Station Beach #4	28 Jul 03	44° 10’ 43”	81° 38’ 44”
Bruce Beach #1	15 Oct 03	44° 06’ 28”	81° 43’ 13”
Bruce Beach #2	15 Oct 03	44° 06’ 30”	81° 43’ 10”
Bruce Beach #3	15 Oct 03	44° 06’ 24”	81° 43’ 20”
Point Clark Beach #1	31 Jul 03	44° 04’ 31”	81° 45’ 27”
Point Clark Beach #2	31 Jul 03	44° 04’ 34”	81° 45’ 30”
Point Clark Beach #3	31 Jul 03	44° 04’ 37”	81° 45’ 33”
Point Clark Beach #4	31 Jul 03	44° 04’ 40”	81° 45’ 36”
Kintail Beach	30 Jul 03	43° 57’ 50”	81° 43’ 46”
Rotary Park Beach #1	14 Oct 03	43°43’ 05”	81° 43’ 37”
Rotary Park Beach #2	14 Oct 03	43°43’ 57”	81° 43’ 34”
Rotary Park Beach #3	14 Oct 03	43°44’ 01”	81° 43’ 34”
Main Beach #1	14 Oct 03	43°44’ 38”	81° 43’ 36”
Main Beach #2	14 Oct 03	43°44’ 41”	81° 03’ 36”
Hamilton Harbour			
Bayfront Beach #1	30 Oct 03	43°16’ 17”	79° 52’ 30”
Bayfront Beach #2	30 Oct 03	43°16’ 18”	79° 52’ 28”
Bayfront Beach #3	30 Oct 03	43°16’ 20”	79° 52’ 29”
Lake Ontario			
Burlington Beach #1	30 Oct 03	43°18’ 48”	79° 48’ 02”
Burlington Beach #2	30 Oct 03	43°18’ 34”	79° 47’ 58”
Burlington Beach #3	30 Oct 03	43°18’ 16”	79° 47’ 52”
Hamilton Beach #1	30 Oct 03	43°17’ 45”	79° 47’ 33”
Hamilton Beach #2	30 Oct 03	43°15’ 08”	79° 45’ 25”
Hamilton Beach #3	30 Oct 03	43°15’ 46”	79° 46’ 05”
Hamilton Beach #4	30 Oct 03	43°16’ 22”	79° 46’ 38”

Table 3. Global positioning coordinates for tributary samples.

Tributaries	Date Sampled	Latitude	Longitude
Penetangore River	28 Jul 03 19 Aug 03	44°10’ 35”	81° 38’ 14”
Royal Oak Creek	31 Jul 03	44°06’ 44”	81° 39’ 46”
Pine River	30 Jul 03	43°05’ 25”	81° 44’ 30”
Clark Creek	31 Jul 03	44°03’ 19”	81° 42’ 28”
Kerry’s Creek	30 Jul 03	43°57’ 26”	81° 42’ 11”
Nine Mile River	30 Jul 03	43°52’ 38”	81° 42’ 19”
Maitland River	30 Jul 03	43°44’ 54”	81° 43’ 24

Table 4. *E. coli* results (MPN/100 mL) collected from nearshore/offshore stations and tributaries southeast shore of Lake Huron

Transect I.D.	Beach & Description	Date	Tributaries *E. coli* results (MPN/100 mL)	Transects Offshore to Nearshore *E. coli* results (MPN/100 mL)							
A	Kincardine north of Penetangore River	28 Jul 03		<3	5	<3		<3			
	Penetangore River	28 Jul 03	49								
	Penetangore River	19 Aug 03	43								
B	Station Beach Transect #1	28 Jul 03		3		<3		8			
C	Station Beach Transect #2	28 Jul 03		3	28	8		3	5	<3	<3	3
D	Poplar Beach	28 Jul 03		<3	3	<3	<3	<3	<3		
	Royal Oak Creek	31 Jul 03	1696								
E	Bruce Beach	28 Jul 03		<3	<3	<3	<3				
F	Bruce Beach	20 Aug 03		8	25						
G	Lurgan Beach	30 Jul 03		<3	<3	36					
	Pine River	30 Jul 03	>2424								
	Pine River	19 Aug 03	33								
H	Point Clark Beach	30 Jul 03		<3	<3	5	16				
	Clark Creek	31 Jul 03	>2424								
I	Amberley Beach	30 Jul 03		<3	<3	119					
	Kerry's Creek	30 Jul 03	>2424								
	Nine Mile River	30 Jul 03	83								
	Maitland river	30 Jul 03	>2424								
J	Rotary Park Beach	30 Jul 03		<3	3	<3					
K	Main Beach	30 Jul 03		22	28	3	11	<3	339		

Table 5. *E.coli* results (MPN/100 ml) from beach transects.

Beach Transect #	Date	A	B	C	D
		Groundwater	Groundwater	Lake water	Lake water
Lake Huron					
Station Beach #1	28 Jul 03	1174	>2424	83	
Station Beach #2	28 Jul 03	>2424	654	194	
Station Beach #3	28 Jul 03	403	119	72	
Station Beach #4	28 Jul 03	72	69	55	
Poplar Beach	21 Aug 03	>2424			
Poplar Beach	15 Oct 03	161			
Bruce Beach #1	15 Oct 03	146	69	19	
Bruce Beach #2	15 Oct 03	46	72		
Bruce Beach #3	15 Oct 03	13	5		
Point Clark #1	31 Jul 03	226	36	36	
Point Clark #2	31 Jul 03	127	79	13	
Point Clark #3	31 Jul 03	102	33	46	
Point Clark #4	31 Jul 03	213	161	166	
Kintail Beach	30 Jul 03	938			
Rotary Park Beach #1	14 Oct 03	403	434	83	127
Rotary Park Beach #2	14 Oct 03	>2424	350	451	59
Rotary Park Beach #3	14 Oct 03	587	587	188	188
Main Beach #1	14 Oct 03	1696	510	119	98
Main Beach #2	14 Oct 03	>2424	858	858	36
Bayfront Beach #1	30 Oct 03	>2424	>2424	200	24
Bayfront Beach #2	30 Oct 03	263	>2424	1174	25
Bayfront Beach #3	30 Oct 03	109	>2424	76	62
Bayfront Beach #1	06 Feb 03	69	510	>2424	>2424
Bayfront Beach #2	06 Feb 03	434	>2424	794	46
Bayfront Beach #3	06 Feb 03	25	22	>2424	2424
Burlington Beach #1	30 Oct 03	123	52	>2424	8
Burlington Beach #2	30 Oct 03	>2424	559	33	3
Burlington Beach #3	30 Oct 03	52	76	5	8
Hamilton Beach #1	30 Oct 03	39	119	5	3
Hamilton Beach #2	30 Oct 03	>2424	510	5	3
Hamilton Beach #3	30 Oct 03	>2424	69	<3	3
Hamilton Beach #4	30 Oct 03	22	19	<3	<3

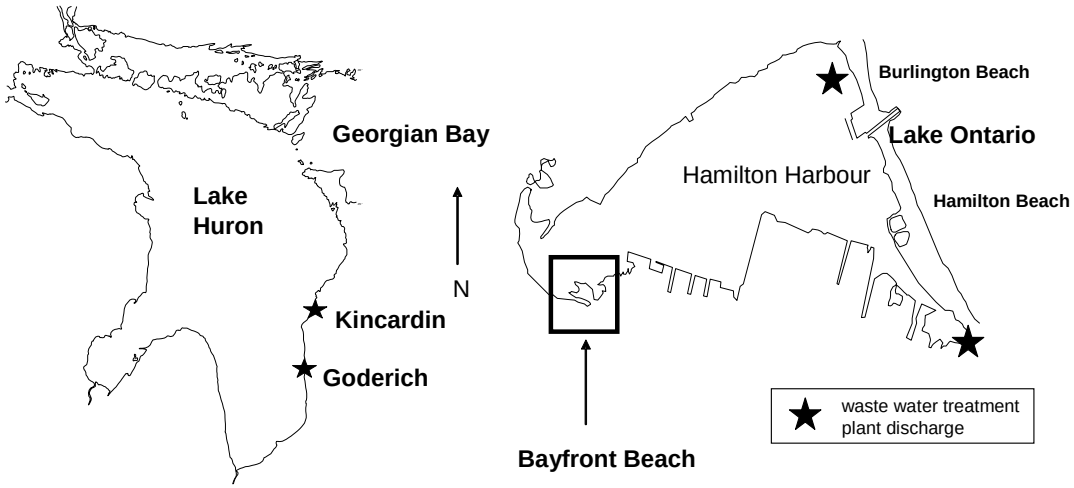

Fig. 1. Lake Huron, Hamilton Harbour and Lake Ontario sampling sites

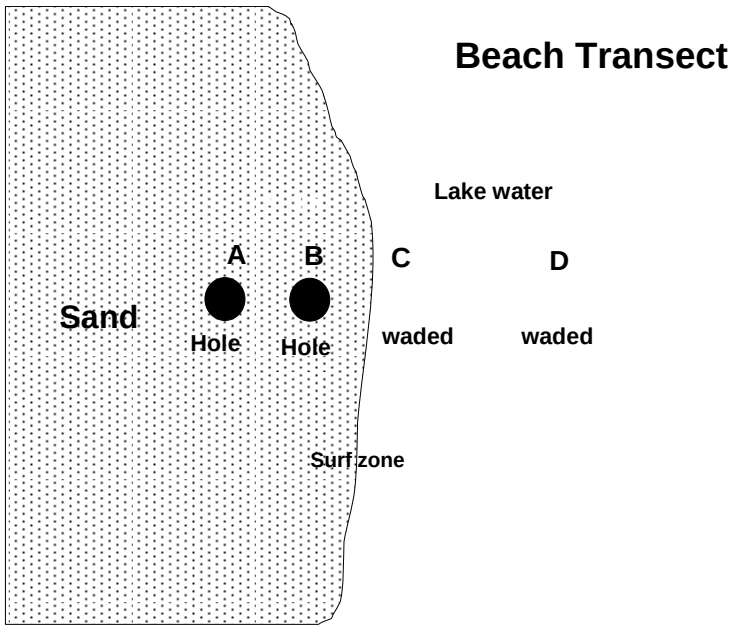

Fig. 2. We collected ground water samples at A and B and we waded to collect nearshore water at C and D.

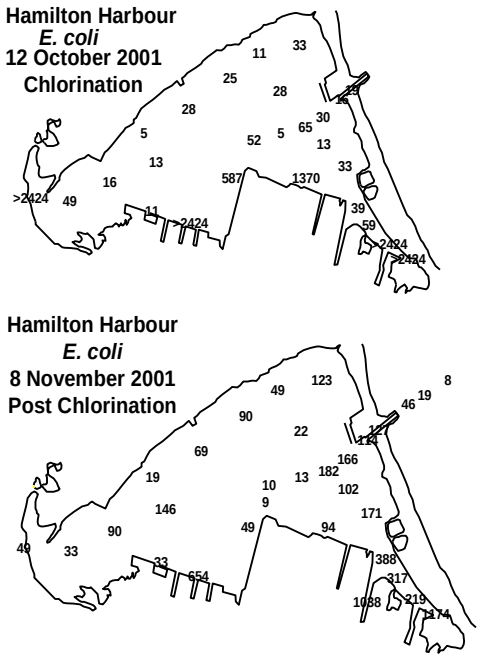

Fig. 3. Spatial *E. coli* results (MPN/100 mL) in Hamilton Harbour pre- and post-chlorination at the waste water plants. Chlorination begins May 15 and ends October 15.

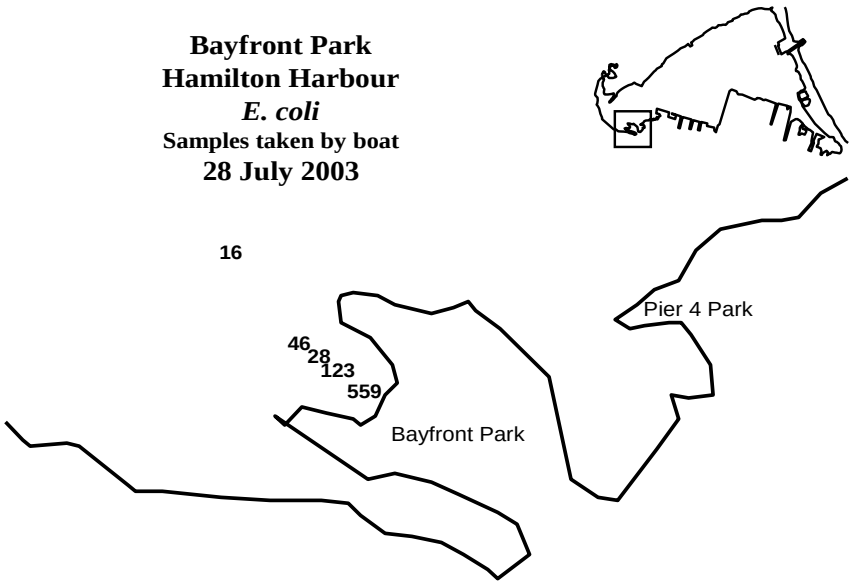

Fig. 4. Offshore/nearshore *E.coli* results (MPN/100 mL) at Bayfront Beach in Hamilton Harbour. Numbers generally increase towards the beach.

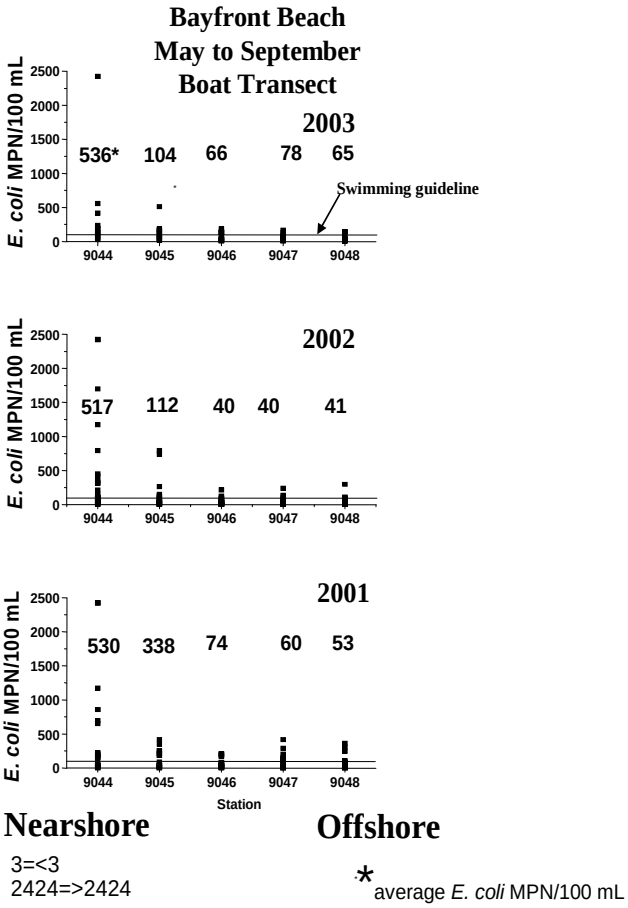

Fig. 5. *E. coli* (MPN/100 mL) and seasonal averages results from nearshore to offshore stations at Bayfront Beach in Hamilton Harbour.

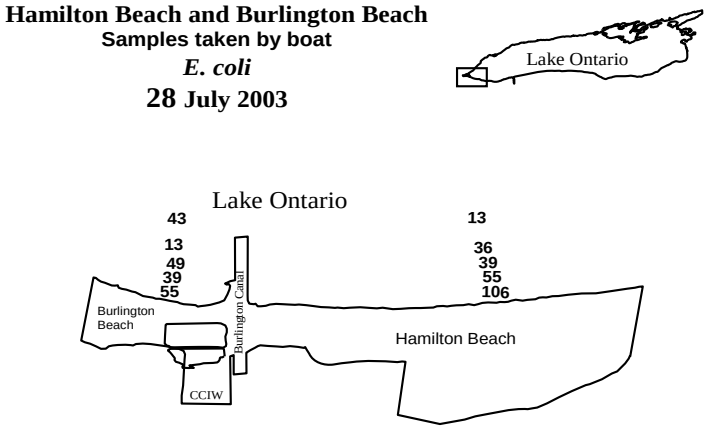

Fig. 6. Offshore/near shore *E. coli* results (MPN/100 mL) at Burlington and Hamilton Beach on Lake Ontario. *E. coli* numbers generally increase towards shore.

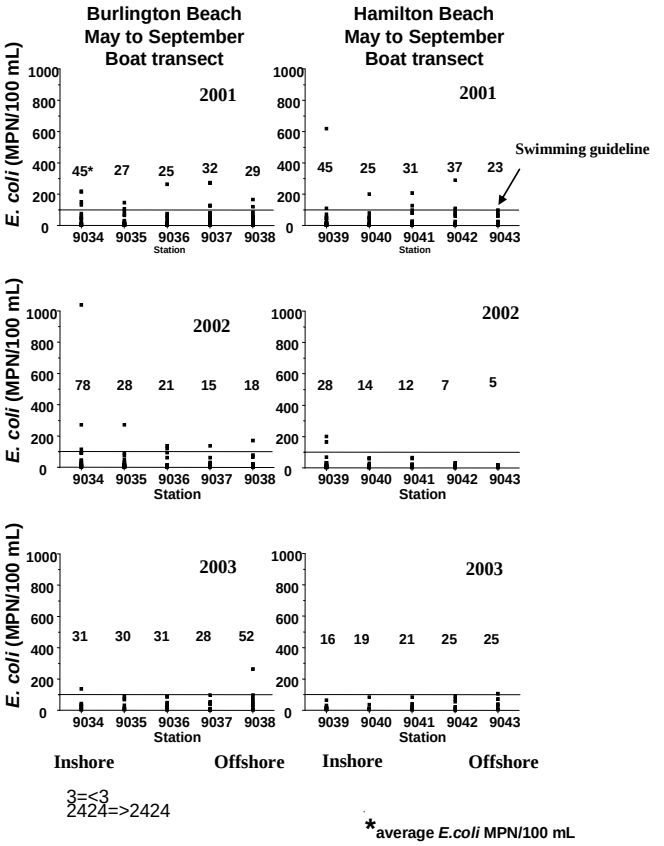

Fig.7. *E. coli* (MPN/100 mL) and seasonal averages results from nearshore to offshore stations at Burlington and Hamilton Beach.