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MICROBIAL SOURCE TRACKING OF FECAL POLLUTION AT TORONTO'S CENTRE ISLAND AND KEW BEACHES IN 2004

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NWRI Contribution No. 06-007

Microbial Source Tracking of Fecal Pollution at Toronto's Centre Island and Kew Beaches in 2004

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Executive Summary

A study was conducted by the National Water Research Institute, Environment Canada to determine the source of fecal pollution responsible for beach postings at two Toronto beaches in the summer of 2004. The study applied a microbial source tracking (MST) approach to determine the source of fecal contamination at Centre Island and Kew Beaches. Since microbial source tracking is still an emerging field, additional lines of evidence were sought from monitoring microbial water quality at the beaches and observing the numbers of animals and their fecal droppings in the beach areas.

The microbial source tracking study was based on a "library-dependent" approach using the water quality indicator bacterium *Escherichia coli*. This indicator is used by the City of Toronto and many other agencies across Canada to detect the presence of fecal contamination, and to make beach posting decisions. The library-dependent MST approach was based on building a library of many *E. coli* isolates collected from "known" sources of fecal pollution near the beach study areas (e.g. bird droppings, municipal wastewater). At the same time, *E. coli* isolates of "unknown" source were collected from beach water and sand, and their similarity was compared to the fecal isolates to make fecal source inferences. The study used antibiotic resistance analysis and rep-PCR DNA fingerprinting MST methods to compare the similarity of *E. coli* isolates.

The numbers of *E. coli* were monitored weekly in wet foreshore sand at the water's edge and in water at different depths (ankle, knee, and chest depths) at several transects at each beach. *E. coli* numbers were generally higher at Centre Island Beach than at Kew Beach over the summer. *E. coli* numbers were highest in ankle depth waters, and these numbers were often 10 to 100 times higher than those in chest depth waters. Beach posting decisions based upon water samples collected at ankle depth, or even knee depth, would have resulted in more beach postings. High numbers of *E. coli* (up to 501 colony forming units / gram dry sand) were found in the wet foreshore sand at the waters edge, particularly at Centre Island Beach. This is consistent with findings at other beaches in the Great Lakes area, and indicates that beach sand may be able to serve as a reservoir for *E. coli*. The implications of higher numbers of *E. coli* in sand and shallow water where children play are starting to be investigated by microbiology researchers. It remains uncertain whether high numbers of *E. coli* in sand are indicative of a public health risk in the same way that high numbers of *E. coli* in water are indicative of public health risks. It is also unknown how wave action to resuspend *E. coli* in sand from previous contamination events may complicate water quality assessments and microbial source tracking studies.

The only animals observed at Centre Island Beach were birds. Each week, there were usually 100-250 gulls, 25-50 Canada geese, and a few mallard ducks on the beach, on the breakwall, or swimming in beach waters. Droppings from gulls and geese were regularly observed within two metres of the waters edge (Canada geese droppings were observed rolling in waves washing onshore at times). The number of gull droppings could reach close to 1000 at times over a two hundred metre stretch of Centre Island Beach. Gulls and Canada geese were also regularly observed swimming and perched on offshore rocks at Kew Beach, although they were fewer in number than at Centre Island Beach. Few bird droppings were observed along Kew beach, although they were regularly deposited on the rock outcroppings just off from Kew Beach. Although dogs were commonly seen in the area around Kew Beach, dog droppings along the beach were rare.

A microbial source tracking library of 2260 *E. coli* isolates was collected from Toronto fecal sources including the droppings from gulls, geese, ducks, swans, cormorants, dogs and cats, and from the municipal wastewater at the Ashbridges Bay Sewage Treatment Plant. Simultaneous weekly water and sand sampling over the summer led to the collection of an additional 3183 waterborne and 1141 sandborne *E. coli* isolates from Centre Island and Kew Beaches. As expected, the *E. coli* from municipal wastewater showed higher frequencies of antibiotic resistance than the *E. coli* from wildlife like gulls and

geese. A discriminant function calculated from antibiotic resistance data for the known fecal source *E. coli* provided an average rate of correct classification of 63 % for discriminating between *E. coli* from bird (gull, Canada geese, duck), pet (dog and cat), and municipal wastewater sources of fecal pollution. While not 100% reliable, this is comparable to what has been used in other microbial source tracking studies, and is much better than a random classification rate of 33%. In parallel, 2696 *E. coli* from the Toronto library were studied by a rep-PCR DNA fingerprinting technique. A cluster analysis of the DNA fingerprint data for the known fecal source *E. coli* provided an average rate of correct classification of 53% (67 % without clones removed) for discriminating between *E. coli* from the three fecal sources: bird, pet, and municipal wastewater.

The results of the antibiotic resistance and DNA fingerprinting analyses were consistent with results from *E. coli* monitoring and animal dropping observations. Both MST methods indicated a predominance of *E. coli* contamination from birds at Centre Island Beach rather than from municipal wastewater or pets. Relative fecal contributions from birds were highest in wet sand and shallow ankle and knee depth waters, particularly at locations along the beach where birds were most common. These fecal contributions coincided with times of presence of increased numbers of birds on the beach. Fecal pollution sources at Kew Beach were more mixed. Although *E. coli* from birds were usually prominent at Kew Beach, *E. coli* from municipal wastewater were more prominent than at Centre Island Beach. Interestingly, at both beaches, the relative proportion of *E. coli* contributions from municipal wastewater often rose with increasing water depth. A nearby stormwater outfall, or other unrecognized municipal wastewater source, may be contributing to fecal contamination of Kew Beach at times. It is possible that *E. coli* from municipal wastewater sources may be entering beach waters sporadically (e.g. wet weather events) from offshore or longshore currents, while *E. coli* from birds may be entering beach waters more continuously from onshore sources of bird droppings.

This microbial source tracking study found that bird droppings were the most prominent contributor of *E. coli* to the sand and beach waters at Centre Island and Kew Beaches in the summer of 2004. As the field of microbial source tracking evolves, it will be important to apply the techniques as part of multiple lines of evidence in determining the basis of Toronto beach postings. It will be important to track scientific advances in this field in order to apply the best available tools for discriminating between diverse fecal pollution sources in the Toronto area. It is likely that fecal pollution sources such as growing populations of gulls and Canada geese, and municipal wastewater sources, will continue to impact beaches along the Toronto waterfront unless the sources are identified and mitigated.

Résumé

L'Institut national de recherche sur les eaux (INRE) d'Environnement Canada a mené une étude pour déterminer la source de contamination fécale responsable des restrictions de baignade affichées à deux plages de Toronto à l'été 2004. Nous avons appliqué une méthode de suivi des sources microbiennes (SSM) aux plages Centre Island et Kew. Le SSM étant un champ d'activité tout nouveau, nous avons cherché à obtenir d'autres sources de données en surveillant la qualité microbienne de l'eau des plages et en observant le nombre des animaux présents et leurs excréments laissés dans le secteur des plages.

L'étude de SSM, avec banque de matériel microbien de référence, a consisté à relever l'indice bactérien de qualité de l'eau *Escherichia coli*. Cet indice est celui qu'utilise la Ville de Toronto et de nombreuses autres administrations au Canada pour déterminer la présence de contamination fécale et pour décider des affichages aux plages. Cette démarche de SSM avec matériel de référence s'est fondée sur l'établissement d'une collection d'un grand nombre d'isolats d'*E. coli* de sources « connues » de pollution fécale à proximité des plages (fientes d'oiseaux, eaux usées, etc.). Concurremment, nous avons examiné divers isolats d'*E. coli* de sources « inconnues » recueillis dans l'eau et le sable des plages afin de trouver les similitudes entre les isolats, pour en déduire les sources fécales. Nous avons procédé à une analyse de la résistance antibiotique et appliqué des méthodes de SSM de détermination des empreintes génétiques par rep-PCR de l'ADN afin de comparer les similitudes des isolats d'*E. coli*.

Nous avons surveillé la présence des bactéries *E. coli* chaque semaine, dans le sable mouillé tout au bord de l'eau et dans l'eau à diverses hauteurs (à la cheville, au genou, à la poitrine) dans divers transects de chaque plage. En règle générale, les quantités d'*E. coli* étaient plus grandes à la plage Centre Island qu'à la plage Kew, et ce, tout au long de l'été. Les concentrations étaient les plus élevées dans les eaux à hauteur de cheville, souvent de 10 à 100 fois plus élevées que celles à hauteur de poitrine. Les décisions de restreindre la baignade fondées sur des échantillons pris à hauteur de cheville ou même à hauteur de genou auraient donc accru le nombre des avertissements aux plages. Nous avons trouvé des concentrations élevées d'*E. coli* (jusqu'à 501 unités formant colonies par gramme de sable sec) dans le sable mouillé à la limite de l'eau, tout particulièrement à la plage Centre Island. Cela est conforme aux constats à d'autres plages des Grands Lacs et indique la possibilité que le sable des plages puisse servir de réservoir pour les bactéries de type *E. coli*. Les chercheurs en microbiologie commencent à s'intéresser aux implications de la présence en fortes concentrations de ces colibacilles dans le sable et l'eau très peu profonde, où jouent les enfants. Il est trop tôt pour savoir si les grandes concentrations d'*E. coli* dans le sable sont indicatrices d'un danger pour la santé publique, de la même façon que les concentrations élevées d'*E. coli* dans l'eau le sont. En outre, nous ne savons pas comment le mouvement des vagues venant remettre en suspension les colonies d'*E. coli* laissées dans le sable par des contaminations antérieures pourrait venir compliquer les évaluations de la qualité de l'eau et les études de SSM.

Les seuls animaux observés à la plage Centre Island étaient des oiseaux. Chaque semaine, nous avons compté habituellement entre 100 et 250 goélands et de 25 à 50 Bernaches du Canada, en plus de quelques Canards colverts, sur la plage, sur la digue ou nageant dans les eaux de la plage. Nous avons régulièrement observé des excréments de goélands et de bernaches à moins de deux mètres de la rive (à l'occasion, on a observé des excréments de bernaches roulant dans les vagues venant mourir sur la plage). À certaines occasions, le nombre des excréments de goélands répertoriés pouvait atteindre le millier, sur une étendue de deux cents mètres de la plage Centre Island. Nous avons aussi régulièrement observé des goélands et des bernaches nageant ou perchés sur des rochers dans l'eau à la plage Kew, même s'il y en avait moins qu'à la plage Centre Island. Le long de la plage Kew, nous avons observé peu d'excréments d'oiseaux, même si les rochers sortant de l'eau près de la plage en étaient régulièrement enduits. Bien que nous ayons couramment vu des chiens dans le périmètre de la plage Kew, les excréments de chiens étaient rares le long de la plage.

Nous avons obtenu une collection de SSM de 2 260 isolats d'*E. coli* à partir de sources fécales de Toronto, y compris les excréments de goélands, de bernaches, de canards, de cygnes, de cormorans, de chiens et de chats, ainsi que des eaux usées de l'usine d'épuration d'Ashbridges Bay. L'échantillonnage hebdomadaire simultané de l'eau et du sable tout au long de l'été nous a permis d'ajouter à la collection 3 183 isolats prélevés dans l'eau et 1 141 isolats prélevés dans le sable des plages Centre Island et Kew. Comme on s'y attendait, les colibacilles provenant des eaux usées municipales démontraient plus souvent de résistance antibiotique que ceux provenant de la faune comme les goélands et les bernaches. Une fonction discriminante calculée à partir des données de résistance antibiotique de la source connue d'*E. coli* nous a fourni un taux moyen de classification correcte de 63 % permettant de discerner entre les souches d'*E. coli* provenant d'oiseaux (goélands, bernaches, canards), d'animaux de compagnie (chiens et chats) et des sources de pollution fécale provenant des eaux usées. Même si ce test n'est pas absolument fiable, il est comparable en qualité à ce qui est utilisé dans d'autres études de SSM; en outre, il est bien meilleur que le taux de 33 % d'une classification aléatoire. En parallèle, nous avons étudié 2 696 isolats d'*E. coli* de la collection de Toronto en nous servant de la technique de détermination des empreintes génétiques par rep-PCR de l'ADN. Une analyse typologique des données d'empreintes génétiques des sources fécales connues d'*E. coli* a révélé un taux moyen de classification correcte de 53 % (67 % sans le retrait des clones) dans la distinction entre les colonies d'*E. coli* des trois sources fécales, soit celles des oiseaux, des animaux de compagnie et des eaux usées municipales.

Les résultats des analyses de résistance antibiotique et des empreintes génétiques allaient dans le même sens que les résultats des relevés des colonies d'*E. coli* et des observations d'excréments animaux. À la plage Centre Island, les deux méthodes de SSM ont indiqué la prédominance de la contamination par *E. coli* provenant des oiseaux sur celle provenant des animaux de compagnie et des eaux usées municipales. La contamination par les oiseaux était à son degré le plus élevé dans le sable mouillé du bord immédiat de la rive et dans les eaux peu profondes, à la hauteur des chevilles et à celle des genoux, tout particulièrement aux endroits de la plage où les oiseaux se tiennent le plus souvent. Ces apports fécaux coïncidaient avec les moments où les oiseaux étaient présents en grand nombre sur la plage. À la plage Kew, les sources de pollution fécale étaient moins polarisées. Même si l'apport d'*E. coli* provenant des oiseaux était habituellement important à la plage Kew, celui provenant des eaux usées municipales était plus important qu'à la plage Centre Island. Curieusement, aux deux plages, la proportion relative des apports d'*E. coli* des eaux usées municipales augmentait souvent de pair avec la profondeur de l'eau. Une décharge d'égout pluvial voisine, ou une autre source d'eau municipale non constatée, pourrait contribuer à la contamination fécale de la plage Kew, à certains moments. Il est possible que des bactéries *E. coli* provenant de sources municipales d'eaux usées entrent sporadiquement dans les eaux des plages (p. ex., en temps de pluie), en provenance de courants littoraux ou de courants d'eaux profondes, alors que les colonies d'*E. coli* provenant d'oiseaux peuvent davantage arriver en continu dans les eaux des plages en provenance de sources terrestres des excréments d'oiseaux.

Cette étude de SSM nous a permis de conclure que les excréments d'oiseaux étaient la principale source d'*E. coli* dans le sable et les eaux des plages Centre Island et Kew, au cours de l'été 2004. À mesure que le domaine du SSM évoluera, il importera d'en conjuguer les techniques avec de multiples autres sources de données pour déterminer les avertissements à afficher aux plages de la région de Toronto. Il sera très important de suivre les percées scientifiques dans ce domaine, pour recourir aux meilleurs outils permettant de discerner entre les diverses sources de pollution fécale de la région de Toronto. On peut s'attendre à ce que certaines sources de pollution fécale, comme celle des populations croissantes de goélands et de bernaches, en plus de celles des eaux usées municipales, continuent d'avoir un impact sur les plages du secteur riverain de Toronto, à moins de trouver les sources et de les limiter.

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1. Introduction

Beach postings resulting from fecal pollution are a growing concern in many areas around the Great Lakes. There is an increasing need to accurately identify the sources of fecal pollution to remediate these sources and prevent future pollution events. Knowing the fecal source is also important for assessing the potential public health risks since different animals and fecal pollution sources can carry different waterborne pathogens.

The National Water Research Institute, Environment Canada conducted a microbial source tracking study in the summer of 2004 to determine the source of fecal contamination responsible for beach postings at two urban Toronto beaches along Lake Ontario. Centre Island Beach and Kew Beach were posted 14 % and 30% respectively of the 2003 swimming season (Environmental Defence, 2004). As a result of the high frequency of postings in 2004, neither beach qualified for designation as Blue Flag beaches for the 2005 swimming season. Despite recent upgrades in municipal wastewater infrastructure along the Toronto waterfront, these beaches have continued to be contaminated by fecal pollution, and the source of this contamination remains uncertain.

Microbial source tracking (MST) is an emerging field that seeks to identify the source of microbial contamination in the environment. The field has been developing rapidly from a growing need to determine the sources of fecal contamination in aquatic environments. Typically, microorganisms collected from aquatic environments like beach waters are characterized by biochemical or genetic methods, and then compared to microorganisms collected from nearby fecal pollution sources. The similarity of the waterborne microorganisms to those from known human or animal fecal sources is used to make inferences about the source of fecal contamination. A variety of methods have been developed for microbial source tracking, and these methods have been recently reviewed (Simpson et al. 2002; Scott et al. 2002; U.S. EPA, 2005a).

Since the field of MST is evolving rapidly, many new methods are also under investigation. The collection of methods for microbial source tracking has often been referred to as a toolbox, with some methods being more relevant to use than others in certain circumstances. At present, there is no single MST method that has emerged as clearly superior to all others (Griffith et al. 2003; Stewart et al. 2003; Stoeckel et al. 2004; U.S. EPA, 2005a; Edge and Schaefer, 2005). The selection of a relevant method will be influenced by factors like the complexity of the environment under study, the number of sources suspected to be implicated in contamination events, and availability of funds and expertise.

While the field of microbial source tracking is growing rapidly, it should be noted that other methods can also be used to track fecal contamination in recreational waters. For example, Martellini et al. (2005) used eukaryotic mitochondrial DNA markers to discriminate between fecal pollution from human, bovine, porcine, and ovine sources in surface waters. This approach was based on detecting the host animal cells that were sloughed off into the gastrointestinal tract with feces. In addition, chemical tracers have been used, most commonly to detect chemicals associated with human wastes (Glassmeyer et al. 2005). As the highest concentration of these chemicals is typically found in wastewater treatment plants, they have been proposed for

tracking human fecal waste pollution. For example, fecal sterols and fecal stanols have been proposed as markers of fecal pollution (Elhmmali et al. 2002). Caffeine, detergents, fragrance materials, and the secretory immunoglobulin A (sIgA) in the intestinal mucosa, and lacrimal and salivary glands are other chemicals suggested as markers (Simpson et al. 2002). In addition, the use of fluorometry to detect laundry brightners has been used to track fecal pollution from municipal wastewater sources. Many of these chemical methods remain to be widely tested and results have uncertain linkages back to *E. coli* and waterborne pathogen concentrations that are the concern for beach posting decisions.

1.1 Microbial Source Tracking Approach

The microbial source tracking study described in this report was conducted based upon a library-dependent approach using the indicator *Escherichia coli*. While there are also library-independent MST methods (e.g. based upon *Bacteriodes* sp. markers), key markers for fecal sources such as birds are still lacking and it was deemed these methods were insufficiently validated in the field to date. Library-dependent MST methods are based upon choosing a fecal indicator microorganism (e.g. *E. coli*) and establishing a reference library of characteristics of individual isolates of *E. coli* obtained from known fecal pollution sources. For example, a library could be a database of DNA fingerprints of *E. coli* isolates obtained from relevant fecal pollution sources such as animal feces, animal waste lagoons, septic tanks, or municipal wastewater effluents. The similarity of DNA fingerprints of *E. coli* isolates obtained from beach waters ("unknowns") can then be compared to the DNA fingerprints in the library ("knowns") to make inferences about the source of the waterborne *E. coli* isolates. Sound taxonomic identification of the fecal and waterborne isolates is necessary in order to ensure similarity comparisons are warranted.

The most common fecal indicator microorganisms used in library-dependent methods to date have been the bacteria *E. coli* and *Enterococcus* spp. *E. coli* is a common inhabitant of warm-blooded animal guts, it is relatively easy to isolate and culture in the lab, and it is recommended for monitoring water quality at freshwater beaches (Health and Welfare Canada, 1992). There are also advantages in using the same indicator for both fecal pollution source tracking and for making water quality decisions pertaining to posting recreational waters. The similarity between isolates of a selected fecal indicator microorganism can be measured by either phenotypic profiling or genotypic fingerprinting methods. The approach described in this report was based upon measuring the similarity of *E. coli* isolates by antibiotic resistance analysis (ARA) and rep-PCR DNA fingerprinting.

Phenotypic library-dependent methods are based upon making cellular or physiological comparisons between isolates of the selected fecal indicator microorganism (usually *E. coli*). Antibiotic resistance analysis (ARA) has been the most common phenotypic approach used to date in MST studies (Kaspar et al. 1990; Wiggins, 1996; Hagedorn et al. 1999; Harwood et al. 2000; Wiggins et al. 2003). In antibiotic resistance profiling approaches, bacterial isolates can be inoculated onto many agar plates, each containing a specific antibiotic concentration. The isolates are incubated overnight on the agar plates, and their growth is compared to their growth on a control plate (i.e. same agar without antibiotics). The growth of each isolate on the agar plates is used to develop a profile of its resistance to many antibiotics. This antibiotic resistance

approach is based on the assumption that human and domestic animal gut bacteria are exposed to different antibiotics in medical and veterinary treatments, and that these gut bacteria will develop different resistance profiles. Since wildlife species do not receive direct antibiotic treatments, their gut bacteria are typically less resistant to antibiotics. ARA has been used in many MST studies since it is relatively inexpensive, and requires less specialized laboratory equipment and expertise. While antibiotic resistance approaches for MST may be useful for discriminating between a few fecal sources (e.g. wastewater and wildlife) in a small area (e.g. local beach), they may not be applicable for resolving complex, multiple host discrimination challenges over larger spatial and temporal domains.

Genotypic library-dependent methods are based on making a DNA sequence or DNA fingerprint comparison between isolates of the selected fecal indicator microorganism (usually *E. coli*). DNA fingerprints for isolates are usually compared using a commercially available software package such as Bionumerics (Applied Maths). A variety of genotypic methods have been applied to microbial source tracking including rep-PCR (Dombek et al. 2000), ribotyping (Carson et al. 2001), and AFLP (Guan et al. 2002). Rep-PCR has been among the most common genotypic library-dependent methods used in MST studies to date. Rep-PCR is based on repetitive element DNA sequences that have been used in PCR assays since the early 1990's to characterize clinical bacteria (Versalovic et al. 1991). Several types of repetitive elements have been identified in bacteria, three of which have been used as PCR primers in MST studies: repetitive extragenic palindromic (REP) sequences, enterobacterial repetitive intergenic consensus (ERIC) sequences, and BOX sequences (Scott et al. 2002). Collectively, these methods are known as rep-PCR. These repetitive elements are short DNA sequences scattered along the bacterial chromosome. Where they occur close enough, they can serve as attachment sites for PCR primers to amplify neighbouring stretches of DNA producing many copies of different sized DNA fragments. The DNA fragments between repetitive elements can then be separated by gel electrophoresis into ladder-like banding patterns, and subjected to digital imaging and DNA fingerprint analysis. Rep-PCR is a relatively simple genotypic library-dependent method for microbiology laboratories, and it does not require expensive equipment other than a PCR cycler. However, reproducibility of rep-PCR fingerprints between laboratories can be problematic and a standardized method has yet to be developed.

The following report summarizes a number of lines of evidence to determine the likely sources of fecal contamination at Centre Island and Kew Beaches in the summer of 2004. These lines of evidence include: *E. coli* monitoring in beach water and sand; observations of animal numbers (and their droppings) in beach areas; and results from ARA and rep-PCR DNA fingerprinting methods of microbial source tracking. The use of multiple lines of evidence will continue to be important as the field of MST evolves and a better understanding of the advantages and limitations of MST methods emerges.

2. Methods

2.1 Water Sampling

Water samples were collected at Kew Beach and Centre Island Beach each Monday morning over the bathing season. Water samples were collected by wading out from the shoreline for ankle and knee depth samples. Chest depth samples were obtained from the City of Toronto boat-based sampling crew on the same morning. All water samples were collected in sterile bottles and returned on ice to the NWRI lab for analysis within several hours of collection. Samples were collected at ankle, knee and chest depths along set transects perpendicular to the shoreline. Two transects (1 and 2) were used at Centre Island Beach that were equivalent to the City's transects 9E and 10E on each side of the pier (see Figure 1). Three transects (1, 2 and 3) were used at Kew Beach that were equivalent to the City's transects 34E, 35E, and 36E (and some 37E) (see Figure 2). While it was believed that there were no longer any discharging combined sewer overflow (CSO) or stormwater outfalls around Kew Beach area as a result of new storage tanks, water samples were also collected at an old stormwater outfall west of transect 1 (roughly equivalent to the City's transect 32E). *E. coli* levels were low at this site but isolates were kept for typing to confirm this outfall was capped. For all water sampling, two water samples were collected at each transect depth location, and *E. coli* counts are presented as the mean of the two replicates.

2.2 Sand Sampling

Sand samples were obtained from the wet foreshore sand within a metre of the water line, and to a depth of about 6 cm, using a sterile plastic core (diameter = 2.5 cm). About 20 grams of wet sand were recovered from the cores and placed in whirlpak bags, and returned to the lab on ice for analysis within several hours of collection. Two sand samples were collected at each transect at the same time as water samples, and *E. coli* counts in sand are presented as the mean of the two core replicates.

2.3 Fecal Sampling

Fecal sampling was conducted weekly over the summer of 2004 on a simultaneous basis with water and sand sampling. Municipal wastewater samples (raw untreated influent and final treated effluent) were collected from the Ashbridges Bay Sewage Treatment Plant. Final treated effluent samples were obtained from a refrigerated composite effluent sample that receives hourly samples of final treated effluent over a continuous 24 hour period. Samples of feces from dogs and birds (gulls, Canada geese, mallard ducks, cormorants, swans) were obtained from fresh fecal droppings on the ground. A number of dog fecal samples were collected in the area around Kew Beach. Canada geese fecal samples were mostly collected from around Ashbridge's Bay Park and on Centre Island Beach. Gull fecal samples were mostly collected from the Leslie Spit gull colony (Tommy Thompson Park) and on Centre Island Beach. Mallard duck fecal samples were mostly collected from a pond near the animal farm on Centre Island. Cormorant fecal samples were obtained from the Leslie Spit colony. Additional samples of feces from fresh droppings of stray dogs and cats were obtained from Toronto's Exhibition Place and Scarborough SPCA facilities. Fecal samples were obtained using sterile culturette cotton swabs (BD Inc.). The swabs were stored on ice and returned to the lab for analysis within several hours of collection.

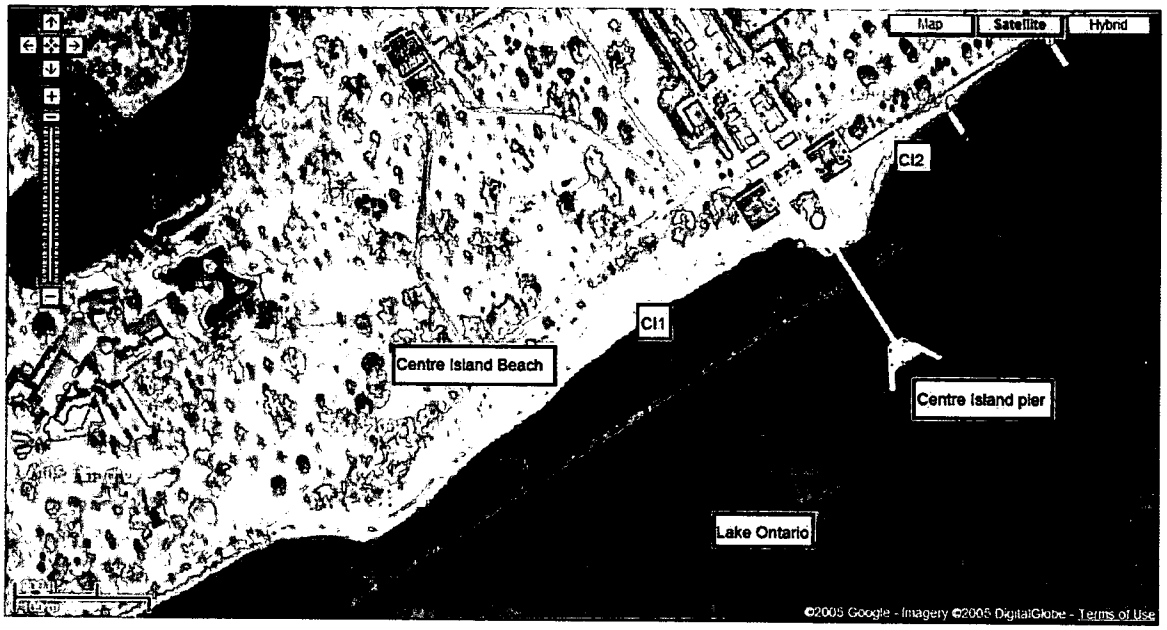
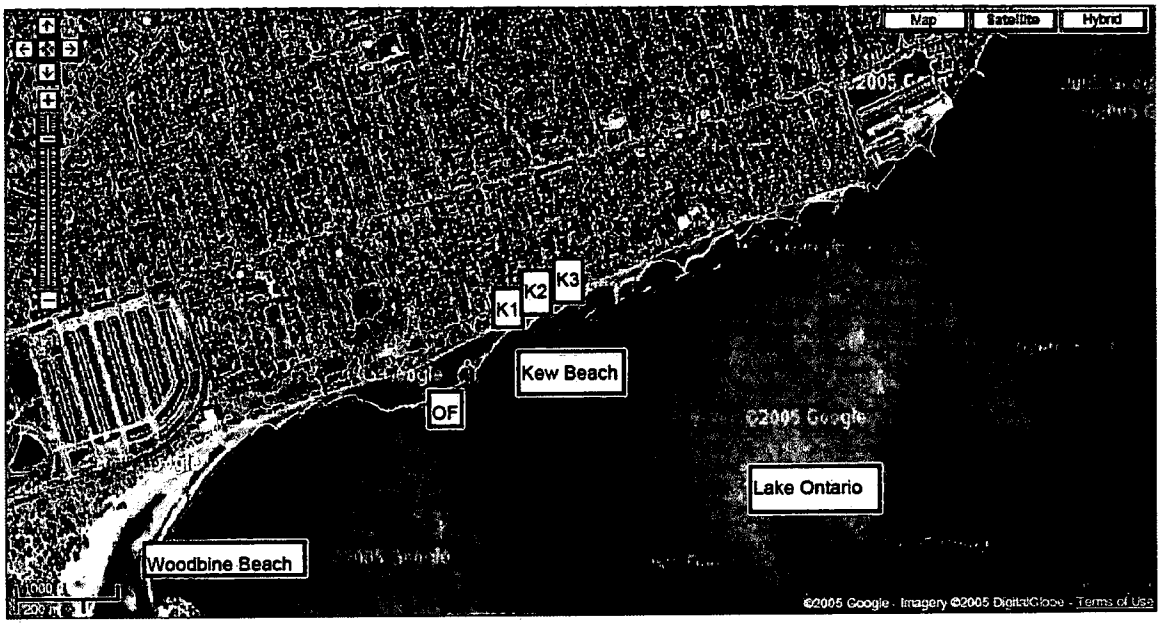


Figure 1. Centre Island Beach from aerial perspective (top) and facing west near the Centre Island Pier (bottom). Aerial perspective shows two transects CI1 and CI2.



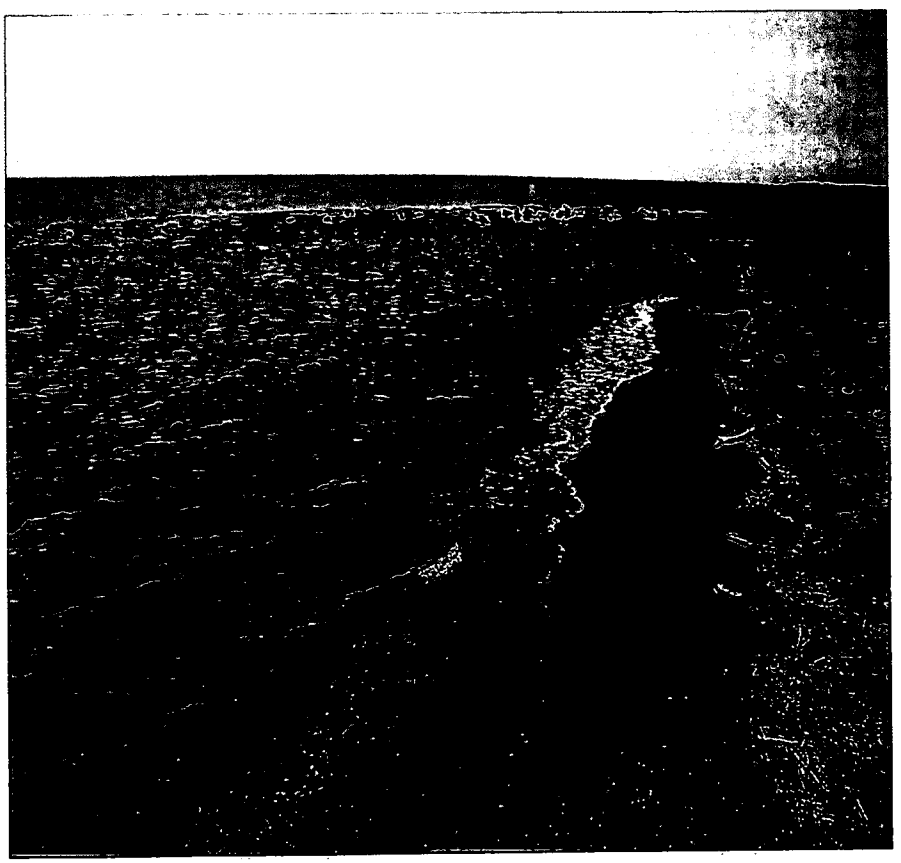


Figure 2. Kew Beach from aerial perspective (top) and facing west near Kew Beach transect 3 (bottom). Aerial perspective shows the location of three transects K1, K2 and K3, and location of stormwater outfall sampled (OF). Birds visible on rock outcroppings between transects 2 and 3 (bottom).

2.4 E. coli Enumeration and Isolation

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

Sand samples were analyzed by a blender-based method and *E. coli* enumeration was expressed as colony forming units / gram of dry sand. Wet sand was weighed to 10 grams and placed into 150 ml of phosphate buffer in a Waring blender. The sand was blended for 1 minute 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 and conversion factor.

Fecal swabs were streaked onto mFC agar (Difco Inc.) and incubated at 44.5°C for 18 hours. Isolates showing a typical dark blue colour on mFC agar were selected for further *E. coli* identification confirmation tests.

Up to 12 *E. coli* isolates were randomly selected from DC agar plates for a given water or sand sample. Between three to five *E. coli* isolates were randomly selected from mFC agar plates for a given fecal swab. These isolates were picked with a sterile toothpick and streak plated 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 at -80°C in tryptic soy broth and 15% (v/v) glycerol. *E. coli* ATCC 29194 and *Klebsiella* ATCC 33495 were used as a positive and negative control respectively during confirmation tests.

2.5 Antibiotic Resistance Analysis

E. coli from 96 well Matrix plates (Matrix Technologies Corp. Hudson, NH) 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 the *E. coli* isolates to the surface of rectangular tryptic soy broth agar plates. The 12 antibiotics (and 3 concentrations of each) used are identified in Table 1. Agar plates were incubated for 18 hours at 37°C and growth of *E. coli* isolates on plates with antibiotics were compared to their growth on control plates without antibiotics. 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. An *E. coli* isolate was considered resistant to an antibiotic if its growth was > 0.73 of its growth on a control plate without the antibiotic. Data were recorded as binary data and a logic model was applied to correct occasional data indicating susceptibility of a strain to a lower concentration of an antibiotic when it was also resistant to a higher concentration.

Negative control wells (blank wells) and positive control wells (wells with other *E. coli* strains with known profiles) were included on antibiotic resistance plates.

Table 1. Antibiotics (and concentrations) used in antibiotic resistance analyses.

Antibiotic	Concentration (µg/ml)	Antibiotic	Concentration (µg/ml)
ampicillin	5, 16, 32	kanamycin	1, 5, 16
cephalothin	5, 16, 32	oxytetracycline	1, 5, 16
chloramphenicol	5, 16, 32	penicillin G	25, 50, 100 U
chlorotetracycline	20, 40, 80	streptomycin	1, 5, 16
erythromycin	25, 50, 100	sulfamethoxazole	50, 200, 512
irgasan (triclosan)	0.01, 0.1, 0.5	tetracycline	1, 5, 16

Prior to statistical analysis of antibiotic resistance profiles, isolates with identical antibiotic resistance profiles (phenotypic clones) from the same individual feces sample were removed to reduce library bias. The resulting Toronto library of fecal *E. coli* antibiotic resistance profiles was analyzed by discriminant function analysis (SAS, 1999 - PROC DISCRIM procedure) to develop a discriminant function for correctly classifying known fecal source *E. coli* isolates. Since the binary data could not be assumed to have a multivariate normal distribution, a nonparametric nearest neighbour (k=5) approach to developing the discriminant function was taken. Ritter et al. (2003) reviewed library-based MST statistical methods and considered this approach to be practical. The discriminant function was calculated to discriminate between three likely sources of urban fecal pollution sources: birds, pets, and municipal wastewater. An *E. coli* isolate was classified as "unknown" when the discriminant function could not classify the isolate to one of the three categories with a probability of greater than 0.6. The relative proportions of the three fecal pollution sources were compared using a variety of different classification probability thresholds ranging between 0.5 and 0.999 to ensure the prominence of a particular fecal source was consistent and not an artifact of setting the probability threshold at 0.6. Other exploratory discriminant functions (e.g. 2-way between birds and wastewater/pets; 4-way between gull, geese, dog, and wastewater; and 8-way) were also calculated, but these functions were considered less useful or reliable.

The Toronto fecal library of antibiotic resistance profiles was evaluated by several techniques (see U.S. EPA, 2005a) prior to applying the discriminant function to classify unknown beach water and sand *E. coli* isolates. Rarefaction analysis of the diversity of *E. coli* antibiotic resistance profiles for each fecal host was used to assess the representativeness of the library. The average rate of correct classification (ARCC) for the discriminant function was calculated using a less biased jackknife-based crossvalidation method rather than a resubstitution method. A minimum detection percentage was calculated following Whitlock et al. (2002) and Wiggins et al. (2003) in order to assess the lower limit for considering that a fecal source was actually being detected in water or sand samples. This limit was calculated based on obtaining an average rate of misclassification for the discriminant function, and then adding a conservative detection factor of 4 times the standard deviation of misclassification rates.

2.6 Rep-PCR DNA fingerprinting

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 hours. 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 minutes at 3050 x 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 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. 15 µl of master mix was created and added to achieve the following concentrations in the final 25 µl solution: 1 X 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 and ultrapure water. The amplification cycling conditions were as follows: initial denaturation of 2 min at 94°C followed by 35 cycles of 20 sec at 94°C, 20 sec 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. 3 µl of sample combined with loading dye were loaded into the wells. 3 µl of a ½ dilution of Promega 1 kb ladder were used as standards 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 hrs). 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 with UV light and image capture 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 was used, however since most analyses were conducted using the lane curve rather than band matchings, manual alterations were not made. DNA fingerprint comparisons were based on using a Pearson coefficient and UPGMA clustering. Isolates that did not have at least one band with a volume of 2000 were removed to exclude failed amplifications. *E. coli* isolates from the same fecal dropping or wastewater sample with greater than 90% similarity were removed to reduce bias due to clones within a sample. The DNA fingerprint clustering approach was evaluated by calculating jackknife-based average rates of correct classification using a maximum similarity measure.

Similar to the ARA analysis, the rep-PCR DNA fingerprinting technique was applied to discriminate between three sources of fecal pollution: birds, pets, and municipal wastewater. The *E. coli* water and sand isolates were then compared to the fecal library isolates using a nearest neighbour similarity method (K=5) to classify them. Where water and sand isolates did not match closest with at least three isolates (out of five nearest neighbours) from a particular fecal source, they were classified as "unknown". Since there was some imbalance in samples size between the fecal source classes (e.g. n= 189 for pets), 2-way source clustering, and other exploratory 3-way clustering analyses were performed using an average and maximum similarity measurement. These analyses gave general source classification results consistent with the nearest neighbour method.

3. Results

3.1 E. coli Water Monitoring

3.1.1 Centre Island

Weekly monitoring results for waterborne *E. coli* at Centre Island are presented in Figure 3. The concentrations of *E. coli* were usually an order of magnitude higher in ankle depth water than chest depth water. This depth trend was consistent at both transects. There were no strong correlations between *E. coli* concentrations at different depths at both Centre Island and Kew beaches (Figure 4).

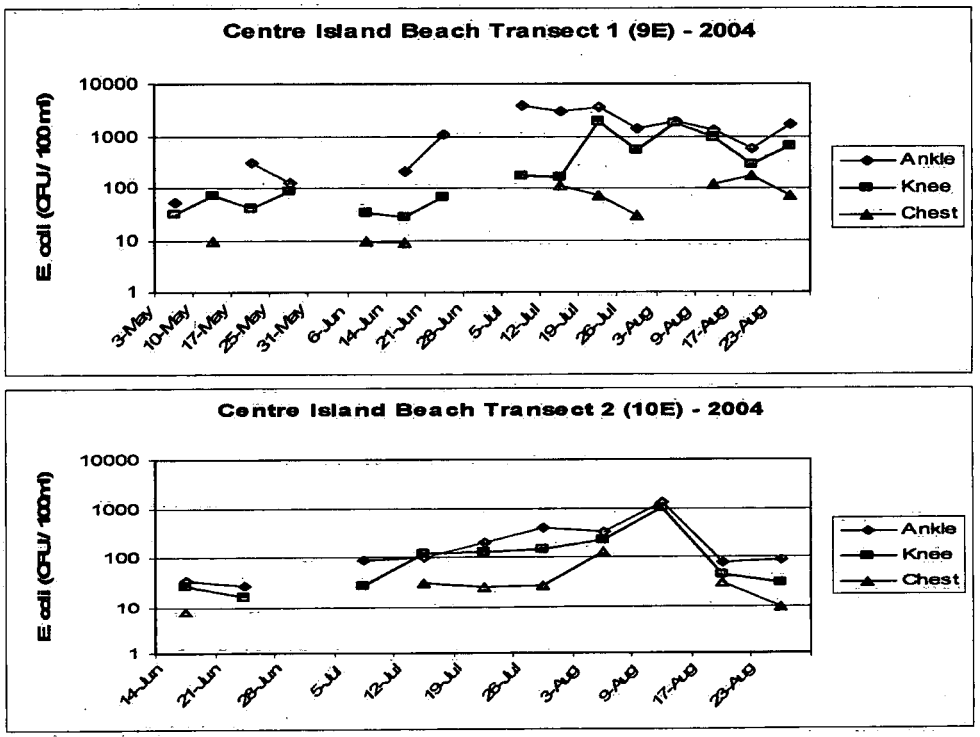


Figure 3. Weekly monitoring of E. coli from surface water samples in different water depth zones at transect 1 (top) and transect 2 (bottom) along Centre Island Beach in 2004.

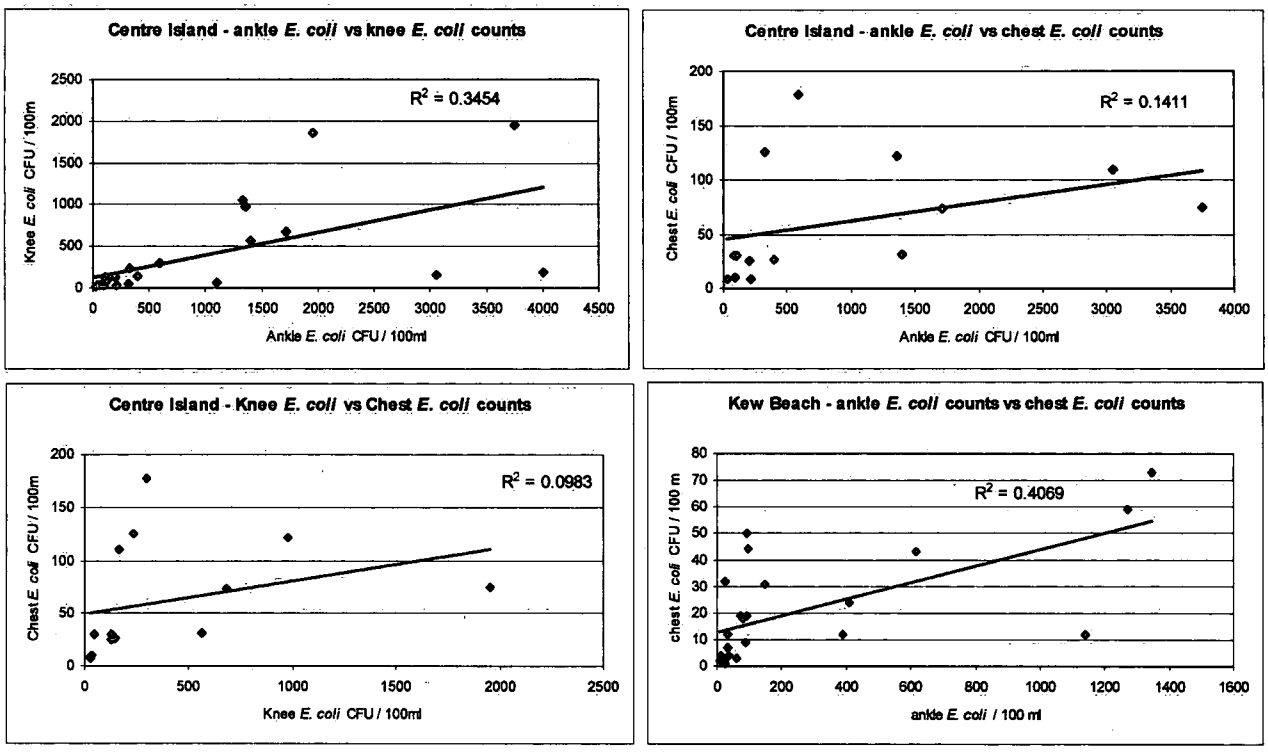


Figure 4. Correlations between E. coli concentrations in water samples from different water depth zones at Centre Island and Kew Beaches in 2004.

Concentrations of *E. coli* at transect 1 along Centre Island Beach west of the pier were generally higher than those at transect 2 east of the pier. In addition, the gradient differences between *E. coli* counts in ankle vs chest depth was greater for transect 1 than transect 2. These two parts of Centre Island Beach are quite different; with transect 1 being more sheltered from offshore currents by the rock breakwall. Transect 2 is likely more exposed to offshore currents from Lake Ontario since the breakwall does not extend far enough to protect the transect location entirely. These two beach transects are likely to have very different physical characteristics (e.g. less wave action and influence of offshore currents at transect 1).

3.1.2 Kew Beach

Weekly monitoring results for waterborne *E. coli* at Kew Beach are presented in Figure 5. In general, *E. coli* numbers were lower at Kew Beach than at Centre Island Beach. Again, the concentrations of *E. coli* were often an order of magnitude higher in ankle depth water than chest depth water. This depth trend was consistent at all three transects. Concentrations of *E. coli* at transect 1 on Kew Beach were generally lower than transects 2 and 3. Transects 2 and 3 differed from transect 1 in having much higher *E. coli* counts at ankle and knee depths in late July and early August. Gulls and geese regularly perched on rock croppings about 10-20 meters offshore near Kew Beach transects 2 and 3.

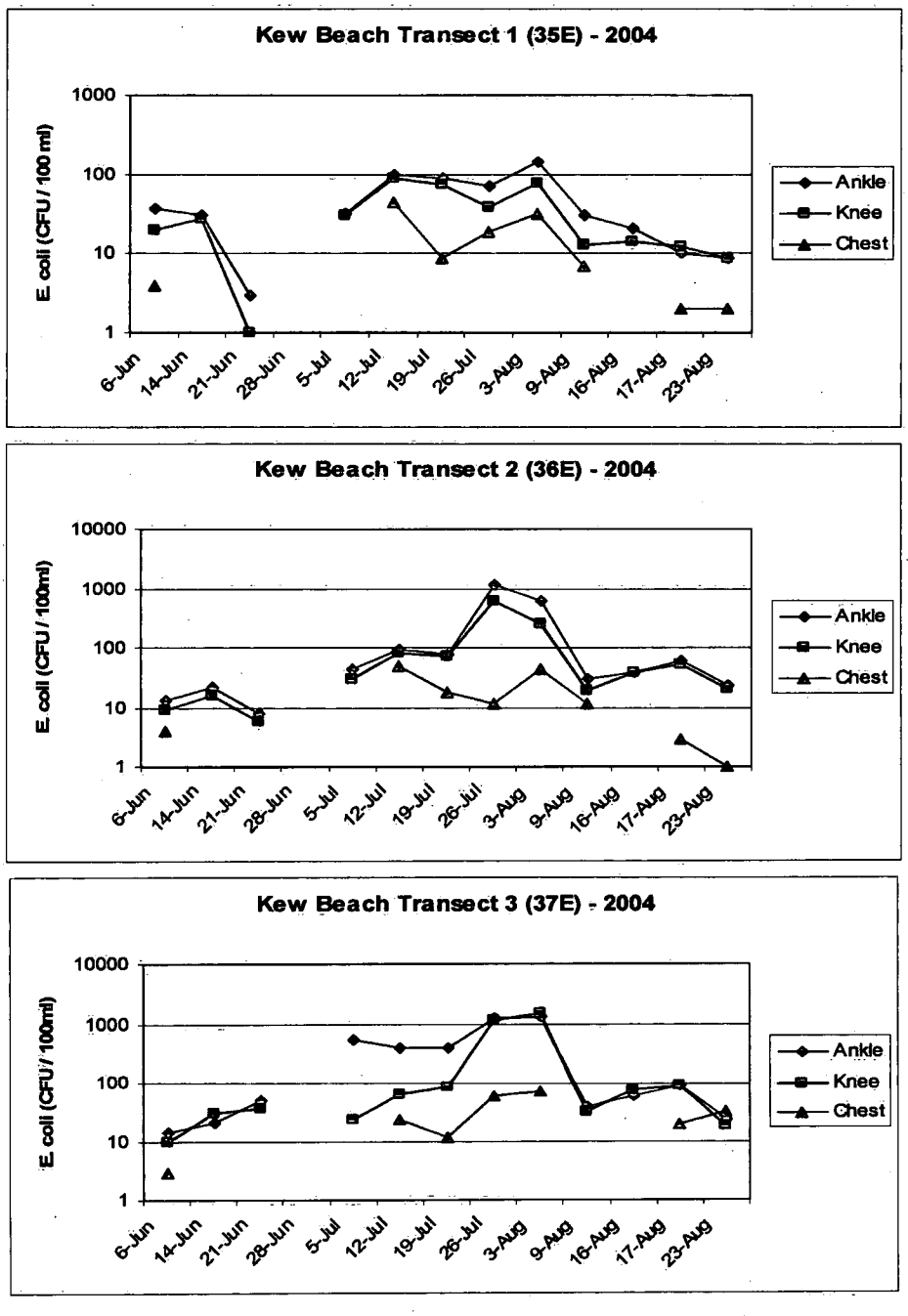


Figure 5. Weekly monitoring of E. coli from surface water samples in different water depth zones at transect 1 (top), transect 2 (middle), and transect 3 (bottom) along Kew Beach in 2004.

3.2 E. coli Sand Monitoring

3.2.1 Centre Island

Weekly monitoring results for *E. coli* in the wet foreshore sand at Centre Island Beach are presented in Figure 6. The concentrations of *E. coli* in the sand followed the same trends between transects at Centre Island Beach as the *E. coli* concentrations in the water. The sand at

transect 1 had higher *E. coli* numbers than transect 2 at Centre Island Beach. As mentioned, previously, these two parts of Centre Island Beach are quite different, with transect 1 being more sheltered from offshore currents by the rock breakwall. These two beach transects also have different sand characteristics (e.g. finer sand particle sizes at transect 1).

3.2.2 Kew Beach

Weekly monitoring results for *E. coli* in the wet foreshore sand at Kew Beach are presented in Figure 7. In general, *E. coli* were less numerous in sand at Kew Beach than Centre Island Beach. The sand at Kew Beach was notably coarser and more pebble-like than the finer sand at Centre Island Beach. The concentrations of *E. coli* in the wet sand followed the same trends between transects at Kew Beach as the *E. coli* concentrations in the water. The sand at Kew Beach transect 1 had lower *E. coli* numbers than at transects 2 and 3. There were no strong correlations between *E. coli* concentrations in the sand and ankle depth waters at Centre Island and Kew beaches (Figure 8).

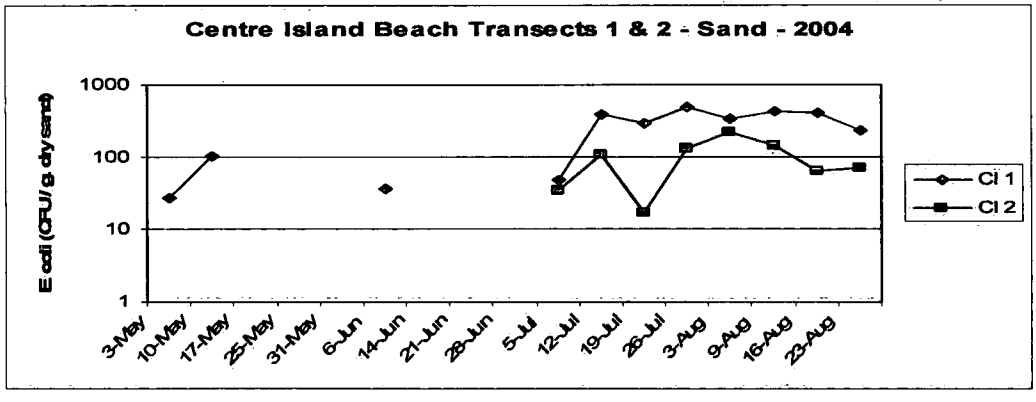


Figure 6. Weekly monitoring of E. coli from foreshore sand samples at two transects along Centre Island Beach in 2004.

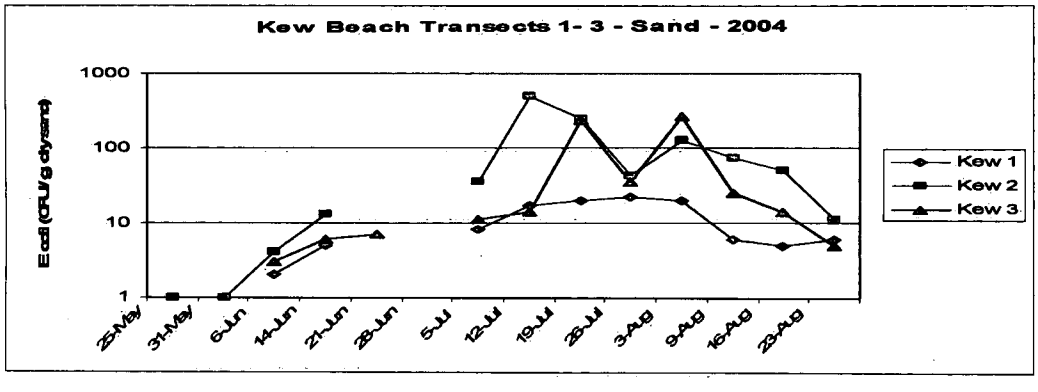


Figure 7. Weekly monitoring of E. coli from foreshore sand samples at three transects along Kew Beach in 2004.

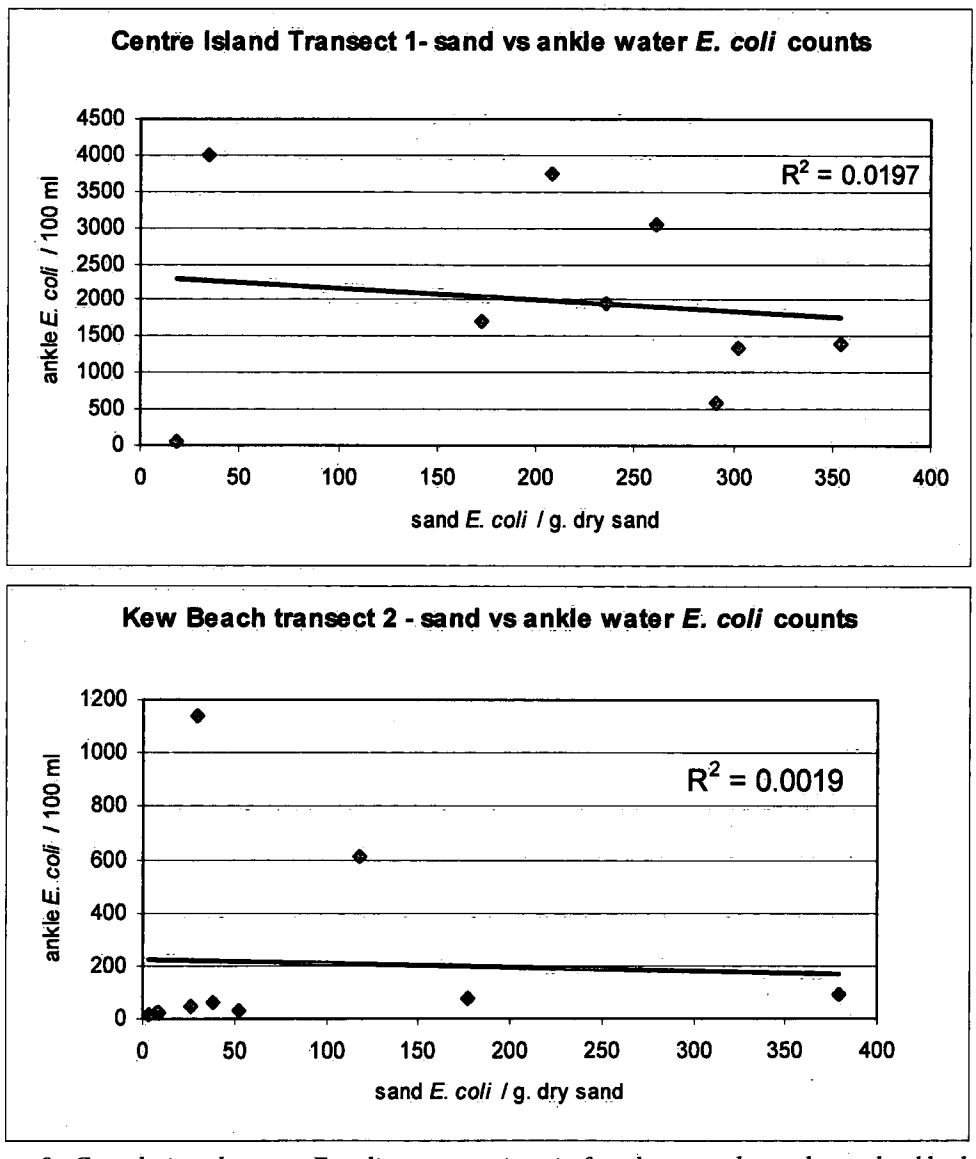


Figure 8. Correlations between E. coli concentrations in foreshore sand samples and ankle depth water samples from transects at Centre Island Beach (top) and Kew Beach (bottom) in 2004.

3.3 Animal Numbers and Droppings

Weekly observations at Centre Island Beach found there were always gulls or Canada geese along the shoreline. It should be noted that the counts of birds and fecal droppings were usually obtained about mid-day on Mondays. It is possible that more birds would have been recorded in the beach area earlier in the morning or at other times during the day when it was cooler and less people were around. Subsequent observations at other Toronto beaches have suggested some beaches can have higher numbers of birds on the beach in the early morning hours.

There were usually significant numbers of gulls along Centre Island Beach west of the pier out towards transect 1. The gulls would be standing along the waters edge or roosting on the sand

10-20 metres in from the shoreline. These gull numbers ranged from about 25 to 150 on the beach, but there were more on the rock breakwall. Gulls were also present east of the pier out towards transect 2, although they were generally less numerous here than west of the pier. Canada geese were rarely present in the beach area at the beginning of the summer, although they started to show up more commonly along the shoreline in early July. Canada geese numbers along the beach or in the beach waters ranged from none on some days, up to about 50 on other days. Canada geese were also numerous on the many grass areas around Centre Island. A few mallard ducks were often observed at Centre Island Beach, although larger numbers of mallard ducks were always found around the pond next to the animal farm on Centre Island. On occasion, several swans were also observed on Centre Island Beach. While cormorants could be seen flying overhead, and were often seen swimming in Toronto harbour, they were never seen on Centre Island Beach.

The only fecal droppings regularly observed along Centre Island Beach were from gulls and Canada geese. A few fecal droppings were noted from time to time from mallard ducks, and occasionally from swans. There did not appear to be any evidence of dog or cat droppings. The numbers of gull and geese droppings along the foreshore sand are presented for Centre Island beach in Figure 9. Gull droppings were more prevalent early in the bathing season, and Canada geese droppings became more prevalent later in the bathing season. This pattern of fecal droppings has been observed at other Lake Ontario beaches (see 2004 results for BayFront Park Beach in Hamilton Harbour for comparison purposes in Edge and Hill, 2005b). Observations of gulls and geese (and their droppings) on the beach were consistent with their breeding behaviour and when they would be more likely to be nesting and less frequent on the beach. Gull droppings near the water line were regularly seen to be washed over by waves rolling up onto the beach. Geese droppings were occasionally seen rolling in the beach's surf. There were no strong correlations found between the number of gull or geese droppings and the number of *E. coli* in sand or ankle depth water at Centre Island Beach.

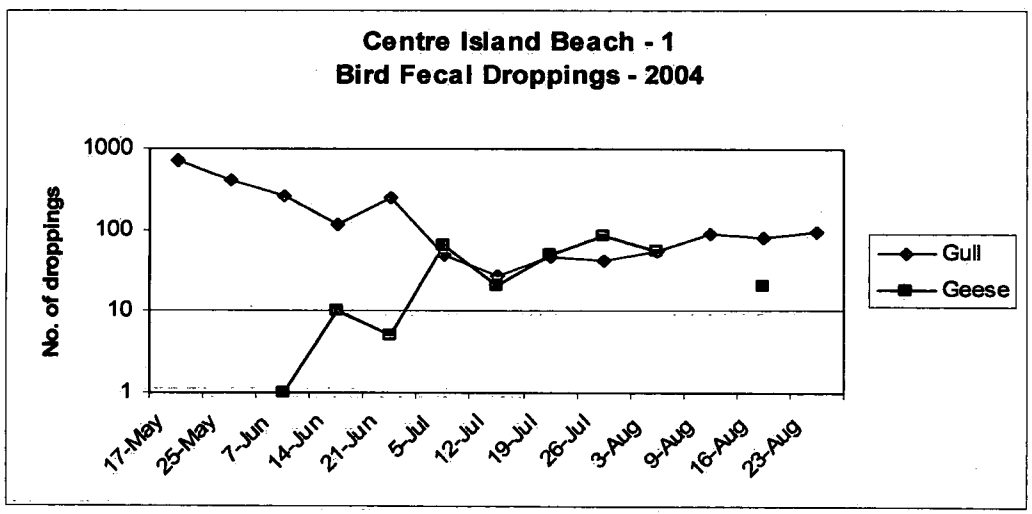


Figure 9. Weekly monitoring of number of bird droppings along 200 metres of shoreline near transect 1 along Centre Island Beach in 2004.

Weekly field visits to Kew Beach found that gulls and geese were rarely observed on the beach early in the morning, but they were regularly present swimming and on rock outcroppings between transects 2 and 3. Each day, 10-50 gulls and 0-25 geese were observed on these outcroppings between transects 2 and 3 over the summer. Dogs were commonly walked along the boardwalk, and also along the beach. These observations were usually made about 8:00am on Monday mornings, so like Centre Island Beach, they may/may not reflect the numbers of birds and dog walkers on different days of the week or at different times during the day.

Unlike Centre Island Beach, few fecal droppings were observed along the shoreline of Kew Beach. Gull and Canada geese droppings were occasionally noted, although the pebble nature of the beach was probably less conducive to preserving the droppings than the finer sand at Centre Island Beach. However, wading out to the rock outcroppings between transects 2 and 3 when water levels dropped later in the summer, revealed numerous gull and geese droppings on submerged and exposed rocks. Unlike Centre Island Beach, dog droppings were observed in the Kew Beach area. Dog droppings were found on the ground at times along the boardwalk, and in the off-the-leash area west of transect 1, however they were less frequent on the beach.

3.4 Microbial Source Tracking Tools

3.4.1 Toronto E. coli Library

A total of 6584 *E. coli* were collected from Toronto area fecal sources and water and sand samples at Centre Island and Kew Beaches in 2004 (Table 2).

3.4.2 Antibiotic Resistance Discriminant Function

Antibiotic resistance data were collected on a total of 6584 *E. coli* isolates from Toronto fecal sources and water and sand samples from Centre Island and Kew Beaches (Figure 10). Isolates with identical profiles from the same fecal dropping or wastewater sample were removed prior to calculating discriminant functions. The resulting numbers of *E. coli* isolates used for discriminant function analyses are presented in Table 2. Discriminant functions were calculated for discriminating between two fecal sources (bird and other), three fecal sources (bird, pet, and municipal wastewater), four fecal sources (gull, geese, dog and municipal wastewater), and eight fecal sources (all). The more detailed discriminant functions (e.g. 4-way and 8-way) provided classification results comparable to those expected at random and were not pursued. The 2-way function was considered less informative than the 3-way, so results are presented in Appendix 1. The 3-way discriminant function calculated to distinguish *E. coli* between birds, wastewater, and pets is evaluated in Table 3. Both 2-way and 3-way discriminant functions were statistically significant at $p<0.0001$. The 3-way discriminant function provided an average rate of correct classification of 63 %, much better than a random classification rate of 33%. Results from rarefaction analyses indicated that the Toronto fecal library of *E. coli* isolates did not represent the potential diversity of ARA phenotypes for each source, although rarefaction curves were not linear and suggested there was some approach towards a plateau of possible ARA phenotypes. The minimum detection percentage was calculated to be 28 % which can serve as a conservative level for ensuring that a given fecal host is actually present in water or sand samples. In general,

wastewater *E. coli* had the highest levels of antibiotic resistance followed by pets, and then birds. The following antibiotics contributed most to the ability to discriminate between these three fecal pollution sources: triclosan (0.01 µg/ml), sulfamethoxazole (512 µg/ml), chloramphenicol (16 µg/ml), tetracycline (5 µg/ml), erythromycin (100 µg/ml), and ampicillin (5 µg/ml).

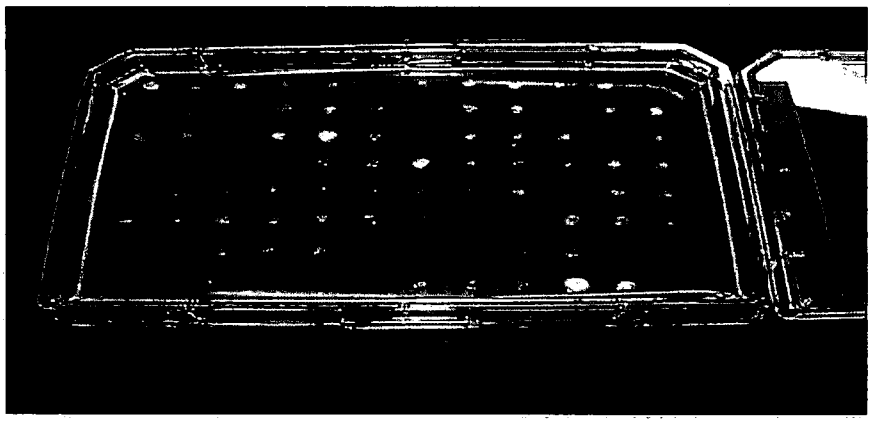


Figure 10. Differential growth of 96 Toronto E. coli isolates on an agar plate with an antibiotic mixed into the agar. Clearly visible isolate colonies are resistant to the antibiotic.

3.4.3 DNA Fingerprint Clustering

Rep-PCR DNA fingerprinting data were collected on a total of 3018 *E. coli* isolates from Toronto fecal sources and water and sand samples from Centre Island and Kew Beaches (Figure 11). Isolates with identical DNA fingerprints (> 0.9 similarity) from the same fecal dropping or wastewater sample, and those with failed amplifications, were removed prior to performing cluster analyses. The resulting numbers of *E. coli* isolates used for clustering analyses are presented in Table 2. Similar to antibiotic resistance analyses, results were calculated for a 3-way discrimination between birds, pets, and municipal wastewater. This 3-way cluster analysis provided an average rate of correct classification of 67%, however, when within-sample clones were removed, the ARCC fell to 53 % (Table 4).

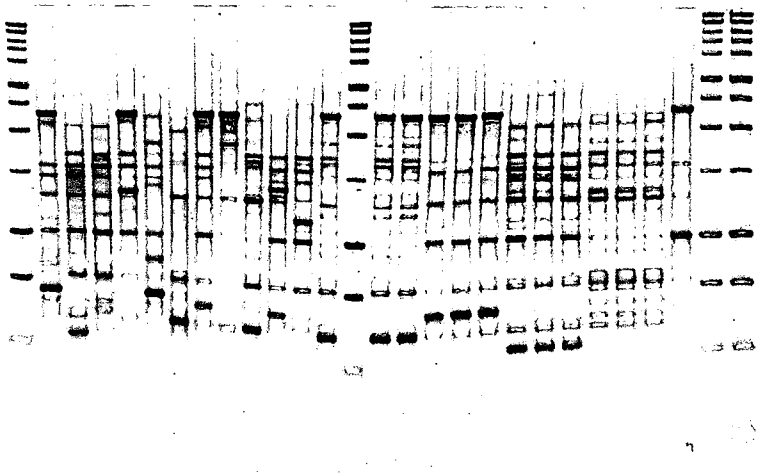


Figure 11. Photo of an electrophoresis gel showing vertical DNA fingerprints of different Toronto E. coli isolates interspersed with four lanes of a standard set of known DNA fragments.

Table 2. Libraries of Toronto E. coli isolates representing the number of isolates: 1) collected in total during 2004; 2) used for antibiotic resistance analyses (ARA) after removing isolates with identical profiles from the same fecal dropping or STP wastewater sample; and 3) used for rep-PCR DNA fingerprinting after removing isolates with identical DNA fingerprints from the same fecal dropping or STP wastewater sample. STP= sewage treatment plant.

Collection source	Total no. of Toronto *E. coli*	ARA analysis size	rep-PCR analysis size
Canada geese	347	332	204
Gull	318	297	152
Cormorant	151	100	77
Duck	225	183	80
Swan	20	17	11
Dog	303	206	128
Cat	243	193	61
STP treated effluent	347	271	250
STP sewage influent	306	268	236
Total fecal library	**2260**	**1867**	**1199**
Sand	1141	1141	184
Ankle water	1144	1144	411
Knee water	1137	1137	423
Chest water	773	773	350
Other water	129	129	129
Overall Total	**6584**	**6191**	**2696**

Table 3. Jackknife evaluation of antibiotic resistance discriminant function classification of Toronto fecal *E. coli* isolates. Values expressed as % of isolates correctly or incorrectly classified into fecal sources. ARCC= average rate of correct classification. STP= sewage treatment plant.

	N	Bird	STP	Pet
Bird	929	60 %	21 %	19 %
STP	539	14 %	68 %	18 %
Pet	399	18 %	20 %	62 %
ARCC = 63 %				

Table 4. Jackknife evaluation of DNA fingerprint clustering (max. similarity) classification of Toronto fecal *E. coli* isolates. Values expressed as % of isolates correctly or incorrectly classified into fecal sources. ARCC= average rate of correct classification. STP= sewage treatment plant.

	N	Bird	STP	Pet
Bird	524	66 %	21 %	13 %
STP	486	28 %	61 %	11 %
Pet	189	44 %	24 %	32 %
ARCC = 53 %				

3.5 Fecal Source Classification – Centre Island Beach

3.5.1 Water and Sand

The results of antibiotic resistance analysis and DNA fingerprinting of *E. coli* isolates from water samples from Centre Island are presented in Figure 12. Both MST methods were consistent in showing birds as being the most prominent contributor of *E. coli* in water at Centre Island Beach. The only fecal source found to exceed the antibiotic resistance minimum detection percentage of 28% were birds.

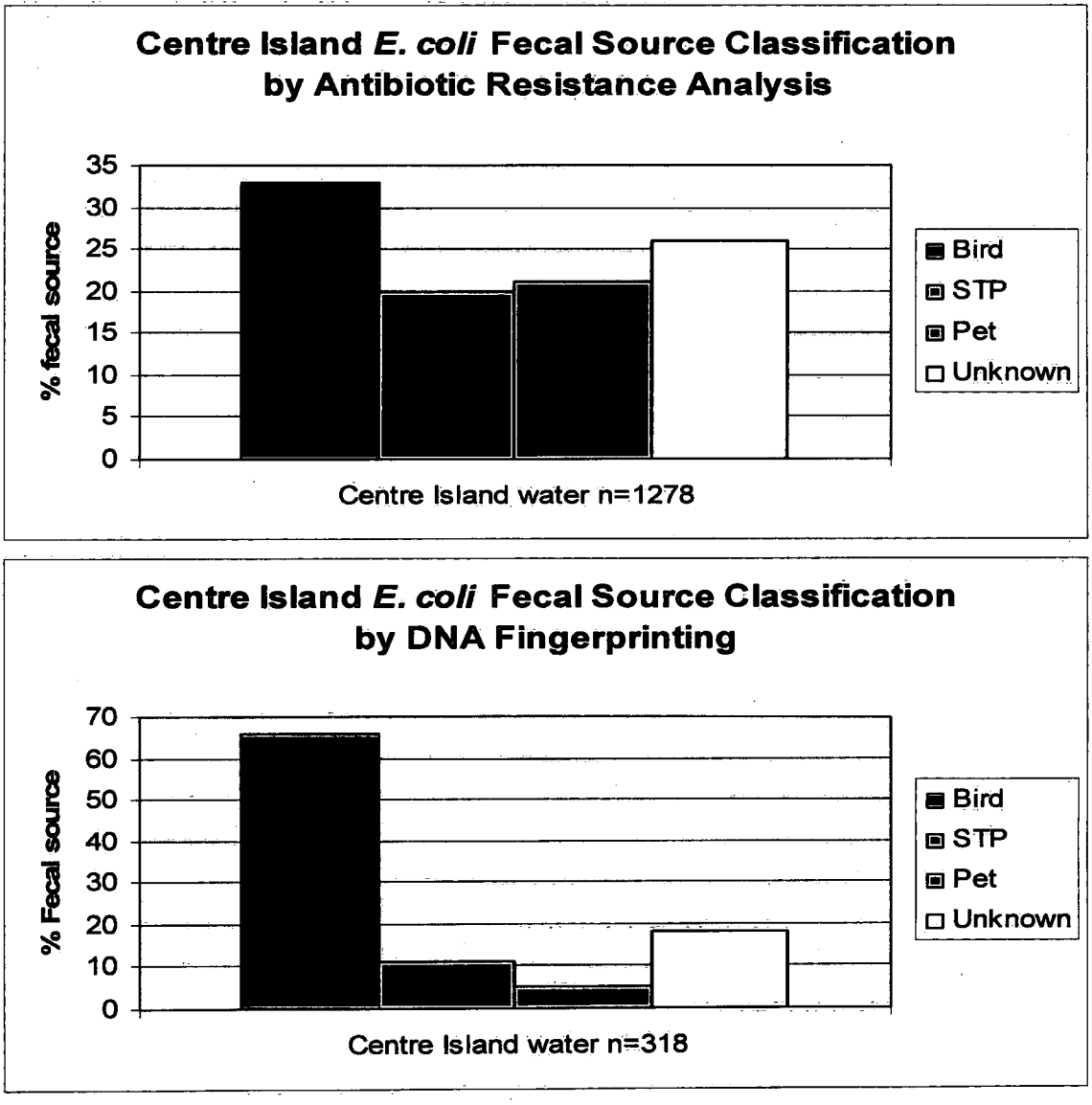


Figure 12. Fecal source classification of E. coli isolates in Centre Island Beach water samples by antibiotic resistance analysis (top) and DNA fingerprinting (bottom).

The results of antibiotic resistance analysis and DNA fingerprinting of *E. coli* isolates from sand samples from Centre Island are presented in Figure 13. Both MST methods showed birds as being a prominent contributor of *E. coli* in sand at Centre Island Beach. Birds were found to exceed the antibiotic resistance minimum detection percentage of 28%. The antibiotic resistance analysis indicated *E. coli* from pets also exceeded the minimum detection percentage. This prominence of pet *E. coli* was not supported by the DNA fingerprinting data. When the probability of correct classification threshold was examined more closely, more stringent thresholds for correct classification indicated that *E. coli* from birds, rather than pets, were more prominent in Centre Island Beach sand (see Figure 14).

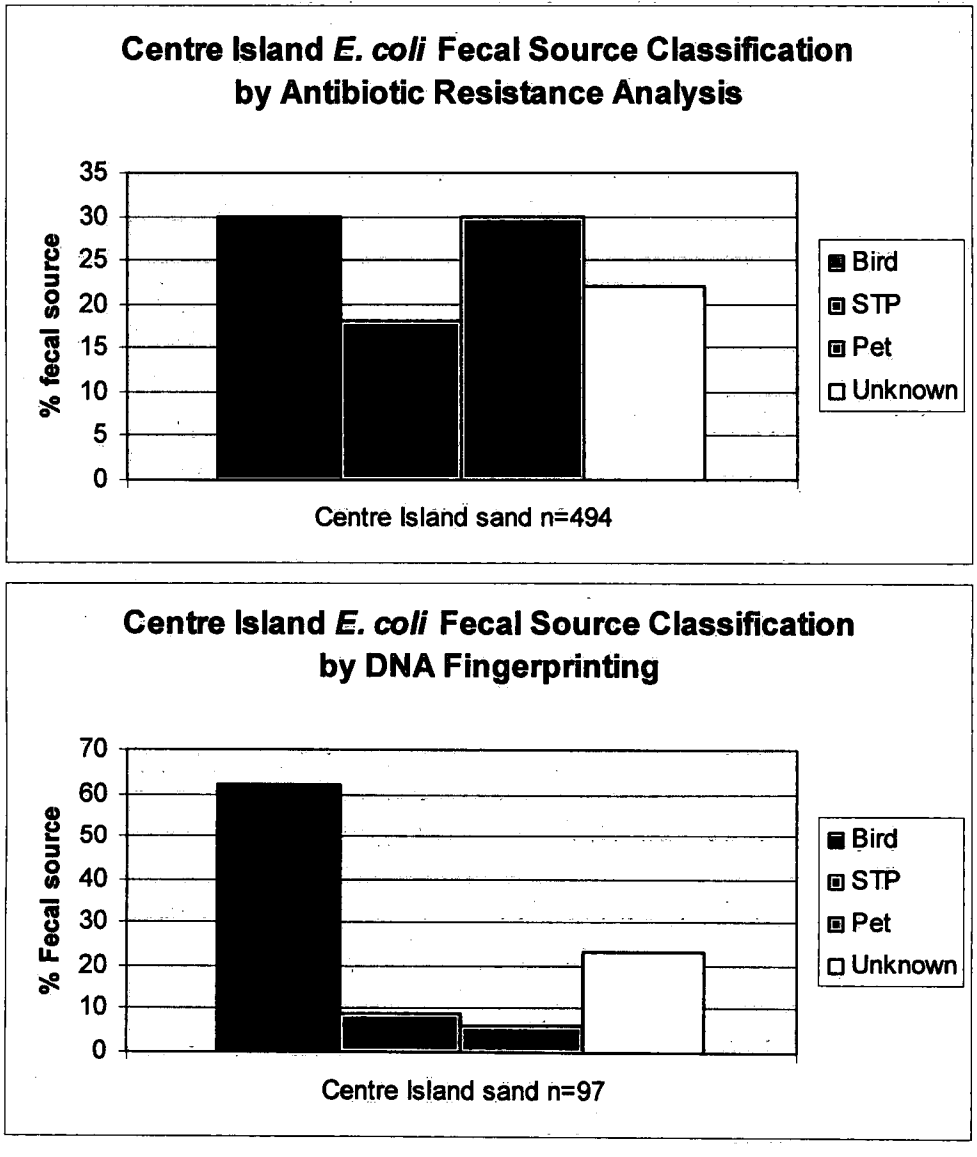


Figure 13. Fecal source classification of E. coli isolates in Centre Island Beach sand samples by antibiotic resistance analysis (top) and DNA fingerprinting (bottom).

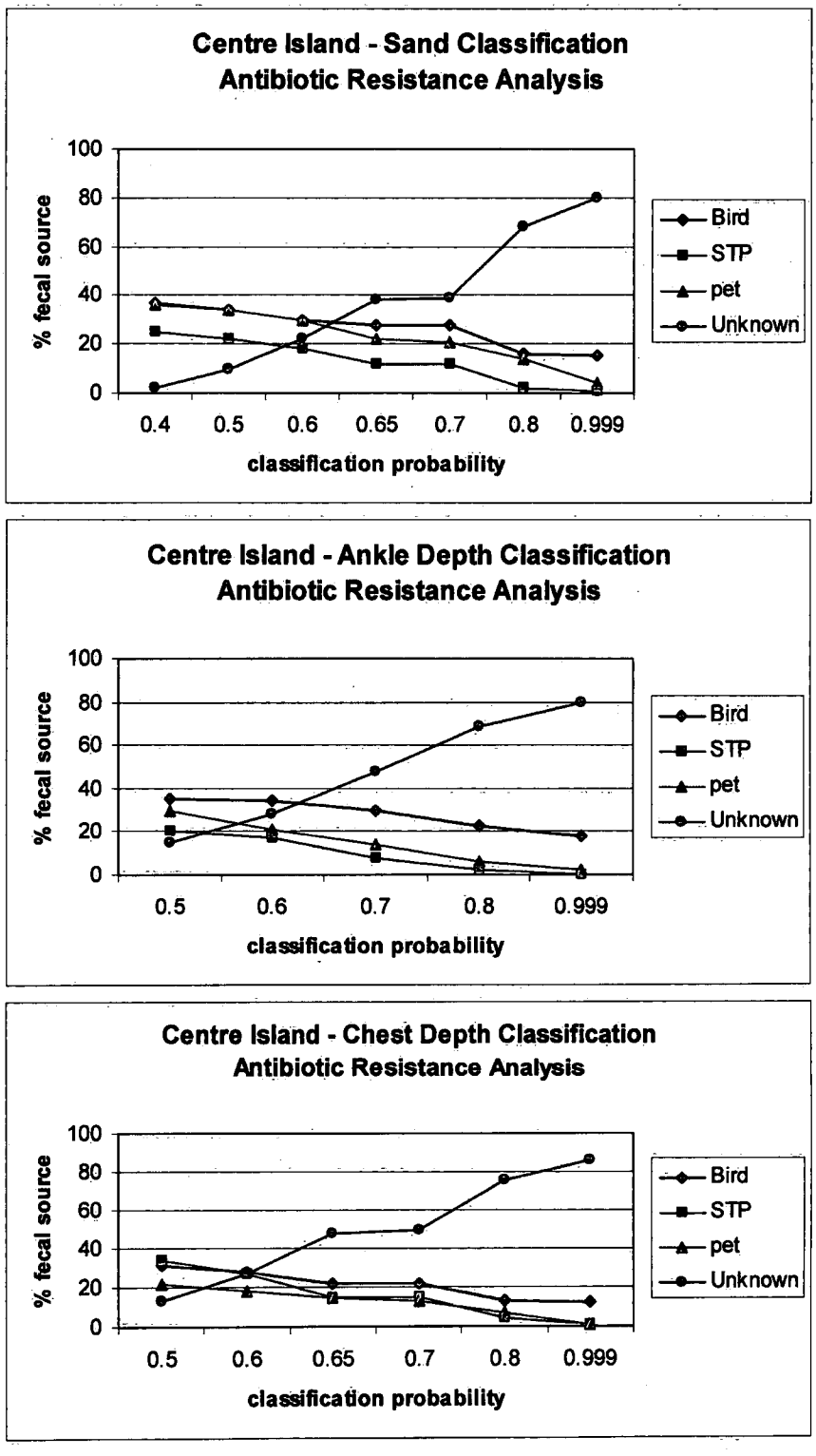


Figure 14. Percent fecal source contributions of E. coli in Centre Island Beach sand (top), ankle water (middle) and chest water (bottom) as the classification probability threshold was varied in discriminant function analyses. Results reported throughout this study used a classification probability threshold of 0.6. Note as probability threshold for correct classification increases towards 0.999, source contributions classified as unknown increases.

3.5.2 Water Depth

A more detailed fecal source classification of *E. coli* isolates collected from different water depth zones at Centre Island Beach is presented in Figure 15. Both MST methods were consistent in showing birds as being the most prominent contributors of *E. coli* at Centre Island Beach. Birds were the only fecal source found to exceed the minimum detection percentage of 28%, although antibiotic resistance data indicated their representation in chest depth water was less significant. Interestingly, both MST methods found that while *E. coli* representation from municipal wastewater was low, it increased with depth of water. When the probability of correct classification threshold was examined more closely, more stringent thresholds for correct classification indicated that *E. coli* from birds, rather than STP sources, were more prominent in chest depth water at Centre Island Beach. (see Figure 14).

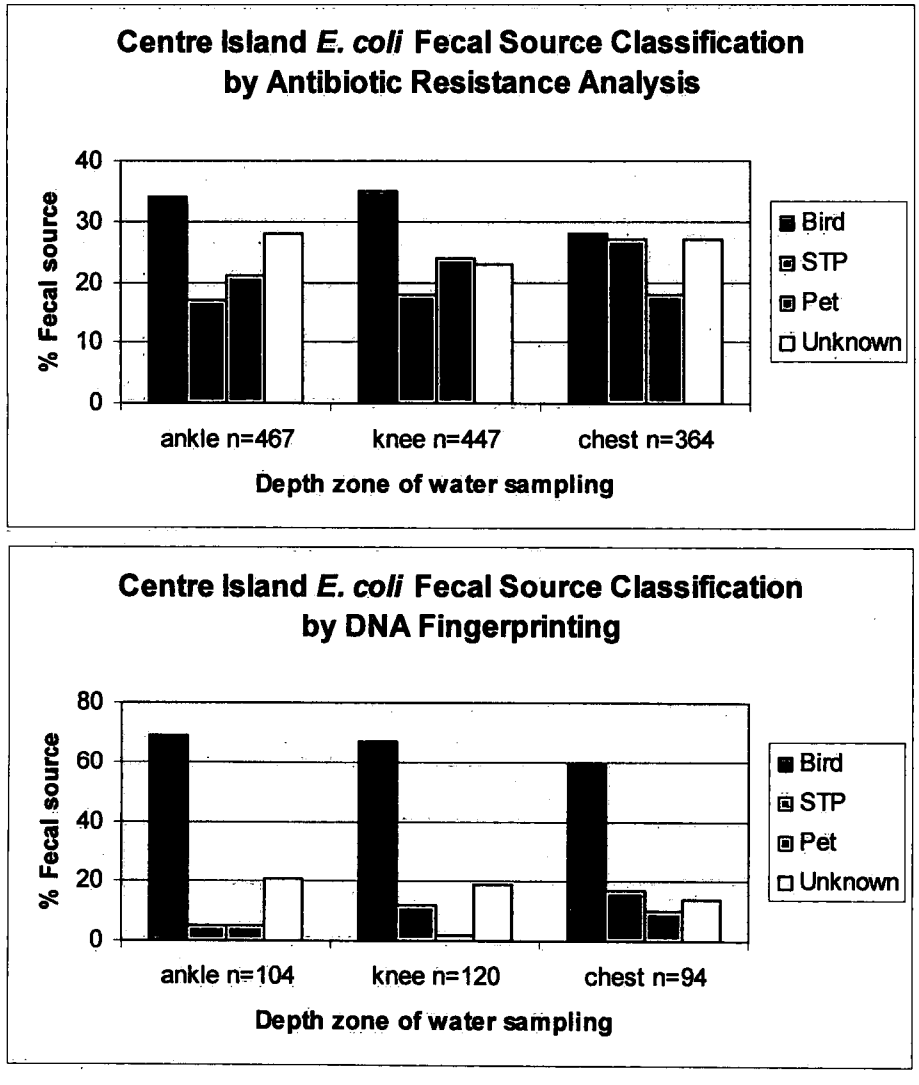
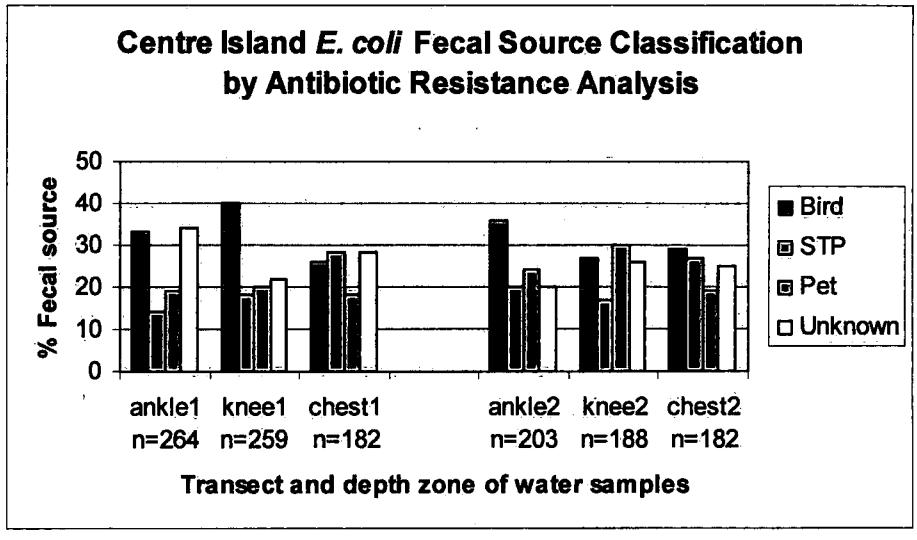


Figure 15. Fecal source classification by antibiotic resistance analysis (top) and DNA fingerprinting (bottom) of E. coli isolates collected from surface water samples in different water depth zones at Centre Island Beach in 2004.

3.5.3 Beach Transect

A further breakdown of the fecal source classification of *E. coli* isolates collected from water at different transects along Centre Island Beach is presented in Figure 16. Both transects show the prominence of *E. coli* contamination from birds. Both antibiotic resistance and DNA fingerprinting data indicate that *E. coli* contamination from birds is more significant at transect 1 west of the pier than transect 2. Interestingly, while *E. coli* contamination from wastewater is less significant, it shows an increasing prominence with water depth across both transects and both MST methods. The slightly increased prominence of STP *E. coli* over bird *E. coli* at transect 1 chest depth, and of pet *E. coli* over bird *E. coli* at transect 2 knee depth, is not supported by DNA fingerprinting results.



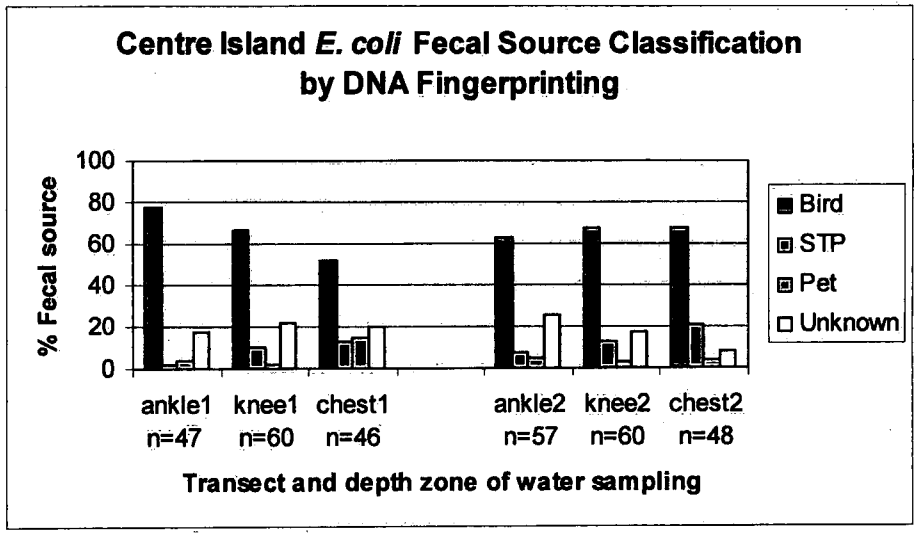


Figure 16. Fecal source classification by antibiotic resistance analysis (top) and DNA fingerprinting (bottom) of E. coli isolates collected from surface water samples at two different transects along Centre Island Beach in 2004.

A further breakdown of the fecal source classification of *E. coli* isolates collected from sand at different transects along Centre Island Beach is presented in Figure 17. Both transects show the prominence of *E. coli* contamination from birds. Consistent with results from water, both antibiotic resistance and DNA fingerprinting data indicate that *E. coli* contamination from birds is more significant in sand at transect 1 west of the pier than in sand at transect 2. The more prominent *E. coli* contamination from pets, particularly at transect 2, is not supported by the DNA fingerprinting results.

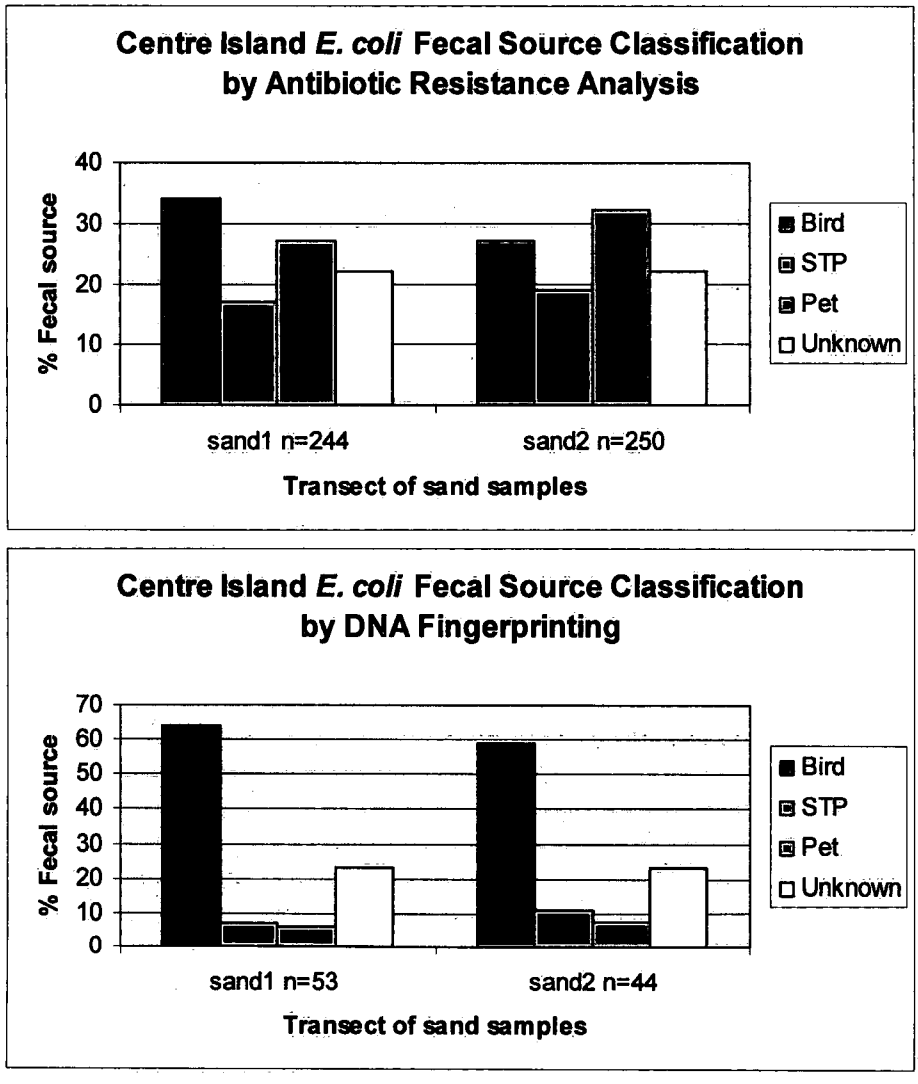


Figure 17. Fecal source classification by antibiotic resistance analysis (top) and DNA fingerprinting (bottom) of E. coli isolates collected from foreshore sand samples at two different transects along Centre Island Beach in 2004.

3.6 Fecal Source Classification – Kew Beach

3.6.1 Water and Sand

The results of antibiotic resistance analysis and DNA fingerprinting of *E. coli* isolates from water samples from Kew Beach are presented in Figure 18. The number of *E. coli* classified as of unknown source was generally higher at Kew Beach than Centre Island Beach. Both MST methods were consistent in showing birds as the most prominent contributor of *E. coli* in water at Kew Beach. However, the *E. coli* from birds, and the other fecal sources, did not exceed the antibiotic resistance minimum detection percentage of 28% suggesting multiple sources of *E. coli* without one that is clearly predominant. DNA fingerprint data indicated *E. coli* from both birds and wastewater were prominent. DNA fingerprinting data indicated that bird *E. coli* contributions were not as high as at Centre Island, although wastewater contributions were higher at Kew.

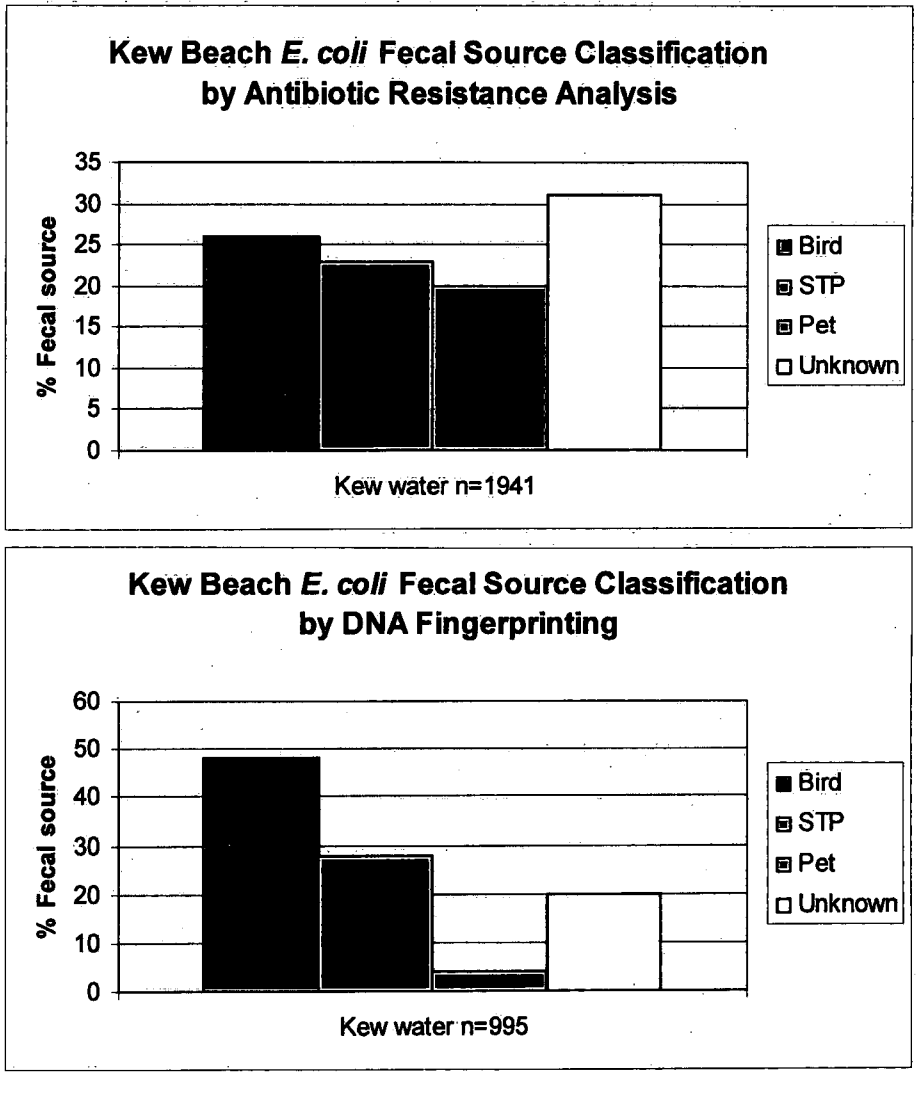


Figure 18. Fecal source classification of E. coli isolates in Kew Beach water samples by antibiotic resistance analysis (top) and DNA fingerprinting (bottom).

The results of antibiotic resistance analysis and DNA fingerprinting of *E. coli* isolates from sand samples from Kew Beach are presented in Figure 19. Both MST methods were consistent in showing birds as being the most prominent contributor of *E. coli* in sand at Kew Beach. *E. coli* from birds was the only source exceeding the antibiotic resistance minimum detection percentage of 28 %. Similar to Kew Beach water, DNA fingerprint data indicated the prominence of *E. coli* from both birds and wastewater in Kew Beach sand. DNA fingerprint data indicated that bird *E. coli* contributions were not as high as at Centre Island, although wastewater contributions were higher at Kew Beach.

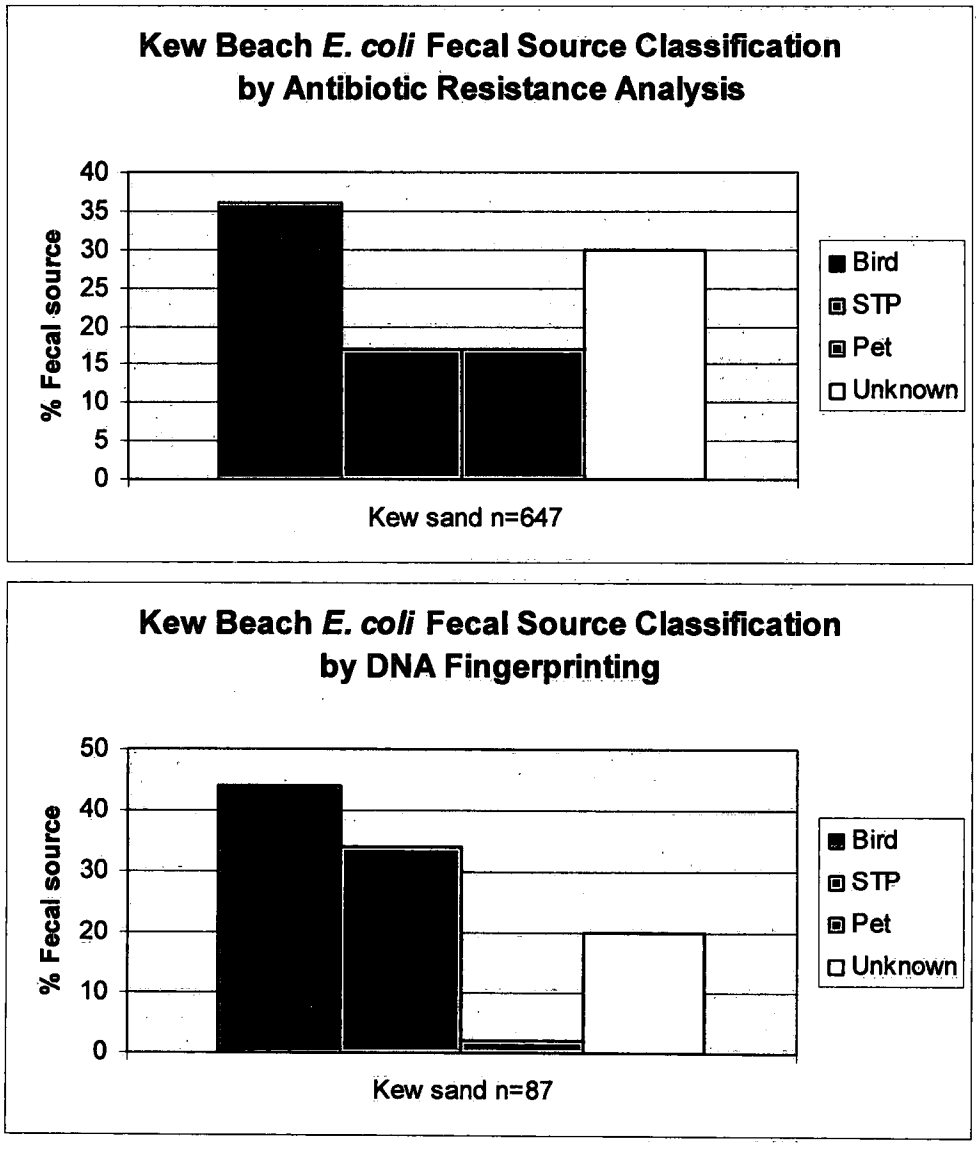


Figure 19. Fecal source classification of E. coli isolates in Kew Beach foreshore sand samples by antibiotic resistance analysis (top) and DNA fingerprinting (bottom).

3.6.2 Water Depth

A more detailed fecal source classification of *E. coli* isolates collected from different water depth zones at Kew Beach is presented in Figure 20. Both MST methods were consistent in showing birds as being the most prominent contributors of *E. coli* in shallow ankle and knee depth waters at Kew Beach. DNA fingerprint data indicated the predominance of bird *E. coli* at all depths. Antibiotic resistance data indicated birds were the only fecal source found to exceed the minimum detection percentage of 28% in shallow water (i.e. knee depth, though not ankle depth). The picture at chest depth was different for antibiotic resistance data, with only *E. coli* from wastewater exceeding the minimum detection percentage of 28%. In contrast, the DNA fingerprinting data indicated the prominence of bird *E. coli* at chest depth. The prominence of pet *E. coli* in ankle depth water according to antibiotic resistance data was not supported by more detailed analysis of probability classification thresholds, nor DNA fingerprinting data. Interestingly, both MST methods indicated that *E. coli* from a wastewater source were clearly predominant in water samples from a stormwater outfall (thought to be capped) just west of Kew Beach transect 1 and the life guard building.

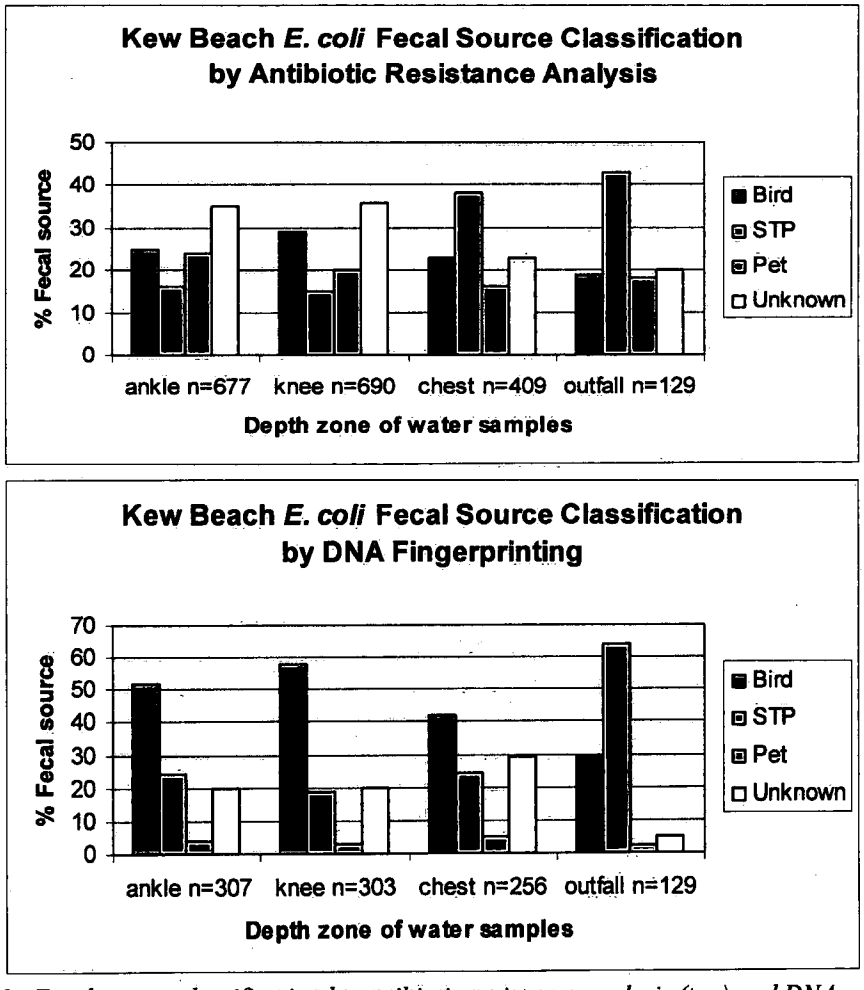


Figure 20. Fecal source classification by antibiotic resistance analysis (top) and DNA fingerprinting (bottom) of E. coli isolates collected from surface water samples in different water depth zones at Kew Beach in 2004.

3.6.3 Beach Transect

A further breakdown of the fecal source classification of *E. coli* isolates collected from water at different transects along Kew Beach is presented in Figure 21. All transects show a prominence of *E. coli* contamination from birds in shallow water, and this fecal source is the only one exceeding the antibiotic resistance minimum detection percentage in shallow water. However, antibiotic resistance data indicate an increased prominence of pet *E. coli* in ankle depth water at transects 1 and 2. This result is not supported by the DNA fingerprinting data. The antibiotic resistance data also indicate the clear predominance of *E. coli* from municipal wastewater in chest depth water across all transects (only fecal source to exceed the minimum detection percentage of 28% at this depth). However, the DNA fingerprinting data only suggest a relative prominence of *E. coli* from municipal wastewater at chest depth at transect 3.

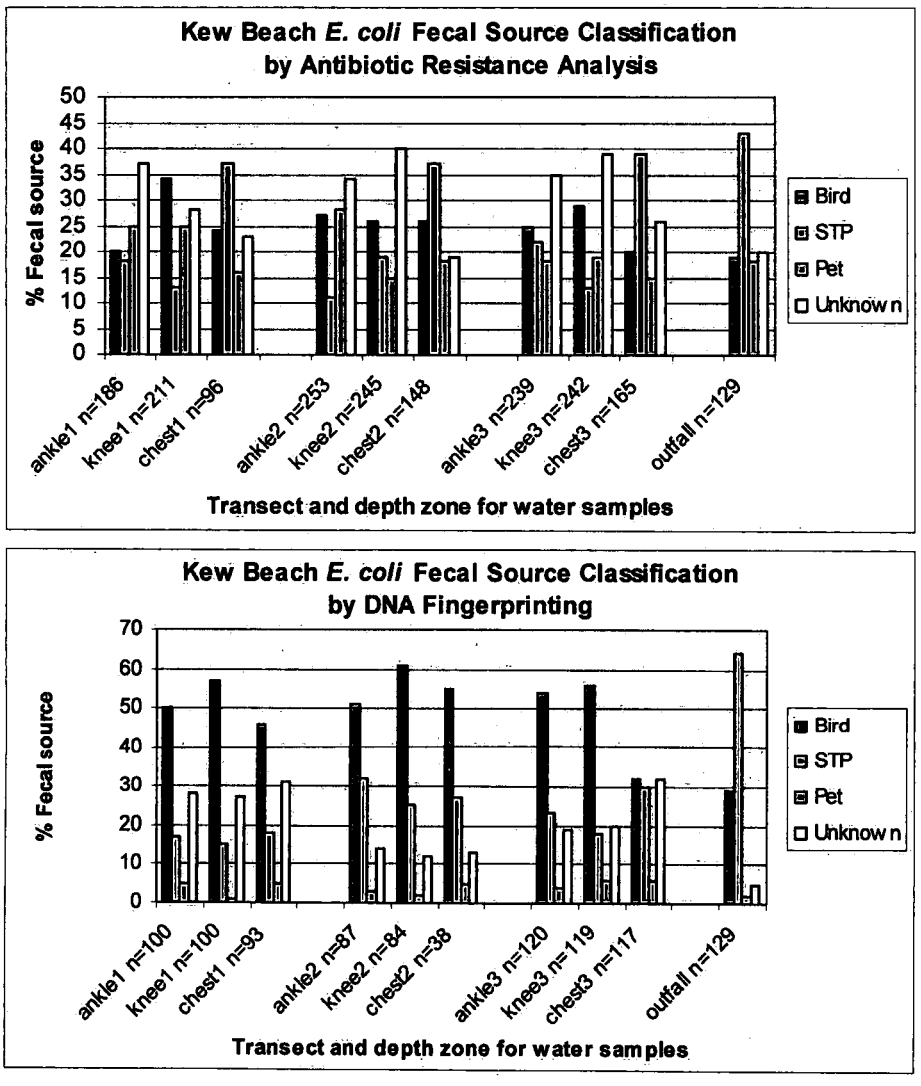


Figure 21. Fecal source classification by antibiotic resistance analysis (top) and DNA fingerprinting (bottom) of E. coli isolates collected from surface water samples at three different transects along Kew Beach in 2004.

A further breakdown of the fecal source classification of *E. coli* isolates collected from sand at different transects along Kew Beach is presented in Figure 22. All transects show a prominence of *E. coli* contamination from birds in sand, except transect 1 as indicated by DNA fingerprinting data. However, it should be noted that the sample size is small for the DNA fingerprinting data at this transect.

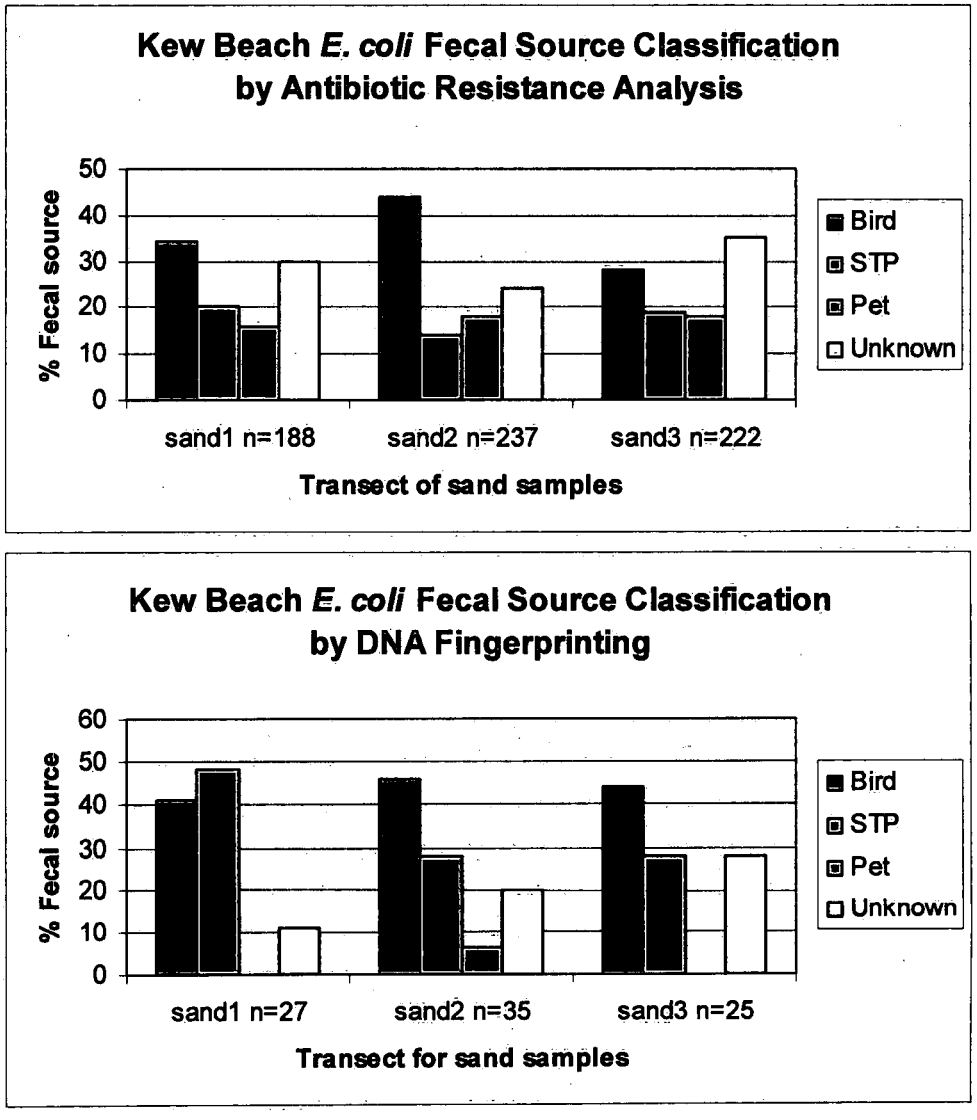


Figure 22. Fecal source classification by antibiotic resistance analysis (top) and DNA fingerprinting (bottom) of E. coli isolates collected from foreshore sand samples at three different transects along Kew Beach in 2004.

4. Discussion and Conclusions

Water quality monitoring is an important aspect of managing public health risks at beaches. It is important to understand the sources and transport dynamics of fecal contamination events in order to prevent waterborne disease outbreaks. While such outbreaks are not common at beaches around the Great Lakes, they do occur. For example, a 2001 outbreak of a harmful strain of *E. coli* O157:H7 at a Montreal beach sent four children (ages 3-7) to hospital (Bruneau et al. 2004). Knowledge about the sources of fecal contamination at beaches can guide mitigation efforts to reduce such public health risks.

Water quality monitoring programs will need to reflect the rapidly evolving scientific knowledge about the ecology of *E. coli* at beaches, and its use for source tracking fecal pollution. While most water quality monitoring programs routinely monitor for *E. coli*, additional surveillance and research is usually needed to determine the source(s) of fecal pollution responsible for beach postings. This microbial source tracking study took an approach based upon multiple lines of evidence, including increased *E. coli* surveillance, regular field observations, and antibiotic resistance profiling and DNA fingerprinting of *E. coli* to determine sources of fecal pollution at Centre Island and Kew Beaches along the Toronto waterfront.

4.1 E. coli Monitoring in Water

Water quality monitoring at chest depth in this study found Centre Island and Kew Beaches exceeding 100 *E. coli* CFU / 100ml on four occasions out of a total of 39 water samples collected over the 2004 bathing season. The numbers of *E. coli* at chest depth were generally higher at Centre Island Beach (season sample maximum – 178 CFU / 100ml) than at Kew Beach (season sample maximum – 73 CFU / 100ml). There were small differences noted between locations along each beach. Numbers of *E. coli* at chest depth were slightly higher to the west of the pier at Centre Island Beach where birds were more common, and eastward along Kew Beach. These *E. coli* levels are not suggestive of direct municipal wastewater contamination at the time of sample collection (e.g. where *E. coli* numbers might be expected in the hundreds to thousands per 100ml).

The situation in shallow ankle and knee depth water at these beaches was much different. The levels of *E. coli* were almost invariably higher closer to the shoreline, being highest in ankle depth water. In ankle depth water, water samples at Centre Island and Kew Beaches were found to exceed 100 *E. coli* CFU / 100ml on 25 occasions out of a total of 59 water samples collected over the 2004 bathing season. This was most prominent at Centre Island Beach (10 out 13 samples) where *E. coli* levels at ankle depth rose above 1000 CFU / 100ml around the end of June, and remained above that level for most of the rest of the bathing season. The ankle depth *E. coli* levels at Centre Island Beach were much higher to the west of the pier where birds were more common, and rose to 4000 *E. coli* CFU / 100ml there on July 5, 2004. Although ankle and knee depth *E. coli* levels were not as high at Kew Beach, they rose above 1000 CFU / 100ml near the end of July and early August at two transects near offshore rock outcroppings where gulls and Canada geese regularly roosted. The highest level at ankle depth at Kew Beach was 1345 *E. coli* CFU / 100ml on August 3, 2004.

The finding of high *E. coli* levels in shallower waters at Centre Island and Kew Beaches is consistent with other recent studies at Great Lakes beaches (Whitman and Nevers, 2003; Haack et al. 2003; Milne and Charlton, 2004 and 2005; Edge and Hill, 2005b; Sampson et al. 2005; U.S. EPA, 2005b). The United States Environmental Protection Agency study (U.S. EPA, 2005b) found the single greatest determinant of *E. coli* levels at a number of U.S. beaches was the depth zone (or roughly distance from shoreline) at which the water sample was collected. *E. coli* densities became substantially lower as one moved from ankle to chest depth water. At present, it is uncertain if high *E. coli* levels in shallow water present an increased public health risk. One might assume the higher *E. coli* levels would be indicative of an increased likelihood of the occurrence of waterborne pathogens. This might be a particular concern for children who play in these shallow waters and generally have weaker immune systems than adult swimmers. However, little research has been conducted to date related to this possible concern. Epidemiology studies conducted to date at beaches have measured indicator densities in waters of swimming depth, and have addressed risks to adult swimmers rather than to infants and toddlers (U.S. EPA, 2005b). Infants and toddlers are more likely to ingest water at ankle depth rather than at waist or chest depths where adults are more likely to immerse their heads. The occurrence of elevated *E. coli* levels in shallow water is unlikely to be a new phenomenon, and the authors are unaware of information indicating that playing in shallow water is a unique contributor to increased incidence of waterborne disease in children. This area warrants additional research.

4.2 E. coli Monitoring in Sand

This study also found relatively high numbers of *E. coli* in the wet foreshore sand at Centre Island and Kew Beaches. The numbers were observed to increase in the sand around the beginning of July at both beaches, although contamination at Centre Island Beach was higher and more sustained over the bathing season. A peak of 501 *E. coli* CFU / g. dry sand was observed at both Centre Island and Kew Beaches. Both beaches exhibited the highest levels of sand contamination at transects where birds were most prevalent. Alm et al. (2003) and Whitman and Nevers (2003) also found high levels of *E. coli* in wet foreshore sand relative to adjacent waters at beaches in Lakes Huron and Michigan. Alm et al. (2003) reported *E. coli* in sand up to around 10 CFU / g. dry sand at times. Whitman and Nevers (2003) reported mean counts of *E. coli* in sand at 1.1×10^4 CFU / 100 ml, and they indicated that proper expression of *E. coli* counts in saturated sand was unresolved. It is difficult to compare these *E. coli* results with the Toronto results since there are no standardized methods for measuring *E. coli* in sand. Both Alm et al. (2003) and Whitman and Nevers (2003) used shaker-based approaches for detaching *E. coli* from sand particles, and getting the cells into suspension. It is possible Toronto beaches had relatively higher *E. coli* levels, or that high counts were the result of using a more rigorous blender-based approach to detach cells from sand particles. The only other comparative data from using this blender-based method found *E. coli* levels above 10,000 CFU / g. dry sand at times in the summer of 2004 in wet foreshore sand at BayFront Park Beach in Hamilton Harbour (Edge and Hill, 2005b). More research will be required to standardize enumeration of *E. coli* in beach sand, and guide assessments of sand quality.

A better understanding is also needed of the potential for beach sand to serve as a reservoir for *E. coli*. Alm et al. (2003) and Whitman and Nevers (2003) have provided data to indicate sand at

freshwater beaches in temperate climates can differentially accumulate fecal indicator bacteria and serve as a reservoir for *E. coli*. It is likely that *E. coli* persists longer in the sand than in the water column, and according to some scientists, may be able to replicate in the wet foreshore sand under suitable temperature and nutrient conditions. One of these conditions in the Great Lakes area could involve beach areas where detached strands and mats of the macro-algae *Cladophora* wash ashore. While such *Cladophora* mats were not observed along Centre Island and Kew Beaches in 2004, Byappanahalli et al. (2003) found that these mats occurred along Lake Michigan beaches and could provide a suitable environment for *E. coli* to persist for months and to grow under natural conditions. Whitman et al. (2003) suggested these *Cladophora* mats can serve as an additional reservoir of *E. coli* in beach environments.

These recent findngs related to the ecology of *E. coli* in beach sand have important implications for beach monitoring programs. If *E. coli* persists in sand for long periods of time, or replicates to any significant extent, then high counts of *E. coli* in water may not necessarily be indicative of recent fecal pollution events. Rather, resuspension of *E. coli* in sand from past fecal contamination events may continue to contribute to impairment of water quality. Palmer (1988) found mechanically resuspending fecal coliforms from beach sediments could have a significant impact on fecal coliform concentrations in the overlying water column at Toronto beaches. Other studies have indicated the importance of wave action for resuspension of fecal indicator bacteria from sediments, and for increasing the levels of these bacteria in the water column to the point where beach water quality guidelines can be exceeded (LeFevre and Lewis, 2003). The resuspension of *E. coli* from sediments is also possible from activities such as wading and bathing, as well as commercial and recreational boating.

While *E. coli* has a long history of use for assessing water quality at freshwater beaches, its use for assessing sand quality is poorly known. Alm et al. (2003) suggested that the presence of fecal bacteria like *E. coli* in beach sand indicated that enteric pathogens may also be found in the sand. Subsequent research has found strains of *E. coli* O157:H7 in sand at a U.S. beach on Lake Huron (Elizabeth Alm, personal communication). Pathogenic campylobacter and salmonella bacteria have also been reported from sand at bathing beaches (Bolton et al. 1999). However, very few studies have been conducted to investigate the occurrence of enteric pathogens in beach sand. At present, there is insufficient understanding to determine whether high levels of *E. coli* in sand is a good indicator of increased likelihood of the presence of waterborne pathogens in sand. It is possible that the ecology of *E. coli* is different from many enteric pathogens, and that its persistence in sand may not necessarily be indicative of the presence of high numbers of harmful pathogens such as *Campylobacter* sp. or *Salmonella* sp. It will be important to investigate the occurrence of common waterborne pathogens such as *Campylobacter* sp. in beach sand in the future to evaluate the potential public health risks. Numerous media reports emerged around the Great Lakes in the summer of 2005 that raised public awareness and concern about the potential for public health risks associated with high levels of *E. coli* in beach sand. This concern was raised in the United States by the Clean Beaches Council (2005).

4.3 Animal and Fecal Dropping Field Observations

Ring-billed gulls were the only animals regularly observed along the shoreline at both Centre Island and Kew Beaches over the entire bathing season. Gulls were always present on field sampling days, either standing on the shoreline or on offshore breakwalls, or swimming in the nearshore waters. The colony of ring-billed gulls on the Leslie Spit numbers over 100,000 gulls (Figure 23). These birds are scavengers, and since their foraging range can be up to 40 km, they can be expected to occur along the length of the Toronto waterfront.

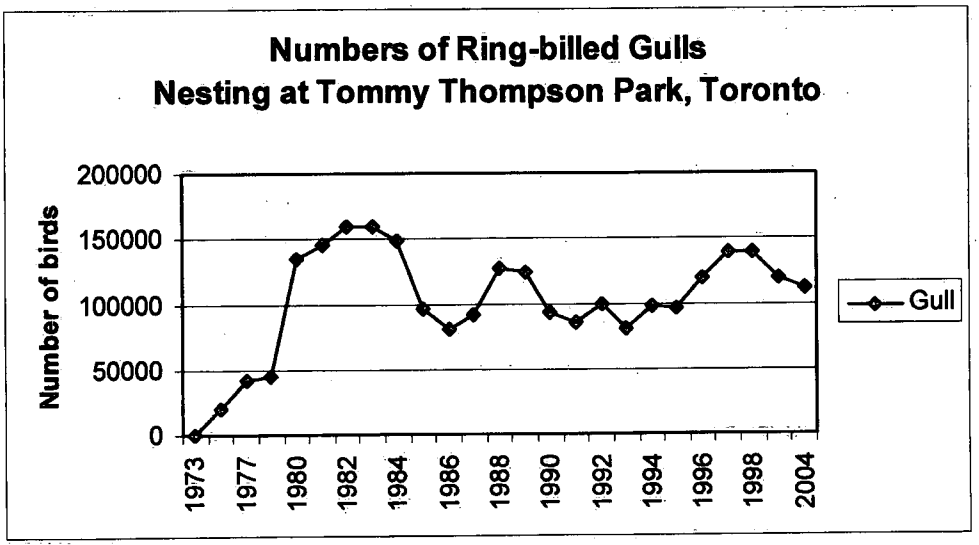


Figure 23. Numbers of Ring-billed gulls nesting at Tommy Thompson Park (Leslie Spit) along Toronto waterfront (Toronto and Regional Conservation Authority).

Canada geese were regularly observed along the shoreline at both beaches starting in June. Adult Canada geese lose their flight feathers around this time, and their grazing areas need to be near water for escape. This kind of habitat was more prevalent near Centre Island Beach than Kew Beach. The numbers of Canada geese around Centre Island is uncertain, although there is a roundup of geese each summer in July for relocation elsewhere as an attempt at bird control. Despite the roundup, Canada geese were still prevalent on Centre Island through July and August. As a result of a management program begun in the late 1960s, the population of Canada geese (*Branta canadensis maxima*) in southern Ontario was suggested to have grown from about several thousand birds to more than 350,000 by August, 1998 (Dennis et al. 2001). An analysis of historical breeding pair data, and more recent data from 2003 are in Figure 24.

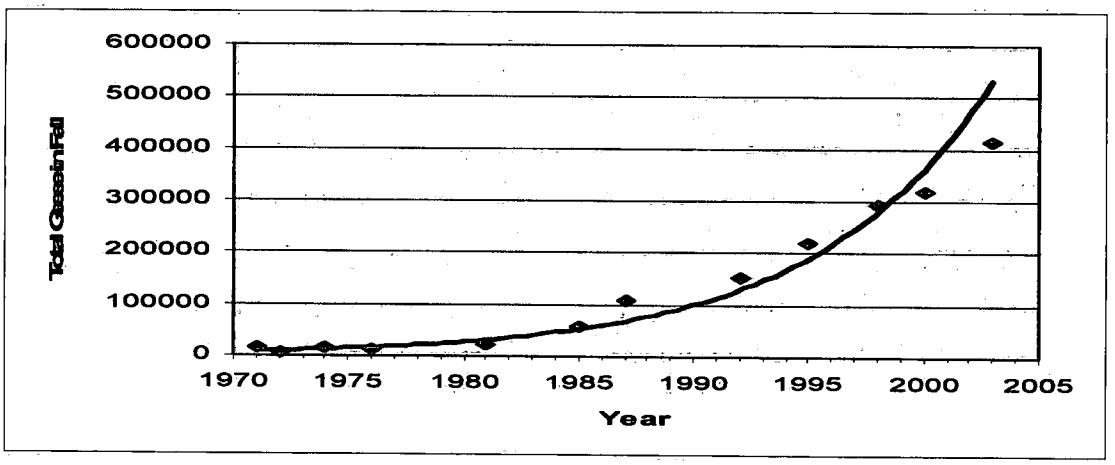


Figure 24. Numbers of Canada geese occurring in Southern Ontario in the fall months. Data courtesy of Jack Hughes, Canadian Wildlife Service, Environment Canada, Ottawa, Ontario.

Other animals occasionally observed on Centre Island Beach were mallard ducks and swans. While dogs were not observed at Centre Island Beach, dog walking was common in the Kew Beach area, and the dogs were occasionally observed swimming in the water. However, dog droppings were only rarely observed along the Kew Beach shoreline, and unlike gull and geese droppings, were not observed directly in the water at both beaches. Any fecal contamination of Kew Beach water from dog droppings would likely be more dependent upon rainfall events to flush *E. coli* into the Lake.

There was no question about *E. coli* from gull and geese droppings contributing to fecal contamination at both Centre Island and Kew Beaches. Gulls were routinely observed to be standing or scavenging right at the waters edge at Centre Island Beach, and their fecal droppings were observed directly in the water or on the wet sand subject to wave action. Early in the spring, many hundreds of gull droppings could be observed within two metres of the waterline at Centre Island Beach. On a number of occasions later in the summer, Canada geese droppings were observed rolling in the surf washing up onto Centre Island Beach. Both gull and geese droppings were observed on the wave swept rock outcroppings between transects 2 and 3 at Kew Beach. The seasonal trends in numbers of gull and geese droppings at Centre Island Beach were the same as observed at BayFront Beach in Hamilton Harbour in 2004 (Edge and Hill, 2005b).

Animals vary in the levels of *E. coli* found in their feces. Seyfried and Harris (1990) compared levels of *E. coli* in feces from humans, wildlife species and domestic animals in the Toronto area. The highest *E. coli* concentrations per gram of feces ($> 10^8$) were found in bird species such as pigeons, ducks, and gulls. High concentrations were also found in raccoons, chickens, dogs, and humans. Lower concentrations were found in feces from muskrats, cats, geese, and horses. Gould and Fletcher (1978) calculated that the weight of daily fecal droppings from gulls, as a percentage of body weight, was about 10 times greater than that of humans. Based upon comparison of their data with published data on other animal species, they concluded that the daily fecal coliform load from some gull species could exceed that from humans. While the daily amount of feces produced by gulls may be considerably less than humans or other animal

species, its high concentration of fecal coliforms may render it a more significant fecal pollution load.

Gould and Fletcher (1978) studied caged gulls to determine the frequency and characteristics of their fecal droppings. Individual gulls produced between 34 and 62 droppings in 24 hours. The total weight of fecal droppings over this period ranged from 11.2 g to 24.9 g per gull. While this study did not measure *E. coli* specifically, individual gull fecal coliform loadings over 24 hours ranged from $3 - 50 \times 10^8$ CFU. The average fecal coliform count (per gram wet weight) ranged from $0.003 - 480.0 \times 10^7$. Fecal droppings that were white with large green or brown (fecal) centers were generally associated with higher fecal bacteria counts that droppings that were mostly white.

Alderisio and DeLuca (1999) studied 249 gull droppings and 236 Canada geese droppings and found gull feces had much higher concentrations of fecal coliforms (3.68×10^8 FC / gram of feces) than the geese (1.53×10^4 FC / gram of feces). Even considering the larger size of geese droppings (mean = 8.35 g.) relative to gull droppings (mean = 0.48 g.), these authors calculated that a gull would contribute an average 1.77×10^8 FC per fecal dropping compared to 1.28×10^5 FC per fecal dropping for geese. Levesque et al. (1993) reported fecal coliform counts (99% of which were estimated to be *E. coli*) in ring-billed gull fecal droppings to range from 1.1×10^6 to 2.4×10^7 CFU / g. of feces. While the average count of *E. coli* in gull feces from a Chicago beach on Lake Michigan was found to be 4.9×10^8 CFU / gram of feces, one fecal dropping had 1.9×10^9 CFU / gram of feces (Fogarty et al. 2003). Based on an average wet weight of feces excreted by gulls ranging from 11.2 to 24.9 grams / day (from Gould and Fletcher, 1978), Fogarty et al. (2003) calculated that the average daily load of *E. coli* from one gull would be 1.2×10^{10}. Hussong et al. (1979) calculated that, over 24 hours, a Canada goose would excrete 10^7 fecal coliforms, while a swan would excrete 10^9 fecal coliforms. Birds can also carry enteric pathogens in their feces such as *Campylobacter* species, *Salmonella* species, and *Aeromonas* species (Fallacara et al. 2001; Fogarty et al. 2003; Levesque et al. 1993) as well as antibiotic resistant bacteria (Cole et al. 2005; Middleton and Ambrose, 2005).

Fecal droppings from birds have been reported to be capable of significantly impairing water quality in aquatic ecosystems. Kirschner et al. (2004) found *E. coli* levels reaching 13,000 CFU / 100 ml in shallow saline pools whose fecal inputs were exclusively from birds such as gulls, geese and ducks. These authors found counts of bird droppings along the shoreline to be a good indicator of recent bird abundance and correlated to *E. coli* levels in the water. Flocks of waterfowl have been shown to cause elevations in fecal coliforms in surface waters of Chesapeake Bay (Hussong et al. 1979). Palmer (1983) found bridges crossing the Rideau River in the city of Ottawa, Ontario had roosting pigeons that were calculated to have a significant impact on fecal coliform levels downstream of the bridges during summer dry weather flows. Benton et al. (1983) found that a serious deterioration in bacterial water quality in a Scottish reservoir could be attributed to an increased number of gulls (*Larus* sp.) roosting on the reservoir. Alderisio and DeLuca (1999) reported that fecal coliform counts decreased significantly in a New York reservoir after a waterfowl mitigation program was implemented.

Fecal droppings from birds have also been implicated in significant impairments to recreational water quality at beaches (Standridge et al. 1979; Levesque et al. 1993 and 2000; Whitman and

Nevers, 2003; Edge and Hill, 2005a; Wither et al. 2005). Levesque et al. (1993) found ring-billed gull droppings to have significantly impaired water quality at an urban beach in Quebec City. Levesque et al. found increases in bird numbers on the beach correlated to increased fecal coliform counts in water, particularly at shallow depths of 0.3 and 0.7 m. These authors found that as soon as food was spread on the sand at a beach with excellent water quality, the numbers of gulls rose rapidly, as did fecal coliforms in the water, to the point where after two days and in the presence of 30 birds, the Canadian recreational water quality guideline of 200 fecal coliforms / 100 ml was exceeded. Far more birds than this were common on Centre Island Beach in the summer of 2004.

4.4 E. coli Source Tracking

This microbial source tracking study applied multiple lines of evidence including an antibiotic resistance analysis approach, supplemented by rep-PCR DNA fingerprinting, to determine the source of *E. coli* at Centre Island and Kew Beaches. The approach received support at a meeting of microbial source tracking experts sponsored by Environment Canada's National Water Research Institute, the City of Toronto, and the Ontario Ministry of the Environment in Toronto on March 7-8, 2005 (Edge and Schaefer, 2005). The field of microbial source tracking is still evolving, and this study is among the most comprehensive conducted at freshwater beaches to date.

The Toronto fecal source library of 2260 *E. coli* isolates is among the larger libraries used in microbial source tracking studies. While a science-based minimum size for a library has not been established to date, it is recognized that libraries of *E. coli* need to be as large as possible to best represent the tremendous diversity of *E. coli* isolates across human and animal hosts (U.S. EPA, 2005a). In this study, the library size was determined by practical limits of the available time and resources to conduct the necessary field sampling and lab work to collect rigorously identified *E. coli* isolates over a bathing season. The discriminant function, calculated based on antibiotic resistance data, provided an average rate of correct classification (ARCC) of 63%. While this tool was not 100% reliable, it was comparable to similar antibiotic resistance discriminant functions used in other microbial source tracking studies (Whitlock et al. 2002; Wiggins et al. 2003; Moore et al. 2005). Many previous MST studies have had inflated ARCC values because they used very small *E. coli* libraries, did not remove identical within-sample antibiotic resistant phenotypes, or used biased resubstitution methods to calculate ARCCs. Similarly, the DNA fingerprinting analysis had an ARCC of 53 % after clone removal, which is comparable to more recent rep-PCR studies that recommend removing within-sample clones to improve accuracy of classifications despite the lower ARCC values (Johnson et al. 2004). Other rep-PCR studies have had higher ARCC values (Dombeck et al. 2000; McClellan et al. 2003) perhaps attributable to small library sizes or not removing these identical DNA fingerprint clone strains.

Both MST methods indicated the prominence of bird *E. coli* in the water and sand at Centre Island Beach rather than wastewater or pet *E. coli*. This was consistent with observations of large numbers of gulls and Canada geese (and their droppings) along the Beach. It was also consistent with the lack of municipal wastewater sources such as CSOs in the Island area (Environmental Defence, 2004). With multiple lines of evidence indicating significant *E. coli*

loadings from birds, a bird control program at Centre Island Beach would likely result in improvements to water quality at the beach.

Unlike DNA fingerprinting data, the antibiotic resistance data suggested a prominent influence of pet *E. coli* in sand and municipal wastewater *E. coli* in chest depth water. When broken down by beach transect, the pet *E. coli* influence was more prominent in sand at transect 2 east of the pier, while the municipal wastewater *E. coli* influence was common across both beach transects. It is possible that the unexplained pet influence is spurious since more detailed analysis of fecal source classifications using more rigorous probability thresholds (e.g. > 0.6) suggested the pet source was less prominent than bird *E. coli*. In addition, when the data was analyzed in the 2-way source classification, birds were a more prominent contributor than pets/wastewater in Centre Island sand. However, it is possible there could be an unrecognized source of pet fecal contamination near the canteen east of the pier, since pet *E. coli* were also more prominent in the water east of the pier than west. It is uncertain why wastewater *E. coli* seems to be relatively more prominent with increasing depth of water. DNA fingerprinting data and 2-way ARA classification data also indicated this trend.

While birds were also prominent *E. coli* contributors to the sand and beach waters at Kew Beach, the fecal contamination sources were more mixed at this beach. The MST results were consistent with the occurrence of birds, and their fecal droppings, on the rock outcroppings between transects 2 and 3. One might have expected more of an influence from the numerous dogs in the Kew Beach area, but this was not the case. A bird control program at Kew Beach would also likely result in some improvements to water quality at the beach.

Both MST methods indicated that municipal wastewater *E. coli* were more prominent at Kew Beach than Centre Island Beach. For example, the water samples collected at a stormwater outfall between Kew Beach transect 1 and the dog-off-the-leash area to the west indicated a very predominant influence of municipal wastewater *E. coli*. Similar to Centre Island, the antibiotic resistance analysis data showed generally similar source contributions at ankle and knee depths that were different from those at chest depth as a result of the relatively increased prominence of wastewater *E. coli* with depth. The basis for these consistent antibiotic resistance results across transects at Kew and Centre Island Beaches remains uncertain. It is possible that *E. coli* from municipal wastewater sources may be entering beach waters sporadically (e.g. wet weather events) from offshore or longshore currents, while *E. coli* from birds may be entering beach waters continuously from onshore sources of bird droppings. Perhaps wastewater *E. coli* is associated with lighter sewage particulates that might settle out of the water column slower than bird *E. coli* associated with heavier sand particulates. As an example, the antibiotic resistance data for Kew Beach classified many of the *E. coli* from ankle, knee, and chest depth collected on July 12, 2004 as from municipal wastewater. It is possible there could have been some form of municipal wastewater release that led to increased and more widespread *E. coli* contamination before that sampling date.

This microbial source tracking study endeavoured to use the best available science to determine the source of fecal contamination at Centre Island and Kew Beaches. However, the weekly Monday sampling regime adopted in the study did not permit sampling to capture sporadic fecal pollution events (e.g. wet-weather events). If municipal wastewater contamination events

occurred that did not coincide with our weekly sampling, then *E. coli* from this source might be underrepresented. Our *E. coli* library thus represented an integration of weekly *E. coli* contamination at Centre Island and Kew Beaches over a whole bathing season. It is also unknown whether birds or other animals are significantly more or less common on beaches than we detected. Use of fecal droppings rather than animal numbers would seem a more stable indicator of potential fecal loading from animal droppings.

The high levels of *E. coli* in sand are a poorly understood complication for applying microbial source tracking tools at beaches. This may complicate MST studies if there is long term persistence of *E. coli* in the sand. If there is differential survival of *E. coli* strains in the sand, the resulting *E. coli* strain composition in the sand may no longer closely reflect the *E. coli* strain composition seen from the original fecal source (e.g. goose gut) entering the environment. Gordon (2001) and Gordon et al. (2002) identified this differential survival of *E. coli* in secondary habitats outside the gut as a potential problem for *E. coli* – based MST studies. In the current study, this concern is minimized compared to many other MST studies by having fewer significant fecal sources to discriminate, and by developing the fecal library on a more restricted spatial and temporal scale.

It remains to be determined whether *E. coli* libraries such as the one developed for Toronto will be relevant for MST studies at beaches away from Toronto, or whether the 2004 library will be relevant for classifying *E. coli* at Toronto beaches in future years. While Wiggins et al. (2003) found some temporal stability for *E. coli* libraries between years in Virginia watersheds, the geographical and temporal stability of the libraries has not been well studied. These are important research questions, since at the extreme, the requirement to build large *E. coli* libraries for each new beach area, or each year at a given beach, would have significant resource implications for beach monitoring programs.

The finding of the importance of bird fecal contamination at Centre Island and Kew Beaches was consistent with findings from source tracking research at other beaches around the Great Lakes area (Levesque et al. 1993; Fogarty et al. 2003; Edge and Hill, 2005a). While birds may not be the most prominent source of fecal contamination at many beaches, the growing populations of gulls and Canada geese in urban areas suggests they will likely be an increasingly important fecal pollution source to address around the Great Lakes in the future. Since each beach is likely to be unique in the prominence of local fecal pollution sources at specific times, or over a bathing season, microbial source tracking tools can be a useful complement to assist ongoing water quality monitoring programs in managing fecal contamination concerns at beaches. For example, some Lake Ontario beaches may have continuous fecal loading from bird or municipal wastewater sources, which is overlaid at times by event-based fecal contamination such as river inputs from a large watershed after a significant rain storm. These situations can present considerable fecal pollution source tracking challenges, and any MST tools must be applied at the appropriate scale to the question posed (e.g. source of contamination in a specific contamination event or over a whole bathing season). The field of microbial source tracking is advancing rapidly, and new MST tools, and a better understanding of the advantages and limitations of existing MST tools will be evolving.

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6. References

Alderisio, K.A. and N. DeLuca. 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., J. Burke and A. Spain. 2003. Fecal indicator bacteria are abundant in wet sand at freshwater beaches. Water Res. 37: 3978-3982.

Benton, C., F. Khan, P. Monaghan, W.N. Richards and C.B. Shedden. 1983. The contamination of a major water supply by gulls (*Larus* sp.). Water Res. 17: 789-798.

Bolton, F.J., S.B. Surman, K. Martin, D.R.A. Wareing and T.J. Humphrey. 1999. Persistence of campylobacter and salmonella in sand from bathing beaches. Epidemiol. Infect. 122: 7-13.

Bruneau, A., H. Rodrigue, J. Ismael, R. Dion and R. Allard. 2004. Outbreak of E. coli O157:H7 associated with bathing at a public beach in the Montreal-Centre Region. Canada Communicable Disease Report Vol. 30-15, August 1, 2004.

Byappanahalli, M.N., D.A. Shively, M.B. Nevers, M.J. Sadowsky and R.L. Whitman. 2003. Growth and survival of *Escherichia coli* and enterococci populations in the macro-alga *Cladophora* (Chlorophyta). FEMS Microbiol. Ecol. 46: 203-211.

Carson, C.A., B.L. Shear, M.R. Ellersieck and A. Asfaw. 2001. Identification of fecal *Escherichia coli* from humans and animals by ribotyping. Appl. Environ. Microbiol. 67: 1503-1507.

Clean Beaches Council. 2005. 2005 State of the Beach Report: Bacteria in Sand - A National Call to Action. July, 2005. Clean Beaches Council, Washington, D.C.16p.

Cole, D., D.J.V. Drum, D.E. Stallknecht, D.G. White, M.D. Lee, S. Ayers, M. Sobsey and J. J. Maurer. 2005. Free living Canada geese and antimicrobial resistance. Emerg Infect. Dis. 11: 935-938.

Dennis, D.G., N.R. North and H.G. Lumsden. 2001. Range expansion and population growth of giant Canada geese in southern Ontario: benefits, drawbacks, and management techniques. p. 159-165 in Towards conservation of the diversity of Canada geese (*Branta canadensis*). K.M. Dickson [Ed]. Canadian Wildlife Service Occasional Paper No. 103. Environment Canada.

Dombek, P.E., L.K. Johnson, S.T. Zimmerley and M.J. Sadowsky. 2000. Use of repetitive DNA sequences and the PCR to differentiate *Escherichia coli* isolates from human and animal sources. Appl. Environ. Microbiol. 66: 2572-2577.

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

Edge, T.A. and S. Hill. 2005b. Preliminary investigations – microbial source tracking with *Escherichia coli* in Hamilton Harbour – 2004. p. 117-121 in Hamilton Harbour Remedial Action Plan Research and Monitoring Report. 2004 Season. Hamilton Harbour Remedial Action Plan, Canada Centre for Inland Waters, Burlington, Ontario. November, 2005.

Edge, T.A. and K.A. Schaefer (ed.). 2005. Microbial Source Tracking in Aquatic Ecosystems: The State of the Science and an Assessment of Needs. National Water Research Institute, Burlington, Ontario. NWRI Scientific Assessment Report Series No. 7 and Linking Water Science to Policy Workshop Series. 26 p

Elhmmali, M., D. Robers and R. Evershed. 2002. Combined analysis of bile acids and sterols/stanols from riverine particulates to assess sewage discharges and other fecal sources. Environ. Sci. Technol. 34: 39-46.

Environmental Defence. 2004. Making waves: An evaluation of Toronto's beaches for the Blue Flag program. Environmental Defence, Toronto, Ontario. 43p.

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

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

Glassmeyer, S.T., E.T. Furlong, D.W. Kolpin, J.D. Cahill, S.D. Zaugg, S.L. Werner, M.T. Meyer and D.D. Kryak. 2005. Transport of chemical and microbial compounds from known wastewater discharges: potential for use as indicators of human fecal contamination. Environ. Sci. Technol. 39: 5157-5169.

Gordon, D.M. 2001. Geographical structure and host specificity in bacteria and the implications for tracing the source of coliform contamination. Microbiology 147: 1079-1085.

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

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

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

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

Haack, S.K., L.R. Fogarty and C. Wright. 2003. *Escherichia coli* and Enterococci at beaches in the Grand Traverse Bay, Lake Michigan: sources, characteristics, and environmental pathways. Environ. Sci. Technol. 37: 3275-3282.

Hagedorn, C., S.L. Robinson, J.R. Filtz, S.M. Grubbs, T.A. Angier and R.B. Reneau Jr. 1999. Determining sources of fecal pollution in a rural Virginia watershed with antibiotic resistance patterns in fecal streptococci. Appl. Environ. Microbiol. 65: 5522-5531.

Harwood, V.J., J. Whitlock and V. Withington. 2000. Classification of antibiotic resistance patterns of indicator bacteria by discriminant analysis: use in predicting the source of fecal contamination in subtropical waters. Appl. Environ. Microbiol. 66 : 3698-3704.

Health and Welfare Canada. 1992. Guidelines for Canadian Recreational Water Quality. Federal-Provincial Working Group on Recreational Water Quality. Minister of Supply and Services Canada. 101 p.

Hussong, D., J.M. Damare, R.J. Limpert, W.J.L. Sladen, R.M. Weiner and R.R. Colwell. 1979. Microbial impact of Canada geese (*Branta canadensis*) and Whistling swans (*Cygnus columbianus columbianus*) on aquatic ecosystems. Appl. Environ. Microbiol. 37: 14-20.

Johnson, L.K., M.B. Brown, E.A. Carruthers, J.A. Ferguson, P.E. Dombek and M.J. Sadowsky. 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.

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

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

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

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

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

Martellini, A., P. Payment and R. Villemur. 2005. Use of eukaryotic mitochondrial DNA to differentiate human, bovine, porcine and ovine sources in fecally contaminated surface water. Water Res. 39: 541-548.

McCLellan, S.L., A.D. Daniels and A.K. Salmore. 2003. Genetic characterization of *Escherichia coli* populations from host sources of fecal pollution by using DNA fingerprinting. Appl. Environ. Microbiol. 69: 2587-2594.

Middleton, J.H. and A. Ambrose. 2005. Enumeration and antibiotic resistance patterns of fecal indicator organisms isolated from migratory Canada geese (*Branta canadensis*). J. Wildlife Dis. 41: 334-341.

Milne, J.E. and M.N. Charlton. 2004. Escherichia coli in water and sand at beaches in Lake Huron, Lake Ontario, and Hamilton Harbour. NWRI Contrinution No. 04-205. National Water Research Institute, Burlington, Ontario.

Milne, J. and M. Charlton. 2005. *E. coli* contamination at beaches on Hamilton Harbour and Lake Ontario. p. 42-45 in Hamilton Harbour Remedial Action Plan Research and Monitoring Report. 2004 Season. Hamilton Harbour Remedial Action Plan, Canada Centre for Inland Waters, Burlington, Ontario. November, 2005.

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

Palmer, M.D. 1983. Fecal coliform loadings from birds on bridges. Can. J. Civ. Eng. 10: 241-247.

Palmer, M. 1988. Bacterial loadings from resuspended sediments in recreational beaches. Can. J. Civ. Eng. 15: 450-455.

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

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

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

Scott, T.M., J.B. Rose, T.M. Jenkins, S.R. Farrah, and J. Lukasik. 2002. Microbial source tracking: current methodology and future directions. Appl. Environ. Microbiol. 68: 5796-5803.

Seyfried, P. and E. Harris. 1990. Bacteriological characterization of feces and source differentiation. Water Resources Branch, Ontario Ministry of the Environment. Queen's Printer for Ontario. 130p.

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

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

Stewart, J.R., R.D. Ellender, J.A. Gooch, S. Jiang, S.P. Myoda and S.B. Weisberg. 2003. Recommendations for microbial source tracking: lessons from a methods comparison study. J. Water Health. 1: 225-231.

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

U.S. EPA. 2005a. Microbial source tracking guide. EPA/600-R-05-064. United States Environmental Protection Agency. Cincinnati, OH. 150p.

U.S. EPA. 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, Ohio. 74p.

Versalovic, J., T. Koeuth and J.R. Lupski. 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucl Acids Res. 24: 6823-6831.

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

Whitman, R.L. and M.B. Nevers. 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., D.A. Shively, H. Pawlik, M.B. Nevers and M.N. Byappanahalli. 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.

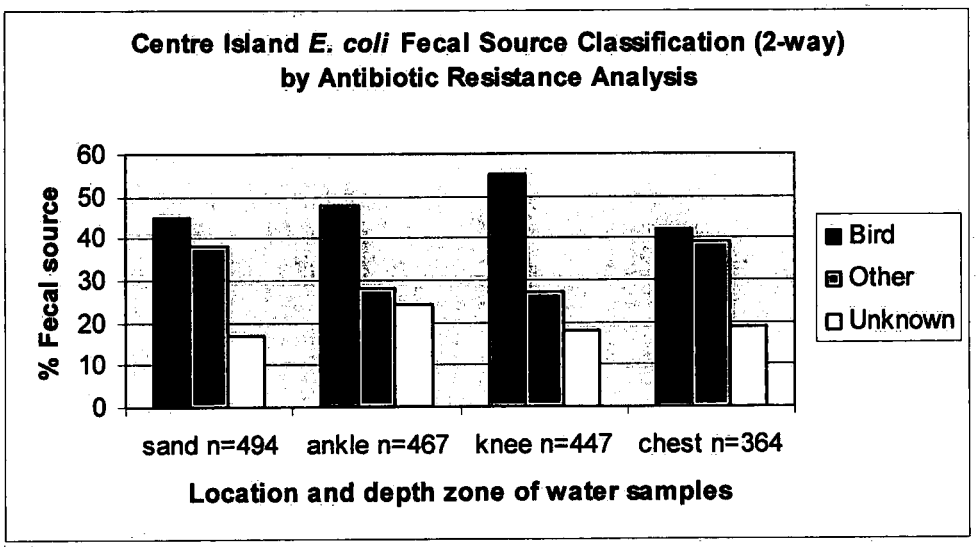
Wiggins, B.A. 1996. Discriminant analysis of antibiotic resistance patterns in fecal streptococci, a method to differentiate human and animal sources of fecal pollution in natural waters. Appl. Environ. Microbiol. 62: 3997-4002.

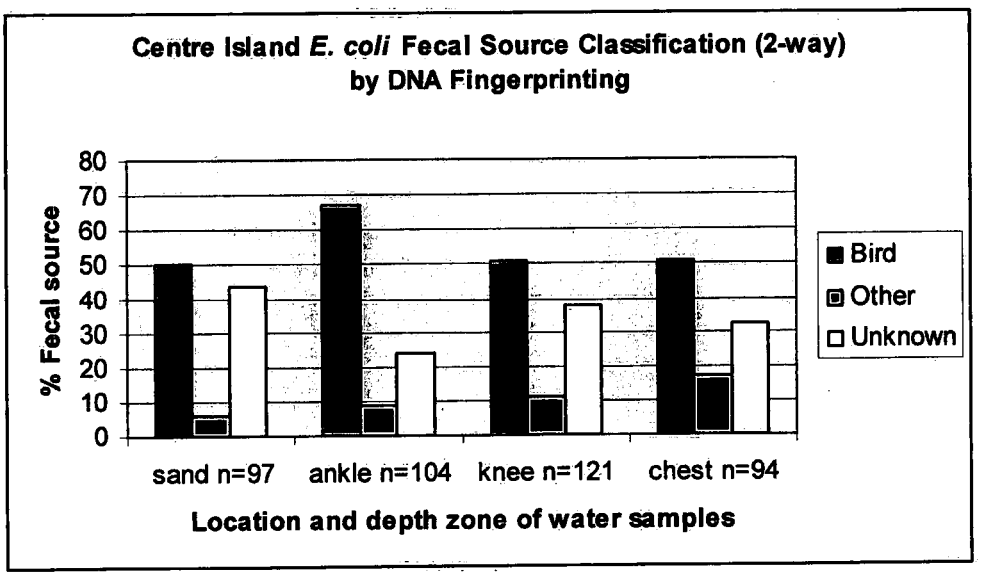
Wiggins, B.A., P.W. Cash, W.S. Creamer, S.E. Dart, P.P. Garcia, T.M. Gerecke, J. Han, B.L. Henry, K.B. Hoover, E.L. Johnson, K.C. Jones, J.G. McCarthy, J.A. McDonough, S.A. Mercer, M.J. Noto, H. Park, M.S. Phillips, S.M. Purner, B.M. Smith, E.N. Stevens and A.K.Varner. 2003. Use of antibiotic resistance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

Wither, A, M. Rehfisch and G. Austin. 2005. The impact of bird populations on the microbiological quality of bathing waters. Water Sci. Technol. 51: 199-207.

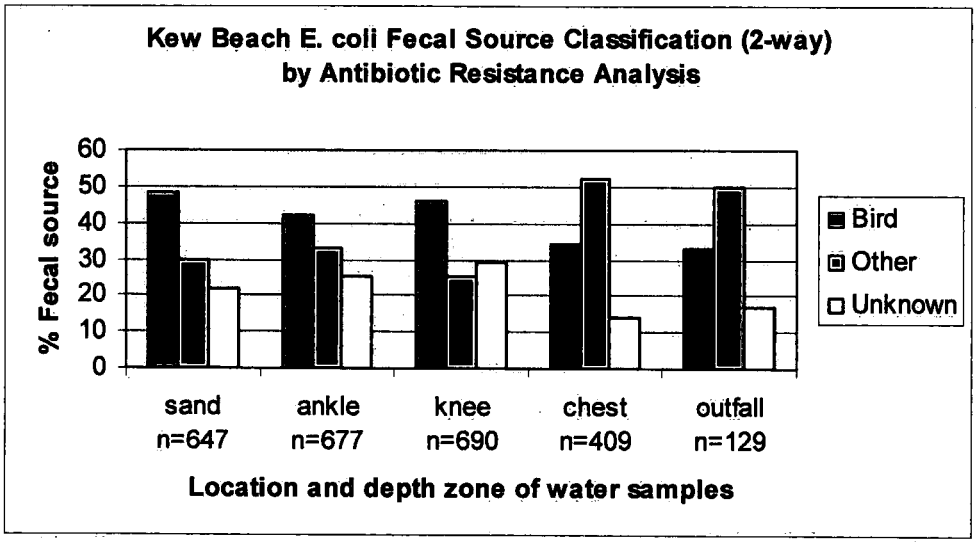
7. Appendix

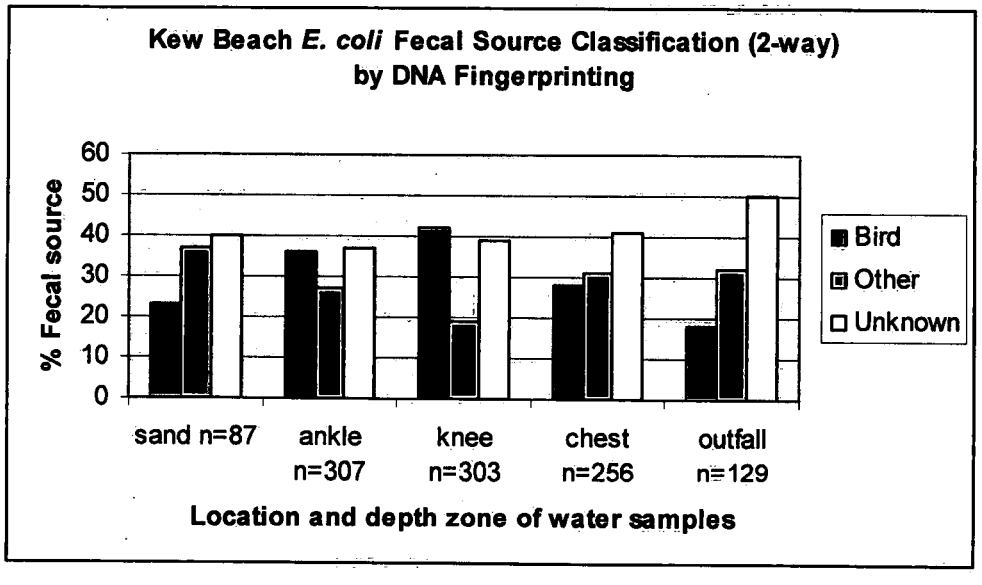
Appendix 1a. 2-way fecal source classification by antibiotic resistance analysis (top) and DNA fingerprinting (bottom) of *E. coli* isolates from Centre Island Beach water and sand samples.





Appendix 1b. 2-way fecal source classification by antibiotic resistance analysis (top) and DNA fingerprinting (bottom) of *E. coli* isolates from Kew Beach water and sand samples.





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