## Environment Canada Water Science and Technology Directorate

# Direction générale des sciences et de la technologie, eau Environnement Canada

Escherichia coli in water and sand at beaches in Lake Huron, Lake Ontario, and Hamilton Harbour By: J.E. Milne & M.N. Charlton NWRI Contribution # 04-205

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04-205

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

#### J.E. Milne and M.N. Charlton

#### Abstract

Escherichia coli has been used as an indicator organism for human faecal material and possible pathogen contamination of beaches for many years. In the public health sense there is not a requirement to understand in all cases the sources of the E.coli that may be causing beach closures or postings. We began to survey E.coli in Hamilton Harbour in 1998 because beaches were not allowed to open despite control of combined sewer overflows which were thought to be the source of contamination. Our surveys led to more detailed work that seemed to indicate that the bacteria actually came from the beach. We repeated this work on beaches of Lake Huron and Lake Ontario with similar results. Moreover, we found a source of E.coli in the pore water of the beach sands. In Hamilton Harbour the beach is heavily littered with goose droppings which provide support to the idea that birds are actually the cause of the beach postings. At other beaches several sources may be present but delineation of them is complicated by the storage and possible growth of E.coli in the beach sand. We suggest that the usefulness of E.coli be re-investigated from the standpoint of which pathogens are actually being indicated.

## Escherichia coli dans l'eau et le sable des plages du lac Huron, du lac Ontario et du port de Hamilton

#### J.E. Milne et M.N. Charlton

#### Résumé

Escherichia coli est utilisé depuis de nombreuses années comme organisme indicateur des matières fécales humaines et de la contamination possible des places par des acents pathogènes. Du point de vue de la santé publique, il n'est pas nécessaire de comprendre dans tous les cas les sources de E. coli qui entraînent la fermeture des plages ou l'affichage d'avertissements aux plages. Nous avons commencé à étudier E. coli dans le port de Hamilton en 1998, parce que l'accès aux plages demeurait interdit malgré la maîtrise des déversoirs d'orage, auxquels on attribuait la source de la contamination. Nos études ont débouché sur des travaux plus détaillés qui ont donné à penser que la bactérie venait en fait des plages. Nous avons répété nos travaux sur les plages du lac Huron et du lac Ontario, et obtenu les mêmes résultats. De plus, nous avons trouvé une source de E, coli dans l'eau interstitielle du sable des plages. Dans le port de Hamilton, la place est très souillée par les excréments des oies, ce qui vient appuyer la thèse que ce sont les oiseaux qui rendent les avertissements nécessaires. À d'autres plages, il se peut qu'il y ait plusieurs sources, mais il est difficile de les délimiter, parce que E. coli s'emmagasine et peut-être prolifère dans le sable de la plage. Nous suggérons de réexaminer l'utilité de E. coli comme indicateur des agents pathogènes.

## NWRI RESEARCH SUMMARY

#### Plain language title

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

### What is the problem and what do scientists already know about it?

Beaches are closed or posted on regular basis due to elevated E. coli numbers.

#### Why did NWRI do this study?

To try and understand the dynamics of bacterial contamination in water and sand.

#### What were the results?

Nearshore water and pore water in sand tended to harbour higher E. coli counts than offshore water. Birds maybe the source of E. coli that cause postings or closures of some beaches.

#### How will these results be used?

These results will be used as part of a larger study including other government agencies and Conservation Authorities.

## Who were our main partners in the study?

Ontario Ministry of the Environment.

## Sommaire des recherches de l'INRE

#### Titre en langage clair

Escherichia coli dans l'eau et le sable des plages du lac Huron, du lac Ontario et du port de Hamilton.

### Quel est le problème et que savent les chercheurs à ce sujet?

Les plages sont fermées régulièrement ou des avertissements y sont affichés en raison du nombre élevé de E. coli.

#### Pourquoi l'INRE a-t-il effectué cette étude?

Chercher à comprendre la dynamique de la contamination bactérienne dans l'eau et le sable.

#### Quels sont les résultats?

L'eau littorale et l'eau interstitielle dans le sable contenaient souvent un plus grand nombre de E. coli que l'eau au large. Il se peut que les oiseaux soient la source de la présence de E. coli qui occasionne l'affichage d'avertissements aux plages ou la fermeture de certaines plages.

#### Comment ces résultats seront-ils utilisés?

Ces résultats serviront dans le cadre d'une étude plus vaste à laquelle participeront d'autres organismes gouvernementaux et des offices de protection de la nature.

## Quels étaient nos principaux partenaires dans cette étude?

Ministère de l'Environnement de l'Ontario.

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

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NWRI Contribution No. 04-205

#### Introduction

Beach postings and closures have been common throughout the Great Lakes during the summer months on beaches in high use areas. Most closures are caused by Escherichia coli (E. coli) from outdated sewage treatment plants (STPs) (RAP for HH, 1992), faulty or old septic systems (Whitman and Nevers, 2003) agriculture (Palmateer et al, 1989) and are usually associated with high precipitation events. A disconcerting observation is that beaches without obvious human E. coli sources, produce levels higher then the Provincial Water Quality Standard (PWQS) of 100 MPN of cfu/100 ml. For example, beaches established in the west end of Hamilton Harbour in 1992 were closed most of the summer season even after the diversion of combined sewage overflows (CSO's) to CSO holding tanks on-line between 1994 and 2003 (Hamilton Harbour RAP Stakeholders, 2003). From a spatial survey done in Hamilton Harbour in 1998, we were surprised to find E. coli concentrations were generally low - below the PWQS and suitable for swimming. With the implementation of the Hamilton Harbour Remedial Action Plan, beach closures remained a problem as there was little understanding of the source of E. coli contamination responsible for beach closures. This study of E. coli includes results gathered from surveys at beaches in Hamilton Harbour, Lake Ontario and Lake Huron.

### Methods and Area of Study

The focus of this study is the east shore of Lake Huron between Goderich and Kincardine Ontario and two beaches on western Lake Ontario between Hamilton and Burlington Ontario (Fig. 1) and Bayfront Beach in Hamilton Harbour. The Kincardine and Goderich area includes cottages, homes, beaches, marinas and agriculture. Hamilton Harbour includes homes, beaches, marinas, heavy industry and 4 sewage treatment plants. The beaches of Burlington and Hamilton are exposed to the full fetch of Lake Ontario. These areas experience beach posting or closures from E. coli greater than 100MPN of cfu/100 mls on a regular basis throughout the summer months but do not have any obvious human E. coli sources.

Samples were collected along transects from inshore to approximately 2 km offshore from a boat, by wading into the water from shore and from beach pore water. Samples taken by boat were collected using a 250 ml sterile bottle attached to a measured pole and submerging to 1 m depth. Beach transect samples were collected by excavating a hole in the sand approximately 0.5 m deep and 2 m from water (A) and 1 m from water (B). Each hole was then left to fill with pore water. Care was taken not to let lake water wash in. Water was then collected by submerging a sterilized plastic bottle into the pore water. Water was also collected along the same transect by wading approximately 1 m (C) from

shore and wading approximately 5 m from shore (D). (Fig. 2). The latter distance depended on depth. Between 1 and 5 transects were completed on each beach.

The samples were kept on ice until plated within 7 hours of collection on Coliplates<sup>™</sup> (EBPI, Brampton, Ontario 1999). The plates consist of previously prepared agar solution. This method was time and cost effective for our purposes. Plates were observed after an incubation period of 24 hours at 35°C. The Most Probable Number (MPN) was derived as per procedures from EBPI, 1999.

Boat transects and Beach sampling at Lake Huron Beaches were done July 30, August 15, October 14, and October 30, 2003 (see Table 1 for sampling summary). Boat transects sampled at Hamilton Harbour, and Lake Ontario Beaches were completed between 2001 to 2003 from April to October. Beach transect samples were collected October 30, 2003 at both Burlington Beach and Hamilton Beach.

#### Results

#### Kincardine to Goderich

Figure 3 shows boat transects off Station beach for both July 20 and August 19, 2003. With the Kincardine STP outflow approximately 1 km south of station beach (44°10'02"N 81°39'17"W), E. coli results were still well under the PWQS for the entire transect from offshore to inshore (<3 MPN/100 ml). A boat transect from approximately 2 km offshore to approximately 1 km upstream in the Penetangore River was completed (Fig. 3). No values above the PWQS were recorded. However, 3 transects on Station Beach (Fig. 4) show very high E. coli in sand (>2424) then decreasing rapidly at or slightly above the PWQS 1 m into the water. The fourth transect samples were all below the PWQS. This shows that there can be considerable variation in E .coli numbers in sand.

Boat transects off Poplar and Bruce Beach had very low E. coli concentrations but two samples of Poplar beach pore water had concentrations of >2424 and 161 cfu/100 ml. Bruce Beach, on October 15, 2003, showed 1 sample of pore water in Transect 3 slightly above the PWQS (Fig. 5). Lower E. coli counts may be attributed to a less activity and dwellings located in the area.

Lurgan Beach results showed low E. coli concentrations offshore (<3 MPN/100 ml) (Fig. 6). However, there was a slight increase nearshore, although still below the PWQS. Royal Oak Creek, a tributary of the Pine River, flows into Lake Huron at the North end of Lurgan Beach. Results from both these rivers were well above the PWQS (1696 and >2424 MPN/100 ml, respectively).

Point Clark results were similar to Lurgan Beach (Fig. 6). Low E. coli was evident in off shore water (<3 MPN/100 ml), but showed a slight increase in the near shore water, although less than the PWQS (16MPN/100 ml). Clark Creek which flows into Lake Huron at Point Clark, had E. coli results well above the PWQS (>2424 MMPN/100 ml). Point Clark beach transects (Fig. 7) showed that the highest E. coli was evident in pore water samples 2 m above the surf zone. E. coli pore water numbers, however, were an order of magnitude less than at Station beach. Pore water samples at 1m above the surf zone and 5m in the water were below the PWQS although still higher than the offshore transect. All samples in transect 4 were above the PWQS and were higher than offshore transect. Again, there was considerable variability along the beach.

Amberley Beach results showed low E. coli concentrations offshore (Fig. 6); however these increased to above PWQS nearshore (119 MPN/100 ml). Beach transects sampled on August 21 show very high E. coli in pore water and lake water (Fig. 8). However these results may not be accurate due to cross contamination in transport from field to laboratory. Another attempt was made October 15 to collect samples from the same stations. However, sampling was performed during an onshore wind storm and unfortunately due to high waves no D samples were collected to determine if elevated bacteria levels were evident. High waves and increased water levels, forced sampling approximately 10 m up the beach from former positions. Figure 9 shows E. coli results during this time. The results are much lower than in August; however a reservoir of E. coli was still evident in the sand.

One pore water sample was taken at Kintail beach (Fig. 10). The result was 938 MPN/100 ml. No off shore/nearshore samples were collected. Kerry's Creek flows into Lake Huron at the south end of Kintail Beach. E. coli was well above the PWQS at >2424 MPN/100 ml. However, Nine Mile River located approximately 5 km south of Kerry's Creek, was also sampled. Results were 83 MPN/100 ml.

Two offshore/nearshore transects were completed in Goderich (Fig. 11). The first was off the STP outflow south of Rotary Park Beach. Results showed low E. coli. The second area was Rotary Park Beach where a significant increase in E. coli was evident from off shore to near shore (339 MPN/100 ml). The mouth of the Maitland River was also sampled with results showing well above the PWQS (>2424 MPN/100 ml). The usual direction of nearshore flow is to the north in the area, thus, it seems difficult to clearly associate the extreme nearshore number at Rotary Park Beach with either the STP or the Maitland River. Rotary Park Beach samples show pore water E. coli above the PWQS (Fig. 12), and remained above the PWQS near surf zone (1 m from sand). Results then rapidly decrease to close to or at the PWQS in lake water. Two beach transects were sampled at Main Beach (Fig. 13). Pore water samples were well the PWQS at 1696 and >2424 MPN/100 ml. Results in the surf zone were still above the

PWQS at 119 and 858 MPN/100 ml, then decreased to below the PWQS 10 m offshore.

#### **Bayfront Park in Hamilton Harbour**

Spatial surveys were begun in 1998. Generally, the E. coli numbers are not high enough to support the notion that the beaches are contaminated by the open waters although evidence can be found of the largest STP in the southeast corner and the second largest in the northeast area. Example spatial results in 2001 of E. coli numbers in the harbour were usually slightly above or below the PWQS during the Skyway and Woodward Ave. STP chlorination period and after chlorination terminated (Fig. 14). An off shore/nearshore transect consisting of 5 stations was sampled weekly by boat between May and September 2001 to 2003. Results of a typical sampling are shown in Fig. 15. E. coli numbers increased from offshore to nearshore. All data between 2001 and 2003 with averages are shown in Fig. 16. The inshore station in 2001, had the highest E. coli count for 54% of the total weeks sampled. The inshore station in 2003 the inshore station had the highest E. coli count 85% of the total weeks sampled.

Pore water samples shows E. coli numbers were highest 1 m from surf zone (>2424 MPN/100 ml) then steadily decreasing at 1 m in lake water to below the PWQS at 10 m into lake water at all three transects (Fig. 17).

#### Burlington Beach and Hamilton Beach

An off shore/inshore transect consisting of 5 stations was sampled weekly by boat between May and September 2001 to 2003 at both Hamilton and Burlington Beach, Figure 18 shows typical results of E. coli numbers. The inshore station in 2001, had the highest E. coli count for 63% of the total weeks sampled. The inshore station in 2002, had the highest E. coli count for 73% of the total weeks sampled and the inshore station in 2003, had the highest E. coli count for 30% of the total weeks sampled. In 2003 the counts were lower (below the PWQS) than previous years therefore no trend from inshore to offshore was noticeable. The inshore station at Hamilton Beach in 2001, had the highest E. coli count for 41% of the total weeks sampled. The inshore station in 2002, had the highest E. coli count for 40% of the total weeks sampled and the inshore station in 2003, had the highest E. coli count for 36% of the total weeks sampled. Most values were at or below the PWQS therefore no trend from inshore to offshore was noticeable. Figure 19 illustrates all data between 2001 and 2003 with averages. All inshore average numbers were higher than offshore except in 2003 Burlington Beach where the offshore station average was 52 MPN/100ml. This could be a result of contamination during sampling. Hamilton Beach in 2003 showed a slight increase to offshore, however, the difference between the in shore and offshore numbers

was an average of only 9 MPN/100 ml and results were below the PWQS, therefore this trend may not be significant.

Three beach transects were sampled on Burlington Beach (Fig. 20). Transect 2 had high E. coli in pore water (>2424 and 559 MPN/100 ml), but decreased to 33 and 3 MPN/100 ml in the lake water. Transect 1 had low E. coli in pore water, but a significant increase to >2424 MPN/100 ml at 1m from surf zone, then rapidly decreasing to 3 MPN/100 ml at 10 m from surf zone. Transect 3 had low E. coli below the PWQS for pore water and lake water.

Four beach transects were sampled on Hamilton Beach (Fig. 21). Transect 2 showed high E. coli results in pore water 2 m above surf zone (>2424 MPN/100 ml) then rapidly decreased at 1 m above surf zone (510 MPN/100ml) to 5 then 3 MPN/100 ml 10 m in lake water. Transect 3 showed high E. coli results in pore water 2 m above surf zone (>2424 MPN/100 ml) then rapidly decreased at 1 m above surf zone (>2424 MPN/100 ml) then rapidly decreased at 1 m above surf zone (>2424 MPN/100 ml) then rapidly decreased at 1 m above surf zone (>2424 MPN/100 ml) then rapidly decreased at 1 m above surf zone (69 MPN/100 ml) then 3 MPN/100 ml 1 m and 10 m in lake water. Transect 1 and 4 results remained below the PWQS in pore water and lake water. Again, there was a great deal of variability along a beach at stations sampled close together temporally.

Overall, average results for all beach transects showed a large decrease from sand to water (Fig. 22).

#### Discussion

## During this study four important factors were noted

1) Even with the influence of sewage outflows and heavy agriculture in the case of Kincardine to Goderich, E. coli numbers in the offshore water were usually at or below the PWQS. Yet beaches are often posted. Observations were similar in Hamilton Harbour. With implementation of HHRAP recommendations and recently installed combined sewer overflow collectors, offshore samples were at or below the PWQS and Bayfront Beach was still posted. Burlington Beach and Hamilton Beach have no direct influence from human E. coli sources yet beach samples numbers were elevated.

2) E. coli numbers tended to increase from off shore to in shore and were sometime highest in beach pore water. Whitman and Nevers (2003) and MOE (1979) found similar results.

3) During the time of this study, creeks sampled were well above the PWQS. Unfortunately, nearshore samples gathered at the creek mouths, were not done on the same day. Creeks tended to have elevated E. coli numbers, but nearshore results in close proximity to the creek mouths were at or below the PWQS. Weather during the time of creek sampling was clear and sunny. Results may have been different during a rain event but, in our sampling, high E. coli numbers in a stream were not predictive of high numbers in the water of adjacent beaches.

4) There is a tendency in our Lake Huron data for the water sample nearest the beaches to have somewhat elevated E. coli numbers. This illustrates the phenomenon that materials introduced at lake shores do not mix offshore readily and that the bacteria may come from the beaches themselves.

#### Why are beaches closed?

With this evidence it appears that sand may act as a storage facility for bacteria. Sediment particles provide a larger surface area for Bacteria to adhere to more readily than free floating bacteria in water (Whitman and Nevers, 2003 and Doyle et al, 1992). E. coli and other pathogens can survive in beach sediment for months (Burton et al, 1987) as opposed to a water environment where pathogens such as Salmonella die off between 6 hours and 3 days (Rudolfs et al, 1950). High bacteria levels in the upper layer of sand may be released into the water through agitation of sediment from wave action and people/animals (Burton et al, 1986 and Whitman and Nevers, 2003). According to the Ontario Ministry of Health and Long Term Care Beach Management Protocol, samples are obtained about 15 to 30 cm below the water surface in 1 and 1.5 m depth. If these samples are taken during or after a turbulent event, E. coli numbers may be elevated. These results may be misconstrued as offshore pathogens coming inshore, i.e., sewage outflow.

#### High levels of bacteria in sand may originate from various sources

1) Faulty and outdated septic systems may be a factor on Lake Huron. There are many seasonal and full time residents lining the beaches – specifically Amberley Beach. However, there are no active septic systems on Burlington Beach and Hamilton Beach and E. coli numbers are still elevated. From a sand replacement study, Whitman and Nevers (2003) found that E. coli increased to pre-sand replacement numbers after only 2 weeks. Therefore, since septic systems at most Lake Huron beaches are located at least 20 and 40 m from the beach sand, contamination from groundwater, which travels approximately 20 to 25 m/year in sand (Dr. Allan Crowe, Environment Canada, personal communication), is not likely a factor.

2) Agriculture runoff may be contributing factor during high water events – high rains and spring runoff. The shoreline between Kincardine and Goderich is in a region with the second highest number of livestock animals in Canada (StatsCan, 2001). The usual elevated E. coli numbers close to shore illustrate that materials introduced to the nearshore do not readily leave. Thus, E. coli from streams

could remain in nearshore water after flow events. At the time of our sampling, however, in Lake Huron, stream velocities were barely noticeable. In addition, the presence of highest numbers in water right at the water's edge seems more consistent with the notion that the beaches themselves are the ongoing source.

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

4) Chladophora may harbour bacteria (Whitman et al, 2003). The south east shore of Lake Huron has had significant algae problems in the past; predominantly Chladophora. Floating Chladophora mats wash up on the beaches leaving malodorous piles. During this study, there were no significant "algal events" observed. Whitman et al (2003) concluded that Chladophora mats can harbor significant numbers of E. coli.

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

In summary, three areas were used for this study. Each area is unique. Lake Huron beaches consist mostly of cottages and small marinas, Hamilton Harbour consists of heavy industry and 4 sewage treatment plants. Burlington Beach and Hamilton Beach are located on the west end of Lake Ontario exposed to the full fetch. From our study, low bacteria levels were evident in off shore areas. Nearshore beach transects showed a dramatic increase in E. coli numbers in beach sand. Why are beaches closed? Perhaps the most important finding for beach management is that beach sand can be a long term reservoir for E. coli. Thus, resuspension events can produce elevated numbers nearshore but, apparently, notable resuspension events are not always required. The original E. coli contamination may come from any or all of the sources but duration of storage and the possibility of multiple low level contamination events or ongoing wildlife contamination may lead to confusion as to where the root problems originate. Whereas formerly sewage contamination was the subject of testing for E. coli as an indicator of potential human pathogens this study and others show the situation is much more complex. Intensive studies are needed of all sources and a determination of whether they are associated with important pathogens would be useful.

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## **List of Tables**

 Table 1
 Sampling summary for Lake Huron Beaches

Location	Date	Hole in sand	Waded	Boa
Horton Pt	28-Jul-03			•
Station Beach	28-Jul-03	•	•	•
	31-Jùl-03	•	•	ļ
	19-Aug-03			•
	21-Aug-03	•	•	
Penetangore transect	28-Jul-03			
Drain penetangore river kin	28-Jul-03			•
Poplar Beach	28-Jul-03	·		۰
	19-Aug-03			•
	21-Aug-03	•		
	15-Oct-03	•		
Poplar Beach small stream	21-Aug-03		•	
	15-Oct-03		•	
Bruce Beach	28-Jul-03			<u>```</u>
	19-Aug-03			
	15-Oct-03	•	•	
Lurgan	19-Aug-03			•
Lurgan mouth of Pine River	19-Aug-03			•
Lurgan S of Pine River	21-Aug-03	•	•	
	15-Oct-03	•	•	
Pt. Clark	28-Jul-03	•	•	
	31-Jul-03	•	•	
	19-Aug-03			· ·
	21-Aug-03	•	•	_
	15-Oct-03	•	•	+
Amberley Beach	19-Aug-03	• ·		
	21-Aug-03	•	•	
	15-Oct-03	•	•	
Rotary Park Beach Goderich	30-Jul-03	•	•	•
1	14-Oct-03	•	•	
S of Rotary Park Beach Goderich	30-Jul-03			•
	14-Oct-03		•	
Main Beach Goderich	<u>30-Jul-03</u>	• • •	•	
	14-Oct-03	•	•	-
Maitland River Goderich	30-Jul-03			•
Nine Mile Creek	30-Jul-03		•	
Kerry's Creek	30-Jul-03		•	
Kintall Beach waded	30-Jul-03		· · · · · · · · · · · · · · · · · · ·	
Pine River	30-Jul-03	· · · · · · · · · · · · · · · · · · ·	•	
Royal Oak Creek	31-Jul-03		•	
South Pine River	31-Jul-03		•	
Clark Creek	31-Jul-03		•	_
Bayfront Beach	30-Oct-03	•	•	
Burlington Beach Hamilton Beach	30-Oct-03	•	•	

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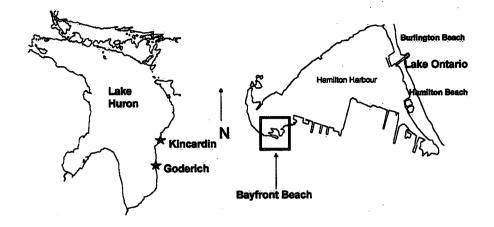
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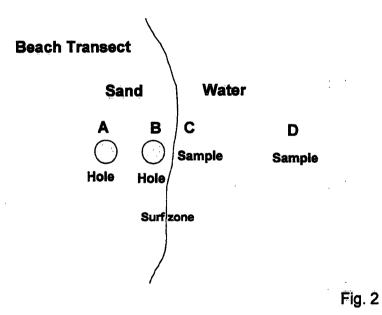
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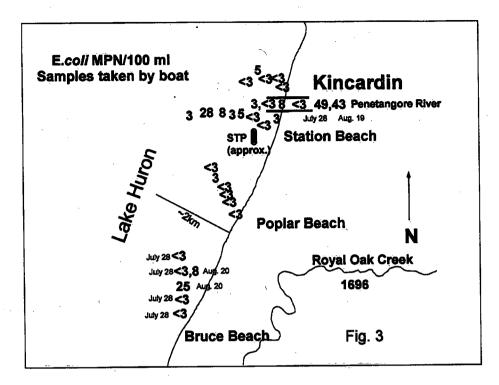
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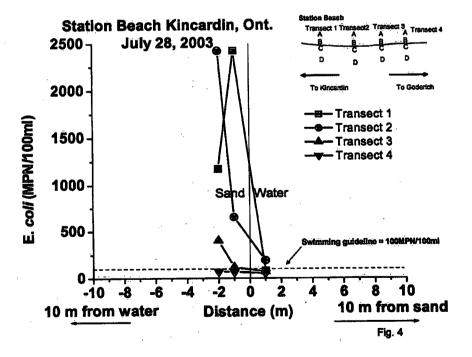
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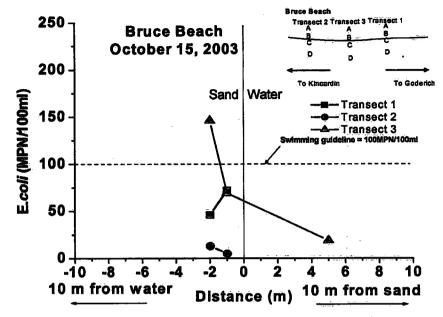




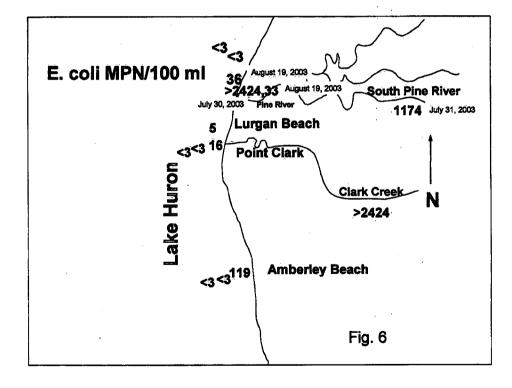


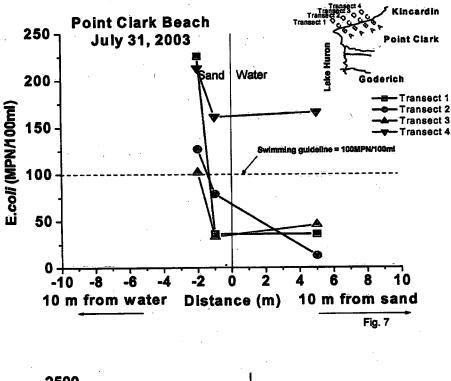












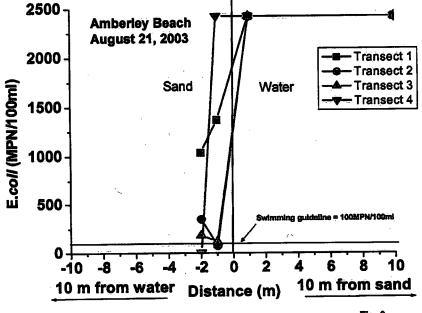
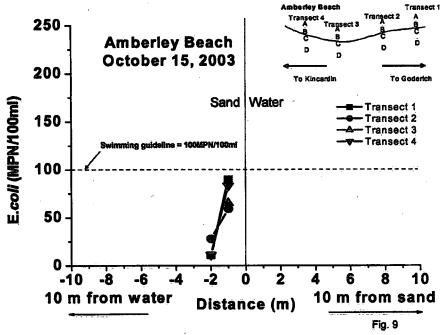
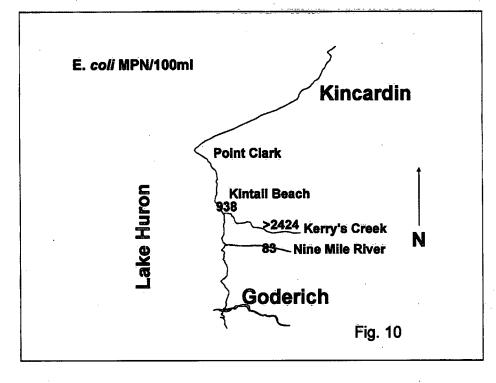
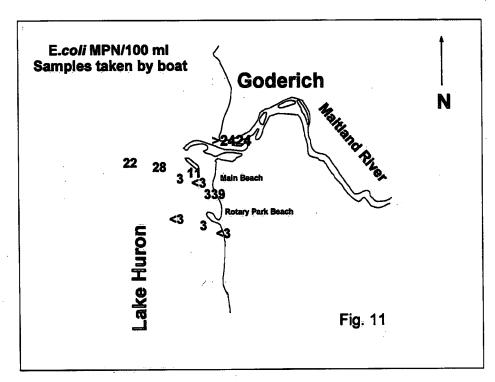


Fig. 8







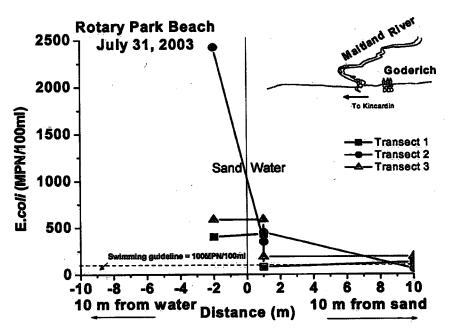
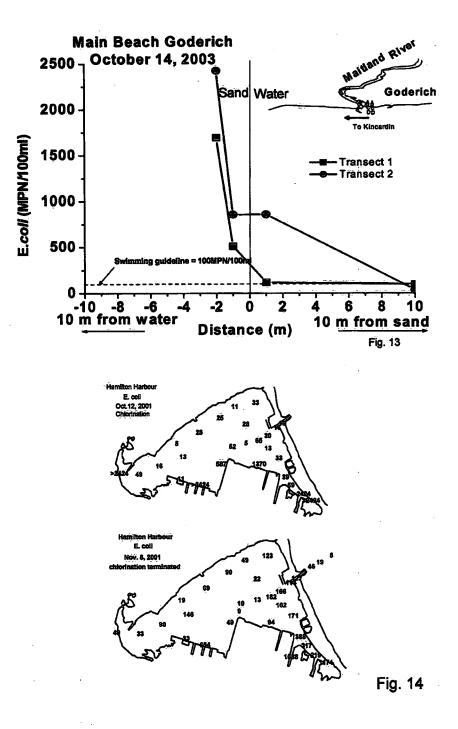
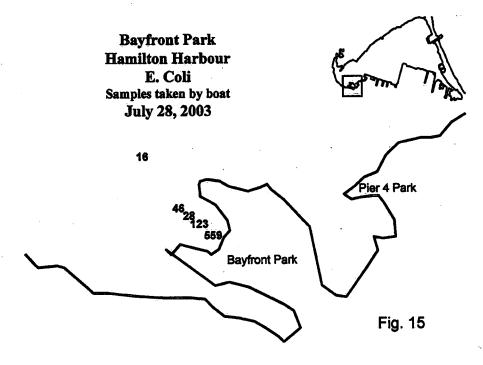
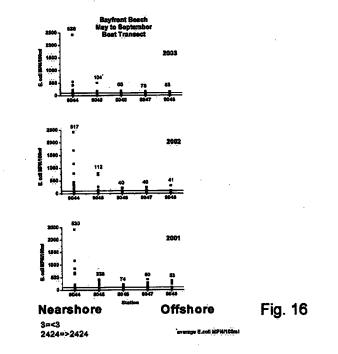
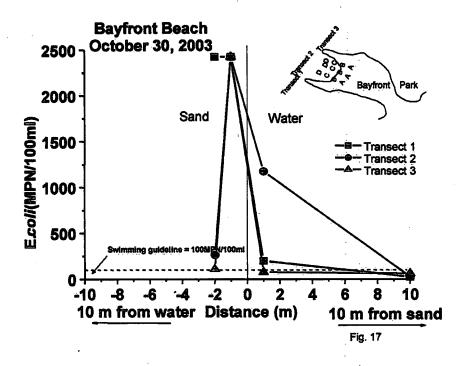


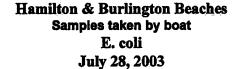
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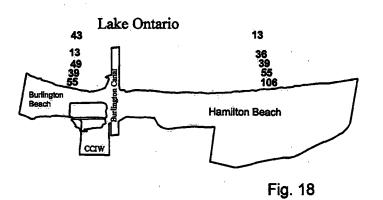


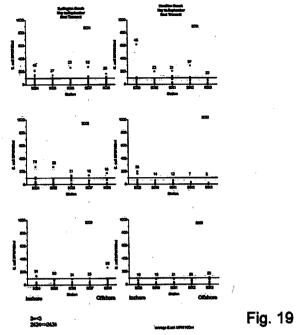


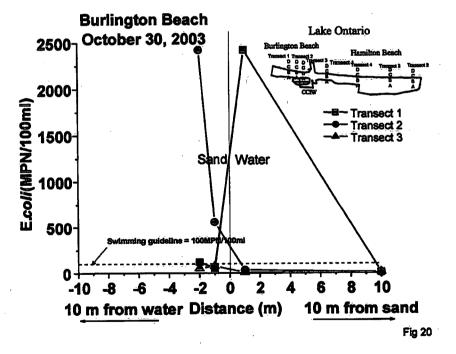


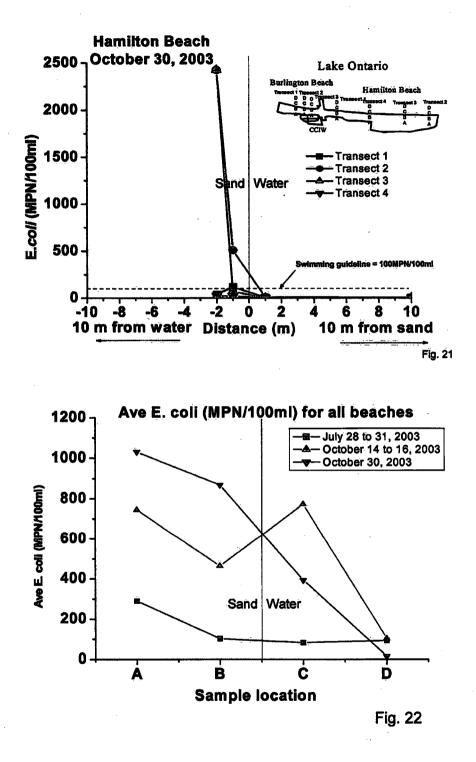
















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