A Review of Hamilton Harbour Beaches: Towards De-listing 2020, Successes and Challenges

Jacqui Milne[1], Sue Gilpin[2], John Fortuna[3]

[1]*Environment Canada, Watershed Hydrology and Ecology Research Division, Water Science and Technology Directorate 867 Lakeshore Rd. Burlington, Ontario, L7S 1A1.*

[2]*Park District, City of Hamilton, 77 James St. N, Suite 351, Hamilton, Ontario*

[3]*City of Hamilton Public Health Services, Health Protection Division, 110 King Street West 2nd Floor, Hamilton, Ontario.*

Abstract

Bayfront and Pier 4 Beaches are located in Hamilton Harbour, Ontario, Canada. Both beaches prior to 2005, experienced health advisory postings >60% of the bathing season. In order to de-list Hamilton Harbour as one of 43 areas of concerns, the water quality at Bayfront and Pier 4 Beaches must meet the provincial water quality objective (PWQO) of 100 cfu of *Escherichia coli* (*E. coli*)/100 ml >=80% of the bathing season for a minimum of three consecutive years.. In 2005, the Hamilton Harbour Beach Committee was formed to guide beach managers on decisions pertaining to beach remediation and *E. coli* mitigation. Here we discuss the various studies, projects and public outreach programs delivered by Environment Canada, the City of Hamilton and the Bay Area Restoration Council (BARC) to improve beach health and move toward de-listing as an Area of Concern by 2020.

Background

Hamilton Harbour, located at the west end of Lake Ontario, was designated as one of 43 Areas of Concern (AOC) in 1986 by the International Joint Commission (IJC) (Hall et al. 2006) (fig.1)**.** In 1987, under the Great Lake Water Quality Agreement (GLWQA), a Remedial Action Plan for Hamilton Harbour (HHRAP) was formalized and 14 Beneficial Use Impairments (BUIs) were identified, including recreational beaches (Ontario Ministry of the Environment 2010). Previous to 1990, the HHRAP committee determined that less than 5% of Hamilton's shoreline was accessible to the public. Support from various stakeholders including the HHRAP and the City of Hamilton lead to a number of successful remediation/implementation of projects including Pier 4 Park Beach and Bayfront Beach which officially opened in 1993 (City of Hamilton 1996) (fig. 1).

These beaches provide greater public access, however, the recreational water quality at Bayfront and Pier 4 Beaches must meet the provincial water quality objective (PWQO) of 100 cfu of *Escherichia coli* (*E. coli)*/100 ml >=80% of the bathing season for a minimum of three consecutive years in order for Hamilton Harbour to delist as an AOC in 2020. In Canada, the legislative responsibility for recreational water quality is provincial, where a province may adopt Health Canada's Canadian Recreational Water Quality Guideline of 200 cfu/100 ml (Health Canada 2012) (Nevers et al. 2014). The province of Ontario, however, has elected to apply a more strict water quality objective of 100 cfu/100ml, which is governed by the Ministry of Environment and Climate Change (MOECC). The Ministry of Health and Long Term Care's Recreational Water Quality Protocol and Beach Management Guidance Document (Ministry of Health and Long-Term Care 2014; Ministry of Health and Long Term Care 2014) are used as a guide by local health departments in Ontario when conducting their beach water sampling programs. It should be noted *E. coli* enumeration by Environment Canada (EC) for research purposes was determined by the widely accepted, cost effective Coliplate-400™ method (Bluewater Biosciences Incorporated 2009) (Lifshitz and Joshi 1998). Results are reported as Most Probable Number (MPN) per 100 ml of sample water. Public Health Ontario, a Crown corporation, analyzes the *E. coli* samples collected by the City of

Hamilton Public Health Services using the membrane filtration technique and reports the *E. coli* results as colony forming units (CFU) per 100 ml of sample water for regulatory purposes (Ministry of Health and Long-Term Care 2008).

In order to guide beach management officials in making informed decisions and in an effort to meet the RAP de-listing criteria, the Pier 4 Beach Committee was formed in 2005 and renamed the Hamilton Harbour Beach Committee (HHBC) in 2011. The committee is chaired by the City of Hamilton and includes scientists from Environment Canada, members of the Hamilton Remedial Action Plan office, the Bay Area Restoration (BARC). BARC represents the public interest in the restoration of Hamilton Harbour and its watershed. BARC is responsible for implementing the Hamilton Harbour RAP and for evaluating the overall performance. It has been instrumental in assisting the HHBC by educating the public on the environmental effects of feeding the gulls and geese at the waterfront through the "Don't Feed the Water Fowl" campaign which was initiated in 2008.

As part of the City of Hamilton's commitment to remediate ecosystem health in Hamilton Harbour, the Public Works Department constructed eight combined sewer overflow (CSO) holding tanks ranging from 1 400 m^3 to 83 500 m^3, which came on line between 1988 and 2007. Storm water is diverted to CSO holding tanks to be later treated at Woodward Wastewater Treatment Plant in Hamilton, Ontario rather than discharging directly to Hamilton Harbour (Routledge 2012). Since the implementation of the CSO holding tanks, the geometric mean (GM) of *E. coli* samples collected at centre station (1001) by Environment Canada (EC) (fig.1) have remained well below the PWQO since 2000 (Min = 7.0 GM MPN/100ml Max = 33 GM MPN/100ml).Thus, in terms of *E. coli*, much of the harbour is suitable for bathing. However, Bayfront Beach and Pier 4 Beach in Hamilton Harbour continued to experienced health advisory postings >60% of the bathing season prior to 2005 (Hamilton Harbour Remedial Action Plan 2011) (fig.2). Where once wastewater was thought to be the primary contributor to increased *E. coli* numbers at the beaches, recent evidence using microbial source tracking (MST) have verified bird fecal material from geese, gulls and ducks to be the most likely source of *E. coli* contamination (Edge and Hill 2005). Environment Canada in collaboration with the City of Hamilton has conducted various experiments to characterize *E. coli* and address potential mitigative techniques at Pier 4 and Bayfront Beaches.

Pier 4 Beach

Pier 4 Beach is a small (<320 m^2), man-made urban beach surrounded by turf grass and paved bike paths. It is located on the southwest shore of Hamilton Harbour (fig 1). Previous to 2005 Pier 4 Beach was open on average 46% of the bathing season (Fortuna 2014). In 2005, the City of Hamilton incorporated mechanical grooming and bird deterrent measures at Pier 4 Beach which consisted of a fence, a vegetation barrier and a row of buoys installed parallel to the beach approximately 30 m offshore, preventing the birds from accessing the beach from the walkway or the water. Mechanical grooming of the beach sand, for aesthetic and public health reasons, was completed by hand using a garden thatch rake to gather and remove unsafe debris**.** As a result, a significant decrease in *E. coli* was evident between 2005 and 2009 (106 GM cfu/100 ml to 44 GM cfu/100 ml p=0.03) resulting in a significant increase in the % days open (40 % to 87% p=0.02) (fig. 2) (Fortuna 2014). However, in recent years (2010, 2012 and 2014), the presence of microcystin, an hepatotoxin released by cyanobacteria harmful algae blooms (CHABs) have been responsible for beach postings resulting in a decline in % days open at Pier 4 Beach (fig.2). Personnel with the City of Hamilton Public Health Services routinely collect water samples for *E. coli* analyses. If a potential CHAB bloom is visually observed or after mid July, the sample water is tested for microcystin using the Abraxis Microcystin Strip Test™ . If one test is >10 ppb additional tests are completed. If the additional tests show samples are >10 ppb a public advisory is issued. Because of health risks to City of Hamilton employees, routine monitoring for *E. coli* is suspended until microcystin levels fall below 10 ppb.

Despite the promising results of the bird deterrent measures and mechanical grooming at Pier 4 Beach, there are instances where *E.coli* levels exceed the PWQO of 100 cfu/100 ml even in the absence of birds and precipitation events (Ministry of Environment and Energy 1994). Recent evidence suggest beach sand may act as a long term storage reservoir for *E.coli* and potentially influence nearshore recreational water quality (Milne and Charlton 2004; Whitman and Nevers 2003; Zehmus et al. 2008). Further to this, Kinzelman (2003; 2004) indicated beach grooming techniques may influence *E.coli* numbers in beach sand. Some grooming techniques can promote *E.coli* growth (Kinzelman et al. 2003; Kinzelman et al. 2004). Deep grooming techniques (7 to 10 cm), however, appeared to be more effective at decreasing *E. coli* numbers in damp beach sand over shallow grooming.

Environment Canada conducted a similar beach grooming study in 2009 and 2010 (Milne et al. 2011b) at Pier 4 Beach. Three beach grooming techniques were applied to evaluate *E. coli* density in beach sand. Treatments included 1. "routine" grooming with a thatch rake; 2. deep grooming with a modified rake and 3. a control with no grooming. The treatments were randomly assigned to eighteen 1 square meter plots resulting in 6 replicates of each treatment. One core sample was collected from each treatment plot for a total of 18 samples. Cores were collected daily for 20 days in 2009 and 22 days in 2010. Results indicated *E. coli* density were not significantly different when comparing deep grooming to routine grooming treatments and the control in 2009 and 2010 (detailed methods and results can be found in Milne et al 2011). This is contrary to results reported by Kinzelman, et al 2004 , where depth of grooming (7 to 10 cm) in damp or wet sand conditions was effective at decreasing *E. coli* concentrations and in turn a decline in beach advisories. Kinzelman et al noted, however, that shallower grooming can in fact promote increases in *E. coli* numbers. Further to this, Mika et al, 2009 reported *E. coli* numbers are not consistently reduced with mechanical mixing. Depth of deep grooming in our study was comparatively shallow (4.5 cm in 2009 and 2.5 cm in 2010). The shallower depth of grooming may be ineffective at aerating the sand resulting in a slower drying rate and less exposure to UV light which may consequently promote a more desirable environment for *E. coli*.

The results additionally showed the beach sand condition was slightly moist, semi-moist or moist 41% of the experimental period. This may indicate the water table was close to the surface potentially protecting the *E. coli* from desiccation. The Hamilton Harbour Beach Management Committee discussed the feasibility of adding or replacing sand to decrease the *E. coli* numbers, however Whitman and Nevers (2003) reported that recolonization of *E. coli* free sand occurred within 2 weeks of sand replacement. Also, depending on the grain size of the additional sand, capillary action from the water table may allow for moisture to penetrate up to the surface of additional sand by hydraulic conductivity providing a suitable environment for *E. coli* (Crowe and Milne 2011).

Bayfront Beach

Bayfront Park and Beach were historically part of a contaminated in-filled industrial operational site from the mid 1960's to the early 1980's. In 1984, the City of Hamilton expropriated the land and in 1988 a study was commissioned to determine the type and extent of contamination. The study revealed elevated levels of cadmium and lead and low levels of PCB's (City of Hamilton 2006; Bouchier and Cruikshank 2008). In 1992, as part of the remediation, approximately 20,000 tonnes of contaminated soil was removed from the site. The remaining industrial waste included foundry sand, coal and cinders as well as glass, wood, concrete and glass (City of Hamilton 2006). Because the remaining waste "did not include extensive layers or zones of highly organic type refuse" and "the potential for significant gas generation is unlikely and the risk of explosive concentrations is minimal" a low permeable cap was installed over the remaining industrial waste in 1993 (City of Hamilton 2006). The cap prevents contact with industrial waste and water infiltration from the surface. The cap thickness is approximately 0.6 m and consists of a clay fraction between 22 and 47% (City of Hamilton 2006). Once the installation of the cap was

complete, the City of Hamilton developed a park system consisting of turf grass, paved bike paths and a beach.

Bayfront Beach is approximately 2400 m^2, 8 times larger than Pier 4 Beach (fig. 1) making it much more challenging to address beach posting issues. The beach is located in a shallow embayment (<3m depth) where two headlands were created on the east and west shore to serve as anchor points for an entertainment barge and/or a chlorine curtain for containment of chlorinated water in the beach area (fig. 1). The successful bird deterrent measures implemented at Pier 4 Beach were applied to Bayfront Beach in 2011.To date the City of Hamilton has planted a vegetation barrier (living fence) to deter geese from accessing the beach from the turf grass and installed a buoy line between the headlands to deter geese from landing in the embayment, but these measures have not achieved the same promising results as at Pier 4 Beach. Bayfront Beach has remained open only 31% on average of the bathing season since 2011 (fig. 2). To address potential avenues of *E.coli* beach contamination at Bayfront Beach, Environment Canada conducted a beach grooming experiment and deployed current meters to characterize flow velocities at the mouth of Bayfront embayment in 2011 (Milne et al. 2011a), and a storm water runoff study in 2012 and .

The beach sand grooming study at Bayfront Beach in 2011 was similar to the Pier 4 Beach sand grooming experiment with the addition of a tiller to increase the effective depth of grooming (a complete description of methods and results can be found in Milne(2011a)) (fig. 3) . However, results indicated a significant increase in *E. coli* density in maximum depth grooming (20.7 cfu/cm^3) compared to medium depth (4.5 cfu/cm^3), shallow depth (4.4 cfu/cm^3) and the control (5.5 cfu/cm^3). Similar to Pier 4 Beach, a significant increase in *E. coli* density was evident in slightly moist (10.2 cfu/cm^3) and moist (35 cfu/cm^3) sand vs dry (6.0 cfu/cm^3) and very dry (2.3 cfu/cm^3) sand. As at Pier 4 Beach, the results indicated that deep grooming increased the *E. coli* likely because the water table was close to the sand surface providing the *E. coli* with optimum growing conditions.

E.coli in storm water runoff was also investigated as another avenue of beach contamination. During heavy precipitation events, runoff from the surrounding parkland onto the beach may transport *E. coli* from the beach sand to the nearshore surface water. The land adjacent to Bayfront Beach slopes toward the beach sand and is primarily turf grass often inhabited by Canada geese. Potentially high levels of *E. coli* in runoff may intercept the the beach and discharge in the nearshore surface water. Environment Canada collected storm water samples during 4 precipitation events: July 26, August 9, September 18 and October 29 and 30, 2012. The number of runoff points where sample water was collected for each event ranged between 4 and 6. The samples were collected where storm water discharges onto the beach adjacent to a paved bike path and turf grass (fig. 4). The volume of storm water intercepting Bayfront Beach was estimated using the USDA Natural Resources Conservation Curve Number (CN) model (United States Department of Agriculture 2004). The *E. coli* load (CFU) was estimated by multiplying the modelled volume by the *E. coli* concentration. Results show *E. coli* loads in runoff to be highly variable ranging from ~250 CFU to ~2700 CFU but surprisingly, orders of magnitude lower compared to other studies. This was due in part to a smaller catchment area (<25,000 m^2), lesser storm intensities, minimal sources of *E. coli* loads (birds only), the implementation of a vegetation barrier adjacent to the beach that may have captured *E.coli* in the runoff and various bird control measures implemented by the City of Hamilton. This study will be repeated in the future to monitor any potential changes is *E. coli* loads.

In 2011 Environment Canada deployed two modular acoustic velocity sensor (MAVS) current meters (Nobksa Development Corporation) at the mouth of the Bayfront embayment. Results indicated current velocities at Bayfront Beach are generally low (<2.0 cm/s) which may infer limited mixing with the outer harbour; therefore *E. coli* entering the nearshore water may not be exchanged with the greater harbour and may have a cumulative effect in the embayment (Milne 2011).

To further support the notion of limited or no mixing in the embayment, mean phosphorus and GM of *E. coli* samples collected weekly in 2014 at ankle depth (70 ugL^{-1}, 726 MPN/100 ml) were significantly greater than waist depth (45 ugL^{-1}, 259 MPN/ 100 ml) and centre station (1001) in Hamilton Harbour (47 ugL^{-1}, 19 MPN/100 ml).

As at Pier 4 Beach, CHABS have been responsible for beach closings at Bayfront Beach every year since 2004, with the exception of 2005 and 2008 (Fortuna 2015, personal communication). The blooms generally appear in August and dissipate by November; however in 2014 the bloom was present from June 23 but remained below the safe level target of 10 ppb until August 20, 2014. Environment Canada continued monitoring *E. coli* September 10 and September 15, 2014 during the CHAB bloom and results showed *E. coli* GM remained below the PWQO (31 and 69 GM MPN/100 ml). According to the RAP protocol, the beaches may be achieving the de-listing criteria of 100 cfu of *E. coli*/100 ml >=80% of the bathing season but the number of days the beach is open is decreasing because of CHAB blooms. As such, in addition to *E.coli,* CHABs are adding an additional challenge to the RAP de-listing target for Bayfront and Pier 4 Beach.

The HHBC had discussed the installation of a curtain connected to the headlands to contain chlorinated water in the beach area to disinfect bathing water. The mean Hamilton Harbour surface water dissolved organic carbon (DOC) is 3.7 mgL^{-1} which is significantly higher than Lake Ontario and Lake Erie (2.6 and 2.3 mgL^{-1} respectively).This may indicate higher dissolved organic matter (DOM) in the water column. When Chlorine reacts with DOM a group of potentially carcinogenic trihalomathanes (THMs) are produced (Richardson et al. 2007; Lee et al. 2013). According to Health Canada Guidelines, the maximum acceptable concentration in drinking water is 0.10 mgL^{-1}. Currently there are no guidelines for THMs in recreational beach water. Because of the risk to public citizens, the City of Hamilton abandoned the chorine curtain method for more safe and cost effective means to control *E. coli*.

Moving Towards Delisting in 2020: What's next?

The City of Hamilton, EC, BARC and the community must work together in order to create safe swimmable beaches in Hamilton Harbour and achieve the de-listing target by 2020.The HHBC will continue to discuss viable options for *E. coli* mitigation at Bayfront and Pier 4 Beach. Current discussions include the feasibility of moving the Bayfront bathing area to an alternate location, converting Bayfront Beach Embayment to a non-bathing recreational beach area, or creating an educational wetland. The creation of a wetland would enhance the fish and wild life habitat in Hamilton Harbour which is also a Beneficial Use Impairment identified under the GLWQA.

The City of Hamilton will continue to provide beach maintenance and bird control while exploring options for alternative beach uses. Bird control mechanisms include egg oiling, street sweeping of paved walkways, canine patrol, bird exclusion structures (fences, and buoys), and installation of visual and moving features such as artificial hawks and periodic use of lasers.

Environment Canada will continue to monitor *E. coli* at Pier 4 and Bayfront Beaches to measure remedial action plan endpoints particularly during CHAB blooms to determine if the beaches would be open in spite of the blooms. Environment Canada and the City of Hamilton will continue researching and testing new and innovative methods of bird control and *E. coli* mitigation.

The Bay Area Restoration Council will continue to engage the community on understanding, promoting and protecting the Hamilton Harbour ecosystem via school programs, volunteer planting events, community workshops, evaluative reports, a website, and a public information kiosk at Bayfront Park.

Acknowledgements

We would like to thank Murray Charlton and an anonymous reviewer for providing expert comments and edits to improve this manuscript, the Technical Operations Division of Environment Canada for assistance with field work, and the dedicated Environment Canada students for their invaluable assistance with laboratory and field work.

References

Bouchier, N. B. and Cruikshank, K. 2008. Remembering the Struggle for the Environment: Hamilton's Lax Lands/Bayfront Park, 1950s-2008. Left History 13:106-128.

City of Hamilton 2006. West Harbour Waterfront Recreational Master Plan Phase 1 Technical Report - Existing Conditions, Issues and Opportunities. pp. 1-320.http://www.hamilton.ca/NR/rdonlyres/3E0C82EB-F1F2-46BD-AB01-988812715B94/0/AppendixA.pdf

Crowe, S. A. and Milne, J. 2011. Relationship between dry and wet beach ecosystems and E. coli levels in groundwater below beaches of the Great Lakes, Canada. Hydrogeology 17:in press.

Fortuna, J. 2014. Hamilton Public Health Services 2014 Beach Monitoring Report.

Hall, J. D., O'Connor, K., and Renieri, J. 2006. Progress toward delisting a Great Lake area of concern: The role of integrated research and monitoring in the Hamilton Harbour remedial action plan. Environmental Monitoring and Assessment 113:227-243.

Kinzelman, J. L., Pond, K. R., Longmaid, K. D., and Bagley, R. C. 2004. The effect of two mechanical beach grooming strategies on *Escherichia coli* density in beach sand at a southwestern Lake Michigan beach. Aquatic Ecosystem Health and Management 7:425-432.

Kinzelman, J. L., Whitman, R. L., Byappanahall, M., Jackson, E., and Bagley, R. C. 2003. Evaluation of beach grooming techniques on Escherichia coli density in foreshore sand at North Beach, Racine, WI. Lake and Reservoir Management 19:349-354.

Lee, J., Kim, E. S., Roh, B. S., Eom, S. W., and Zoh, K. D. 2013. Occurrence of disinfection by-products in tap water distribution systems and their associated health risk. Environmental Monitoring and Assessment 185:7675-7691.

Mika, K. B., Imamura, G., Chang, C., Conway, V., Fernandez, G., Griffith, J. F., Kampalath, R. A., Lee, C. M., Lin, C.-C., Moreno, R., Thompson, S., Whitman, R. L., and Jay, J. A. 2009. Pilot- and bench-scale testing of faecal indicator bacteria survival in marine beach sand near point sources. Journalof Applied Microbiology 107:72-84.

Milne, J., Gilpin, S., Gatozzi, A., Guay, S., and Kiela, S. 2011a. Characterizing E. Coli density in beach sand using three grooming techniques at Bayfront Beach, Hamilton, Ontario. National Water Reseach Institute.

Milne, J. E., Butcher, A., Delaney, K., Gilpin, S., Guay, S., Lee, J., Mendoza, J., Packull, J., and Radisic, S. 2011b. Characterizing E. coli density in beach sand using two grooming techniques. 11-006.

Milne, J. E. and Charlton, M. N. 2004. Escherichia coli in water and ground water at beaches in Lake Huron, Lake Ontario, and Hamilton Harbour. NWRI, Environment Canada, Burlington. 04-205.

Milne, J. 2011. Quantifying water circulation at Bayfront Beach Hamilton, Ontario.

Ministry of Environment and Energy 1994. Water management policies guidelines of the Ministry of Environment and Energy. Queen's Printer for Ontario. PIBS 3303B.

Ontario Ministry of the Environment 2010. Canadian Great Lakes Areas of Concern. Status of Beneficial Use Impairments. Environment Canada and the Ontario Ministry of the Environment. En164-22/2011E-PDF.

Richardson, S. D., Plewa, M. J., Wagner, E. D., Schoeny, R., and DeMarini, D. M. 2007. Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. Mutation Research/Reviews in Mutation Research 636:178-242.

Routledge, I. 2012. City of Hamilton Wastewater Treatment Facitlities 2011 Annual Report: CSO Tanks report. pp. 1-270. Hamilton: City of Hamilton. http://www.hamilton.ca/NR/rdonlyres/DC190C97-DA66-4B8E-B511-89701CBBA5FE/0/2011WWTPAnnualReport.pdf

United States Department of Agriculture 2004. Hydrologic Soil-Cover Complexes. In National Engineering Handbook pp. 9-i-9-14. Washington D.C..

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

Zehmus, T. T., McDermott, C. M., and Kleinheinz, G. T. 2008. Microbial concentrations in sand and their effect on beach water in Door County, Wisconsin. Journal of Great Lakes Research 34:524-534.

Fig. 1 Bayfront and Pier 4 Beaches are located in Hamilton Harbour at the westerly point of Lake Ontario.

Fig. 2 Long term trend results of the annual percentage of days open during the bathing season at Bayfront and Pier 4 Beaches.

Fig. 3 Beach grooming treatment plots at Bayfront Beach 2011. Treatments included shallow grooming with a thatch rake; medium depth grooming with a modified rake with extended prongs, maximum depth grooming using a tiller and a control with no grooming.

Fig. 4 Location of runoff sampling points (stars) at Pier 4 Beach 2012.

Fig. 1

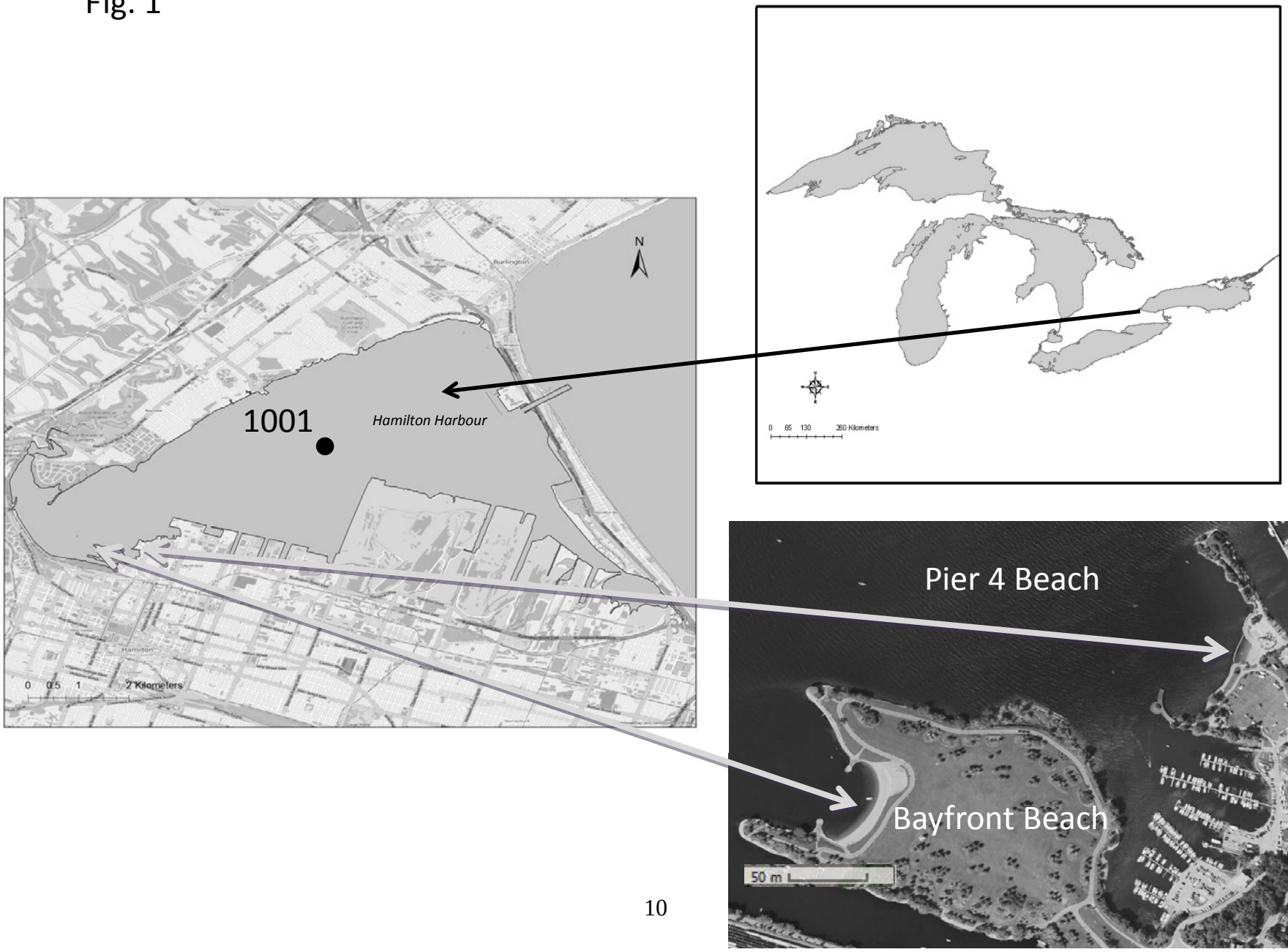

N
1001
Hamilton Harbour
0 0.5 1 2 Kilometers
0 65 130 260 Kilometers
Pier 4 Beach
Bayfront Beach
50 m

Fig. 2

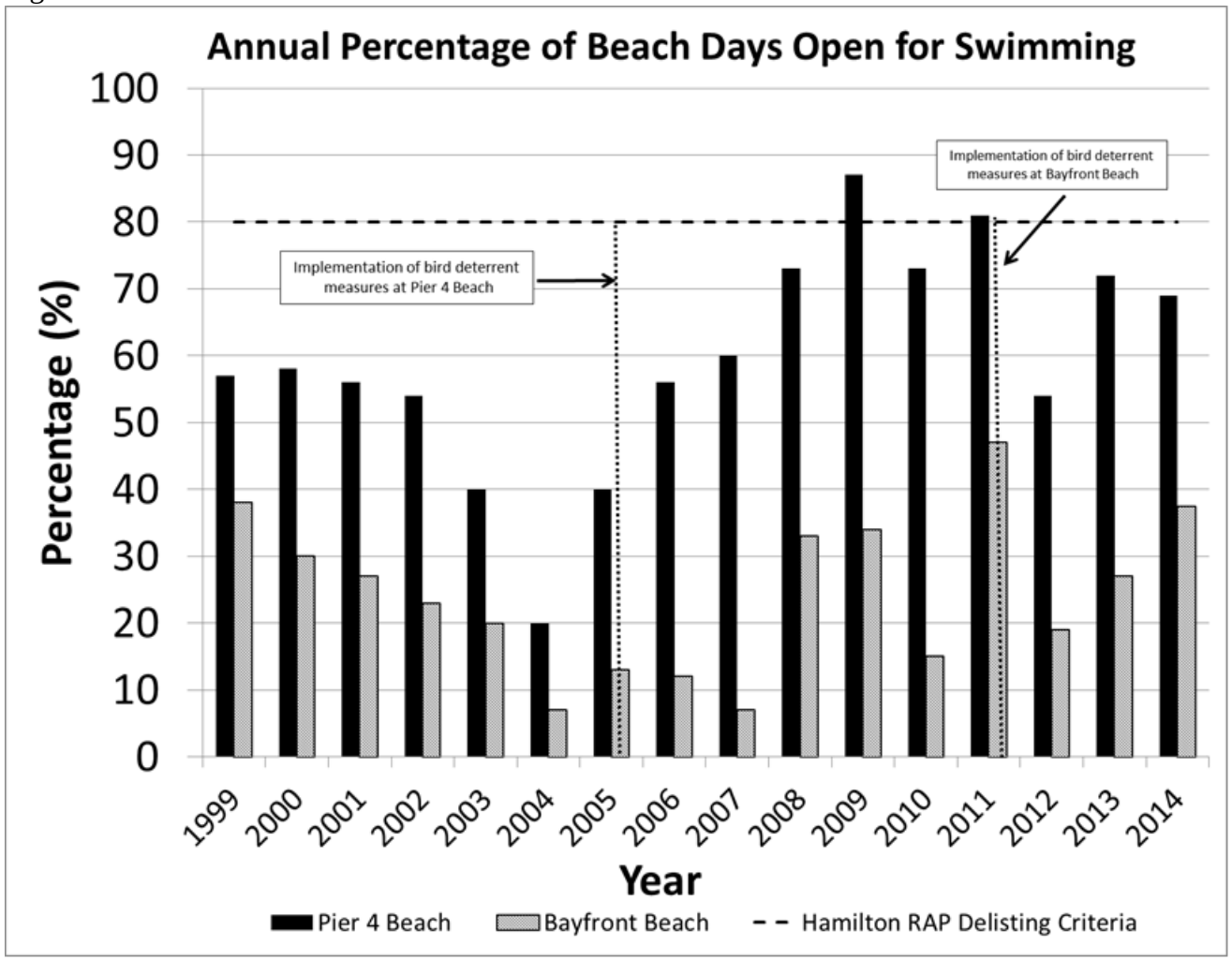
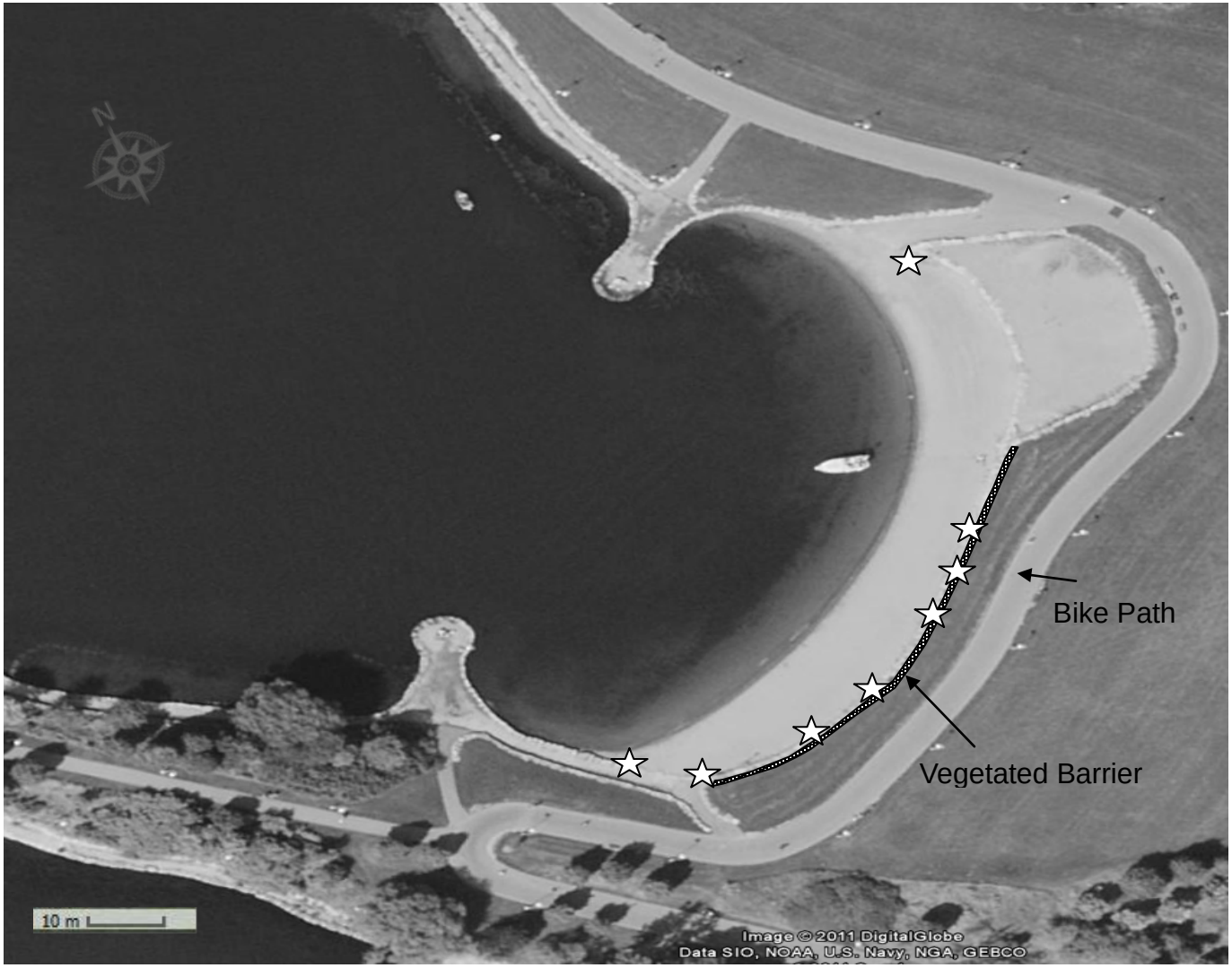

Annual Percentage of Beach Days Open for Swimming
Percentage (%)
100
90
80
70
60
50
40
30
20
10
0
Implementation of bird deterrent measures at Pier 4 Beach
Implementation of bird deterrent measures at Bayfront Beach
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
Year
Pier 4 Beach
Bayfront Beach
Hamilton RAP Delisting Criteria

Fig. 3

Fig. 4


N
Bike Path
Vegetated Barrier
10 m
Image © 2011 DigitalGlobe
Data SIO, NOAA, U.S. Navy, NGA, GEBCO

Characterizing *E .coli* and phosphorus in nearshore surface water at Bayfront Beach, Hamilton Harbour, Ontario.

Jacqui Milne[1], Sue Gilpin[2]

[1]*Environment Canada, Aquatic Ecosystem Management Research Division, Water Science and Technology Branch, 867 Lakeshore Rd. Burlington, Ontario, L7R 4A6.*

[2]*Park District 3 City of Hamilton, 77 James St. N, Suite 351, Hamilton, Ontario*

Introduction

Bayfront Beach, located in the City of Hamilton on the southwest shore of Hamilton Harbour. Health advisories are posted on a regular basis during swimming season. Hamilton Harbour was designated as one of 43 areas of concern (AOC) in 1986 by the IJC (Hall et al. 2006)). Under the Great Lake Water Quality Agreement (GLWA), a Remedial Action Plan for each Hamilton Harbour was established in 1987. In order to de-list as an AOC, confirmation of restoration of uses including beaches must be achieved. Delisting is targeted for 2020 (Hall et al. 2006) In order to de-list the *E .coli* numbers must remain under the Provincial Water Quality Objective (PWQO) of 100 cfu/100ml for 80% of the swimming season. During precipitation events *E. coli* in beach sand may be transported to the surface water by infiltration and overland flow causing exceedances of the PWQO of 100 cfu/100ml in the nearshore water. This is potentially exacerbated by limited flows in the bay that inhibit exchange of water with Hamilton Harbour during the swimming season (Milne 2011). The objectives of the following study are to 1) collect surface water samples at Bayfront Beach to characterize *E .coli* and nutrient concentration variability in the nearshore area during ice free period 2) to determine if a significant relationship exists between *E .coli* vs nutrients, precipitation and wind speed data at Bayfront Beach 3) compare results at Bayfront Beach with centre station Hamilton Harbour. The information from this study will be used by beach managers to make informed decisions on *E .coli* mitigation at Bayfront Beach. Data collected post mitigation will be compared to this study to measure improvements in *E .coli* and nutrients at Bayfront Beach.

Methods

Study Site

Bayfront Beach is a man-made urban beach in Hamilton Harbour, western end of Lake Ontario. The beach is approximately 120 m long by 20 m wide and is bordered by armor stone and a 2 m wide vegetation zone. This in turn is adjacent to a paved walkway and turfgrass (Fig. 1). It is located in a small bay approximately 2 to 3m deep. Flow in the bay is limited where Milne (2011) estimated velocities to be <2.0 m/s for 80% of the swimming season.

To deter water fowl from occupying the beach and nearshore water, a row of buoys was installed approximately 60 m from shore and vegetation planted around the perimeter of the beach (fig. 1).Centre Station in Hamilton Harbour is located approximately 3 km east of Bayfront Beach (fig. 2).

Study Design

Nearshore surface water samples were collected weekly from April 24 to Oct 7, 2013 for a total of 23 days for *E .coli* and nutrient samples were collected at 5 locations by dipping an acid washed 250 ml and 1000 ml bottle by hand in ankle depth water at Bayfront Beach (fig. 3). Water samples from centre station in Hamilton Harbour were collected at 1 m depth by boat weekly from April 23 to Oct 7, 2013. *E .coli* data from centre station was collected weekly in 2009. Daily precipitation and wind data was downloaded from the Government of Canada's Climate website for Hamilton Airport climate station # 6153193. Forty-eight hour cumulative precipitation previous to the date of sampling was used in the data analyses.

Laboratory Method

Samples were kept cool and transported to the lab within 30 minutes of collection. *E .coli* were analyzed by the Coliplate-400™ method (Bluewater Biosciences Incorporated 2009). The plates consist of 96 wells containing a previously prepared agar solution designed to stimulate *E .coli* growth. Water from a sample is poured over the plate, filling each well (Fig. 4A). Plates were observed after an incubation period of 24 hours at 35°C. Following incubation, an *E .coli* count is determined by counting the number of wells that exhibit fluorescence under a long wavelength UV light (Fig. 4B), and converting this count to the Most Probable Number (MPN) of colony forming units per 100 mL of water following the procedures from EBPI, 1999.

Fig. 1 Location of buoys and vegetation barrier at Bayfront Beach, Hamilton, Ontario.

Fig. 2 Location of Bayfront Beach and Centre Station in Hamilton Harbour.

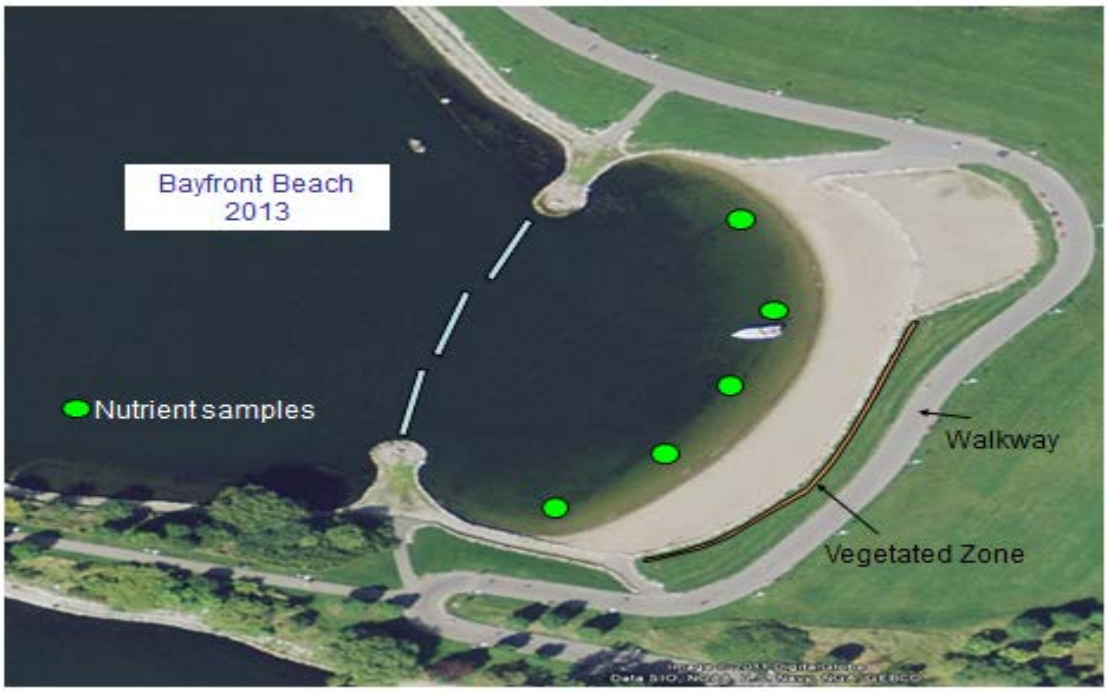
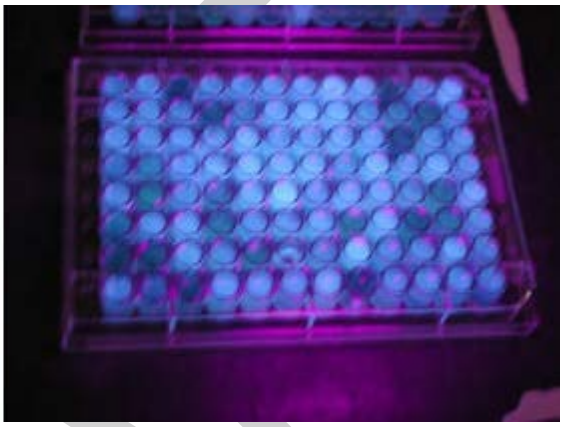


Fig. 3 Sample locations of nearshore surface water for phosphorus and *E .coli* analyses at Bayfront Beach, Hamilton Harbour, Ontario.

Nearshore surface water samples and samples from centre station were prepared following the National Laboratory Environmental Testing (NLET) protocols for soluble reactive phosphorus (SRP), filtered phosphorus (TFP), Chlorophyll a (Chla), Nitrate/Nitrites ($NO_{3/2}$) and Ammonia (NH_{4+3}) (Operational Analytical Laboratories and Research Support Water Science and Technology Directorate Environment Canada 2010). An aliquot of unfiltered sample water was fixed with 30% sulphuric acid for total phosphorus (TP) analyses. Chlorophyll a (Chla) samples were prepared by filtering 1 liter of sample water through a 47 mm glass fiber filter. The prepared samples were analyzed by the NLET at the Canada Centre for Inland Waters, Burlington, Ontario.

A



B

Fig. 4A and B Sample water is poured onto plates consisting of 96 wells with agar (A). Samples are incubated for 24 to 30 hours then fluorescent cells are counted and converted into MPN cfu/100ml (B).

Statistical Method

E .coli data for each sampling day was averaged using a geometric mean (GM) and log transformed to perform statistical analyses. Pearson's Correlation Coefficient and ANOVA's were applied to determine if a significant relationship existed between *E. coli* and nutrients at Bayfront Beach and centre station.

Results

E .coli results at Bayfront Beach were variable ranging between 23.5 MPN/100ml to 976 MPN/100ml with a GM of 167 MPN/100 ml (GMSD 4 MPN/100ml). Mean $NO_{3/2}$, NH_{4+3}, Chla, SRP, TFP and TP at Bayfront Beach were 2.2 mg/L, 0.07 mg/L, 15.3 ug/L, 5.1 ug/L, 16.3 ug/L and 59.0 ug/L respectively results including means and SD are located in table 1. At centre station the GM *E .coli* was 13 MPN/100 ml (GMSD 4 MPN/100 ml). Mean $NO_{3/2}$, NH_{4+3}, Chla, SRP, TFP and TP were 2.4 mg/L, 0.06 mg/L, 11.56 ug/L, 3.4 ug/L, 15.3 ug/L and 40.3 ug/L

respectively. Total precipitation on or 48 hours previous to sampling dates during the study period was 103.2 mm. The highest precipitation was 32 mm and the lowest was 0 mm and the mean was 4.5 mm (table 1). There were no significant correlations between *E .coli* vs nutrients, precipitation or wind gusts, however a highly significant relationship was evident with TP vs Chla (R^2=0.83). *E .coli* and TP means at Bayfront Beach were significantly higher when compared to centre station (p=<0.05, p=<0.05 respectively) (table 2).

Discussion

Bayfront Beach is located in a small enclosed bay approximately 2 to 3 m deep oriented NNW. The area adjacent to Bayfront Beach is primarily turf grass with paved bike paths sloped toward the beach. Canada Geese frequently inhabit the grassy areas of the park depositing fecal material where during precipitation events runoff may intercept the beach causing elevated *E .coli* in beach sand and nearshore surface water.

Flow in the bay is limited where Milne (2011) estimated velocities to be <2.0 m/s for 80% of the swimming season. For example, it appears from this and a previous study (Milne 2011), the embayment likely has a low flushing rate; therefore *E .coli* and nutrients entering the nearshore water is not exchanged with the greater harbour and may have a cumulative effect in the embayment (Bigg and Webber 2003;Sanger et al. 2012) This is indicated by significantly higher *E .coli* and TP compared to centre station for the duration of the study. In fact, *E .coli* numbers remained below the PWQO for only 40% of the time and the TP remained above RAP initial target of 34 ug/L for 100% of the study period, whereas, centre station *E .coli* remained below the PWQO for 80% of the time and the TP remained above RAP initial target for 70% of the study period. Further to this City of Hamilton and Environment Canada did indeed observed filamentous algal growth and cyanobacteria blooms in the embayment from July to the end of September. Precipitation did not appear to significantly impact *E .coli* or nutrient data in this study. However, it should be noted that it was a relatively dry summer. High E. coli in the Low flow in the embayment may be exacerbated by the population of geese and gulls which frequently inhabit the beach and nearshore surface water contributing excess fecal contamination.

High E. coli in the embayment may be may be as a result of populations of geese and gulls which frequently inhabit the beach and nearshore surface water contributing excess fecal contamination. This maybe exacerbated by low flow and limited flushing in the embayment.

Conclusion

E .coli was highly variable and exceeded the PWQO 60 % of the study period and was significantly higher than *E .coli* numbers at centre station. Total Phosphorus at Bayfront Beach remained above the initial RAP target 100% of the time and was significantly higher than centre station in Hamilton Harbour.

No significant correlation was evident between *E .coli* and nutrients, wind speed or precipitation at Bayfront Beach..

Studies in 2014 will include the deployment of current meters in the embayment, weekly sample collection for analyses of *E .coli* and nutrients, and collection of wind and precipitation data at Bayfront Beach.

Table1. *E .coli*, nutrient, rainfall and wind gust data collected at Bayfront Beach April to October 2013.

Date	*E .coli**	$NO_{3/2}$	NH_{4+3}	CHLA	SRP	TPF	TP
24-Apr-13	7.4	2.5	0.15	4.0	1.6	10.1	33.5
1-May-13	23.5	2.3	0.03	16.2	2.0	12.4	44.5
8-May-13	N/A	2.1	0.05	8.7	35.4	45.9	62.5
15-May-13	28.0	2.9	0.20	7.7	3.8	15.2	39.7
21-May-13	76.5	2.3	0.04	86.6	11.1	34.5	168.0
28-May-13	170.2	2.9	0.02	9.9	1.9	13.0	43.9
6-Jun-13	976.3	2.8	0.10	4.6	9.1	21.7	72.6
12-Jun-13	99.0	2.4	0.11	7.8	9.0	23.9	51.0
18-Jun-13	224.0	3.1	0.11	13.1	6.3	17.7	66.2
26-Jun-13	611.1	2.9	0.03	11.3	1.2	13.1	50.0
3-Jul-13	N/A	2.6	0.05	16.5	1.4	14.1	60.1
11-Jul-13	620.6	2.2	0.05	17.9	1.7	11.8	77.8
17-Jul-13	439.9	2.1	0.03	9.9	2.9	13.9	61.4
24-Jul-13	563.0	2.1	0.02	16.3	1.8	12.7	56.4
31-Jul-13	129.2	2.1	0.02	16.6	1.5	11.8	50.8
8-Aug-13	467.6	1.9	0.01	27.8	2.0	11.8	94.9
13-Aug-13	378.8	1.6	0.02	N/A	3.0	11.9	55.8
5-Sep-13	89.2	1.5	0.04	5.5	1.6	10.0	38.4
9-Sep-13	56.1	1.5	0.01	14.7	N/A	17.7	55.5
19-Sep-13	69.1	1.4	0.01	17.6	1.4	12.3	44.5
24-Sep-13	298.1	1.4	0.01	10.1	1.0	10.5	45.1
1-Oct-13	560.8	1.4	0.13	4.2	4.9	14.8	36.1
7-Oct-13	262.7	2.5	0.29	8.6	6.8	14.9	48.8
Mean*	167	2.2	0.07	15.3	5.1	16.3	59.0
STD	3.6	0.5	0.07	16.9	7.4	8.4	27.7

* *E .coli* was calculated as Geometric Mean

Table 2. Results of ANOVA of Bayfront Beach (BF) means vs Centre Station (CS) means for *E .coli* and nutrients. Significance (sig) is denoted by the number 1.

Variable	Mean Difference	SEM*	t-value	Prob^	sig	LCL[@]	UCL[#]
BF $NO_{3/2}$ vs CS $NO_{3/2}$	0.20029	3.0183	0.06636	1	0	-10.3314	10.73199
BF NH_{4+3} vs CSN NH_{4+3}	0.13593	3.0183	0.04504	1	0	-10.3958	10.66763
BFCHLa vs CSCHLA	-4.41853	3.05512	-1.44627	1	0	-15.0787	6.24163
BFSRP vs CSSRP	0.03461	3.05512	0.01133	1	0	-10.6256	10.69478
BFTPF vs CSTPF	0.1067	3.0183	0.03535	1	0	-10.425	10.63839
BFTP vs CSTP	-18.7686	3.0183	-6.21825	1.37E-07	1	-29.3003	-8.23688
BF*E.coli* vs CS*E.coli*	-1.109	0.1934	-5.7317	1.92E-06	1	-1.50187	-0.7157

*Sum of error means
^ Probability
[@]Lower confidence interval
[#]Upper confidence interval

References

Bigg, G. R. and Webber, D. F. 2003. The impact of coastline change and urban development on the flushing time of a coastal embayment, Kingston Harbour, Jamaica. Bulletin of Marine Science 73:291-305.

Hall, J. D., O'Connor, K., and Renieri, J. 2006. Progress toward delisting a Great Lake area of concern: The role of integrated research and monitoring in the Hamilton Harbour remedial action plan. Environmental Monitoring and Assessment 113:227-243.

Milne, J. 2011. Quantifying water circulation at Bayfront Beach Hamilton, Ontario.

Operational Analytical Laboratories and Research Support Water Science and Technology Directorate Environment Canada 2010. NLET schedule of services., Burlington, Ontario.

Sanger, D. M., Smith, E. M., Voulgaris, G., Koepfler, E. T., Libes, S. M., Riekerk, G. H. M., Bergquist, D. C., Greenfield, D. I., Wren, P. A., McCoy, C. A., Viso, R. F., Peterson, R. N., and Whitaker, J. D. 2012. Constrained enrichment contributes to hypoxia formation in Long Bay, South Carolina (USA), an open water urbanized coastline. Marine Ecology Progress Series 461:15-30.

Characterizing *E. coli* loads in storm water runoff and phosphorus in nearshore surface water at Bayfront Beach, Hamilton Harbour, Ontario.

Jacqui Milne[1], Ilona Feldmann[2], Sue Gilpin[3]

[1]*Environment Canada, Aquatic Ecosystem Management Research Division, Water Science and Technology Branch, 867 Lakeshore Rd. Burlington, Ontario, L7R 4A6.*

2 Bay Area Restoration Council B130F Life Sciences Building 1280 Main St. W., Hamilton,, Ontario L8S 4K1

[3]*Park District 3 City of Hamilton, 77 James St. N, Suite 351, Hamilton, Ontario*

Abstract

Bayfront Beach has experienced health advisory postings on a regular basis during summer months. The land adjacent to Bayfront Beach slopes toward the beach sand and is primarily turf grass often inhabited by Canada geese. Potentially high levels of *E. coli* in runoff intercept the beach sand and discharge in the nearshore surface water. We have attempted to estimate *E. coli* loads in storm water intercepting Bayfront Beach using the USDA Natural Resources Conservation Curve Number (CN) model and characterize *E. coli* and phosphorus concentrations in nearshore water during wet and dry events. Results show *E. coli* loads in runoff to be highly variable ranging from ~250 CFU to ~2700 CFU however, magnitudes lower compared to other studies. Total phosphorus and soluble reactive phosphorus concentrations in the nearshore surface water were significantly higher in dry events compared to wet events. Information from this study will assist beach managers in making informed decisions on *E. coli* mitigation.

Acknowledgements

We would like to thank the City of Hamilton and BARC for their assistance with this study. We would also like to thank Charlie Talbot of the technical operations section of Environment Canada for providing the elevations at Bayfront Park.

Introduction

Bayfront Beach, located on the southwest shore of Hamilton Harbour in an enclosed bay, has experienced health advisory postings on a regular basis during summer months. Recent studies have revealed fecal contamination by birds may be a significant source of *E. coli* (Edge and Hill 2005). The land adjacent to Bayfront Beach slopes toward the beach sand and is primarily turf grass often inhabited by Canada geese. Potentially high levels of *E. coli* in runoff intercept the beach sand and discharge in the nearshore surface water. Under optimum conditions beach sand may act as a storage reservoir for *E. coli* (Sampson et al. 2006; Whitman and Nevers 2003). During precipitation and wind events *E coli* in beach sand may be transported to the surface water by infiltration and wave agitation causing exceedances of the Provincial Water Quality Objective (PWQO) in nearshore water. This is potentially exacerbated by limited flows in the bay that inhibit exchange of water with Hamilton Harbour during the summer season. The objectives of the following study are to 1) collect runoff samples adjacent to the beach to characterize *E. coli* concentrations; 2) implement the USDA Natural Resources Conservation Curve Number (CN) model to estimate volume of runoff during precipitation events and total *E. coli* load intercepting Bayfront Beach during precipitation events; and 3) collect surface water samples at Bayfront Beach to characterize *E. coli* concentrations and nutrients in the nearshore area during dry and wet events. The information from this study will be used by beach managers to make informed decisions on *E. coli* mitigation at Bayfront Beach.

Methods

Study Site

Bayfront Beach is a man-made urban beach approximately 120 m long by 20 m wide. The beach is bordered by armor stone and an approximately 1 m wide vegetation zone. This in turn is adjacent to a paved walkway and turfgrass (Fig. 1). It is located in a small bay approximately 2 to 3m deep with extensive macrophyte growth.

To deter water fowl from occupying the beach and nearshore water, a row of buoys was installed approximately 60 m from shore and vegetation planted around the perimeter of the beach (fig. 1).

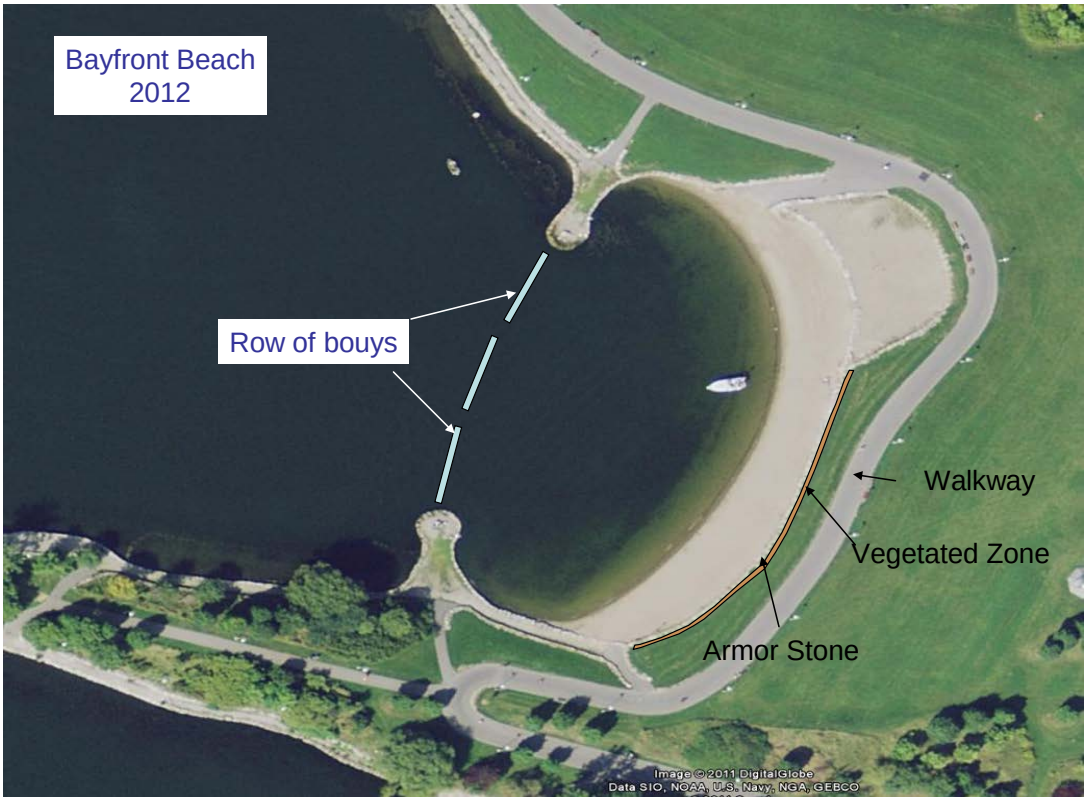


Fig. 1

Study Design

Runoff samples were collected at Bayfront Beach during 4 precipitation events: July 26, August 9, September 18 and October 29 and 30, 2012. The number of runoff points where sample water was collected for each event ranged between 4 and 6 (fig. 2). Samples were collected by capturing runoff in a sterilized 250 ml plastic bottle. In some instances where flow was very low samples were collected using a sterilized 50 ml syringe and transferred to a sterilized 250 ml plastic bottle.

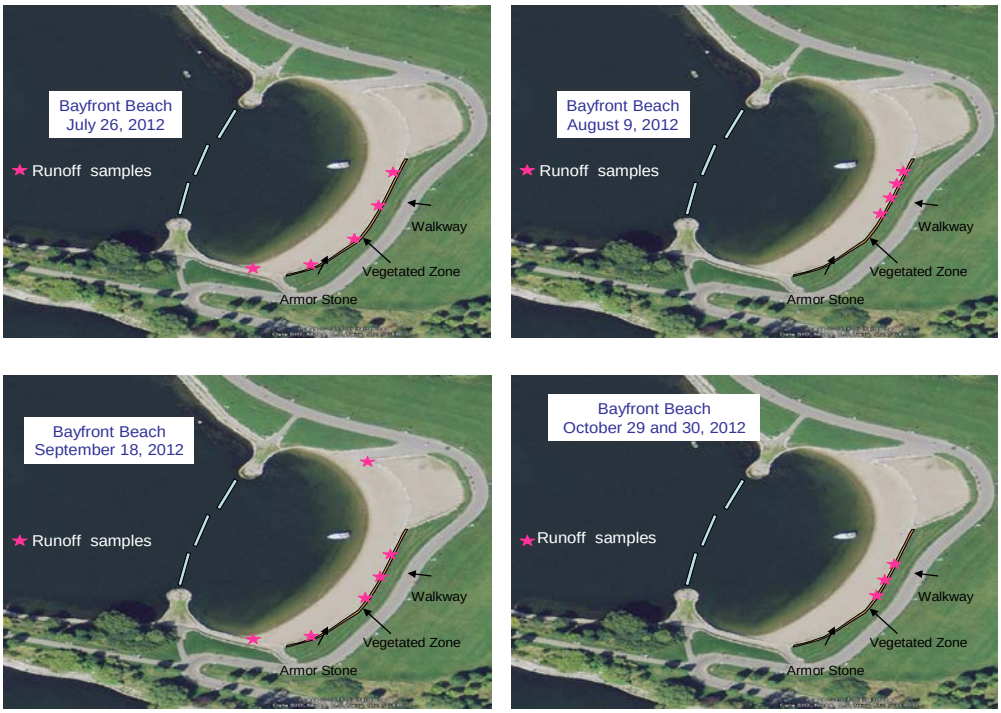


Fig. 2 Runoff sample locations for *E. coli* analyses at Bayfront Beach in Hamilton Harbour, Ontario.

Nearshore surface water samples were collected for nutrient and *E. coli* analyses at 5 locations by dipping an acid washed 250 ml bottle by hand in ankle depth water. Sample water for nutrient analyses was collected during 2 precipitation events on July 26, September 18 and 1 dry event on September 13, 2012 (fig.3). Sample water for *E. coli* analyses was collected September 13 (dry event) and September 18, 2012 (wet event).

E. coli results were log transformed to perform t-tests.

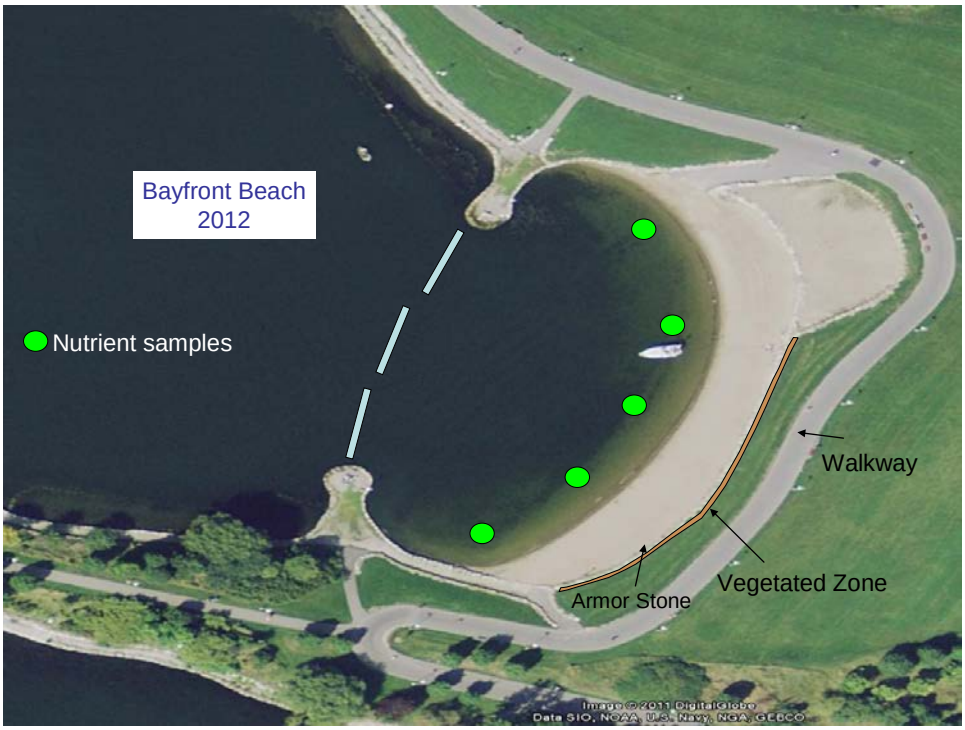
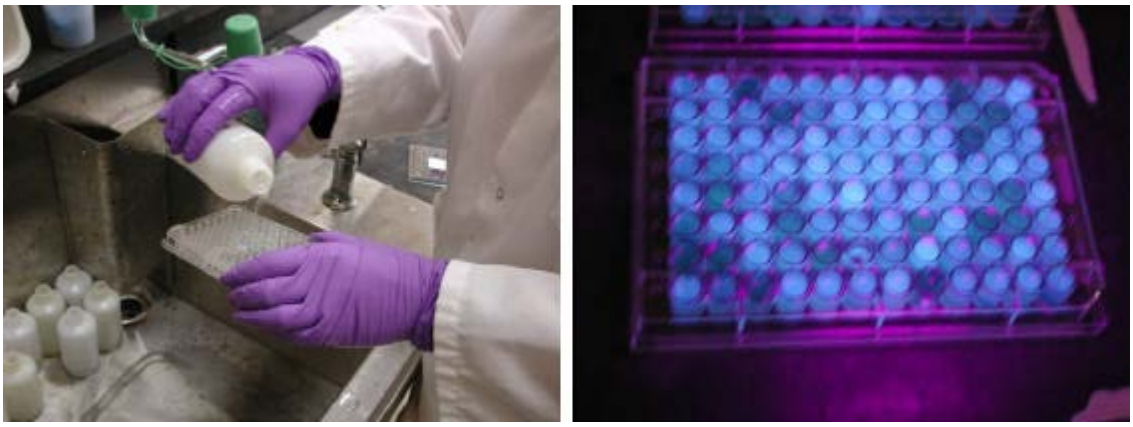


Fig. 3 Sample locations of nearshore surface water for phosphorus analyses at Bayfront Beach, Hamilton Harbour, Ontario.

Laboratory Method

Samples were kept cool and transported to the lab within 30 minutes of collection. *E. Coli* were analyzed by the Coliplate-400™ method (Bluewater Biosciences Incorporated 2009). The plates consist of 96 wells containing a previously prepared agar solution designed to stimulate *E. Coli* growth. Water from a sample is poured over the plate, filling each well (Fig. 4A). Plates were observed after an incubation period of 24 hours at 35°C. Following incubation, an *E. Coli* count is determined by counting the number of wells that exhibit fluorescence under a long wavelength UV light (Fig. 4B), and converting this count to the Most Probable Number (MPN) of colony forming units per 100 mL of water following the procedures from EBPI, 1999. The geometric mean (GM) of *E. coli* values were used in the CN model.

Nearshore surface water samples were prepared following the National Laboratory Environmental Testing (NLET) protocols for soluble reactive phosphorus (SRP) and filtered phosphorus (TFP) (Operational Analytical Laboratories and Research Support Water Science and Technology Directorate Environment Canada 2010). An aliquot of unfiltered sample water was fixed with 30% sulphuric acid for total phosphorus (TP) analyses. The prepared samples were analyzed by the NLET at the Canada Centre for Inland Waters, Burlington, Ontario.



A B

Fig. 4A and B Sample water is poured onto plates consisting of 96 wells with agar (A). Samples are incubated for 24 to 30 hours then fluorescent cells are counted and converted into MPN cfu/100ml (B).

Modeling Methodology

The runoff area of Bayfront Beach is approximately 25,000 m^2. ArcGis 10 was used to determine the area of the catchment by analyzing elevation data recorded by Environment Canada on July 4 and 9, 2012. Most predictive catchment models have been developed for watersheds >26,000 m^2, for example TR-55, CanWet, SWAT and AGNPS. Because the runoff area in this study is <26,000 m^2, we chose to implement the USDA Natural Resources Conservation Curve Number (CN) model to estimate storm water runoff volume (United States Department of Agriculture 2004):

$$Q = \frac{(P - I_a)^2}{P - I_a + S}$$

S = (1000/CN)-10
Ia = S X 0.2 (20% of surface storage)

Where:

Q = estimated volume (mm/m^2)
P = precipitation (mm/m^2)
I_a= Initial abstraction (the maximum amount of rainfall infiltrated without causing runoff) (mm/m^2)
S = Surface storage (mm/m^2) maximum retention after runoff begins
CN = Curve number (non dimensional)

Daily precipitation (P) was downloaded from the National Climate Data and Information Archive of Environment Canada for Hamilton, Ontario, Royal Botanical Garden Climate ID 6153301 43°17'30.000" 79°54'30.000". A CN of 69 was used in the model

representing >50 to 75% turf grass coverage (United States Department of Agriculture 2004). The estimated volume, Q, was multiplied by the total area of runoff to give a total volume in litres. The *E. coli* load (GM of cfu) was estimated by multiplying the total runoff volume by the observed *E. coli* concentration (cfu/l) giving a total load of *E. coli* GM of cfu. *E. coli* loads were estimated for specific precipitation events on July 26, August 9, September 18 and October 29/30 2012 and total monthly precipitation for July, August, September and October 2012.

Results

Precipitation during the study period was highly variable ranging from < 10 mm to 54 mm. Monthly precipitation ranged from <40 mm in July to >150 mm in October. *E. coli* values in the runoff were also highly variable ranging from < 70 GM cfu/100ml to ~2500 GM cfu/100ml (table 1). The GM of *E. coli* in the nearshore surface water on September 13, 2012 (dry event) was 463 cfu/100ml whereas the GM of *E. coli* on September 18, 2012 (wet event) was 114 cfu/100ml, however was not significantly different from the dry event (p=0.2).

The mean TP concentration in the nearshore surface water on September 13, 2012 (dry event) of 40 ug/L was significantly greater than mean total phosphorus concentrations on July 26 and September 18, 2012 (wet events) 28 and 40 ug/L (table 2). Mean TFP showed no significant difference between wet and dry events, however, mean SRP on September 13, 2012 (dry event) of 1.24 ug/L was significantly higher than the wet events on July 26 and September 18 , 2012 (0.32 and 1.06 ug/L) (table 2). GM of *E. coli* concentrations were higher during the dry event (September 13, 2012) than the wet event on September 18, 2012 (table 2) but not significant.

Table 1. Geometric mean of *E. coli* concentration in runoff, precipitation, total estimated runoff volume and total estimated *E. coli* load.

Date	GM E. coli (GM cfu/100ml)	Precipitation (mm)	Estimated Volume (l)	Estimated E. coli load (GM cfu)
July 26, 2012	2588	13	223, 883	5795
August 9, 2012	283	9.1	134,425	380
September 18 , 2012	62	20	392,311	243
October 29/30, 2012	74	53.8	1,237,002	915

GM= geometric mean

Table 2. Mean phosphorus and geometric mean of *E. coli* concentrations during wet and dry events in the nearshore surface water.

Date	TP (ug/L)	TFP (ug/L)	SRP (ug/L)	*E. coli* (mpn/100ml)
July 26, 2012 (Wet event)	28 ±1.8	11±0.33	0.32±0.34	
September 13, 2012 (Dry event)	48*±9.0	10±0.89	1.20*±0.30	463±5.45
September 18, 2012 (wet event)	40±4.60	10±5.40	1.00±0.60	91 ±2.73

*significantly greater than wet events; ± Standard deviation; TP=total phosphorus; TFP=total filtered phosphorus; SRP=soluble reactive phosphorus.

The CN model was applied to each precipitation event to estimate volume. The total estimated volume of runoff from the precipitation event on July 26, 2012, August 9, September 18 and October 29/30 was 223,883; 134,425; 392,311; and 1,237,002 litres respectively. The corresponding *E.coli* loads were 5,795; 379; 243; and 915 cfu respectively (fig. 5). The model was then used to simulate *E. coli* loads on a monthly basis. Using the observed *E. coli* concentrations, the loads for July, August, September, and October were 20,626; 3,026; 1,372; 2,885 respectively.

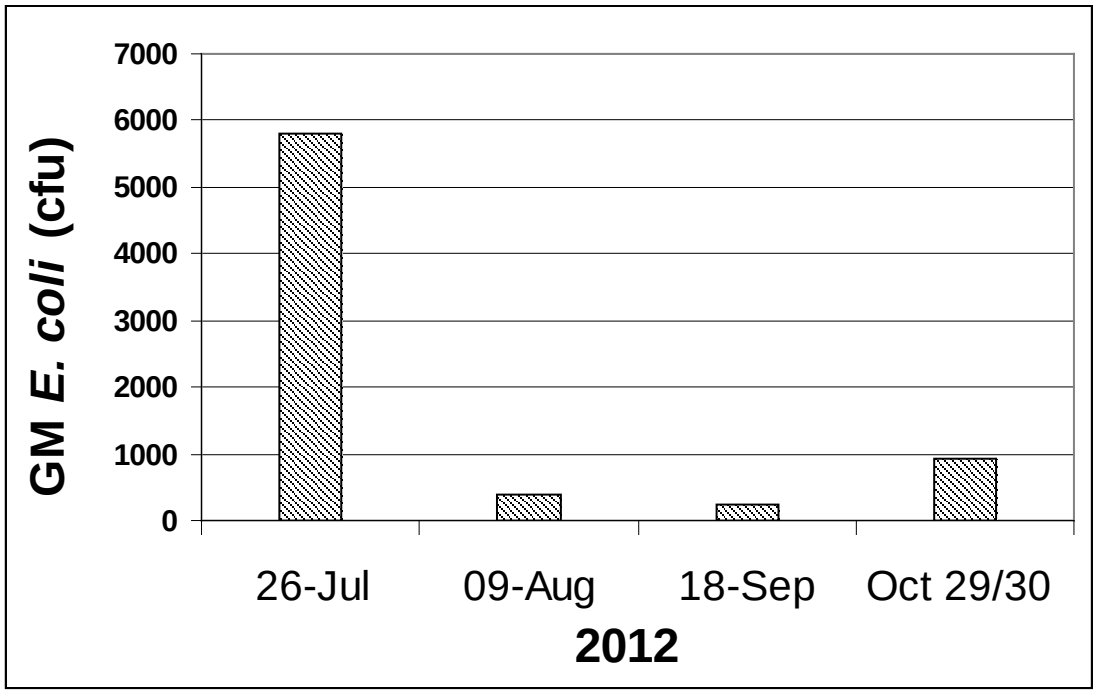


Fig. 5 Estimated load of *E. coli* (cfu) for each precipitation event at Bayfront Beach, Hamilton, Ontario.

Discussion

The area adjacent to Bayfront Beach is primarily turf grass with paved bike paths sloped toward the beach. Geese frequently inhabit the grassy areas of the park depositing fecal material where during precipitation events runoff may intercept the beach causing elevated *E. coli* in beach sand and nearshore surface water.

In this study, the highest *E. coli* load and concentration was evident in the precipitation event on July 26. The load from this event was 15 times higher then the August 9 event. The precipitation event previous to July 26, >5mm event, occurred on June 21, 2012 nearly 1 month before. The elevated *E. coli* load on July 26 may be due to the timing of the event. For example, *E. coli* in the form of goose feces, may have accumulated in the area adjacent to the beach for the month previous resulting in a flush of elevated *E. coli* load on July 26. McKergow and Davies (2010) found similar results. They monitored 12 rain events where several of the events had significantly higher *E. coli* concentrations than others. They attributed this to the distribution of the rainfall within the catchment and timing of the events where "microbial stores have been built up to high levels".

Contrary to this, the GM of *E. coli* results from the events on Sept 18 and Oct 29/30 (62 and 74 GM cfu/100ml) were well below the PWQO of 100 cfu/100ml. September and October experienced a greater frequency of rain events compared to July and August. Less accumulation of fecal material may have been built up between rain events resulting in lower *E. coli* concentrations during precipitation events. It is also very likely we are missing the first flush of the precipitation event where the highest *E. coli* may loads occur.

With the exception of the July 26 event, *E. coli* loads were generally magnitudes lower when compared to other studies (McKergow and Davies-Colley 2010). This is due to comparatively lower *E. coli* concentrations in this study then reported in the literature (McLellan and Salmore 2003; McKergow and Davies-Colley 2010). This is because the catchment areas in other studies are magnitudes larger and have more sources of *E. coli* whereas birds are the primary source of *E. coli* in this study (Edge and Hill 2005). However, when the runoff catchment area in this study was increased to simulate a larger watershed, the *E. coli* load for each rain event was still 1 to 2 magnitudes lower than that reported by McKergow and Davies (2010). In 2011, shrubs were planted around the perimeter of the beach adjacent to the bike path, referred to as a "living fence" which may help in intercepting *E. coli* in the runoff and provide a barrier to geese attempting to access the turf grass.

Mean TP, SRP and *E. coli* in the nearshore surface water were significantly greater during dry weather events compared to wet weather events which contrary to other reports. This particular embayment is known to have low flows during the summer months of <2.0 cm/s (Milne (2011), unpublished data). During precipitation events, runoff and wind may temporarily increase flows in the embayment thereby exchanging

higher nutrient and lower *E. coli* embayment water with lower nutrient water from the outer harbour. Current meter data collected 2011 shows small increases in flow during precipitation and/or wind events providing some evidence that an exchange may be happening. The total phosphorus in the nearshore surface water ranged between 28 and 48 ug/L. Generally, undesirable algal growth can occur at TP concentrations of 20 ug/L (spring mean) (Dillon and Rigler 1975), therefore the Bayfront Beach embayment may experience localized algal blooms during long term dry events. It has also been reported that algal mats can harbour large numbers of *E. coli* populating the foreshore sand and nearshore surface water with *E. coli* (Whitman et al. 2003).

Conclusion

In summary, we found *E. coli* loads to be magnitudes less than other studies. This is due in part to a smaller catchment area, minimal sources of *E. coli*, the implementation of vegetation barrier adjacent to the beach and various goose control measures. TP and SRP were significantly higher during dry events compared to wet events. Precipitation and wind events may increase current velocities and exchange embayment water with outer harbour water resulting lower TP and SRP in the embayment.

Recommendations:

1) The vegetation barrier or "living fence" will continue to grow and may increase efficiency in capturing *E. coli* in the storm water runoff.
2) Increase circulation in the embayment to decrease *E. coli* and nutrients in the nearshore water.
3) Continue goose control: egg oiling, canine patrol, fencing, buoy lines, pyrotechnics and implement new technologies as they become available.
4) Public outreach programs conducted by BARC such as the "Don't Feed the Water Fowl" campaign.

References

Bluewater Biosciences Incorporated 2009. ColiPlates. 14 Abacus Road, Brampton, Ontario, L6T 5B7:

Dillon, P. J. and Rigler, F. H. 1975. A simple method for predicting the capacity of a lake for development based on lake trophic status. Journal of the Fisheries Research Board of Canada 32:1519-1531.

Edge, T. A. and Hill, S. 2005. Occurrence of antibiotic resistance in Escherichia coli from surface waters and fecal pollution sources near Hamilton, Ontario. Canadian Journal of Microbiology 51:501-505.

McKergow, L. A. and Davies-Colley, R. J. 2010. Stormflow dynamics and loads of Escherichia coli in a large mixed land use catchment. Hydrol. Process. 24:276-289.

McLellan, S. L. and Salmore, A. K. 2003. Evidence for localized bacterial loading as the cause of chronic beach closings in a freshwater marina. Water Research 37:2700-2708.

Operational Analytical Laboratories and Research Support Water Science and Technology Directorate Environment Canada 2010. NLET schedule of services. Burlington, Ontario:103 p.

Sampson, W. R., Swiatnicki Sarah, A., Osinga Viki, L., Supita Jamie, L., McDermott Colleen, M., and Kleinheinz, G. T. 2006. Effects of temperature and sand on E. coli survival in a northern lake water microcosm. Journal of Water and Health 4:389-393.

United States Department of Agriculture 2004. Hydrologic Soil-Cover Complexes. In National Engineering Handbook pp. 9-i-9-14. Washington D.C..

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

Whitman, R. L., Shively, D. A., Pawlik, H., Nevers, M. B., and Byappanahall, M. 2003. Occurrence of Escherichia coli and Enterococci in Cladophora (Chlorophyta) in Nearshore Water and Beach Sand of Lake Michigan. Applied and Environmental Microbiology 69:4714-4719.

Characterizing *E. Coli* density in beach sand using three grooming techniques at Bayfront Beach, Hamilton, Ontario.

Jacqui Milne[1], Alexandra Gattozzi[1], Sue Gilpin[2], Stephen Guay[1], Simon Kiela[1],

[1]*Environment Canada, Aquatic Ecosystem Management Research Division, Water Science and Technology Branch, 867 Lakeshore Rd. Burlington, Ontario, L7R 4A6.*

[2]*Park District 3 City of Hamilton, 77 James St. N, Suite 351, Hamilton, Ontario*

NWRI Contribution no. 11-006

Abstract

Bayfront Beach is located on the south west shore of Hamilton Harbour. The beach has experienced health advisory postings on a regular basis during the summer months. Recent studies report beach grooming to influence *E. Coli* density in beach sand where deep grooming may decrease *E. Coli* density. Three beach grooming techniques were applied to evaluate *E. Coli* density in beach sand in 2011 at Bayfront beach. Treatments included 1) shallow grooming with a thatch rake; 2) medium depth grooming with a modified rake with extended prongs 3) maximum depth grooming using a tiller and 4) a control with no grooming. The treatments were randomly assigned to twenty-four 1 square meter plots resulting in 6 replicates of each treatment. One core sample was collected from each treatment plot for a total of 24 samples. Cores were collected daily for 20 days. Results indicated a significant increase in *E. Coli* density with maximum depth grooming and moist vs dry sand. Results of this study will 1.) provide insight into the fate of *E. Coli* in beach sand using different grooming techniques and 2.) will allow for sound decisions in beach management strategies thereby providing the public with safe and aesthetically pleasing beaches 3.) provide valuable information for HH RAP de-listing in 2015.

Acknowledgments

We would like to thank Dr. Bruce Newbold and Kyle Empringham from McMaster University; Eric Mathews and Ramona Maharaj from the City of Hamilton for assistance with this study.

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 wastewater treatment plants (WWTPs) (Remedial Action Plan for Hamilton Harbour 1992), faulty or old septic systems (Whitman and Nevers 2003), agriculture (Palmateer *et al*. 1989), goose and gull faeces (Whitman and Nevers 2003), and *Cladophora* (Whitman *et al* 2003).

Hamilton Harbour was designated as one of 43 areas of concern (AOC) in 1986 by the IJC (Hall et al. 2006). Under the Great Lake Water Quality Agreement (GLWA) Remedial Action Plans for each AOC was established where in 1987 a Remedial Action Plan for Hamilton Harbour (HHRAP) was formalized. In order to de-list as an AOC, confirmation of restoration of uses including beaches must be achieved. Delisting is targeted for 2015 (Hall et al. 2006).

Bayfront Beach in Hamilton Harbour (Fig. 1) in 2009 and 2010 has experienced health advisory postings >66% of the swimming season (Hamilton Harbour Remedial Action Plan 2011). Recent evidence suggest beach sand may act as a long term storage reservoir for *E.coli* and potentially influence nearshore recreational water quality (Milne and Charlton 2004; Whitman and Nevers 2003; Zehmus et al. 2008). Wastewater was once considered to be the key source of *E.coli* contamination but recent studies have revealed fecal contamination by birds may be a significant source (Edge and Hill 2005).

The success of a bird deterrent system implemented at nearby Pier 4 Beach (Milne et al. 2011; Hamilton Harbour Remedial Action Plan 2011) prompted the City to install a similar system at Bayfront Beach in 2011. The system consists of a row of buoys installed parallel to the beach approximately 60 m offshore with vegetation planted around the perimeter of the beach adjacent to the walkway. These actions were intended to prevent the birds from accessing the beach from the water and/or walkway.

Recent studies from Lake Michigan indicated beach grooming techniques may influence *E.coli* numbers in beach sand (Kinzelman et al. 2004; Kinzelman et al. 2004; Kinzelman et al. 2003). In fact, it has been reported that some grooming techniques promote *E.coli* growth (Kinzelman et al. 2003; Kinzelman et al. 2004) Deep grooming techniques (7 to 10 cm), appeared to be more effective at decreasing *E. Coli* numbers in damp beach sand than shallow grooming.

A similar study was conducted on Pier 4 Beach in Hamilton Harbour where deep grooming *E. Coli* densities were compared to shallow grooming. No significant difference was evident between the treatments. This was likely because the beach is located close to the water table and remains moist most of the season which provides suitable conditions for *E. Coli*. In fact a significant increase in *E. Coli* density was evident in wet vs dry sand (Milne et al. 2011).

It was agreed by the Beach Management Committee to conduct a beach grooming study on Bayfront Beach because the beach is larger and appears to be drier. We, therefore, hypothesize that deeper grooming at Bayfront will decrease *E. Coli* levels in beach sand. To compliment the beach grooming experiment, runoff samples were to be collected during precipitation events to characterize *E. Coli* in overland flow adjacent to and intercepting the beach.

Other ongoing beach management programs by the City of Hamilton and BARC are 1) goose and gull control on a daily basis by canine patrol and egg oiling and 2) the "don't feed the water fowl" campaign.

The objective of this study is to evaluate *E.coli* density in beach sand by application of shallow grooming using a typical garden thatching rake, medium depth grooming using a modified rake with extended prongs and maximum depth grooming using a gas-powered tiller vs. controls where no treatment is applied. If results from this study show a decrease in *E.coli* numbers in beach sand with deeper grooming, current beach management will be modified to include deep grooming on a daily basis during the bathing season. Results of this study will 1) provide insight into the fate of *E. Coli* in beach sand using different grooming techniques and 2) will allow for sound decisions in beach management protocols thereby providing the public with safe and aesthetically pleasing beaches 3) provide valuable information for HH RAP de-listing in 2015 4) technique may be applied to other beaches.

Fig. 1 Bayfront Beach is located near the west end of Hamilton Harbour, Ontario.

Methods

Study Site

Bayfront Beach is a man-made urban beach approximately 120 m long by 20 m wide. The beach is bordered by armor stone and an approximately 1 m wide vegetation zone. This in turn is adjacent to a paved walkway and turfgrass (Fig. 2) It is located in a small bay approximately 2 to 3m deep with extensive macrophyte growth.

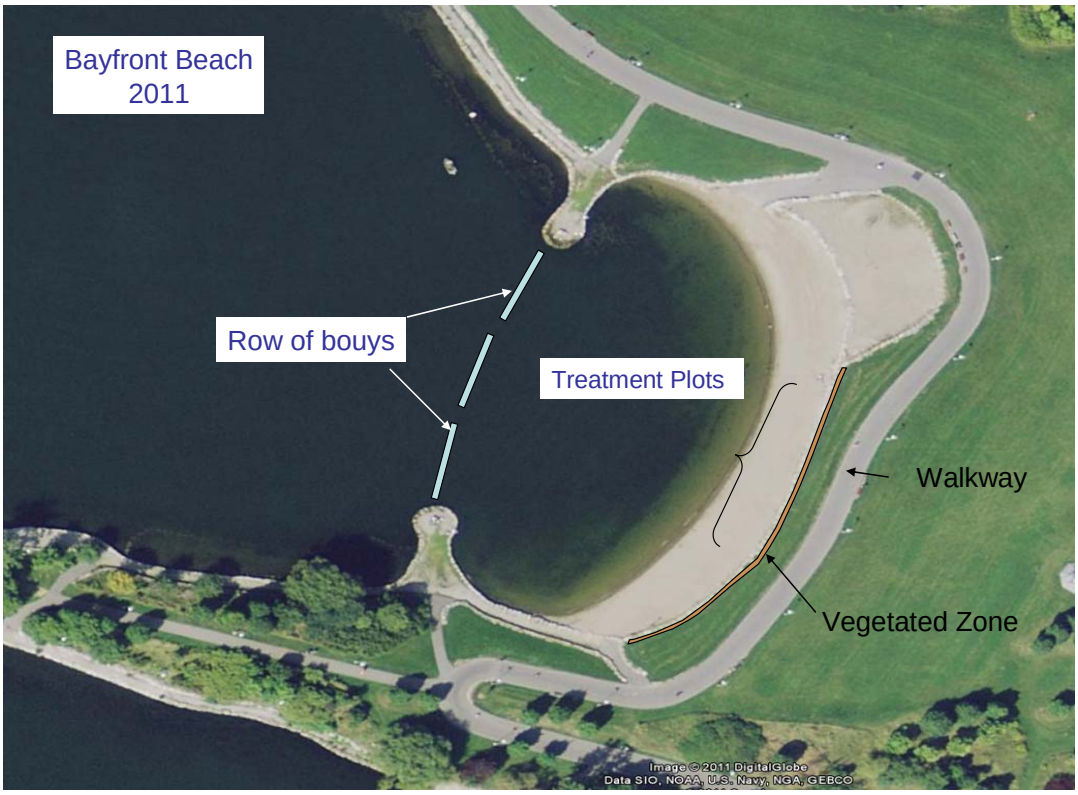


Fig. 2 The bird exclusion system at Bayfront Beach consisted of a row of buoys installed approximately 60 m from shore and vegetation planted around the perimeter of the beach.

Study Design

In 2011 the study began on June 20 and finished on July 25 for a total of 20 days. Weather conditions were recorded daily including precipitation events, sun, wind and air temperature. Beach conditions were observed including wave height (no waves, minimum, or moderate), number of birds, condition of beach sand (bird feces, feathers, prints, algae, macrophytes, refuse and other debris) and sand dampness. Sand dampness was determined by visual inspection where criteria included very dry, dry, slightly moist, and moist.

A completely randomized block design was implemented. Four treatments were randomly assigned to twenty-four 1 square meter plots resulting in 6 replicates of each treatment (fig. 3). Treatments included 1) shallow grooming with a thatch rake; 2) medium depth grooming with a modified rake with extended prongs; 4) maximum depth grooming with a tiller and 5) control with no grooming (fig. 4). The effective depth of grooming for each treatment was: 1) thatch rake = 7 cm 2) rake with extended prongs = 17 cm and 3) tiller = 10 cm (fig. 5). Treatments were applied by hand before 9:00 am each morning for the duration of the experiment for a total of 24 samples per day. Furrow measurements were recorded in centimeters using a ruler. The total sample area was ~25 m^2.

Fig. 3 Three grooming treatments were applied to $1m^2$ plots parallel to shore.

Beach sand was sampled using a sterilized Eijkelkamp soil core sampler with 5 cm by 30 cm butyrate liners. One core was randomly collected from each treatment plot for a total of 24 cores. All cores were collected within 30 min of application of the grooming treatment.

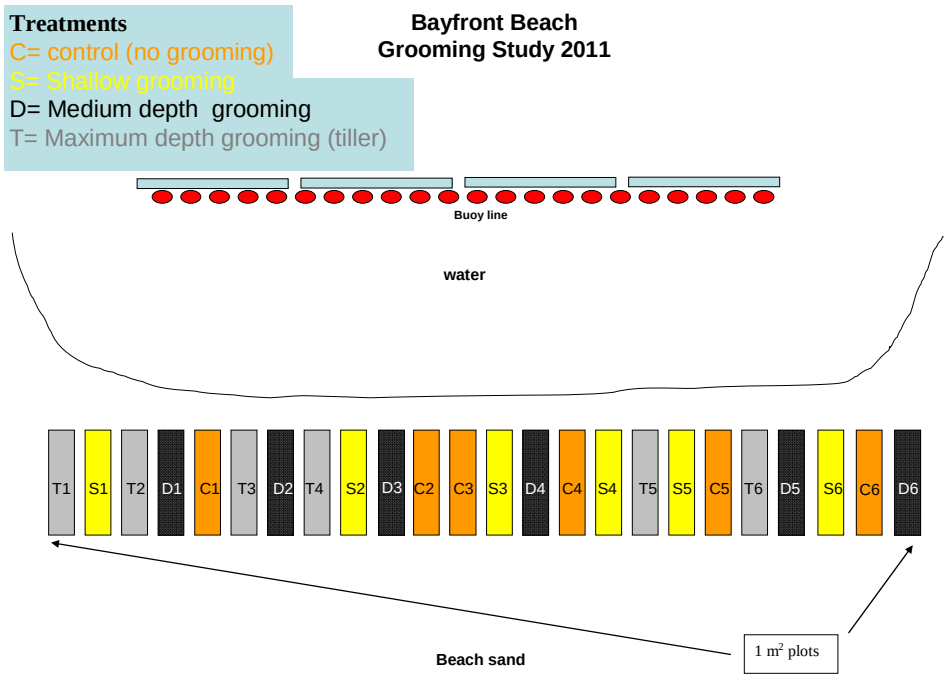
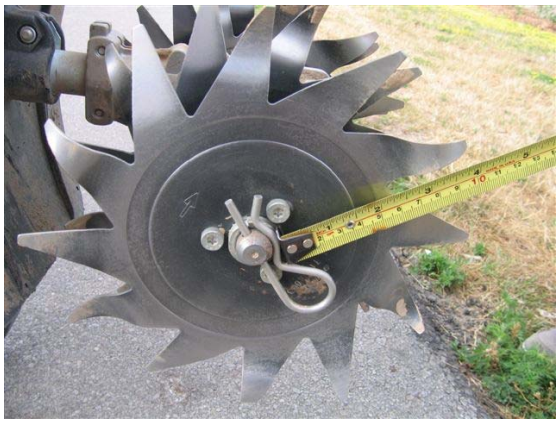


Fig. 4 Three grooming treatments and a control were applied to 24 one m^2 plots for a total of 6 replicates of each treatment.

A B



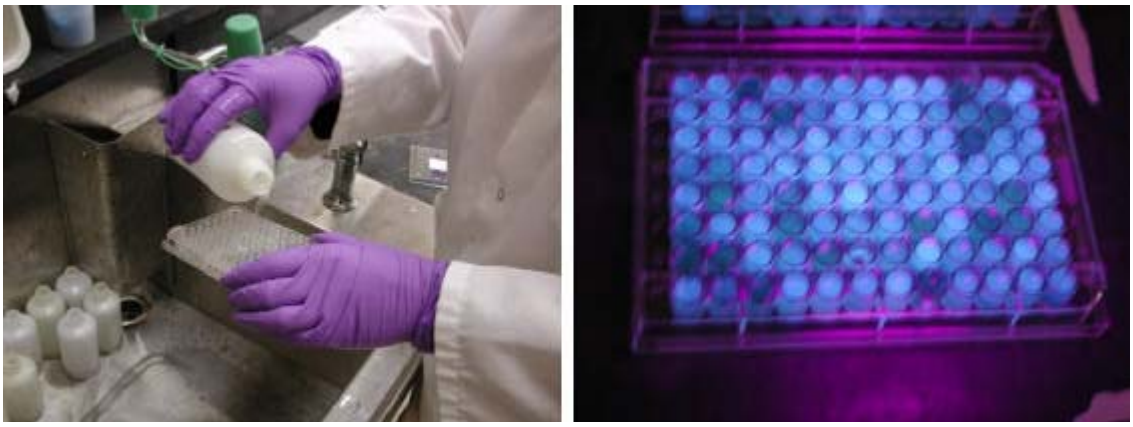
C

Fig. 5 Effective depth of grooming: A) garden thatch rake (shallow grooming) B) rake with extended prongs (medium depth grooming) C) tiller (maximum depth grooming).

No precipitation events occurred during the study period, therefore no runoff samples were collected.

Laboratory Methods

Sand samples were returned to the laboratory within 30 minutes of collection. Wet weights (gm) and lengths (cm) were recorded for each core. Core contents were emptied into sterilized 500 ml polypropylene bottles and 100 ml of phosphate buffer was added. Samples were shaken vigorously for 30 seconds by hand. Previous to the start of this study, sand samples were collected to determine the appropriate dilution for *E. Coli* counts; therefore 1:10 and 1:300 dilutions were used for the study respectively. *E. Coli* were analyzed by the Coliplate-400™ method (Bluewater Biosciences Incorporated 2009). The plates consist of 96 wells containing a previously prepared agar solution designed to stimulate *E. Coli* growth. Water from a sample is poured over the plate, filling each well (Fig. 6A). Plates were observed after an incubation period of 24 hours at 35°C. Following incubation, an *E. Coli* count is determined by counting the number of wells that exhibit fluorescence under a long wavelength UV light (Fig. 6B), and converting this count to the Most Probable Number (MPN) of colony forming units per 100 mL of water following the procedures from EBPI, 1999. Results were reported as cfu per cm^3 of wet weight.



A B

Fig. 6A and B Sample water is poured onto plates consisting of 96 wells with agar (A). Samples are incubated for 24 to 30 hours then fluorescent cells are counted and converted into MPN cfu/100ml (B).

Statistical Methods

All results were log transformed to ensure normally distributed data. A one-way ANOVA with Scheffe test was applied to compare grooming treatment means, overall sand dampness means and sand dampness means within treatments.

Results

Prior to the study, 24 sand cores were randomly gathered to test for homogeneity of *E. Coli* density throughout the treatment area. There were no significant differences of *E. Coli* density across the beach at the 5% level (p=0.23).

During the study period of 2011, 57 mm of precipitation fell, far less than 2009 and 2010 where 207 mm and 147 mm of rain fell respectively. The surface of the beach was dry or very dry during the entire experiment. After application of grooming treatment, sand was wet/moist or slightly moist 3 to 15 of the 20 experimental days. Large runoff rills were evident running the width of the beach through the treatment plots indicating interception of past surface runoff adjacent to the beach. Evidence of bird activity including prints, feathers and feces were observed 20 of the 20 experimental days. On six occasions birds were observed occupying the beach in spite of the bird deterrent system.

Grooming treatments were applied between 9:00 and 9:15 am each sampling day. Mean actual depths of grooming for each treatment and effective depths are located in table 1. Shallow grooming ranged between 0.2 and 4.5 cm with a mean of 1.6; medium grooming 0.5 and 7cm mean, 3.7; maximum depth 4 and 14 cm, mean 7.6. Mean depth of the sand cores was 9.5 cm (+-1.72).

Table 1. Mean depth of furrows for shallow, deep and tiller grooming. SD = Standard Deviation

Year	Treatment	Effective Depth	Actual Mean Depth of Furrow(cm)	S.D.
2009	Shallow	7	1.6	0.7
	Deep	17	3.7	1.7
	Tiller	10	7.6	2.2

Data sets were subdivided based on overall treatment effect and sand dampness. Geometric Mean of *E. Coli* density were control = 5.5 cfu/cm^3 (+-13 cfu/cm^3), shallow = 4.4 cfu/cm^3 (+-11 cfu/cm^3) medium depth grooming = 4.5 cfu/cm^3 (+- 13 cfu/cm^3), and maximum depth grooming = 20.7 cfu/cm^3 (+- 17 cfu/cm^3) (fig. 7). A significant increase in *E. Coli* density was evident in maximum depth grooming (tiller) vs control, shallow and medium depth grooming (p= <0.005). No significant difference was evident in shallow grooming vs control or medium depth grooming (p=0.9) and medium depth grooming vs control (p=0.9) (table 2).

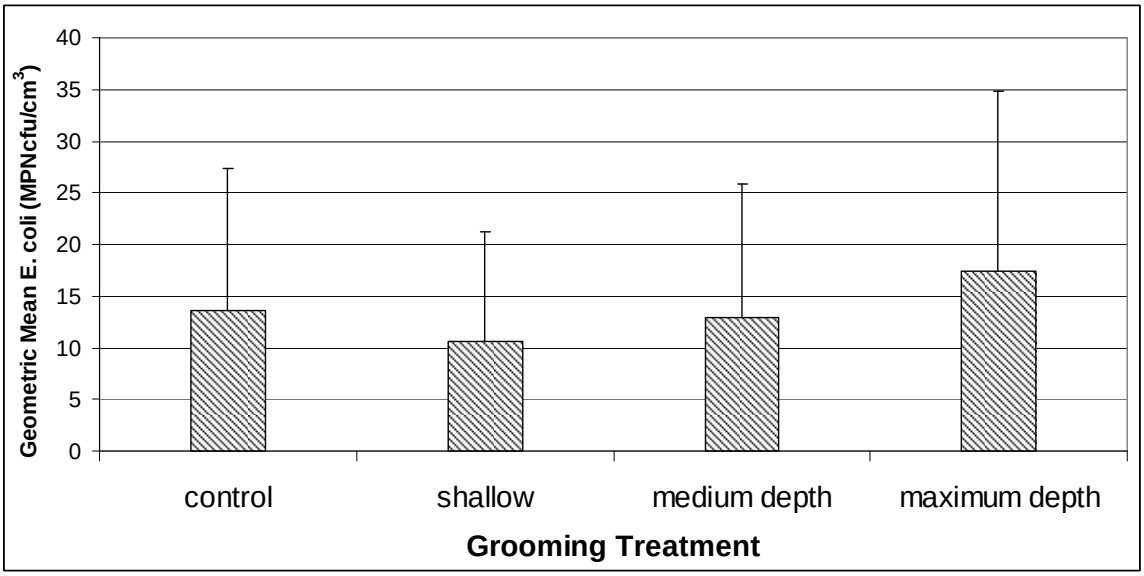


Fig 7. Geometric Mean *E.coli* density for each grooming treatment at Bayfront Beach 2011.

Generally, significantly more *E.coli* was evident in moist sand conditions vs very dry or dry conditions (Table 3) (fig. 8). When comparing *E. Coli* density means and sand dampness within each treatment group, we found a significantly higher *E. Coli* density in dry vs very dry sand in the control group; significantly higher *E.coli* density in moist vs slightly moist sand in the shallow and maximum depth treatment group; no significant difference in sand dampness in the medium depth treatment group (Table 4).

Table 2 Comparison of E. coli density means for each grooming treatment. NS= Not Significant.

P value for each comparison				
2011	Grooming Treatment			
	Shallow	Med depth	Max depth	Control
Shallow	----------	NS	<0.001	NS
Med depth		----------	<0.001	NS
Max depth			---------	0.001

Table 3. Comparison of *E. Coli* density means with levels of sand dampness. NS = Not Significant.

P value for each comparison				
2011	Sand Condition			
	Very Dry	Dry	Slightly moist	Moist
Very Dry	----------	0.01	<0.001	<0.001
Dry		--------	NS	<0.001
Slightly moist			----------	NS
Moist				----------

Table 4. A comparison of sand dampness *E. Coli* density means within each grooming treatment. NS = Not Significant

P value for each mean comparison

Shallow Grooming

Sand Condition

	Very Dry	Dry	Slightly moist	Moist
Very Dry	----------	NS	0.01	0.02
Dry		--------	NS	NS
Slightly moist			----------	NS
Moist				--------

Medium Depth Grooming

Sand Condition

	Very Dry	Dry	Slightly moist	Moist
Very Dry	----------	NS	NS	NS
Dry		--------	NS	NS
Slightly moist			----------	NS
Moist				-------

Maximum Depth Grooming

Sand Condition

	Very Dry	Dry	Slightly moist	Moist
Very Dry	----------	NS	0.002	0.009
Dry		--------	<0.001	0.007
Slightly moist			----------	NS
Moist				------

Control

Sand Condition

	Very Dry	Dry	Slightly moist	Moist
Very Dry	----------	0.01	NS	NS
Dry		--------	NS	NS
Slightly moist			----------	NS
Moist				------

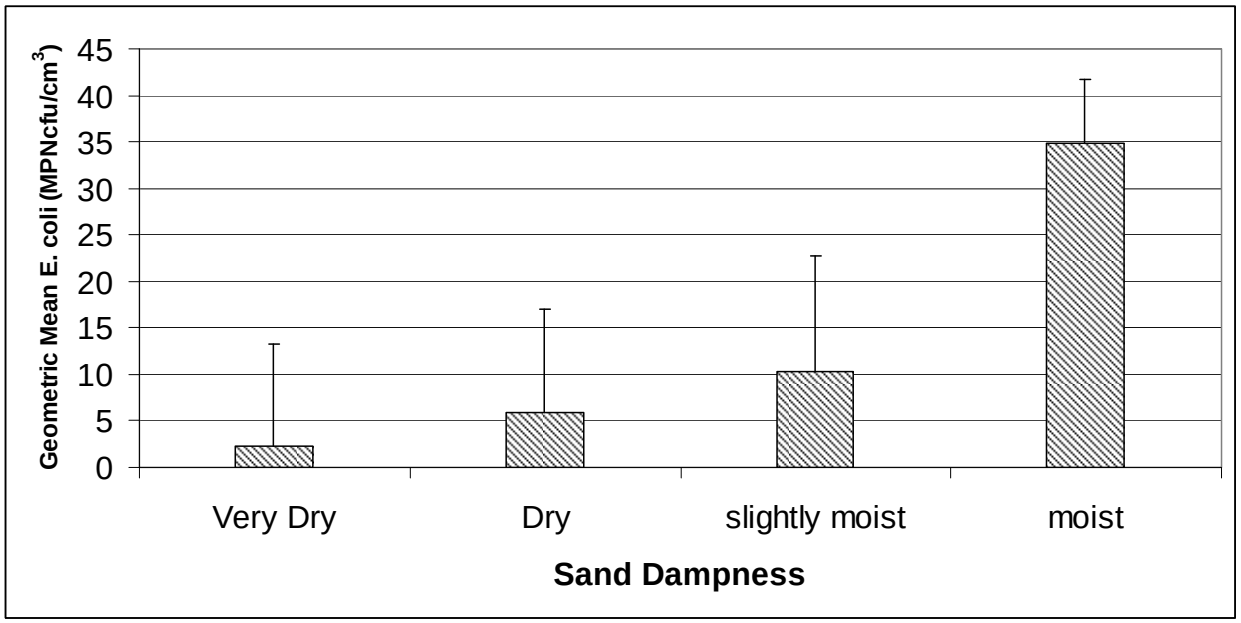


Fig 8. Generally moist sand had significantly greater *E. Coli* density than dry or very dry sand.

Discussion

E. Coli density was significantly increased when comparing maximum depth grooming to shallow and medium depth grooming treatments and the control. This is contrary to results reported by Kinzelman, et al 2004 , where depth of grooming (7 to 10 cm) in damp or wet sand conditions was effective at decreasing *E. Coli* concentrations and in turn a decline in beach advisories. Kinzelman et al noted, however, that shallower grooming can in fact promote increases in *E. Coli* numbers. Further to this, Mika et al, 2009 reports that *E. Coli* numbers are not consistently reduced with mechanical mixing.

Generally, in this study, maximum depth grooming resulted in slightly moist or moist conditions 50% of the experimental days. This indicates the water table is close to the surface. This may lead to *E. Coli* survival in the sand depending on predation, nutrient availability and competition (Alm et al. 2006; Davies et al. 1995). Thus *E. Coli* populations in sand may be transported via run-off during precipitation events to surface waters adjacent to the beach resulting in beach advisories. Kinzelman and McLellan (2009) found that beach slope or grade decreases *E. Coli* numbers. The authors also found that by 1) eliminating standing water; 2) sloping beach to promote adequate drainage and 3) maintaining a defined berm crest (the point at which waves no longer influence beach sand), a notable decrease in *E. Coli* numbers was evident. By implementing these beach management measures along with beach grooming allowed for desiccation of wet sand thereby reducing *E. Coli*.

Bayfront Beach is a component of a park system that was originally designed to provide people with a place to recreate and for the City to occasionally host various events. As a result, the park consists of turf grass and paved paths. The park was engineered to direct runoff away from the

park towards Hamilton Harbour. During heavy or persistent rains impervious areas flooded so that a portion of the runoff flowed across the beach to reach the harbour. Runoff in urbanized areas generally has elevated bacteria numbers (Dorsey 2009). Geese and gulls frequent the turf grass contributing to increased *E. Coli* levels well above the PWQO in the runoff. *E. Coli* is carried from the park area to the beach sand via storm runoff. Infiltration of runoff into the beach sand may continually repopulate *E.coli* within the beach sand. Unfortunately, no precipitation events occurred during the study period, however samples were collected in 2010 at Pier 4 beach within the same park system(Milne et al. 2011). *E. Coli* numbers in surface runoff samples were well above the PWQO (490 to >900 MPN/100ml). Recent evidence suggest a potential seasonal link between decreases in Total Phosphorus concentrations and an increase in *E.coli* numbers and ammonia concentrations signifying *E.coli* may be persisting in beach sand at Pier 4 Park (Milne and Hiriart-Baer 2007). Further to this, Byappanahall et al (2006) , suggests that bacteria deposited in the sand from runoff may be protected from UV radiation, desiccation or freezing allowing for growth and persistence.

Generally, *E. coli* and other fecal indicators are associated with suspended particles in urban runoff. Most BMP's include filtration, infiltration, and retention to remove suspended particles (Kinzelman and Hiller 2007; Koski and Kinzelman 2010). Techniques used to mitigate *E. coli* in urban runoff may include artificial wetlands; vegetated buffer strips; vegetated swales or sand filters. These may implemented as a stand alone BMPs or in combination. The type of BMP(s) employed will depend largely on availability of funding, land use and desired outcomes.

In addition to runoff, birds may directly contribute to increased *E. Coli* density in sand. Birds (gull and/or geese), bird feces, prints and/or feathers were observed during the entire study period inferring direct deposition of *E. Coli* on the beach sand. Gull feces may contain up to ten times more pathogenic bacteria than geese (Ricca and Cooney 1998), primarily due to the gulls opportunistic behavior. In fact, Fogarty et al. (2003) estimated an average daily load of up to 4.2 X 10^8 *E. Coli* per gull. *E. Coli* in the foreshore sand may be reintroduced to nearshore surface water by wave run-up and perturbation (Milne and Crowe 2007).

This has prompted bird exclusion measures to be implemented at beaches in Canada and the US (Associated Medical Officer of Health 2000; Di Gironimo et al. 2006; Colaco 2009; City of Los Angeles Harbour Department and Kinnetic Laboratories Inc. 2003). Most of these include gull netting erected over the beach area to prevent gulls and other water fowl from roosting and defecating on the sand. All reports indicate an improvement in nearshore bacteria numbers, however, the City of Los Angeles, 2003 reported that "sand samples collected near the tide-line indicated that the beach sands are contaminated with bacteria" in spite of the bird exclusion experiment. Other bird exclusion techniques may include 1) the use of dogs where harassment of geese/gulls deter occupation in specific areas; 2) naturalizing shorelines by planting grasses/sedges, shrubs and trees; 3) Population removal/reduction where geese are gathered and transported away from area and/or egg oiling (Koski and Kinzelman 2010).

Recommendations

We have provided the following recommendations to further improve beach management strategies: 1) Further studies on characterizing *E. Coli* in storm water runoff at Bayfront Beach and related BMP approaches; 2) continual use and maintenance of the bird exclusion structures; harassment and population reduction techniques 3) sloping/grading of beach sand to provide drier conditions therefore decreasing *E. Coli* in beach sand and nearshore surface water.

The information from this study will aid beach managers in decisions regarding public health and safety.

Conclusion

We evaluated *E.coli* density in beach sand where shallow grooming using a typical garden thatching rake, medium depth grooming using a modified rake with extended prongs and maximum depth grooming using a gas powered tiller was applied daily vs s control where no grooming was applied. Overall, there was a significant increase in *E. Coli* density in maximum depth grooming compared to medium depth, shallow depth and control. A significant increase in *E. Coli* density was also evident in slightly moist and moist sand vs dry and very dry sand.

References

Alm, E. W., Burke, J., and Hagan, E. 2006. Persistence and Potential Growth of the Fecal Indicator Bacteria, Escherichia coli, in Shoreline Sand at Lake Huron. Journal of Great Lakes Research 32:401-405.

Associated Medical Officer of Health 2000. Region of Ottawa-Carlton: 2000 Beach Recommendations.

Bluewater Biosciences Incorporated 2009. ColiPlates. 14 Abacus Road, Brampton, Ontario, L6T 5B7.

Byappanahall, N. M., Whitman, R. L., Shively, D. A., Ting, W. T. E., and Tseng, C. 2006. Seasonal persistence and population characteristics of Escherichia coli and enterococci in deep backshore sand of two freshwater beaches. Journal of Water and Health 4:313-320.

City of Los Angeles Harbour Department and Kinnetic Laboratories Inc. 2003. Task 1 final report. Compliation of pertinent data. Inner Cabrillo Beach water quality improvement project.

Colaco, T. 2009. Kelso Beach.

Davies, C. M., Long, J. A. H., Donald, M., and Ashbolt, N. J. 1995. Survival of fecal microorganisms in marine and freshwater sediments. Applied and Environmental Microbiology 61:1888-1896.

Di Gironimo, L., Librecz, B., and Mckeown, D. 2006. Beach postings in 2005 and actions to improve water quality at City beaches (wards 6, 13, 14, 20, 30, 32, and 44)., Toronto, Ontario.

Dorsey, J. H. 2009. Improving water quality through California's Clean Beach Initiative: an assessment of 17 projects. Environmental Monitoring and Assessment.

Edge, T. A. and Hill, S. 2005. Occurrence of antibiotic resistance in Escherichia coli from surface waters and fecal pollution sources near Hamilton, Ontario. Canadian Journal of Microbiology 51:501-505.

Fogarty, L. R., Haack, S. K., Wolcott, M. J., and Whitman, R. L. 2003. Abundance and characteristics of the recreational water quality indicator bacteria Escherichia coli and enteroocci in gull faeces. Journal of Applied Microbiology 94:865-878.

Hall, J. D., O'Connor, K., and Ranieri, J. 2006. Progress toward delisting a Great Lake Area of Concern: The role of integrated research and monitoring in the Hamilton Harbour. Environmental Monitoring and Assessment 113:227-243.

Hall, J. D., O'Connor, K., and Renieri, J. 2006. Progress toward delisting a Great Lake area of concern: The role of integrated research and monitoring in the Hamilton Harbour remedial action plan. Environmental Monitoring and Assessment 113:227-243.

Hamilton Harbour Remedial Action Plan 2011. Research and Monitoring Report 2009 Season.

Kinzelman, J. L., Pond, K. R., Longmaid, K. D., and Bagley, R. C. 2004. The effect of two mechanical beach grooming strategies on *Escherichia coli* density in beach sand at a southwestern Lake Michigan beach. Aquatic Ecosystem Health and Management 7:425-432.

Kinzelman, J. and Hiller, J. 2007. Incorporating education and outreach of a storm water outfall impacting recreational water quality at public beaches. The Journal of Marine Education 23:13-21.

Kinzelman, J. L. and McLellan, S. L. 2009. Success of science-based best management practices in reducing swimming bans - a case study from Racine, WIsconsin, USA. Aquatic Ecosystem Health and Management 12:187-196.

Kinzelman, J. L., Whitman, R. L., Byappanahall, M., Jackson, E., and Bagley, R. C. 2003. Evaluation of beach grooming techniques on Escherichia coli density in foreshore sand at North Beach, Racine, WI. Lake and Reservoir Management 19:349-354.

Koski, A. and Kinzelman, J. 2010. A review of best management practices benefiting Great Lake recreational waters: Current success stories and future innovations. International Joint Commission.

Mika, K. B., Imamura, G., Chang, C., Conway, V., Fernandez, G., Griffith, J. F., Kampalath, R. A., Lee, C. M., Lin, C.-C., Moreno, R., Thompson, S., Whitman, R. L., and Jay, J. A. 2009. Pilot- and bench-scale testing of faecal indicator bacteria survival in marine beach sand near point sources. Journalof Applied Microbiology 107:72-84.

Milne, J. E., Butcher, A., Delaney, K., Gilpin, S., Guay, S., Lee, J., Mendoza, J., Packull, J., and Radisic, S. 2011. Characterizing E. coli density in beach sand using two grooming techniques. 11-006.

Milne, J. E. and Charlton, M. N. 2004. Escherichia coli in water and ground water at beaches in Lake Huron, Lake Ontario, and Hamilton Harbour. NWRI, Environment Canada, Burlington. 04-205.

Milne, J. E., Hiriart-Baer, V. 2007. An update on *E. coli* contamination at Bayfront and Pier 4 Beach in Hamilton Harbour. In Research and Monitoring Report 2007 Season, ed. Remedial Action Plan for Hamilton Harbour, pp. 36-43. Burlington, Ont..

Milne, J. and Crowe, A. 2007. Investigation of Former Septic Systems as a Potential Source of *E. coli* at Burlington Beach (Beachway Park). 07-317.

Ricca, D. M. and Cooney, J. J. 1998. Coliphages and indicator bacteria in birds around Boston Harbor. Journal of Industrial Microbiology and Biotechnology 21:28-30.

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

Zehmus, T. T., McDermott, C. M., and Kleinheinz, G. T. 2008. Microbial concentrations in sand and their effect on beach water in Door County, Wisconsin. Journal of Great Lakes Research 34:524-534.

WATER RESEARCH 45 (2011) 3960–3968

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/watres

Distribution and potential significance of a gull fecal marker in urban coastal and riverine areas of southern Ontario, Canada

Jingrang Lu [a,1], *Hodon Ryu* [a,1], *Stephen Hill* [b], *Mary Schoen* [a], *Nicholas Ashbolt* [a], *Thomas A. Edge* [b], *Jorge Santo Domingo* [a,*]

[a] *Office of Research and Development, U.S. Environmental Protection Agency, 26 W. MLK Dr., Cincinnati, OH 45268, USA*
[b] *National Water Research Institute, Environment Canada, Burlington L7R 4A6, Canada*

ARTICLE INFO

Article history:
Received 22 December 2010
Received in revised form
19 April 2011
Accepted 3 May 2011
Available online 13 May 2011

Keywords:
Microbial source tracking
Gull-targeted fecal assay
Fecal contamination
Lake Ontario

ABSTRACT

To better understand the distribution of gull fecal contamination in urban areas of southern Ontario, we used gull-specific PCR and qPCR assays against 1309 water samples collected from 15 urban coastal and riverine locations during 2007. Approximately, 58% of the water samples tested positive for the gull-assay. Locations observed to have higher numbers of gulls and their fecal droppings had a higher frequency of occurrence of the gull marker and a higher gull marker qPCR signal than areas observed to be less impacted by gulls. Lower gull marker occurrence and lower qPCR signals were associated with municipal wastewater (7.4%) and urban stormwater effluents (29.5%). Overall, there were no statistically significant differences in gull marker occurrence at beach sites for pore water, ankle, and chest-depth samples, although signals were generally higher in interstitial beach sand pore water and ankle-depth water than in chest-depth water samples. Overall, the results indicated that gull fecal pollution is widespread in urban coastal and riverine areas in southern Ontario and that it significantly contributes to fecal indicator bacterial loads.

Published by Elsevier Ltd.

1. Introduction

A large number of Great Lakes beaches in North America (49% in Canada and 73% in the United States) had swimming advisories, postings, or closures during 1998–2007, significantly impacting local economies (Environment Canada and USEPA, 2005). Diverse fecal sources could contribute to these beach advisories, including point sources (e.g., municipal wastewater outfalls) and non-point sources (e.g., agricultural runoff and wildlife), in particular, waterfowl (Edge and Hill, 2007). From a public health perspective, prevention of waterfowl pollution may be important as several studies have shown that waterfowl excrete human waterborne pathogens (Baudart et al., 2000; Makino et al., 2000; Kullas et al., 2002; Slodkowicz-Kowalska et al., 2006; Waldenström et al., 2002; Zhou et al., 2004). Aquatic birds are also natural reservoirs of influenza viruses (Krauss et al., 2007) and therefore are an important link in the evolution and environmental dispersal of these viruses. Modeling of recreational waters with negligible human fecal contamination suggests one-to-two orders of magnitude lower gastrointestinal illness risk from seagull-impacted sites at current water quality criteria (Schoen and Ashbolt, 2010; Soller et al., 2010). Yet such assessments rely on fecal indicator bacteria (FIB) numbers, pathogen data for excreta and a very limited amount of waterborne pathogen data, most of which have been measured using widely criticized culture-based techniques. While gulls have been implicated as primary sources of fecal contamination in the Great Lakes (Edge and Hill, 2007), the relative abundance of their fecal inputs in environmental waters has not been accurately

* *Corresponding author*. Tel.: +1 513 569 7085; fax: +1 513 569 7328.
E-mail address: santodomingo.jorge@epa.gov (J.S. Domingo).
[1] These authors contributed equally to this study.
0043-1354/$ – see front matter Published by Elsevier Ltd.
doi:10.1016/j.watres.2011.05.003

assessed due to the lack of quantitative host-specific techniques.

In a recent study, Lu et al. (2008) developed a PCR assay targeting the 16S rRNA gene of *Catellicoccus marimammalium*. Thus far, this assay has shown high specificity toward gull feces and has generated positive signals from water samples collected in various locations with a history of gull fecal pollution (Lu et al., 2008; Shibata et al., 2010). However, data on the prevalence and distribution of the bacterial species targeted by this assay in environmental waters is scarce. Moreover, the relationship between this assay and FIB such as *Escherichia coli* and enterococci is poorly understood. The main goal of this study was to further evaluate the gull marker assay by studying the prevalence of the proposed marker within a geographic location receiving different sources of pollution, including gull feces. Additionally, the significance of gull contamination was studied by assessing the abundance of the gull marker in relation to the presence of fecal bacteria and observations of gull fecal dropping impacts.

2. Materials and methods

2.1. Study sites and sampling

A total of 1309 water and wastewater samples were collected between May and October of 2007 from 12 locations (50 sub-locations; Table S1) around the cities of Toronto, Ottawa, and Hamilton, Canada, and challenged against the gull marker assay. Samples were collected in sterile 500 ml bottles, and returned on ice to the Burlington lab for filtration (0.45 μm) within 6 h of collection. Water samples at beaches were collected weekly over the bathing season along transects perpendicular to the shore from interstitial sand pore water (from a hole dug in foreshore sand), and by wading out to collect surface water at ankle and chest-depth. Samples were collected from Toronto locations at Ashbridges Bay Sewage Treatment Plant (AHS), Bluffers Park (BL), Don River (DON), Humber River (HUM), Kew Beach (KW), Marie Curtis Park (MC), Rouge Park (RG), and the Western (Sunnyside) Beaches (WB); from Hamilton locations at Bayfront Park (BP), Eastport (EP), Hamilton harbor (HH); and from Ottawa locations near Petrie Island (PI) (Fig. 1).

Observations of the number of gulls and their fecal droppings were made at water sampling locations in an attempt to provide a qualitative assessment of low-to-high impacts from gull fecal droppings. This assessment was more rigorous at beach locations based on previous microbial source tracking studies (Edge et al., 2007a, 2007b, 2010) and weekly enumeration of the numbers of gulls and their fecal droppings each time water samples were collected. The number of gulls was counted in the immediate vicinity of the sampling location, and the number of gull fecal droppings was enumerated by walking along the shoreline near the sampling location and counting fresh droppings on the foreshore sand within 2 m of the waterline. Since beaches varied slightly in length, the gull fecal dropping results were standardized to 100 m of beach shoreline, and the mean number of droppings from weekly observations at a beach location was used to calculate an estimate of the total cumulative number of gull fecal droppings at that beach location from each day over the bathing season sampling period from May to September.

2.2. Molecular methods

All water samples were processed (100–300 ml) as previously described (Lu et al., 2008) with the following modifications.

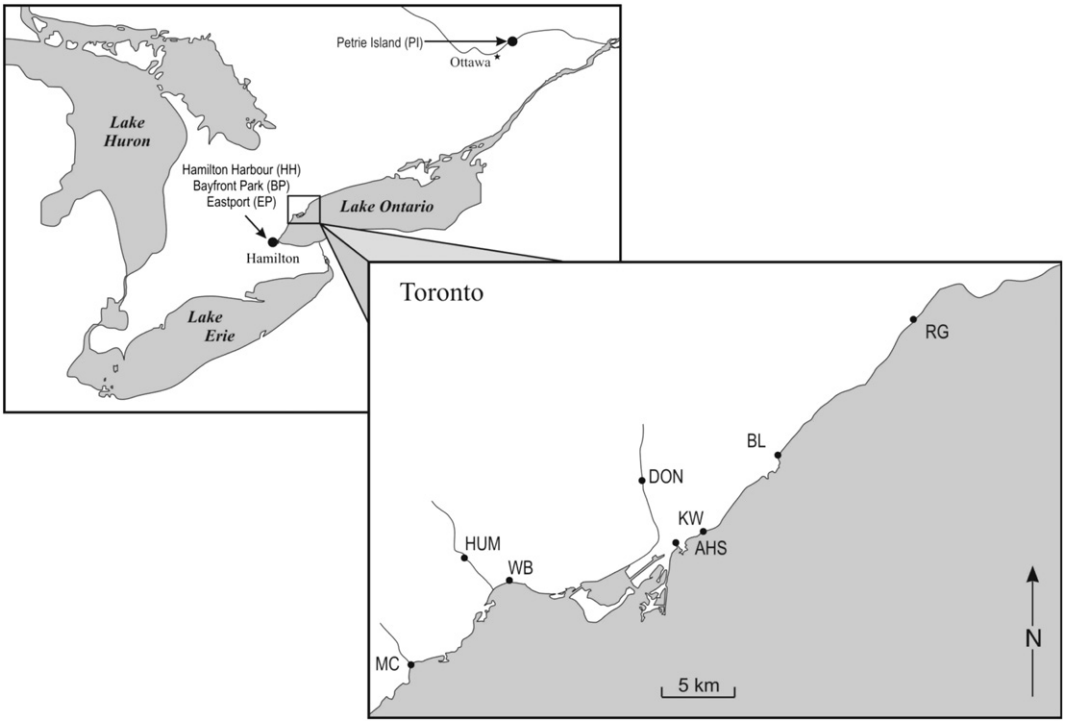


Fig. 1 – Sites used in this study. Labels are described in the Material and Method section.

DNA extracts (2 μl) were used as template in SYBR green-based gull PCR assays, which were performed using a 7900 HT Fast Real-Time Sequence Detector (Applied Biosystems). Gull-specific PCR data were analyzed using ABI's Sequence Detector software (version 2.2.2) and a 0.2 threshold. PCR signals were recorded as presence/absence data and signal quantity values. Disassociation curves were examined to determine the presence of potential primer–dimers and other non-specific reaction products. Data points with artifacts (e.g., double peaks) that resulted in signal overestimation were not used in statistical analyses. Signal intensity values were recorded for those reactions showing one corresponding amplification peak within the disassociation curves. Serial dilutions of *C. marimammalium* DNA (1 ng–10 fg) in duplicate were used to generate a standard curve. Two no-template controls per PCR plate were used to check for cross-contamination. A ten-fold dilution of each DNA extract was used to test for PCR inhibition. Real-time PCR (qPCR) units were calculated as fg/100 ml of filtered water sample. PCR products were visualized using 2% agarose gel electrophoresis and GelStar as the nucleic acid stain (FMC BioProducts, Rockland, ME) to confirm the size of amplification products. Ten randomly selected water samples that tested positive for the presence of the gull-targeted marker were used to develop clone libraries to examine the identity and molecular diversity of environmental products (Lu et al., 2008). General *Bacteroides* PCR-based assay (Bac32) was tested against water DNA extracts using 32F and 708R primers (Bernhard and Field, 2000.) and the cycling conditions described by Lamendella et al. (2007). *E. coli* (EC) counts were determined by membrane filtration as described by Edge and Hill (2007); where two replicate samples were collected, the counts were expressed as the mean in CFU/100 ml units.

2.3. Statistics

Statistical analyses were performed using Statistical Analysis Software v8.2 (SAS Institute Inc., Cary, NC). Logistic analysis was used to predict the probability of occurrence of gull-targeted marker (response variable) to assess the relative importance of effect variables such gull-impacted (GI) and fecal indicator data such as *E. coli* counts (EC) and presence of general *Bacteroides* (Bac32). The Wald chi-square test was used to determine if relationships between the gull-targeted marker and effect variables were statistically significant ($p \leq 0.05$). Quantitative analysis was conducted using the PROC GLM analysis with the F-statistical significance test at $\alpha = 0.05$ between the response variable (Gull: gull qPCR assay). The Logistic model was conducted using the odds ratio which is the ratio of presence to absence of gull marker between two levels of a categorical variable such as Bac32 and gull impacts, or numerical variables such as EC. Point estimate (PE) obtained from the Logistic analysis is the coefficient of the prediction model and used to determine the relationship between gull marker and predictor variables. Multiple comparisons (contrast) between the gull impact (GI) levels (L, M, and H) were also conducted. Duncan's multiple range test, Tukey's Studentized Range (HSD) Test, Bonferroni (Dunn) t-Tests and Scheffe's Test were used to assess if qPCR data for the gull-targeted assay was statistically different among different sites. Correspondence analysis with two dimensions was performed to determine the association between the quantity of gull-targeted assay and other independent variables (categories of sub-locations, GI and FIB). In addition, cluster analysis was used to further assess the value of the gull marker assay in classifying gull fecal contamination at study sites.

3. Results

Of the 1309 samples tested, 58% of the samples were positive for the gull marker and the identity of PCR products was confirmed by sequencing analysis (Table 2 and Table S1). The mean frequency of occurrence of the gull marker in sub-locations ranged from 0 to 77% of the total samples collected across all 12 sampling locations. The gull marker was detected at almost all sampling locations, but there were significant differences among the categories of sites ($p < 0.0001$). Specifically, the gull marker was most often detected at beach and urban lake shoreline sites where relatively high numbers of gulls and gull fecal droppings were also observed (i.e., medium and high GI). For example, the Eastport (EP) location was immediately adjacent to a large colony of gulls in Hamilton Harbor, and higher numbers of gulls were observed at

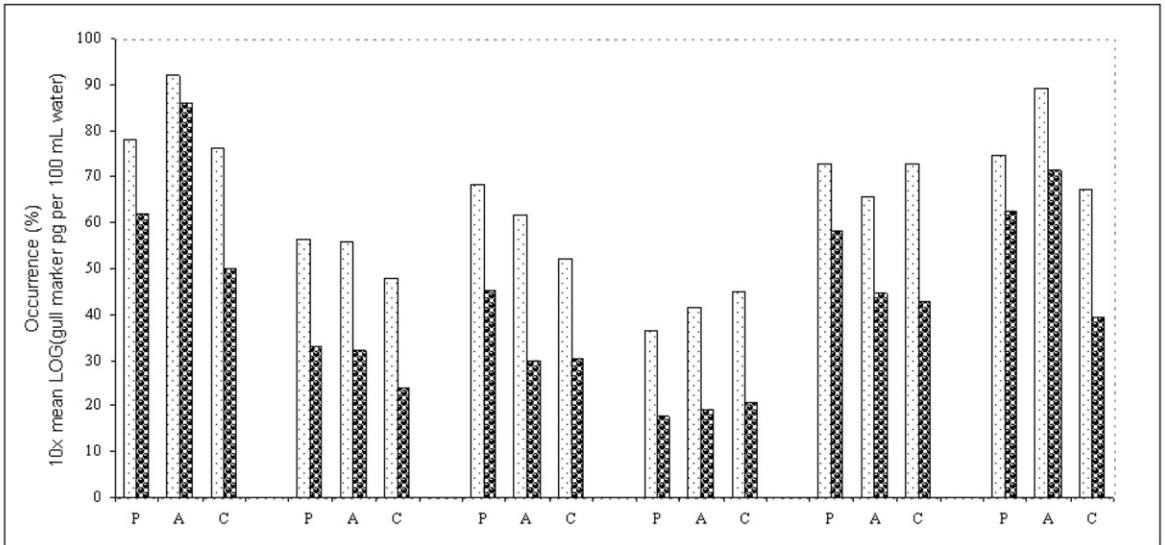
Table 1 – Identity of clone sequences from Ontario beach water samples to original gull marker.

Sample type	Sampling locations[a]	Sub-locations	Number of sequences			Observed gull impacts
			Total	100% identity[a]	99% identity[a]	
Beach water, Ontario	MC	W	23	16	7	Moderate
		E	23	15	8	Moderate
	WB	1	23	13	10	High
		2	24	20	4	High
		4	22	11	11	High
	KW	1	24	13	11	High
		2	24	12	12	High
		3	23	15	8	High
	RG	53E	24	18	6	High
Total			210	133	77	

a See Fig. 1 for identification of locations.

Bluffers, Western and Rouge Beaches (mean = 108, 90, and 88 gulls per sampling day respectively) than Petrie Island, Marie Curtis, and Kew Beaches (mean = 41, 40, and 37 gulls per sampling day respectively). In addition, two of three samples from Bayfront Park Beach were positive for the gull marker and associated with high qPCR values (6.13 Log_{10} mean value; data not shown). These sites are known to be heavily impacted by gulls throughout the bathing season. Similarly, the number of gull fecal droppings over the sampling season at beach locations (per 100 m shoreline) was estimated to range from as high as 10,422 droppings (Rouge Beach) and 6044 droppings (Bluffers Beach) to as low as about 254 droppings (Kew Beach) and 272 droppings (Marie Curtis Beach). In contrast, a significantly lower occurrence of the gull-targeted marker ($p < 0.05$) was associated with municipal wastewater effluents and stormwater outfalls. At individual beaches, the differences in gull marker occurrence between transects (i.e., sand pore water, ankle, and chest samples) were not significant ($p = 0.233, n = 1009$) (Fig. 2). The mean gull marker qPCR signals across categories of sampling locations ranged from 0 to 1901 pg/100 ml with beaches and urban lake shorelines yielding significantly higher qPCR signals than most creek, river, stormwater or municipal wastewater samples (Table 2). The highest mean levels of the gull marker were measured at beaches where higher numbers of gulls and their fecal droppings were observed. The lowest mean values of the gull marker were measured at sites where there were very few observations of gulls or their fecal droppings in the immediate sampling vicinity such as municipal wastewater effluents, stormwater outfalls, and some creek/river sites. Across all beach transects in which samples were taken in sand pore water, and at ankle and chest-depths, gull qPCR signals were significantly different based on ANOVA results ($p = 0.015, n = 1009$). Pairwise tests showed that qPCR signal intensity for sand pore water samples was significantly higher than for chest-depth water samples ($p = 0.009$), but not significantly different from ankle-depth water samples ($p = 0.771$). There were significant differences in signal intensity between ankle- and chest-depth water samples at beaches (BL and WB) with the highest observed gull impacts.

Logistic analysis provided additional insights on the geographical distribution of the gull marker. Overall, the presence of the gull marker was significantly associated with observed gull fecal impacts and fecal indicators (EC and Bac32) (Table 3). Sites with observations of a high gull impact (GI = *H*) were 4.7 times more likely to be positive for the gull marker than those with observations of a low gull impact (GI = *L*), while only 2.5 times when compared to moderately-impacted



Location	BL	KW	MC	PI	RG	WB
Sample number	125	190	137	196	168	190
Probability associated with PCR	0.157	0.546	0.297	0.581	0.376	0.021*
Probability associated with qPCR	0.029*	0.444	0.308	0.896	0.28	0.009*
Observed gull impact level	High	Low~Moderate	Low~Moderate	Low~Moderate	Moderate~High	High

Fig. 2 – Distribution and multiple probability test of gull-targeted marker in beach sand and different water depth zones at Ontario beaches. Light bars represent the percentage (mean values) of samples positive for gull-targeted marker (as determined by percent from total samples). Darker bars represent the qPCR results (mean values) for the samples. Samples were grouped by collection location along a beach transects: sand interstitial pore water (P); ankle-depth zone (A); chest-depth zone (C). Asterisk indicates differences are statistically significant at a 5% significance level.

Table 2 – Overall occurrence and quantity of gull marker for beach, creek/river and municipal wastewater sampling locations.

Categories	Location[a]	Number of samples	Positive gull signals (%)	Log_{10} mean gull qPCR values (STD)	Average LOG gull qPCR value of each category[b]	Observed gull impact level
Beach	BL	125	102 (81.6%)	6.44 (4.6)	4.47	High
	KW	190	101 (53.2%)	2.93 (3.9)		Low ~ Moderate
	MC	137	83 (60.6%)	3.44 (4.3)		Low ~ Moderate
	PI	196	80 (40.8%)	1.93 (3.3)		Low ~ Moderate
	RG	168	118 (70.2%)	4.77 (4.4)		Moderate ~ High
	WB	190	145 (76.3%)	5.63 (4.7)		High
Creek/river	BL	16	4 (25.0%)	1.52 (3.2)	1.69	Moderate
	DON	17	7 (41.2%)	0.87 (2.1)		Low ~ Moderate
	HUM	88	51 (58.0%)	2.74 (3.4)		Low
	MC	25	11 (44.0%)	2.34 (3.5)		Moderate
	PI	17	5 (29.4%)	1.29 (2.7)		Moderate
	RG	10	3 (30.0%)	1.37 (2.7)		Moderate
Municipal wastewater	AHS	22	2 (9.1%)	0	BDL	Low
Stormwater	BL	31	9 (29.0%)	1.07 (2.6)	1.15	Low
	HUM	13	4 (30.8%)	1.23 (2.8)		Low
Urban lake shoreline	EP	4	4 (100.0%)	7.55 (0.3)	4.89	High
	KW	47	24 (51.1%)	2.22 (3.4)		Low ~ High

a See Fig. 1 for identification of locations.
b Average was estimated using data not shown for sites ($n = 4$) for which ≤3 samples were collected.

sites (GI = *M*). The analysis also showed that the occurrence of the gull-targeted marker was positively associated with Bac32 detection. For example, the odds of gull marker detection at sites negative to Bac32 (*Bacteroides*) were 0.256 times less likely than at sites where Bac32 was detected. With respect to *E. coli* counts, and with all other parameters constant, a natural log increase in *E. coli* count corresponded to about a 1.2 times increase in the gull marker assay.

To further examine potential associations among variables, correspondence analysis was performed using two-dimension Chi-square data analysis for site categories (Fig. 3). Correspondence analysis showed that Chi-square values were significantly different across site categories. Over 73% of the total Chi-square was explained by one dimension (i.e., the horizontal dimension), indicating that association of the variables over site categories was dominated by gull contamination as determined by the detection of the gull marker. The gull marker was strongly associated with beaches and urban lake shoreline sites. In general, creek/river and stormwater sites were poorly associated with the gull marker.

Quantitatively, general linear analysis showed that gull marker was positively associated significantly with observed gull impacts (GI, $p < 0.0001$), *E. coli* counts (EC, $p < 0.0001$) and Bac32 ($p = 0.0032$). Comparisons of gull marker signal between GI levels were also highly significant ($p < 0.0001$ for all three pairs of comparisons). There was about one natural log scale increase of gull marker quantity from one GI level to another (*L* to *M* or *M* to *H*; calculated in Least Squares Means). Based on gull marker quantity, cluster analysis was conducted to see whether gull-assay signal levels could be used as

Table 3 – Results from statistical logistic analyses performed on presence of PCR gull marker assay signals (sample size $n = 1254$).

Analysis of effects[a]	Effect variables		Degree freedom (df)	Wald Chi-Square[a]	Pr > *ChiSq* (*p* value)
	Fecal source	Observed gull impact	2	62.68	<0.0001
	Fecal indicator	*E. coli* count Bac32	1	12.46	0.0004
			1	35.14	<0.0001

Odds Ratio Estimates	Effect variable		Point Estimate (PE)[b]	Wald Confidence Limits[c]	
	Fecal source: Observed Gull Impact	Low vs High	0.213	0.123	0.369
		Moderate vs High	0.408	0.310	0.535
	Fecal indicator	*E. coli* counts	1.195	1.082	1.319
		Bac32: absence vs presence	0.256	0.163	0.401

a Relationship between the gull-targeted marker and effect variables was statistically significant when Wald Chi-Square is >12.71 (df = 2) or 4.30 (df = 1).
b Lower PE values for observed gull impacts mean a greater difference between variables. The higher PE values for *E. coli* counts when compared to presence/absence of Bac32 data suggests a weaker positive relationship between gull marker and *E. coli* than gull marker and Bac32.
c Lower and upper confidence limits used to determine if estimated PE was within confidence levels at 95%.

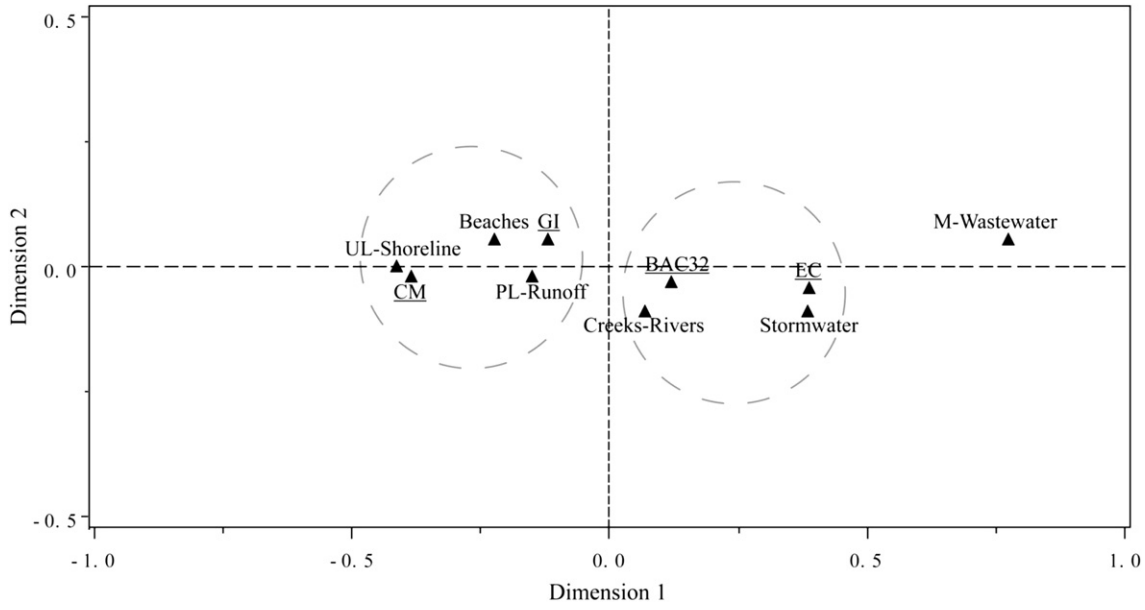


Fig. 3 – Correspondence analysis showing associations among categories of sampling locations (Beaches, Creek/River, Municipal wastewater (M-Wastewater), Parking Lot runoff (PL-Runoff), Stormwater, Urban lake shoreline: UL-Shorline), observed gull impact (GI) and fecal indicators (gull marker qPCR signals: CM; presence/absence of general *Bacteroides* marker: BAC32; *E. coli* counts: EC). The first circled cluster indicates the higher association between gull marker, presumed GI, and UL-Shoreline, Beaches and PL-Runoff locations. The second circled cluster indicates a lesser association between gull marker fecal indicators (BAC32 and EC) and locations like Creek-Rivers and Stormwater location. M-Wastewater location showed a poor associated with the gull marker while a stronger association with fecal indicators EC and BAC32.

a classification for the extent of gull fecal contamination. Sub-locations with observations of high gull fecal impacts grouped together, most of which were located at beaches and urban lake shorelines (Fig. 4). Sub-locations with observations of moderate-to-low gull impacts grouped within corresponding categories.

4. Discussion

With over 1300 samples tested, this represents the first large-scale study involving the field application of the *C. marimammalium* (gull-targeted) qPCR assay. The samples were collected from various urban locations around the cities of Toronto, Ottawa, and Hamilton, providing the opportunity of studying the distribution and prevalence of the gull marker in urban areas in proximity to large colonies of gulls. There are large ring-billed gull colonies on the Toronto and Hamilton waterfronts, with approximately 55,000 breeding pairs located at Tommy Thompson Park in Toronto. In general, we showed that the gull marker was detected across all sampling locations, suggesting that gull fecal contamination is widespread and highly prevalent in these urban areas. The occurrence of the gull marker in water samples from stormwater outfalls and parking lot runoff indicates the presence of gull fecal contamination in runoff from impervious surfaces in urban areas.

Sequence analysis of 210 clones from nine different water types showed that the sequences generated with the gull qPCR assay were identical or nearly identical (sequence identity $\geq$ 99%) to the *C. marimammalium* 16S rRNA gene (Table 1) confirming the identity of the PCR signals. The lower occurrence of the gull marker in municipal wastewater and other areas with observations of lower numbers of gulls and their fecal droppings provided additional evidence suggestive of the specificity of the gull-targeted assay. Specifically, the results revealed the following features for the gull-targeted assay: (1) the occurrence of the gull marker was positively associated with observed gull impacts (gull numbers or gull fecal droppings); (2) the association between the levels of the gull marker (i.e., intensity of qPCR signals) and observed gull impacts was statistically significant; (3) statistical analysis showed that there was a positive association between gull signals and fecal indicators (*E. coli* counts and occurrence of *Bacteroidetes*). Altogether, the results from this study suggest that the gull-assay is a good predictor of the presence of gull fecal contamination and, to some extent, of gull fecal pollution levels. This was particularly noticeable as the highest levels of the gull marker were measured at sampling locations where the highest numbers of gulls and their fecal droppings were observed (e.g. Eastport gull colony location in Hamilton, and Bluffers Park Beach and Western Beaches in Toronto). Lower levels of the gull marker were measured in wastewater samples and at beaches observed to be less impacted by gulls (e.g. Petrie Island and Kew Beaches).

An important objective in this study was to investigate the prevalence of the gull marker across sampling locations frequented by varying numbers of gulls. We found that the gull marker was more prevalent at sites with higher observations of gulls and their fecal droppings; however, additional

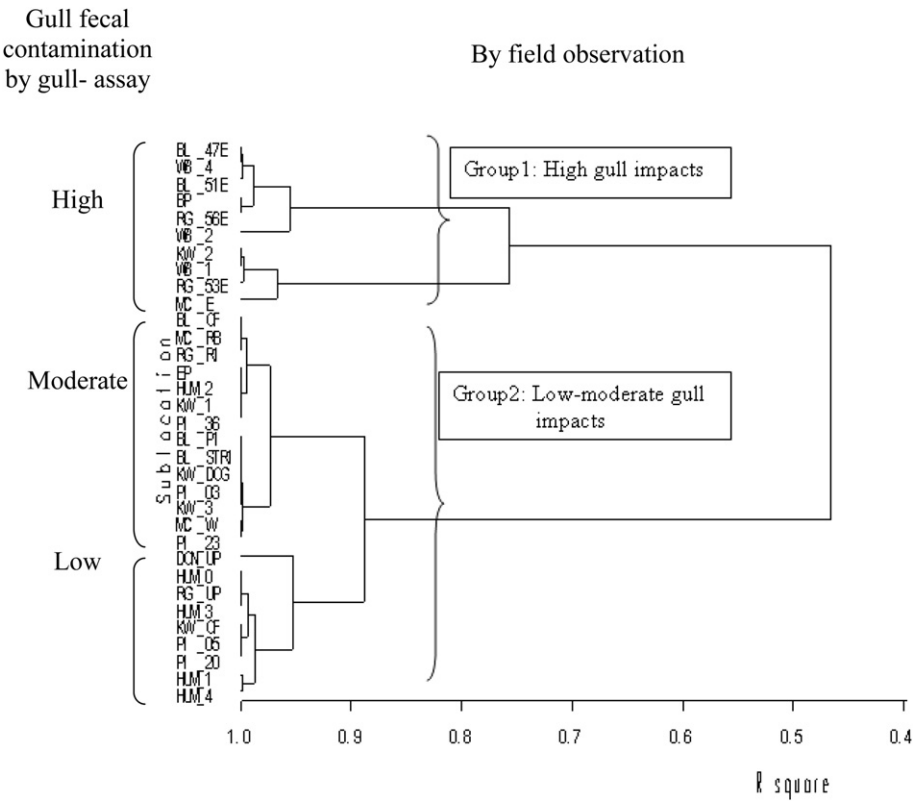


Fig. 4 – Cluster analysis for sampling sub-locations at Lake Ontario based on gull-targeted qPCR signal intensity.

research will be required to evaluate the ability to use the gull marker or bird observations to predict *E. coli* or other FIB concentrations in specific water samples. It should be noted that bird numbers and indicator bacteria might not always correlate in waters where there are other fecal sources. This is due to the significant differences in the levels of FIB in different hosts. For example, as noted recent studies one fecal dog sample could represent thousands of gull fecal events when using enterococci densities as the standard (Wright et al., 2009; Wang et al., 2010). Thus a strictly linear correlation between FIB and host-specific markers may not exist for most of the currently available assays used for fecal source identification as they do not use FIB as the targeted population. There are other variables associated with the lag time between bird observations and FIB enumeration in water (i.e., wave action, wind speed/direction, and precipitation) and with bacterial fate (i.e., inactivation and/or die off due to sunlight and desiccation) that could preclude a simple correlation between bird observations and *E. coli* concentrations. Indeed, several studies have not shown a good quantitative relationship between numbers of birds or bird droppings and E. coli numbers at Great Lakes beaches (Kleinheinz et al., 2006; Edge and Hill, 2007).

The incidence of the gull marker at locations observed to have low-to-moderate gull impacts could be influenced by the level of survival of *C. marimammalium* in environmental waters and secondary habitats such as sediments and sands. Little is known about the ecology of *C. marimammalium*. In fact, little is known about this organism besides limited biochemical characterization data and its phylogenetic relatedness with catalase-negative genera such as *Enterococcus*, *Melissococcus*, *Tetragenococcus* and *Vagococcus* (*Enterococcaceae* family) (Lawson et al., 2006). Other fecal members of the *Enterococcaceae* family are presumed to survive longer in water than other fecal bacteria such as members of the *Enterobacteriaceae* and *Bacteroidetes* (Fiskal et al., 1985; Sinton et al., 1998; Noble et al., 2003; Haller et al., 2009). The presence of *C. marimammalium* in areas of presumed low gull fecal pollution suggest that this organism may survive in the environment to some extent, On the other hand, host-specificity data (Lu et al., 2008) suggest that the gull gastrointestinal tract is a preferred habitat of *C. marimammalium* and it is also possible that its occurrence in environmental waters is the product of a recent contamination event. Future studies need to be conducted to better understand the survival potential and seasonal variation of this bacterial species in environmental waters. Similarly, fecal bacteria have been isolated in secondary habitats, implicating them as potentially important reservoirs of fecal pathogens. No data on the occurrence of *C. marimammalium* in sediments or sand has been reported, highlighting a general area that needs to be addressed in future studies if the assay is going to be used effectively as a source tracking tool.

The presence and levels of gull fecal pollution in the areas studied, as determined by the gull marker assay, can partly explain the levels of *E. coli* and *Bacteroidetes* detected. In addition to gull feces, sewage discharges, urban runoff, and other waterfowl (i.e., Canada geese) are possible pollution

sources. Indeed, Canada geese were also commonly observed on many of the beaches examined in this study. Interestingly, *Bacteroidetes* represented 2–18% of the sequences in geese 16S rRNA gene clone libraries, while only 1% in gull counterparts (Lu et al., 2008, 2009). While avian feces tend to harbor lower densities of *Bacteroidetes* than mammal feces, some of the bacterial populations detected by the Bac32 assay could be of geese origin in those areas in which geese are important pollution sources. Although a *Bacteroidetes*-based marker was recently developed as a goose-specific assay (Fremaux et al., 2010), to this date there is no *Bacteroidetes*-based marker for gulls, making it difficult to understand the contribution of each avian species to this bacterial order. Additionally, differences in *Bacteroidetes* composition may exist, even within the same bird type. For example, Jeter et al. (2009) showed considerable differences in *Porphyromonadaceae* abundance in gull feces collected from different locations around Lake Michigan. Similar findings were observed for *Prevotella* and *Bacteroides* spp. in gull samples collected from other locations in or nearby the Great Lakes (Lu et al., 2009). These issues are not exclusive to efforts tracking waterfowl sources, and highlight the difficulties in developing source tracking markers that can correlate with fecal indicator bacteria, particularly when the targeted populations belong to different bacterial groups. Also, for MST tools to be more useful in quantitative microbial risk assessments, markers should also be correlated to reference pathogens of concern.

5. Conclusions

- Several lines of evidence suggest that gull feces can play an important role in contaminating urban waters in areas near where these birds establish large colonies. The pollution of southern Ontario urban coastal and riverine waters was characterized by: (1) the widespread detection of a putative gull marker at almost all sampling sites; (2) positive association between *E. coli* counts and presence of *Bacteroidetes* with the occurrence of the gull marker; (3) correspondence analysis showed that other sources might also be contributing to the overall levels of *E. coli* and *Bacteroidetes*, while cluster analysis indicated the gull-assay could be used for the identification of locations with significant impacts from gull fecal contamination.
- The gull-targeted assay may be useful in a 'waterfowl pollution toolbox'. However, additional studies are needed to better understand the ecology of the targeted population and the value of this assay in quantitative risk analysis models. In this regard, when mixed sources are present in recreational waters for which high gull contamination is expected and a low percentage of the indicator bacteria are coming from human sources, it would be of practical value if a gull-assay can demonstrate that most of the other fecal indicator bacteria are coming from gulls given the significantly lower risks associated for such scenarios.
- Future studies should be performed to better establish the ratio between fecal indicator bacteria, pathogens and source tracking markers using a large number of fecal samples collected from different geographic locations.

Acknowledgments

This research was funded in part by a New Start Award from the EPA National Center for Computational Toxicology to J.S.D, and funding from the Cities of Toronto and Ottawa, and Environment Canada's STAGE genomics program to T.A.E. The U.S. Environmental Protection Agency, through its Office of Research and Development, funded and managed, or partially funded and collaborated in, the research described herein. It has been subjected to the Agency's administrative review and has been approved for external publication. Any opinions expressed in this paper are those of the author (s) and do not necessarily reflect the views of the Agency, therefore, no official endorsement should be inferred. Any mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.watres.2011.05.003.

REFERENCES

Baudart, J., Lemarchand, K., Brisabois, A., Lebaron, P., 2000. Diversity of *Salmonella* strains isolated from the aquatic environment as determined by serotyping and amplification of the ribosomal DNA spacer regions. Applied and Environmental Microbiology 66 (4), 1544–1552.

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

Edge, T.A., Hill, S., 2007. Multiple lines of evidence to identify the sources of fecal pollution at a freshwater beach in Hamilton Harbour, Lake Ontario. Water Research 41 (16), 3585–3594.

Edge, T.A., Crowe, A., Hill, S., Seto, P., Marsalek, J., 2007a. Surveillance for Potential Sources of *E. Coli* at Toronto's Bluffers Park Beach 2005–06. Environment Canada, Burlington, Ontario. WSTD Technical Note No. AEP-TN07-003.

Edge, T.A., Hill, S., Seto, P., Marsalek, J., 2007b. Experience with the antibiotic resistance analysis and DNA fingerprinting in tracking faecal pollution at two lake beaches. Water Science Technology 56 (11), 51–58.

Edge, T.A., Hill, S., Seto, P., Marsalek, J., 2010. Library-dependent and library-independent microbial source tracking to identify spatial variation in faecal contamination sources along a Lake Ontario beach (Ontario, Canada). Water Science Technology 62 (3), 719–727.

Environment Canada and USEPA, 2005. State of the Great Lakes 2005. In: State of the Lakes Ecosystem Conference. Environment Canada, Quebec, Canada, and U.S. Environmental Protection Agency, Washington, DC. 15–19.

Fiksdal, L., Maki, J.S., LaCroix, S.J., Staley, J.T., 1985. Survival and detection of *Bacteroides* spp., prospective indicator bacteria. Applied and Environmental Microbiology 49 (1), 148–150.

Fremaux, B., Boa, T., Yost, C.K., 2010. Quantitative real-time PCR assays for sensitive detection of Canada goose-specific fecal pollution in water sources. Applied and Environmental Microbiology 76 (14), 4886–4889.

Haller, L., Amedegnato, E., Poté, J., Wildi, W., 2009. Influence of freshwater sediment characteristics on persistence of fecal indicator bacteria. Water, Air, and Soil Pollution 203 (1–4), 217–227.
Jeter, S.N., McDermott, C.M., Bower, P.A., Kinzelman, J.L., Bootsma, M.J., Goetz, G.W., McLellan, S.L., 2009. *Bacteroidales* diversity in ring-billed gulls (*Laurus delawarensis*) residing at Lake Michigan beaches. Applied and Environmental Microbiology 75 (6), 1525–1533.
Kleinheinz, G.T., McDermott, C.M., Chomeau, V., 2006. Evaluation of avian waste and bird counts as predicators of *Escherichia coli* contamination at Door County, Wisconsin beaches. Journal of Great Lakes Research 32 (1), 117–123.
Krauss, S., Obert, C.A., Franks, J., Walker, D., Jones, K., Seiler, P., Niles, L., Pryor, S.P., Obenauer, J.C., Naeve, C.W., Widjaja, L., Webby, R.J., Webster, R.G., 2007. Influenza in migratory birds and evidence of limited intercontinental virus exchange. PLoS Pathogens 3, e167.
Kullas, H., Coles, M., Rhyan, J., Clark, L., 2002. Prevalence of *Escherichia coli* serogroups and human virulence factors in faeces of urban Canada geese (*Branta canadensis*). International Journal Environmental Health Research 12 (2), 153–162.
Lamendella, R., Santo Domingo, J.W., Oerther, D.B., Vogel, J.R., Stoeckel, D.M., 2007. Assessment of fecal pollution sources in a small northern-plains watershed using PCR and phylogenetic analyses of *Bacteroidetes* 16S rRNA gene. FEMS Microbiology Ecology 59 (3), 651–660.
Lawson, P.A., Collins, M.D., Falsen, E., Foster, G., 2006. *Catellicoccus marimammalium* gen. nov., sp. nov., a novel Gram-positive, catalase-negative, coccus-shaped bacterium from porpoise and grey seal. International Journal of Systematics and Evolutionary Microbiology 56 (2), 429–432.
Lu, J., Santo Domingo, J.W., Lamendella, R., Edge, T., Hill, S., 2008. Phylogenetic diversity and molecular detection of gull feces. Applied and Environmental Microbiology 74 (13), 3969–3976.
Lu, J., Santo Domingo, J.W., Hill, S., Edge, T.A., 2009. Microbial diversity and host-specific sequences of Canadian goose feces. Applied and Environmental Microbiology 75 (18), 5919–5926.
Makino, S., Kobori, H., Asakura, H., Watarai, M., Shirahata, T., Ikeda, T., Takeshi, K., Tsukamoto, T., 2000. Detection and characterization of Shiga toxin-producing *Escherichia coli* from seagulls. Epidemiology and Infection 125 (1), 55–61.
Noble, R.T., Moore, D.F., Leecaster, M.K., McGee, C.D., Weisberg, S.B., 2003. Comparison of total coliform, fecal coliform, and enterococcus bacterial indicator response for ocean recreational water quality testing. Water Research 37 (7), 1637–1643.
Schoen, M.E., Ashbolt, N.J., 2010. Assessing pathogen risk to swimmers at non-sewage impacted recreational beaches. Environmental Science and Technology 44 (7), 2286–2291.
Shibata, T., Solo-Gabriele, H.M., Sinigalliano, C.D., Gidley, M.L., Plano, L.R., Fleisher, J.M., Wang, J.D., Elmir, S.M., He, G., Wright, M.E., Abdelzaher, A.M., Ortega, C., Wanless, D., Garza, A.C., Kish, J., Scott, T., Hollenbeck, J., Backer, L.C., Fleming, L.E., 2010. Evaluation of conventional and alternative monitoring methods for a recreational marine beach with nonpoint source of fecal contamination. Environmental Science and Technology 44 (21), 8175–8181.
Sinton, L.W., Finlay, R.K., Hannah, D.J., 1998. Distinguishing human from animal faecal contamination in water: a review. New Zealand Journal Marine and Freshwater Research 32 (2), 323–348.
Slodkowicz-Kowalska, A., Graczyk, T.K., Tamang, L., Jedrzejewski, S., Nowosad, A., Zduniak, P., Solarczyk, P., Girouard, A.S., Majewska, A.C., 2006. Microsporidian species known to infect humans are present in aquatic birds: implications for transmission via water? Applied and Environmental Microbiology 72, 4540–4544.
Soller, J.A., Schoen, M.E., Bartrand, T., Ravenscroft, J.E., Ashbolt, N.J., 2010. Estimated human health risks from exposure to recreational waters impacted by human and non-human sources of faecal contamination. Water Research 44 (7), 4674–4691.
Waldenström, J., Broman, T., Carlsson, I., Hasselquist, D., Achterberg, R.P., Wagenaar, J.A., Olsen, B., 2002. Prevalence of *Campylobacter jejuni*, *Campylobacter lari*, and *Campylobacter coli* in different ecological guilds and taxa of migrating birds. Applied and Environmental Microbiology 68 (12), 5911–5917.
Wang, J.D., Solo-Gabriele, H.M., Abdelzaher, A.M., Fleming, L.E., 2010. Estimation of enterococci input from bathers and animals on a recreational beach using camera images. Marine Pollution Bulletin 60 (8), 1270–1278.
Wright, M.E., Solo-Gabriele, H.M., Elmir, S., Fleming, L.E., 2009. Microbial load from animal feces at a recreational beach. Marine Pollution Bulletin 58 (11), 1649–1656.
Zhou, L., Kassa, H., Tischler, M.L., Xiao, L., 2004. Host-adapted *Cryptosporidium* spp. in Canada geese (*Branta canadensis*). Applied and Environmental Microbiology 70 (7), 4211–4215.

Quantifying water circulation at Bayfront Beach Hamilton, Ontario 2011

Jacqui Milne[1]

[1]Environment Canada 867 Lakeshore Rd Burlington, Ont.

Introduction

Water circulation in embayments with beaches can be an important factor in movement of *E. coli* from nearshore to offshore. Beaches located within enclosed basins are effective at reducing erosion however they tend to have longer flushing times with minimal exchange with offshore water causing retention of contaminants such as *E. coli* (Ge et al. 2012). Bayfront beach in Hamilton Harbour has experienced *E. coli* levels well above the PWQO of 100 cfu/100 ml on a regular basis during the bathing season. The land adjacent to Bayfront Beach slopes toward the beach sand and is primarily turf grass often inhabited by Canada geese. Potentially high levels of *E. coli* in runoff intercept the beach sand and discharge in the nearshore surface water. This is potentially exacerbated by limited flows in the bay that inhibit exchange of water with Hamilton Harbour during the summer season.

The beach in this study is in a bay with two headlands constricting flow in the nearshore area. The purpose of this study is to quantify velocities in the embayment adjacent to the beach and compare to current velocities from centre station in Hamilton Harbour. Information from this study will aid in future decisions on beach management.

Methods

Bayfront Beach is located on the southwest shore in Hamilton Harbour (Fig.1). Three MAVS current meters (East, Mid, West) were deployed on June 14, 2011 at equal distance parallel to shore between the headlands at Bayfront Beach at a depth of ~3.0 m (fig. 2). They were preset to log at 1 hour intervals in cm/s. Periodically throughout the summer, macrophytes were removed adjacent to the current meters to unobstructed flow. The current meters were retrieved November 4, 2011 and downloaded at Environment Canada in Burlington, Ont. An Acoustic Doppler Current Profiler (ADCP) was deployed at the centre station in Hamilton Harbour on May 12, 2011 and retrieved November 9, 2011 and downloaded at Environment Canada in Burlington, Ont.

Results

When downloading data from the “west” current meter it was determined that a malfunction had occurred and data was compromised, therefore was not included in the analyses.

Current velocities on the east side of the embayment were generally very low. Velocities were < 2.0 cm/s for 85% of the deployment period, (fig. 3). The predominant direction of flow was towards the west (fig. 4).

Current velocities from "mid" were very similar to "east". Velocities were <2.0 cm/s for 81% of the deployment period, (fig. 5). The predominant direction of flow was towards the west and southwest (fig. 6). It appears water movement near Bayfront Beach flows in a clockwise direction.

For comparison, current velocities at the centre station in Hamilton Harbour were generally higher than Bayfront Beach ranging from 2 to 20 cm/s for 77% of the deployment period (fig. 7).

Discussion and Conclusions

We have found in this study that current velocities at Bayfront Beach are generally low (<2.0 cm/s). Other complex hydrodynamic studies have shown that nearshore circulation regimes in embayments can have significant impacts on *E. coli* concentrations (Ge et al. 2010; Ge et al. 2012). For example, it appears from this preliminary study, the embayment likely has a low flushing rate; therefore *E. coli* entering the nearshore water from adjacent runoff during precipitation events is not exchanged with the greater harbour and may have a cumulative effect in the embayment. This is exacerbated by the population of geese and gulls which frequently inhabit the beach and nearshore surface water.

Future proposals for *E. coli* mitigation may include the use of nearshore models to investigate the use of circulation systems and beach restructuring by running various scenarios to determine the best option. Environment Canada has a high resolution model that may be sufficient for this purpose.

In conclusion, current velocities at Bayfront Beach are minimal (<2.0 cm/s) compared to centre station in Hamilton Harbour (2 to 20 cm/s) and appear to move in a clockwise direction (towards the west). Further studies, such as modeling, are needed to assess the best case scenario for improving water movement at Bayfront Beach.

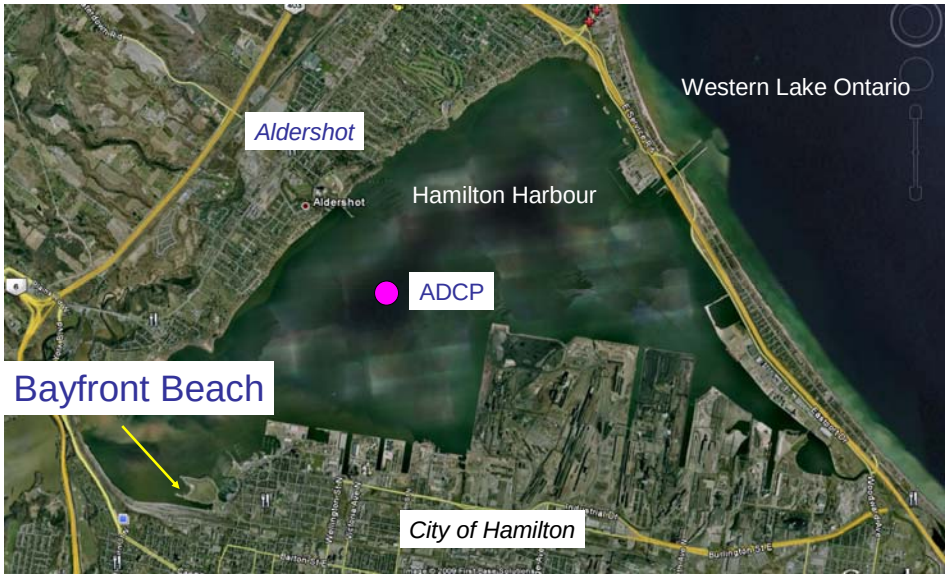


Fig. 1 Bayfront Beach is located near the west end of Hamilton Harbour, Ontario. The ADCP was located in the Centre of the harbour.

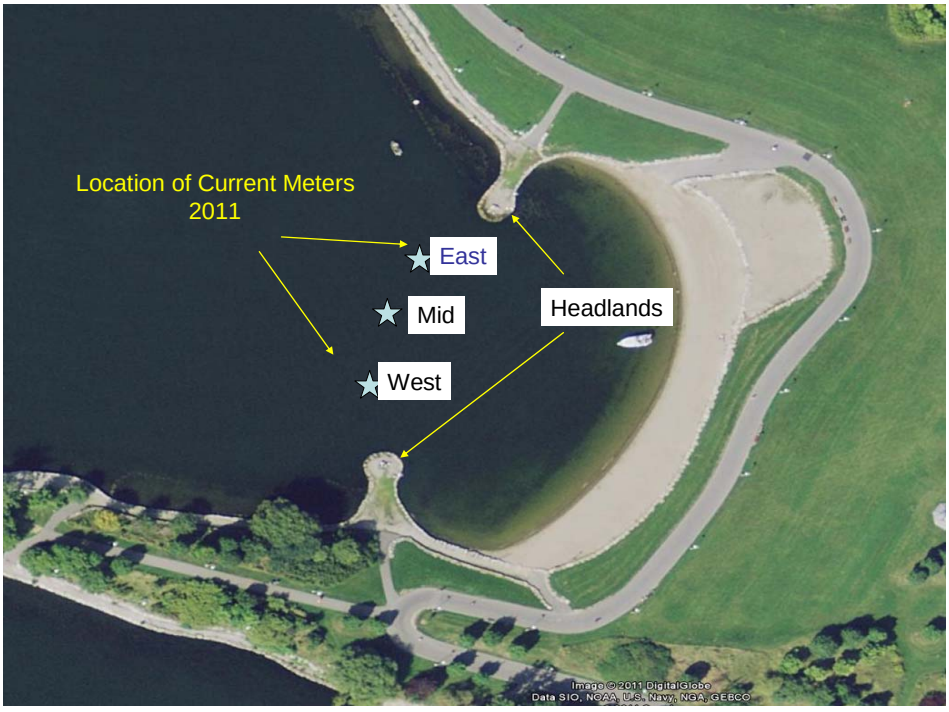


Fig. 2 Location of current meters at Bayfront Beach, Hamilton, Ont.

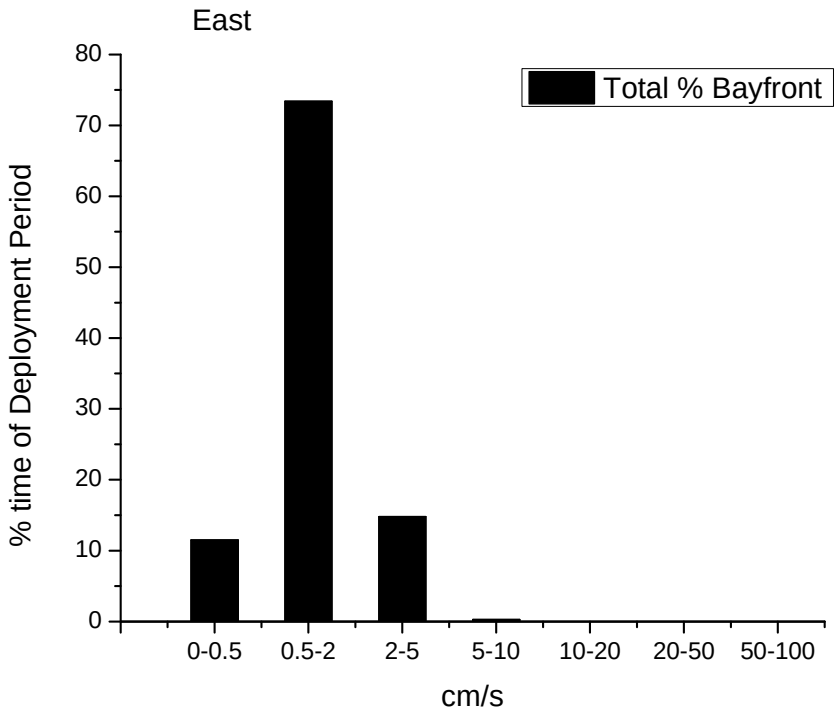


Fig. 3 Illustrates % time for each velocity bin for current meter "east" at Bayfront Beach.

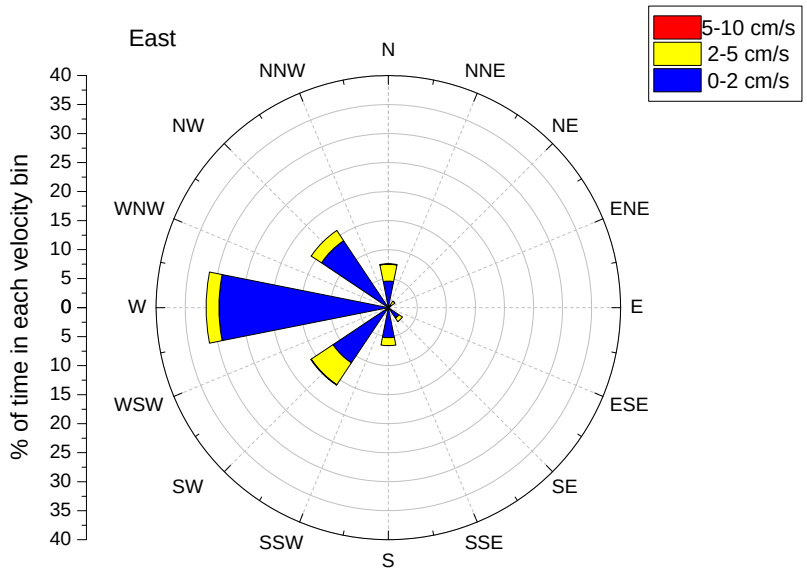


Fig. 4 Shows the predominant current direction and velocity for current meter "east" at Bayfront Beach.

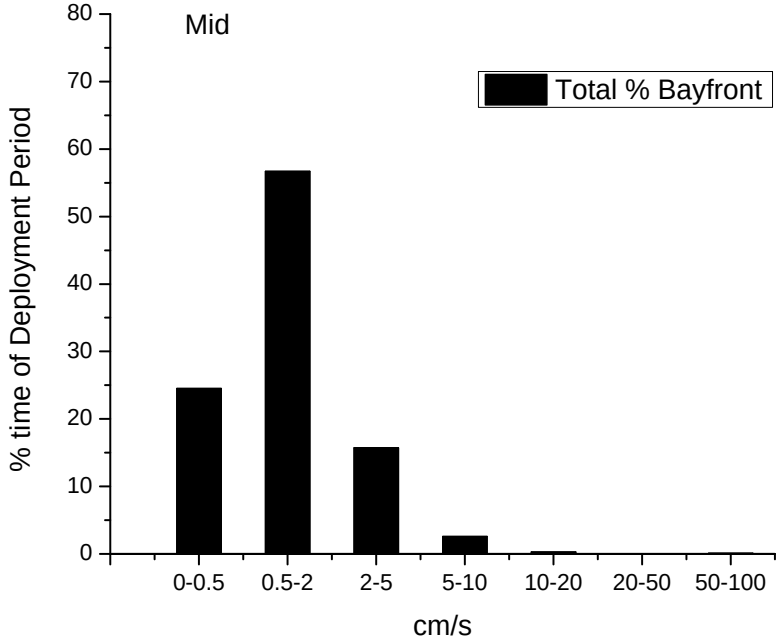


Fig. 5 Illustrates % time for each velocity bin for current meter "mid" at Bayfront Beach .

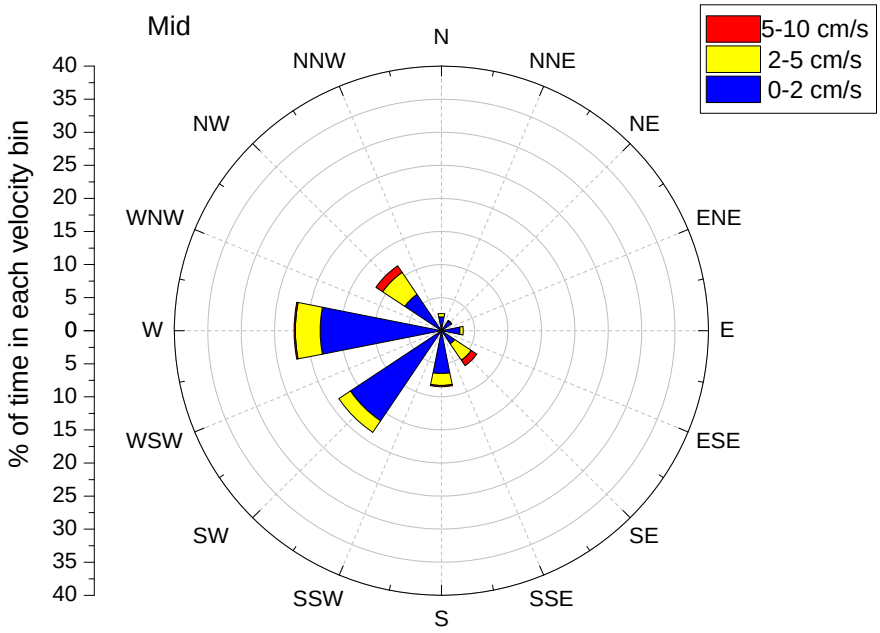


Fig. 6 Shows the predominant current direction and velocity for current meter "mid" at Bayfront Beach.

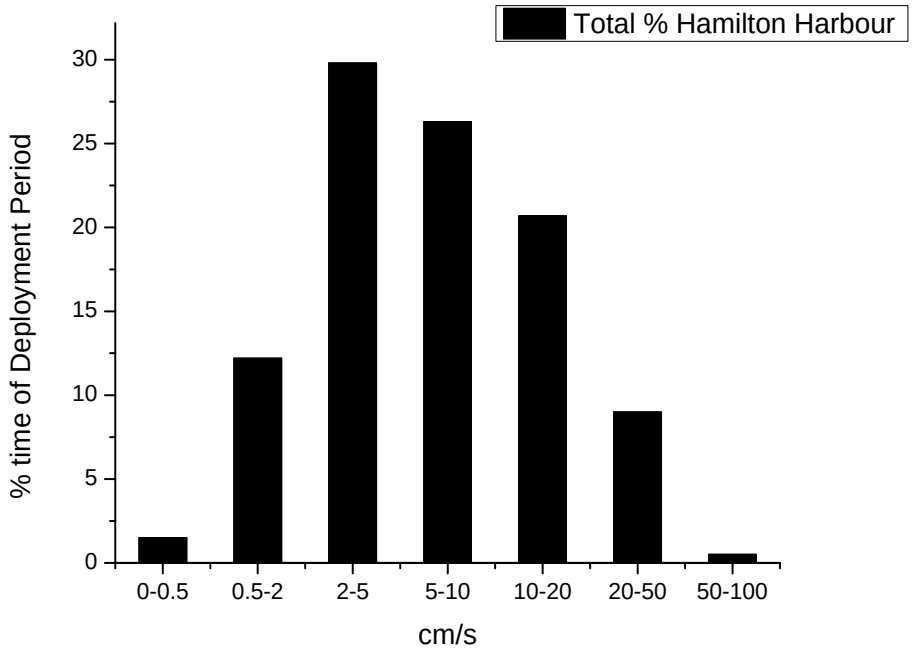


Fig. 7 Illustrates % time for each velocity bin for ADCP at centre station Hamilton Harbour.

References

Ge, Z., Nevers, M. B., Schwab, D. J., and Whitman, R. L. 2010. Coastal Loading and Transport of Escherichia coli at an Embayed Beach in Lake Michigan. Environmental Science & Technology 44:6731-6737.

Ge, Z., Whitman, R. L., Nevers, M. B., Phanikumar, M. S., and Byappanahall, M. N. 2012. Nearshore hydrodynamics as loading and forcing factors for Escherichia coli contamination at an embayed beach. Limnology and Oceanoraphy 52:362-381.

WATER RESEARCH 41 (2007) 3585–3594

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/watres

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

Thomas A. Edge, Stephen Hill*

National Water Research Institute, Water Science & Technology Directorate, Environment Canada, 867 Lakeshore Road, Burlington, Ont., Canada L7R 4A6

ARTICLE INFO

Article history:
Received 18 January 2007
Received in revised form
1 May 2007
Accepted 7 May 2007
Available online 17 May 2007

Keywords:
Escherichia
Beach
Source tracking
Antimicrobial resistance

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

*Corresponding author. Tel.: +905 319 6932; fax: +905 336 6430.
E-mail address: tom.edge@ec.gc.ca (T.A. Edge).
0043-1354/$ - see front matter
doi:10.1016/j.watres.2007.05.012

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

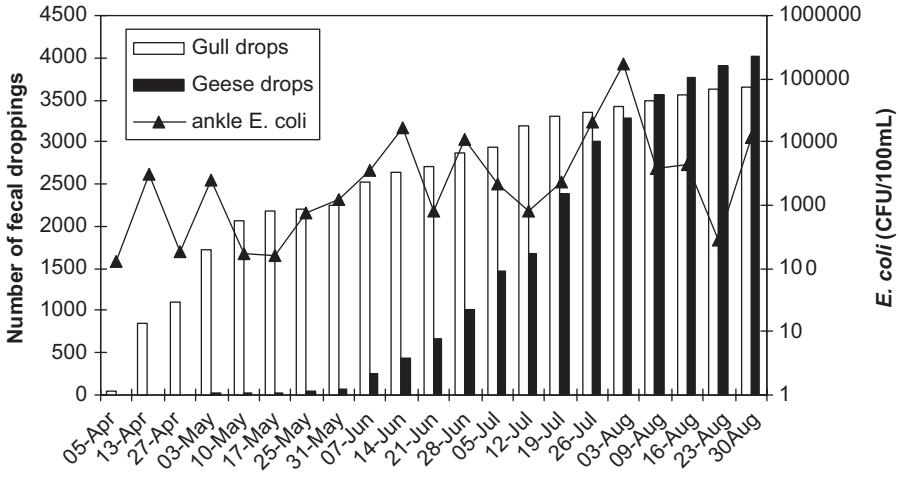


Fig. 1 – Cumulative numbers of bird fecal droppings and concentration of *E. coli* in ankle depth water at Bayfront Park Beach in 2004.

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.

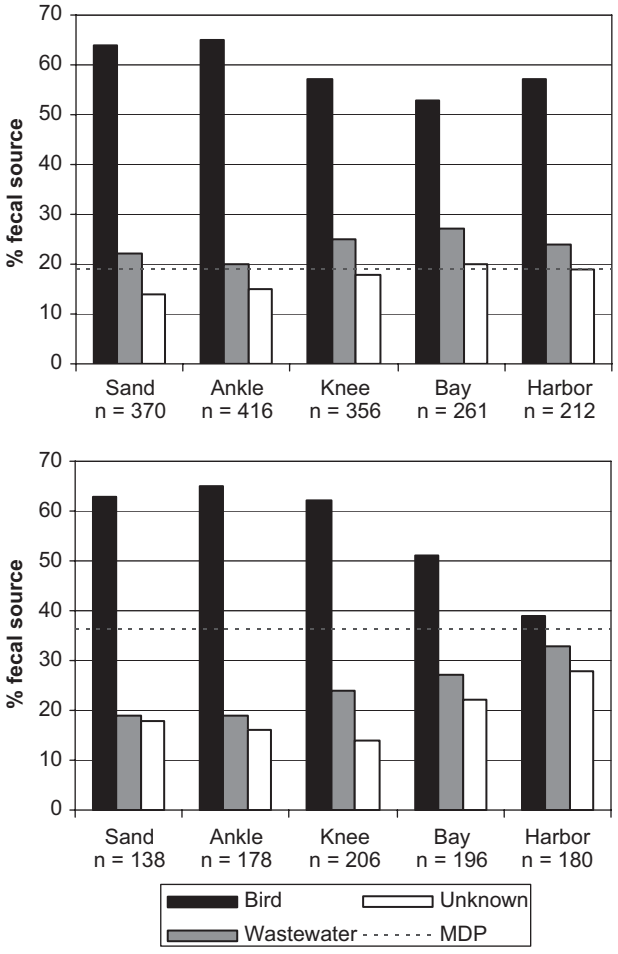


Fig. 2 – Two-way assignment of *Escherichia coli* isolates in Bayfront Park Beach sand and water samples to bird or wastewater fecal sources by antimicrobial resistance (top) and rep-PCR DNA fingerprinting (bottom) analyses. MDP = minimum detection percentage.

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.

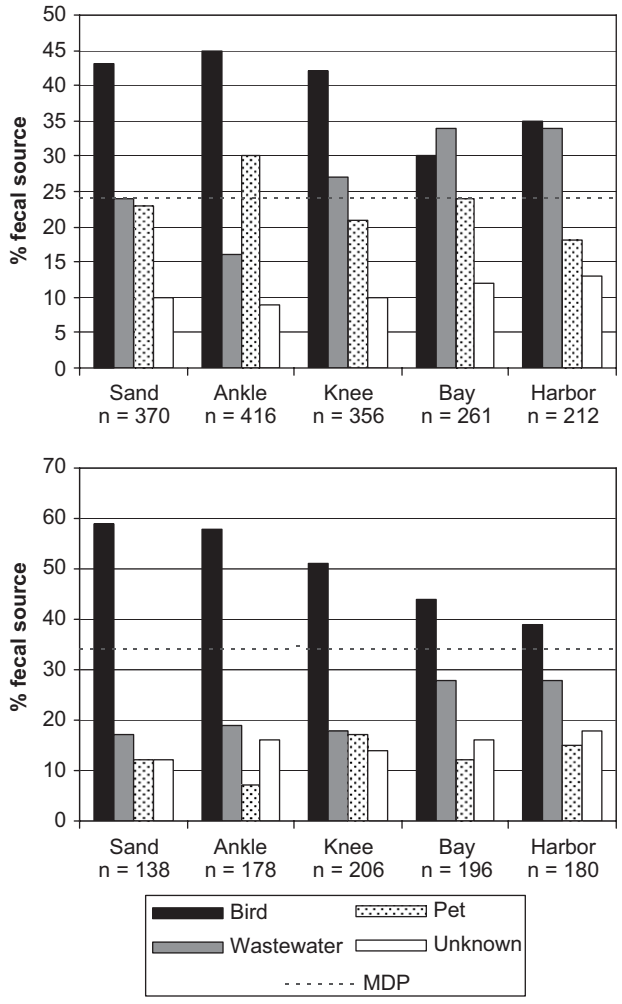


Fig. 3 – Three-way assignment of *Escherichia coli* isolates in Bayfront Park Beach sand and water samples to bird, wastewater, or pet fecal sources by antimicrobial resistance (top) and rep-PCR DNA fingerprinting (bottom) analyses. MDP = minimum detection percentage.

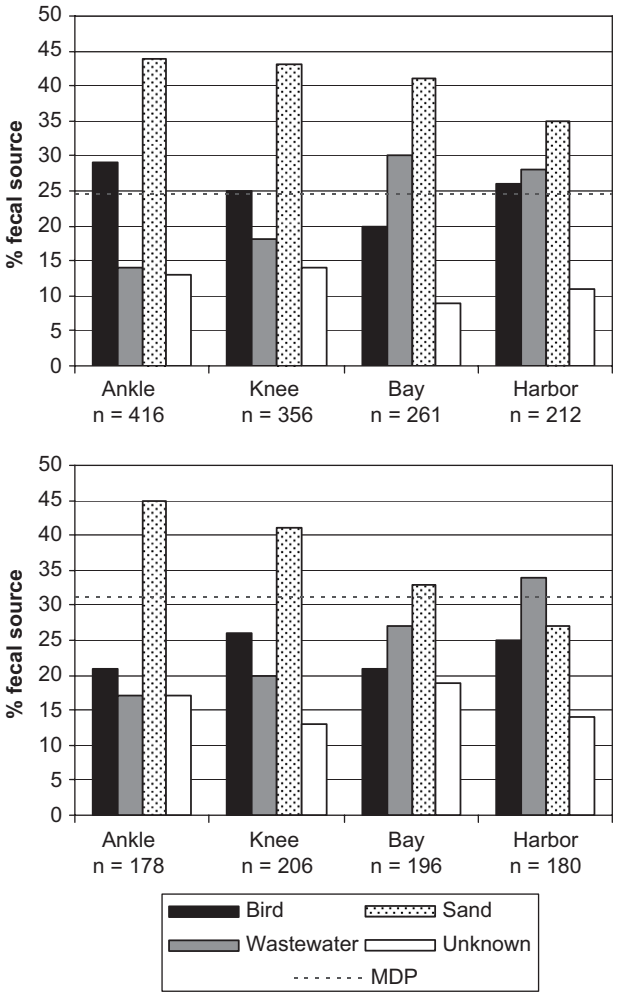


Fig. 4 – Three-way assignment of *Escherichia coli* isolates in Bayfront Park Beach water samples to bird, wastewater, or sand sources by antimicrobial resistance (top) and rep-PCR DNA fingerprinting (bottom) analyses. MDP = minimum detection percentage.

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.

Acknowledgments

Many thanks to Diana Benetteraj, Murray Charlton, Charlotte Curtis, Bailey Davis, Jason Demelo, Fargol Firouzchian, Jeannine Guindon, Tammy Kim, Leichelle Little, Alyssa Loughborough, Lauren McShane, Gabriela Midence, Jacqui Milne, Keira Pereira, Kinga Smolen, Flora Suen, and Laura Swystun from the National Water Research Institute, Environment Canada, and to Carol Dunn and Greg Gowing from the City of Hamilton.

REFERENCES

Abulreesh, H.H., Paget, T.A., Goulder, R., 2004. Waterfowl and the bacteriological quality of amenity ponds. J. Water Health 2, 183–189.

Alderisio, K.A., DeLuca, N., 1999. Seasonal enumeration of fecal coliform bacteria from the feces of ring-billed gulls (*Larus delawarensis*) and Canada geese (*Branta canadensis*). Appl. Environ. Microbiol. 65, 5628–5630.

Alm, E.W., Burke, J., Spain, A., 2003. Fecal indicator bacteria are abundant in wet sand at freshwater beaches. Water Res. 37, 3978–3982.

Bower, P.A., Scopel, C.O., Jensen, E.T., Depas, M.M., McLellan, S.L., 2006. Detection of genetic markers of fecal indicator bacteria in Lake Michigan and determination of their relationship to *Escherichia coli* densities using standard microbiological methods. Appl. Environ. Microbiol. 71, 8305–8313.

Charlton, M., Milne, J., 2004. Hamilton Harbour water quality update. RAP Technical Team, Research and Monitoring Report: 2003 Season. May 2004, Burlington, Ont., Canada, pp. 60–65.

Dorfman, M., Stoner, N., Merkel, M., 2004. Swimming in sewage. Natural Resources Defense Council and the Environmental Integrity Project. February, 2004, 68pp. 〈www.nrdc.org〉.

Edge, T.A., Hill, S., 2004. Microbial source tracking with *Escherichia coli* in Hamilton Harbour. RAP Technical Team, Research and Monitoring Report: 2003 Season. May 2004, Burlington, Ont., Canada, pp. 90–91.

Edge, T.A., Hill, S., 2005. Occurrence of antimicrobial resistance in *Escherichia coli* from surface waters and fecal pollution sources near Hamilton, Ontario. Can. J. Microbiol. 51, 501–505.

Edge, T.A., Schaefer, K.A. (Eds.), 2006. Microbial source tracking in aquatic ecosystems: the state of the science and an assessment of needs. National Water Research Institute, Burlington, Ont. NWRI Scientific Assessment Report Series No. 7 and Linking Water Science to Policy Workshop Series, 26pp.

Environment Canada and US Environmental Protection Agency, 2006. Beach advisories, postings and closures—Indicator #4200. State of the Great Lakes 2005. Cat. No. En161-3/0-2005E-PDF, pp. 161–164.

Fogarty, L.R., Haack, S.K., Wolcott, M.J., Whitman, R.L., 2003. Abundance and characteristics of the recreational water quality indicator bacteria *Escherichia coli* and enterococci in gull faeces. J. Appl. Microbiol. 94, 865–878.

Gordon, D.M., Bauer, S., Johnson, J.R., 2002. The genetic structure of *Escherichia coli* populations in primary and secondary habitats. Microbiology 148, 1513–1522.

Gould, D.J., Fletcher, M.R., 1978. Gull droppings and their effects on water quality. Water Res. 12, 665–672.

Griffith, J.F., Weisberg, S.B., McGee, C.D., 2003. Evaluation of microbial source tracking methods using mixed fecal sources in aqueous test samples. J. Water Health 1, 141–151.

Guan, S., Xu, R., Chen, S., Odumeru, J., Gyles, C., 2002. Development of a procedure for discriminating among *Escherichia coli* isolates from animal and human sources. Appl. Environ. Microbiol. 68, 2690–2698.

Hall, J.D., O'Connor, K., Ranieri, J., 2006. Progress toward delisting a Great Lakes area of concern: the role of integrated research and monitoring in the Hamilton Harbour remedial action plan. Environ. Monit. Assess. 113, 227–243.

Hamelin, K., Bruant, G., El-Shaarawi, A., Hill, S., Edge, T.A., Bekal, S., Fairbrother, J.M., Harel, J., Maynard, C., Masson, L., Brousseau, R., 2006. A virulence and antimicrobial resistance DNA microarray detects a high frequency of virulence genes in *Escherichia coli* from Great Lakes recreational waters. Appl. Environ. Microbiol. 71, 4200–4206.

Johnson, L.K., Brown, M.B., Carruthers, E.A., Ferguson, J.A., Dombek, P.E., Sadowsky, M.J., 2004. Sample size, library composition, and genotypic diversity among natural populations of *Escherichia coli* from different animals influence accuracy of determining sources of fecal pollution. Appl. Environ. Microbiol. 70, 4478–4485.

Jones, K., 2005. Flying hazards: birds and the spread of disease. Microbiol. Today 32, 174–178.

Kinzelman, J., McLellan, S.L., Daniels, A.D., Cashin, S., Singh, A., Gradus, S., Bagley, R., 2004. Non-point source pollution: determination of replication versus persistence of *Escherichia coli* in surface water and sediments with correlation of levels to readily measurable environmental parameters. J. Water Health 2, 103–114.

Kirschner, A.K.T., Zechmeister, T.C., Kavka, G.G., Beiwl, C., Herzig, A., Mach, R.L., Farnleitner, A.H., 2004. Integral strategy for evaluation of fecal indicator performance in bird-influenced saline inland waters. Appl. Environ. Microbiol. 70, 7396–7403.

Kleinheinz, G.T., McDermott, C.M., Leewis, M.C., Englebert, E., 2006. Influence of sampling depth on *Escherichia coli* concentrations in beach monitoring. Water Res. 40, 3831–3837.

LeFevre, N.M., Lewis, G.D., 2003. The role of resuspension in enterococci distribution in water at an urban beach. Water Sci. Technol. 47, 205–210.

Levesque, B., Brousseau, P., Simard, P., Dewailly, E., Meisels, M., Ramsay, D., Joly, J., 1993. Impact of the ring-billed gull (*Larus delawarensis*) on the microbiological quality of recreational water. Appl. Environ. Microbiol. 59, 1228–1230.

Levesque, B., Brousseau, P., Bernier, F., Dewailly, E., Joly, J., 2000. Study of the bacterial content of ring-billed gull droppings in relation to recreational water quality. Water Res. 34, 1089–1096.

McLellan, S.L., 2004. Genetic diversity of *Escherichia coli* isolated from urban rivers and beach water. Appl. Environ. Microbiol. 70, 4658–4665.

McLellan, S.L., Salmore, A.K., 2003. Evidence for localized bacterial loading as the cause of chronic beach closings in a freshwater marina. Water Res. 37, 2700–2708.

Moore, D.F., Harwood, V.J., Ferguson, D.M., Lukasik, J., Hannah, P., Getrich, M., Brownell, M., 2005. Evaluation of antimicrobial resistance analysis and ribotyping for identification of faecal pollution sources in an urban watershed. J. Appl. Microbiol. 99, 618–628.

O'Connor, K.M., 2003. Remedial action plan for Hamilton Harbour: Stage 2 Update 2002. Prepared by Hamilton Harbour RAP Stakeholder Forum. Hamilton Harbour Remedial Action Plan Office, Canada Centre for Inland Waters, Burlington, Ont., Canada.

Ritter, K.J., Carruthers, E., Carson, C.A., Ellender, R.D., Harwood, V.J., Kingsley, K., Nakatsu, C., Sadowsky, M.J., Shear, B., West, B., Whitlock, J.E., Wiggins, B.A., Wilbur, J.D., 2003. Assessment of statistical methods used in library-based approaches to microbial source tracking. J. Water Health 1, 209–223.

Rochelle, P., De Leon, R., 2006. Workshop on microbial source tracking. Final Report 03-HHE-3. Water Environment Research Foundation, Alexandria, VA, 106pp.

Salmore, A.K., Hollis, E.J., McLellan, S.L., 2006. Delineation of a chemical and biological signature for stormwater pollution in an urban river. J. Water Health 4, 247–262.

Sampson, R.W., Swiatnicki, S.A., McDermott, C.M., Kleinheinz, G.T., 2005. *Escherichia coli* at Lake Superior recreational beaches. J. Great Lakes Res. 31, 116–121.

SAS Institute Inc., 1999. SAS/STAT User's Guide, Version 8. SAS Institute Inc., Cary, NC, 3884pp.

Scopel, C.O., Harris, J., McLellan, S.L., 2006. Influence of nearshore water dynamics and pollution sources on beach monitoring outcomes at two adjacent Lake Michigan beaches. J. Great Lakes Res. 32, 543–552.

Simpson, J.M., Santo Domingo, J.W., Reasoner, D.J., 2002. Microbial source tracking: state of the science. Environ. Sci. Technol. 36, 5279–5288.

Standridge, J.H., Delfino, J.J., Kleppe, L.B., Butler, R., 1979. Effect of waterfowl (*Anas platyrhynchos*) on indicator bacteria populations in a recreational lake in Madison, Wisconsin. Appl. Environ. Microbiol. 38, 547–550.

Stoeckel, D.M., Harwood, V.J., 2007. Performance, design, and analysis in microbial source tracking studies. Appl. Environ. Microbiol. 73, 2405–2415.

Stoeckel, D.M., Mathes, M.V., Hyer, K.E., Hagedorn, C., Kator, H., Lukasik, J., O'Brien, T.L., Fenger, T.W., Samadpour, M., Strickler, K.M., Wiggins, B.A., 2004. Comparison of seven protocols to identify fecal contamination sources using *Escherichia coli*. Environ. Sci. Technol. 38, 6109–6117.

Topp, E., Welsh, M., Tien, Y.-C., Dang, A., Lazarovits, G., Conn, K., Hong, Z., 2003. Strain-dependent variability in growth and survival of *Escherichia coli* in agricultural soil. FEMS Microbiol. Ecol. 44, 303–308.

US Environmental Protection Agency, 2005a. Microbial Source Tracking Guide, EPA/600-R-05-064. Office of Research and Development. United States Environmental Protection Agency, Cincinnati, OH, 150pp.

US Environmental Protection Agency, 2005b. The EMPACT Beaches Project: Results from a Study on Microbiological Monitoring in Recreational Waters, EPA 600/R-04/023, August, 2005. Office of Research and Development, United States Environmental Protection Agency, Cincinnati, OH, 74pp.

Whitlock, J.E., Jones, D.T., Harwood, V.J., 2002. Identification of the sources of fecal coliforms in an urban watershed using antimicrobial resistance analysis. Water Res. 36, 4273–4282.

Whitman, R.L., Nevers, M.B., 2003. Foreshore sand as a source of *Escherichia coli* in nearshore water of a Lake Michigan beach. Appl. Environ. Microbiol. 69, 5555–5562.

Whitman, R.L., Shively, D.A., Pawlik, H., Nevers, M.B., Byappanahalli, M.N., 2003. Occurrence of *Escherichia coli* and enterococci in *Cladophora* (Chlorophyta) in nearshore water and beach sand of Lake Michigan. Appl. Environ. Microbiol. 69, 4714–4719.

Wiggins, B.A., Cash, P.W., Creamer, W.S., Dart, S.E., Garcia, P.P., Gerecke, T.M., Han, J., Henry, B.L., Hoover, K.B., Johnson, E.L., Jones, K.C., McCarthy, J.G., McDonough, J.A., Mercer, S.A., Noto, M.J., Park, H., Phillips, M.S., Purner, S.M., Smith, B.M., Stevens, E.N., Varner, A.K., 2003. Use of antimicrobial resistance analysis for representativeness testing of multiwatershed libraries. Appl. Environ. Microbiol. 69, 3399–3405.

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-

Received 21 October 2004. Revision received 21 March 2005. Accepted 29 March 2005. Published on the NRC Research Press Web site at http://cjm.nrc.ca on 12 July 2005.

T.A. Edge[1] and S. Hill. National Water Research Institute, Environment Canada, 867 Lakeshore Road, Burlington, ON L7R 4A6, Canada.

[1]Corresponding author (e-mail: Tom.Edge@ec.gc.ca).

 doi: 10.1139/W05-028

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 μg·mL^{-1}; amp, ampicillin 50 μg·mL^{-1}; cep, cephalothin 25 μg·mL^{-1}; chlo, chlorotetracycline 80 μg·mL^{-1}; cip, ciprofloxacin 20 μg·mL^{-1}; ery, erythromycin 90 μg·mL^{-1}; gen, gentamicin 20 μg·mL^{-1}; kan, kanamycin 40 μg·mL^{-1}; neo, neomycin 50 μg·mL^{-1}; oxy, oxytetracycline 60 μg·mL^{-1}; pen, penicillin 100 U·mL^{-1}; strp, streptomycin 60 μg·mL^{-1}; sulf, sulfamethoxazole 50 μg·mL^{-1}; tet, tetracycline 30 μg·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.

Acknowledgements

Thanks to Carol Dunn and Greg Gower, City of Hamilton, who provided assistance in obtaining *E. coli* from municipal wastewater sources. Thanks also to Murray Charlton and Jacqui Milne, National Water Research Institute, for their collaborative work on *E. coli* surveillance in Hamilton Harbour. Funding provided by the Great Lakes 2020 program.

References

Ash, R.J., Mauck, B., and Morgan, M. 2002. Antibiotic resistance of gram-negative bacteria in rivers, United States. Emerg. Infect. Dis. **8**: [serial online] 2002 Jul [cited April 2004]. Available from: URL: http://www.cdc.gov/ncidod/EID/vol8no7/01-0264.htm.

Blake, D.P., Hillman, K., Fenlon, D.R., and Low, J.C. 2003. Transfer of antibiotic resistance between commensal and pathogenic members of the Enterobacteriaceae under ileal conditions. J. Appl. Microbiol. **95**: 428–436.

Fallacara, D.M., Monahan, C.M., Morishita, T.Y., and Wack, R.F. 2001. Fecal shedding and antimicrobial susceptibility of selected bacterial pathogens and a survey of intestinal parasites in free-living waterfowl. Avian Dis. **45**: 128–135.

Gorbach, S.L. 2001. Antimicrobial use in animal feed – time to stop. N. Engl. J. Med. **345**: 1202–1203.

Guan, S., Xu, R., Chen, S., Odumeru, J., and Gyles, C. 2002. Development of a procedure for discriminating among *Escherichia coli* isolates from animal and human sources. Appl. Environ. Microbiol. **68**: 2690–2698.

Harwood, V.J., Wiggins, B., Hagedorn, C., Ellender, R.D., Gooch, J., Kern, J., Samadapour, M., Chapman, A.C.H., Robinson, B.J., and Thompson, B.C. 2003. Phenotypic library-based microbial source tracking methods: efficiency in the California collaborative study. J. Water Health, **1**: 153–166.

Karlowsky, J.A., Jones, M.E., Thornsberry, C., Friedland, I.R., and Sahm, D.F. 2003. Trends in antimicrobial susceptibilities among Enterobacteriaceae isolated from hospitalized patients in the United States from 1998 to 2001. Antimicrob. Agents Chemother. **47**: 1672–1680.

Kaspar, C.W., Burgess, J.L., Knight, I.T., and Colwell, R.R. 1990. Antibiotic resistance indexing of *Escherichia coli* to identify sources of fecal contamination in water. Can. J. Microbiol. **36**: 891–894.

Livermore, D.M., Warner, M., Hall, L.M.C., Enne, V.I., Projan, S.J., Dunman, P.M., Wooster, S.L., and Harrison, G. 2001. Antibiotic resistance in bacteria from magpies (*Pica pica*) and rabbits (*Oryctolagus cuniculus*) from west Wales. Environ. Microbiol. **3**: 658–661.

McKeon, D.M., Calabrese, J.P., and Bissonnette, G.K. 1995. Antibiotic resistant gram-negative bacteria in rural groundwater supplies. Water Res. **29**: 1902–1908.

O'Connor, K.M. 2003. Remedial action plan for Hamilton Harbour: stage 2 update 2002. *Prepared by* Hamilton Harbour RAP stakeholder forum. Hamilton Harbour Remedial Action Plan Office, Canada Centre for Inland Waters, Burlington, Ontario.

Osterblad, M., Hakanen, A., Manninen, R., Leistevuo, T., Peltonen, R., Meurman, O., Huovinen, P., and Kotilainen, P. 2000. A between-species comparison of antimicrobial resistance in Enterobacteria in fecal flora. Antimicrob. Agents Chemother. **44**: 1479–1484.

Parveen, S., Murphree, R.L., Edmiston, L., Kaspar, C.W., Portier, K.M., and Tamplin, M.L. 1997. Association of multiple-antibiotic-resistance profiles with point and nonpoint sources of *Escherichia coli* in Apalachicola Bay. Appl. Environ. Microbiol. **63**: 2607–2612.

Reinthaler, F.F., Posch, J., Feierl, G., Wust, G., Haas, D., Ruckenbauer, G., Mascher, F., and Marth, E. 2003. Antibiotic resistance of *E. coli* in sewage and sludge. Water Res. **37**: 1685–1690.

Roe, M.T., Vega, E., and Pillai, S.D. 2003. Antimicrobial resistance markers of class 1 and class 2 integron-bearing *Escherichia coli* from irrigation water and sediments. Emerg. Infect. Dis. **9**: [serial online] 2003 Jul [cited April 2004]. Available from: URL: http://www.cdc.gov/ncidod/EID/vol9no7/02-0529.htm.

SAS Institute Inc. 1999. SAS/STAT User's Guide, Version 8. SAS Institute Inc. Cary, N.C. pp. 3884

Simpson, J.M., Santo Domingo, J.W., and Reasoner, D.J. 2002. Microbial source tracking: state of the science. Environ. Sci. Technol. **36**: 5279–5288.

Summers, A.O. 2002. Generally overlooked fundamentals of bacterial genetics and ecology. Clin. Infect. Dis. **34**: S85–S92.

Whitlock, J.E., Jones, D.T., and Harwood, V.J. 2002. Identification of the sources of fecal coliforms in an urban watershed using antibiotic resistance analysis. Water Res. **36**: 4273–4282.