

IMPACT OF RAINFALL ON *ESCHERICHIA COLI* CONCENTRATIONS AT BEACHES IN DOOR COUNTY, WISCONSIN

By Amanda M. Griesbach

Rainfall and its associated storm water runoff have been associated with transport of many pollutants into beach water. Fecal material, from a variety of animals (humans, pets, livestock, and wildlife), can wash into beach water following rainfall and result in microbial contamination of the beach. Many locales around the world issue pre-emptive beach closures associated with rainfall. This study looked at eight beaches located in Door County, Wisconsin, on Lake Michigan to determine the impact of rainfall on *E. coli* concentrations in beach water. Water samples were collected from beach water and storm water discharge pipes during rainfall events of 5 mm in the previous 24 hours. Six of the eight beaches showed a significant association between rainfall and elevated beach water *E. coli* concentrations. The duration of the impact of rainfall on beach water *E. coli* concentrations was variable (immediate to 12 hours). Amount of rainfall in the days previous to the sampling did not have significant impact on the *E. coli* concentrations measured in beach water. Presence of storm water conveyance pipes adjacent to the beach did not have a uniform impact on beach water *E. coli* concentrations. This study suggests that each beach needs to be examined on its own with regard to rain impacts on *E. coli* concentrations in beach water.

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by

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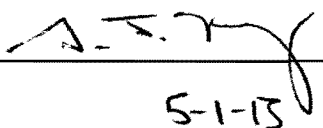
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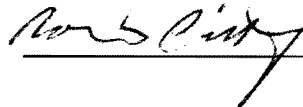
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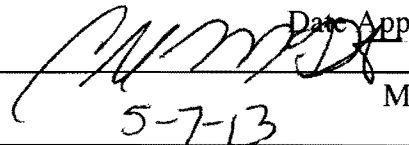
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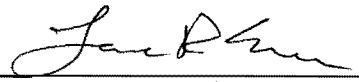
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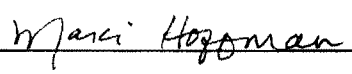
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Introduction

Wisconsin is a leader when it comes to freshwater reservoirs. Wisconsin's surface water is important for drinking water and recreation. It is surrounded by two Great Lakes (Lake Superior and Lake Michigan) and contains over 15,000 inland lakes (Wisconsin Department of Natural Resources [WDNR], 2007). Unfortunately, the abundance of freshwater in Wisconsin increases the potential of fecal contamination of these waterways from agriculture, gull or human inputs. It is essential to evaluate contaminants that pose a threat to human health.

In the last ten years there has been increased concern involving fecal contamination at recreational beaches. In 2000, the United States Congress passed the Beaches Environmental Assessment and Coastal Health Act (BEACH Act) which provided specific criteria for beach monitoring, better public notification, and increased funding for state and local health departments to develop and continue beach monitoring programs (United States Environmental Protection Agency [USEPA], 2006). Since the onset of this program, there has been both an abundance of data collected on fecal indicator bacteria (FIB) concentrations in the Great Lakes, including *Escherichia coli*. Likewise, a variety of research related to the potential sources of contamination has been conducted (Nobel et al., 2003). Naturally occurring microbes are ubiquitous in water and include such organisms as *Escherichia coli* (*E. coli*) (Whitman, Nevers, & Byappanahalli, 2006).

It is determining the difference and significance between the natural cycling of these organisms and the extraneous inputs of these organisms that has confounded beach managers. When microbial concentrations exceed historical levels, concerns are raised regarding public health risks. Fecal contaminated water can host a variety of non-native organisms; including pathogenic bacteria. Generally, the bacteria that are of most concern with regard to fecal contamination are thought to be those that reside in mammalian intestines (Joao & Cabral, 2010). Once mammalian feces are defecated into the environment, the microbes existing in the feces have the potential to cause disease. Bacteria capable of causing disease, or pathogens, are the primary concern when evaluating water quality and the risk of microbial content to public health (Benskin, Wilson, Jones, & Hartley, 2009; USEPA, 1984). In general, pathogens can be difficult to monitor and both expensive and time consuming to examine. Recreational water quality is generally measured using indicator organisms or bacteria that have traditionally been thought to behave similarly to pathogens (Colford et al., 2007; USEPA, 1986). These organisms are much easier to test for and are more cost effective.

E. coli has been conventionally used as a microbial indicator of recent fecal pollution due to its perceived presence or absence when pathogens are present or absent. Additionally it was thought to have growth characteristics and survivability similar to that of bacterial pathogens, and it survives in similar numbers to that of pathogenic organisms (Jagals, Grabow, & de Villiers, 1995).

The water quality standards in the state of Wisconsin and according to the Beach Act have been implemented since 2003. When the fecal indicator bacteria, *E. coli* reaches a concentration of 235 MPN/100 mL of beach water an advisory sign is posted at the beach indicating a potential health risk to those swimming in the near-shore water. If *E. coli* concentrations exceed 1000 MPN/100 mL of beach water a closure sign is posted signifying that bathers should stay out of the water due to elevated *E. coli* concentrations until further notification. A limiting factor associated with conventional testing of indicator organisms like *E. coli* or enterococci is the inability to distinguish the source of fecal contamination. That is, one can test for the presence or absence of an indicator organism but there is not a reliable rapid method for determining the source of these indicators (USEPA, 2002 & 2006).

Across the nation, closures of recreational swimming beaches due to microbial (fecal) contamination of water have prompted research into the source of elevated microorganism concentrations. Fecal pollution may result from point and non-point sources (Hagedorn et al., 1999; McLellan, 2004). Point sources such as sewage overflows, agricultural runoff, urban storm water, and streams have been linked to increases in microbial loads to natural bodies of water and swimming beaches (McLellan, 2004). Fecal bacteria identified in water are generally derived from either human and/or animal sources (Stevenson, 1953) and enter recreational swimming beaches through a variety of pathways. This research focuses on source identification (human, cow, dog, cat

or birds) from polluted beach water and how fecal contamination moves from host to beach water.

Microbial Source Tracking (MST) refers to the categorizing of various microorganisms according to their host. It can also be used to differentiate between different lineages of bacteria found within a variety of human hosts, which is important in identifying where *E. coli* may be coming from after a rainfall (Stoeckel & Harwood, 2007).

An aspect that was examined in this study includes additional characterization (DNA fingerprinting) of *E. coli* isolates from beach water, to further help in determining a source of microbial contamination at these beaches. The results may be used to help assess risk posed by *E. coli* found at a beach. Bacteria can form various subgroups depending on their environmental conditions including pH and specific hosts. The genetic components established from the adapted bacteria should show genetic similarity in continuing generations. These genetic similarities can be used like bacterial “fingerprints” in uniquely identifying differences from bacteria with different hosts or environments (Dombek, Johnson, Zimmerley, & Sadowsky, 2000).

Studies rely on either library dependent and independent source tracking methods. A library dependent study relies on a collection of isolates from known source samples (Hansen, Ishii, Sadowsky, & Hicks, 2009). From these known source isolates, unknown source isolates can be compared with specific source characteristics and classified. Analysis of similarity between known isolates has been determined through a library self-

cross, which shows a value of similarity between isolates and can predict percentage rates of correct classification (Hagedorn et al., 1999; Stoeckel & Harwood, 2007).

This technique presents a way to differentiate *E. coli* based on the amplification of the DNA between repetitive extragenic elements. A BOX primer is used to construct a vast pattern of detailed fingerprints. These strain specific DNA fingerprints can be identified and compared to unknown isolates through computer generated software. Once many sources have been correctly identified via the computer program, unknown sources can be entered for correct source determination (Dombek et al., 2000). The program used in this experiment was Gel ComparII. This technique was chosen due to its accuracy and speed. Other methods such as ribotyping include more time and manipulation with the DNA (Dombek et al., 2000).

Storm water runoff across impervious surfaces such as roads, roofs, lawns, and construction sites, has been identified as the greatest pollution source causing beach closures and advisories (Dorfman & Rosselot, 2008). Streets and parking lots are responsible for over 54% of the total runoff volume in residential areas, and 80% of the total runoff volume in commercial areas (Bannerman, Owens, Dodds, & Hornewer, 1993). As storm water flows over these impervious surfaces, the water can pick up a variety of pollutants including oil, grease, nutrients, pesticides, phosphorus, copper, zinc, and fecal bacteria (Bannerman et al., 1993; Papiri et al., 2003). The fecal bacteria in storm water may be from domestic animals such as cattle, horses, dogs, and cats, or wild animals such as deer and waterfowl. Eventually, these contaminated waters reach surface

waters, and if near a swimming beach, can result in elevated bacterial concentrations and increased health risks for swimmers (Dorfman & Rosselot, 2008; McLellan, 2004; USEPA, 2004).

Heavy rainfall and runoff has been implicated in increases in bacterial contamination at beaches along many coastlines (Ackerman & Weisberg, 2003; Haack, Fogarty, & Wright, 2003). Along the southern California shoreline, swimming beaches are automatically closed or restricted after a rainfall event greater than 2.5 mm, even without microbiological testing of water (Ackerman & Weisberg, 2003; Nobel et al., 2003). Although storms are fairly infrequent in southern California, rainfall events have precipitated microbial contamination exceedances due to storm water runoff (Schiff, Morton, & Weisberg, 2003). The increase in bacterial concentrations is associated with almost all storms with rainfall greater than 6 mm and with every storm with rainfall greater than 25 mm. There is little effect on microbial contamination of beaches following storms of less than 2.5 mm (Ackerman & Weisberg, 2003).

Studies of the contamination of recreational waters by storm water and nonpoint source runoff have been conducted in a few locations along highly urbanized coastlines (Ackerman & Weisberg, 2003; Nobel et al., 2003). While this research has been focused on urban marine shorelines, the contamination of rural and semi-urban freshwater swimming beaches by nonpoint source runoff has not been extensively studied. At several urban beaches in southeastern Wisconsin, the beaches are automatically closed due to increased microbial concentrations in water following a rain event and increased

storm water runoff (Kleinheinz, McDermott, & Chormeau, 2006; McLellan, 2004). Data on microbial loading of swimming beaches due to rainfall and runoff have been collected at several Lake Michigan and Lake Superior beaches in Wisconsin (Kleinheinz et al., 2006; McLellan & Salmore, 2003; Sampson, Swiatnicki, McDermott, & Kleinheinz, 2006) however, data quantifying the microbial loads during rainfall and storm water runoff events have not been studied at the mainly rural beaches located in Door County, Wisconsin.

Door County is a 75-mile long peninsula located in northeastern Wisconsin. The county is bordered by Lake Michigan to the east and the Bay of Green Bay to the west, and has over 300 miles of shoreline with over 30 swimming beaches. Door County is one of the greatest tourist destinations in the Midwest, with more than two million visitors per year. Because many of these tourists visit the beaches in Door County, a minimum of 31 beaches along both sides of the Door County peninsula, at Washington Island, within the Sturgeon Bay Canal, (Figure 1) and at three inland lakes have been sampled for fecal bacteria on a regular basis during the summer swimming season (June 1-August 31), since 2003. In addition, daily rainfall data was collected at multiple locations on the peninsula using rain gauges. Rain gauges were spaced throughout the county to account for differences in meteorological effects from the northern end of the county to the southern end, and to provide data for more than one beach (Figure 1).

While the beaches in Door County have been monitored for fecal bacteria, there is little information on the extent rainfall impacts the water quality of these beaches. Due to

the lack of available data related to rainfall impacts on rural beaches, eight beaches were monitored for *E. coli* concentrations following rain events greater than 0.5 cm (Figure 1).

The overall objective of this project was to determine what impact rainfall had on the *E. coli* concentrations at the selected beaches in Door County, WI. Underlying objectives were to evaluate *E. coli* concentrations in beach water 1, 2, 3, 4, 8, 12, and 24 hours after a rainfall event of 0.5 cm within 24 hours, to assess *E. coli* concentrations of storm water outfall sites surrounding the selected beach containing storm water, and to utilize microbial source tracking (MST) techniques to assist in determining the source of *E. coli* contamination.

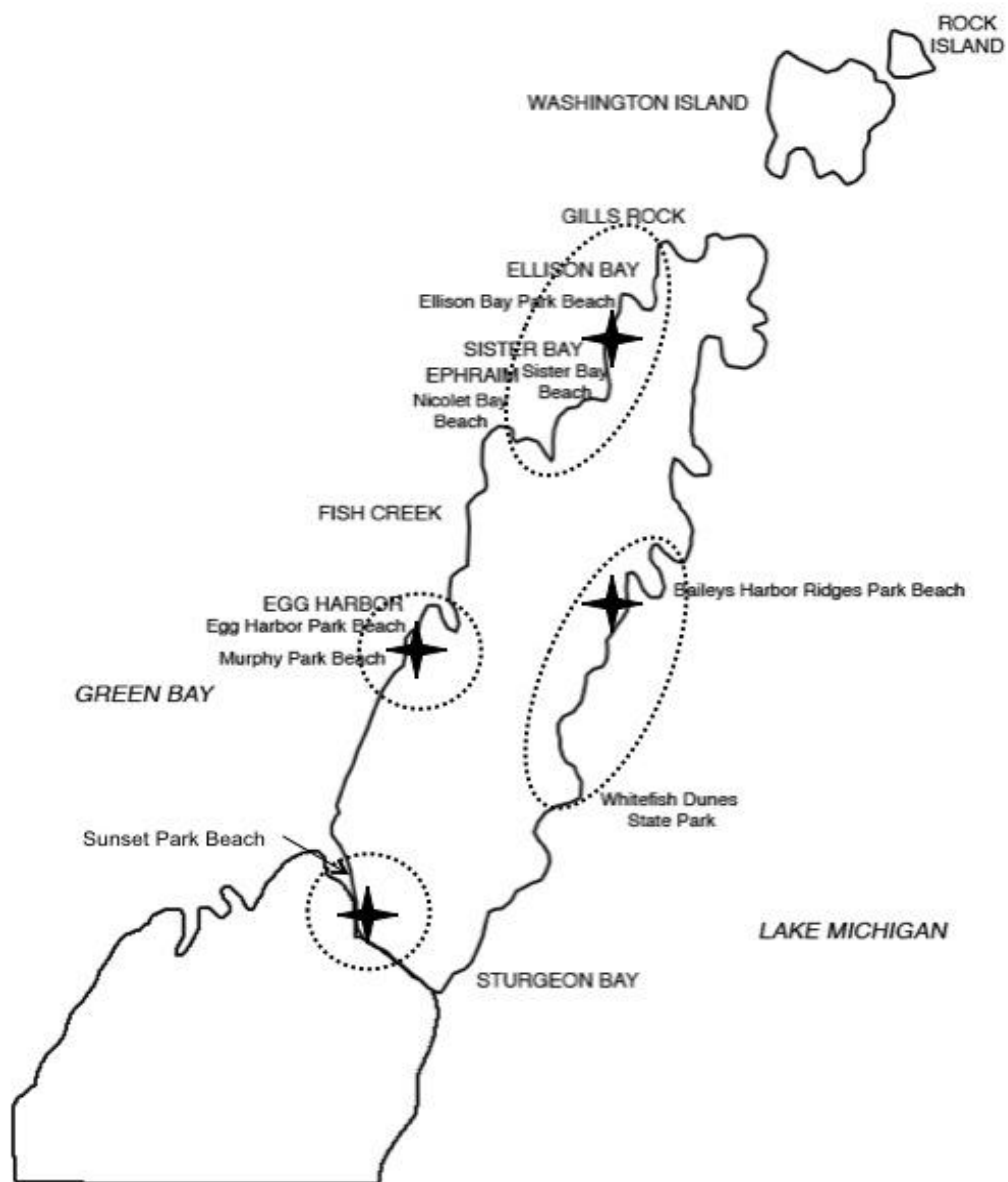


Figure 1. Beaches utilized in this study were located along the Lake Michigan and Green Bay coastlines in Door County, Wisconsin. Stars indicate location of the rain gauges and the dashed lines show the beaches that were studied based on the rainfall at those locations.

Materials and Methods

Water Sample Collection

Beaches were monitored for *E. coli* contamination after a significant rainfall event of 0.5 cm or more in a 24 hour period from mid-May through the end of August, during 2005 and 2006. The rainfall collection amount was an arbitrary number that was selected through the help of The University of Wisconsin Oshkosh and the Door County Public Health Department. To help determine the effect of rainfall on *E. coli* concentrations in beach water and the source of fecal contamination during rainfall events in Door County, WI, beach water samples were collected from 13 selected beaches. Beaches included: Whitefish Dunes, Lakeside Park, Anclam Beach, Bailey's Harbor, Sunset Park, Otumba Park, Egg Harbor, Murphy Park, Fish Creek, Nicolet Bay, Ephraim, Sister Bay, and Ellison Bay (Figure 3). To compare the microbial loads at these beaches during rainfall and runoff to microbial loads during dry beach conditions, the beaches and nearby outfalls were sampled within one hour of the rainfall event.

There were four automated rain gauges located throughout the peninsula in Sturgeon Bay, Whitefish Dunes, Egg Harbor, and Sister Bay (Figure 1). Each gauge was centrally located between certain beaches. Each rain gauge was monitored 24 hours a day by Critical Services Inc. (Green Bay, WI) where an automated phone call alerted samplers when a designated amount of rainfall was reached in a 24 hour period. Water

sampling began within the first hour of the initial phone call. Samples continued to be collected during the 2nd, 3rd, 4th, 8th, 12th, and up to 24th hour.

The water samples were taken from beach water at a depth of 60-78 cm at the center of each beach and approximately 30 cm below the water surface, as required by the Wisconsin Department of Natural Resources (WIDNR) water quality standards for the WI BEACH Act. In addition, samples were taken from various storm sewer basins, pipes, runoff areas, and streams. Designated pipes and streams, in proximity to selected beaches were sampled at 1, 2, and possibly 3 hours after the rainfall trigger, depending on runoff water flow. Duplicate samples were collected at each site for each hour into 100 mL polystyrene sterile bottles (IDEXX Corp., Portland, ME). Samples, upon collection were labeled and immediately kept on ice until lab analysis could be performed. Lab analyses took place at a certified lab in Sturgeon Bay, WI. *E. coli* concentrations at various times and locations post-rainfall event were compared to determine if rainfall was associated with fecal contamination of beach water.

There were 31 beaches in Door County and two beaches in Kewaunee County that were routinely monitored (non-rainfall) for *E. coli* during the summer months, in compliance with the BEACH Act (USEPA, 2006). These water samples were collected according to the priority of each beach, based upon microbial loading and popularity of individual beaches. *E. coli* concentrations from routine monitoring data was collected and analyzed at 24, 48, and 72 hours prior to a significant rainfall and compared with routine *E. coli* concentrations for each beach after a significant rainfall. Routine monitoring data

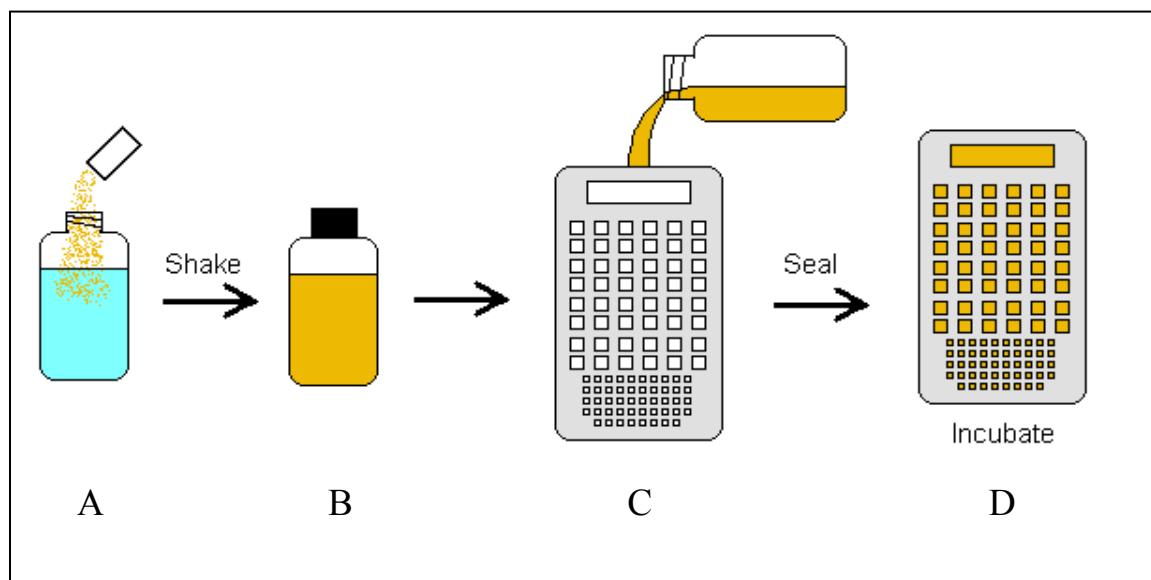
were analyzed using Pearson correlations ($\alpha=0.05$) on an individually beach basis, to more accurately predict a relationship between pre and post rainfall data at each beach (Appendix N, Table 14).

Defined Substrate Method

A defined substrate test, Colilert® (IDEXX Corp., Portland, ME), was used to enumerate *E. coli* from all samples (Figure X-2). All results were recorded as Most Probable Number (MPN) of *E. coli* per 100 mL of water. The defined substrate test contained reagents composed of β -D-glucuronide bound to 4-methyl-umbelliferyl (MUG) and two nutrient indicators, ONPG and MUG which are the major carbon sources. In the presence of the *E. coli* enzyme β -glucuronidase, MUG will be cleaved from β -D-glucuronide and will fluoresce when viewed under an ultraviolet light, indicating a positive result for *E. coli*. As coliform bacteria grow in Colilert® they use β -galactosidase to metabolize ONPG, resulting in a color change from clear to yellow (American Public Health Association [APHA], American Water Works Association [AWWA], & Water Environment Federation [WEF], 1999).

Colilert® was added to 100 mL sample of beach water and shaken until contents dissolved. The contents of the bottle were poured into sterile Quanti-trays (IDEXX) and heat sealed prior to incubation. Coliforms were enumerated after a 24 hour incubation period at 35°C, by counting the number of yellow colored wells and calculating the coliform number according to an MPN table. Fluorescence wells were then counted

under an ultraviolet light and *E. coli* concentrations were expressed and calculated as MPN per 100 mL water (Figure 2) (APHA, AWWA, & WEF, 1999).



E



Figure 2. Defined substrate test using Colilert® and Quanti-Tray/2000. **A.** Add Colilert® to 100 mL of water sample. **B.** Close cap tightly and shake to completely dissolve reagent. **C.** Pour sample into Quanti-Tray/2000. **D.** Seal the Quanti-Tray/2000 with a sealer and incubate for 24 hours. **E.** After 24 hours observe and record color changes with and without fluorescent light.

Rain Gauge Locations

Thirteen beaches were selected for sampling and were grouped based on four automated rain gauges. The beaches were grouped accordingly:

Lake Michigan Side

1. Whitefish Dunes Beach (Appendix M, Figure Q-2) (1-4, Rain gauge #1)
2. Lakeside Park Beach (Appendix G, Figure L-1)
3. Anclam Beach (Appendix A, Figure A)
4. Bailey's Harbor Beach (Appendix B, Figure G)

Green Bay Side

5. Sunset Park Beach (Appendix L, Figure N-2) (5-6, Rain gauge #2)
6. Otumba Park Beach (Appendix J, Figure A-2)
7. Egg Harbor Beach (Appendix C, Figure O) (7-8, Rain gauge #3)
8. Murphy Park Beach (Appendix H, Figure R-1)
9. Fish Creek Beach (Appendix F, Figure E-1) (9-13, Rain gauge #4)
10. Nicolet Bay Beach (Appendix I, Figure W-1)
11. Ephraim Beach (Appendix E, Figure Y)
12. Sister Bay Beach (Appendix K, Figure G-2)
13. Ellison Bay Beach (Appendix D, Figure T)

Additional water samples were collected (post-rainfall) from designated pipes, runoff areas, streams, and storm sewer basins. Some samples were collected using an extending pole, which a sterile sampling bag was attached, for sampling hard to reach areas. After collection, samples were stored in a cooler at 4°C until they were processed. All samples were processed within 4 hours of collection and analyzed after 24 hours and up to 28 hours.

Fecal samples were obtained for human, cow, dog, cat, goose, duck, and gull at designated Door County, WI beach locations. The samples were obtained through raw sewage, manure, known dog and cat samples, as well as bird droppings at beaches. These samples followed the same isolation and genetic procedures as unknown *E. coli* isolates (see below), which were used to construct a library of known *E. coli* isolates, in order to compare sequences to unknown beach water samples.

Isolation and Identification of *E. coli* Isolates

When beach water results showed elevated *E. coli* concentrations, a duplicate sample underwent membrane filtration in the hopes of isolating multiple *E. coli* colonies. A 0.45µm filter was placed on a filtering apparatus attached to a vacuum. A solution of buffered water along with the duplicate sample was then filtered through the micro-filter. The 0.45µm filter was then placed onto modified mTEC agar. Modified mTEC is a selective media specific for *E. coli* and halts the growth of other bacteria (USEPA, 2002). The mTEC plates were labeled and incubated at 44.5 ± 0.2 °C for 24 to 48 hours. *E. coli* isolates can be identified after incubation by their purple appearance on the media (Figure 4). The positive mTEC plates were then refrigerated at 4°C to slow further replication before transport back to UW Oshkosh lab for isolate identification.

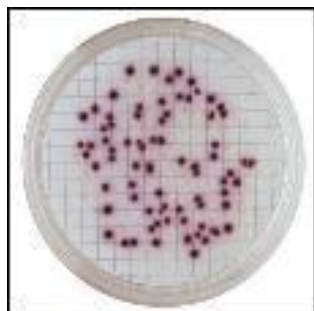


Figure 4. Modified mTEC Agar is a selective culture medium used for the detection and enumeration of *Escherichia coli* in water by the membrane filtration technique. *E. coli* is identified by the purple colonies formed after incubation. From here, isolated colonies can be labeled and identified (APHA, AWWA, & WEF, 1999).

At the UW Oshkosh lab, *E. coli* samples were then prepared for rep-PCR DNA-fingerprinting. This technique is used to differentiate between sources of *E. coli* (Dombek et al., 2000).

Unknown *E. coli* isolates were labeled and a portion of each colony was placed on a nutrient agar slant. The slants were incubated at 35 °C for 24 to 48 hours. A loopful of culture was then transferred onto another nutrient agar slant and the process was repeated. Culture slants were then stored at 4°C. A loopful of culture from the 2nd nutrient agar slant was then transferred into 7 mL of sterile nutrient broth. With extra culture samples: a 500 µL aliquot of sample broth was extracted with a micropipette and mixed with glycerol nutrient broth (50:50) and stored at -80°C until horizontal fluorophore enhanced Repetitive Polymerase Chain Reaction (rep-PCR), (HFERP) could be performed.

A loopful of culture, from individual *E. coli* isolates, was transferred into separate 7 mL sterile Nutrient Broth (NB) tubes, and placed on a shaker (200 rpm) for 24 hours at

37°C. Four microcentrifuge tubes were labeled for each isolate, two with the isolate name, and the remaining two with isolate name/DNA. 1000µl was transferred from NB/culture tubes into microcentrifuge tubes labeled DNA, while 500µl was transferred from NB/culture tubes into microcentrifuge isolate only tubes. 500µl of glycerol was added to the latter two microcentrifuge tubes. These steps were repeated for all isolates. All microcentrifuge tubes were centrifuged at 14,000 RPM for 6 minutes, supernatants were eliminated and then resuspended with 20µl of 95% ethanol and 200 µl distilled water. Each microcentrifuge tube was vortexed and placed into a boiling water bath for 5 minutes, before cooling on ice for 3-5 minutes.

PCR/Gel Electrophoresis

After the *E. coli* cells were lysed, the free-DNA was added to a combination of buffers, primers, and Taq polymerase to form a master mix for successful amplification of DNA. All reagents (listed below) used the following equation based on the number of isolates undergoing amplification (this equation takes into account positive/negative controls and margin of error):

Table A. Recipe for Electrophoresis Gel

Master mix = x(reagent) + 3(reagent) x=number of isolates

5x Gitscher Buffer	5ul
DMSO	2.5ul
6FAM-Box Primer*	1ul
100uM dNTP's	1.25ul
BSA	0.2ul
ddH ₂ O	12.65ul
Taq polymerase (5u/ul)	0.4ul

(*)1 µl of 6FAM-BOX primers consists of a mixture of 0.09 µg of unlabeled Box A1R primer per µl and 0.03 µg of 6-FAM fluorescently labeled Box A1R primer per µl (Integrated DNA Technologies, Coralville, IA).

23µl of reagent master mix was added to each sterile microcentrifuge tube, before 2µl of DNA (lysed cells) were added to each tube (2µl distilled water was added to negative control tube). To initiate the amplification process, all tubes were placed in a 9600 thermocycler (set to RADEMAKER protocol):

Table B. Rademaker Thermocycler Regime

Rademaker Thermocycler Regime

1. Incubation	95°C	2 min	(30 cycles of Steps 2 – 4)
2. Denaturation	94°C	3 sec	
	92°C	30 sec	
3. Annealing	50°C	1 min	
4. Extension	65°C	8 min	
5. Final Ext.	65°C	8 min	
6. End Run	4°C	infinite	

After the thermocycler had completed its cycle an internal standard (6.6 μ L Rox reagent) was added to each microcentrifuge tube, ensuring the validity of each gel. All samples were refrigerated at 4°C until a gel was prepared to run. An agarose gel was prepared by measuring 3.375g of agarose into a flask and set aside. 20 mL of 50x TAE buffer solution was placed in a 2000 mL flask and then filled with Milli-Q water (creating a 0.5 x TAE solution). 225 mL of 0.5 x TAE buffer was then added to the agarose, microwaved for 2-3 minutes and slightly cooled before poured. A plastic comb was placed in the gel box for DNA lane distinction before the agarose solution was slowly poured into gel box. The comb was removed after the gel became solid to create individual wells. In a cold room the remaining 0.5 x TAE buffer was added to the gel before the DNA product/ROX mixture was injected into individual wells. All gels included a positive control (DNA: *E. coli* ATCC 25922) and negative control (no DNA). The positive control, with an internal standard and the negative control were the last two lanes of the gel.

After the power supply was connected to the gel box each gel ran for 14 hours. The completed gel was then placed into a Tupperware container and transported to an imaging room where it was scanned (FX Pro Plus scanner), uploaded (GelComparII), and saved onto a computer.

Statistical and Genetic Analysis

Gels were uploaded into a computer alongside a library of known sources for genetic identification. Genetic similarities were based on the percent similarity of an internal control (ROX) used as a standard on each gel. The standard estimated an amount of error on each gel, giving the ability to calculate a method limit of discrimination. The method limit of discrimination determined for all processed gels was 86% (S.D. +/- 7), indicating any isolate $\geq 86\%$ was considered an identical match to a known source (Appendix N, Table 18).

Average seasonal *E. coli* means were calculated for each beach by averaging *E. coli* concentrations, as required in the BEACH Act (4 samples/week per beach; approximately Memorial Day to Labor Day). Statistical analyses were performed through Gel ComparII (fingerprint and gel analysis software) including Jackknife and Principal Component Analysis (PCA). Scheffe Matrices and Analysis Of Variance (ANOVA) were performed using Systat 11.0. Cluster analysis (dendograms), variability, bias, standard error, and significance between *E. coli* concentrations and post-rainfall sampling times were interpreted from these analyses.

Results

Post Rainfall *E. coli* Concentrations

During the summer swimming seasons in Door County, WI in 2005 and 2006, all beaches studied (post-rainfall) exceeded the advisory threshold of 235 *E. coli*/100 mL of beach water sample, except Nicolet Bay Beach. The closure threshold of 1000 *E. coli* /100 mL of beach water was exceeded at all beaches in either 2005 or 2006, except for Nicolet Bay Beach. The effects of rainfall on *E. coli* were determined through an ANOVA between post-rainfall and non-rainfall seasonal *E. coli* means between 2005 and 2006 for all available beach data. Rainfall and *E. coli* concentrations exhibit a similar pattern throughout Sturgeon Bay, Egg Harbor and Sister Bay County's (Appendix N, Figures V-2 and W-2).

Genetic Analysis of *E. coli* Rain Isolates by Beach

The method limit of discrimination amongst all gels (*E. coli* rain isolates) was 86% (S.D. +/- 7). Appendix N, Table 17 shows the number of *E. coli* isolates, from post-rainfall samples identified by source (human, cow, goose, gull, duck, total avian, dog, or cat). It also shows isolates exceeding the correlation coefficient and isolates remaining within the standard deviation. Appendix N, Table 18 shows the percentage of isolates (from each source) above the method limit of discrimination based on the total number of isolates in Appendix N, Table 17.

Anclam Beach (Appendix A, Figure A).

Beach water collected after a rainfall during the summers of 2005 and 2006, had *E. coli* concentrations spiking on the 24th hour of water collection. In three out of six samples, *E. coli* concentrations were above the (*E. coli*) closure threshold (1000 MPN/100 mL) after a 24 hour period. Of the remaining three samples one sample had *E. coli* concentrations exceeding the (*E. coli*) advisory threshold (235 MPN/100 mL) with a value of 260 MPN/100 mL, after a 24 hour period (Appendix A, Figures B & C).

Stormwater discharge from Anclam's Pipe 1, during the swimming season of 2006, had *E. coli* concentrations exceeding the closure threshold by five to eight times. *E. coli* concentrations ranged from 5,239 MPN/100 mL in the first hour to 8,397 MPN/100 mL in the second hour post-rainfall. The third sampling hour post-rainfall had *E. coli* concentrations of 358 MPN/100 mL, still exceeding the advisory threshold (Appendix A, Figure D).

In both 2005 and 2006 seasonal *E. coli* concentrations in beach water (no rainfall) were significantly less than post-rainfall ($\alpha=0.05$) with p-values of 0.000 and 0.002, respectively (Appendix N, Tables 15 & 16). Due to significant differences between Anclam's seasonal *E. coli* concentrations and *E. coli* concentrations post-rainfall, a Scheffe Matrix analysis was performed on all of Anclam's data, to show if and where significance had occurred. Significance occurred during the 8th hour ($p=0.023$) and 24th hour ($p=0.000$) post-rainfall when compared with the seasonal mean. *E. coli*

concentrations were not significantly different in post-rainfall sampling times between hours 1-4 and hour 12, versus the non-rainfall seasonal mean (Appendix A, Table 1).

A total of 433 *E. coli* isolates were collected post-rainfall from Anclam's beach water. A total of 106 *E. coli* isolates from Anclam's beach water, were identified as from an avian source. Other *E. coli* isolates were identified as cow and human totaling 47 and 45, respectively (Appendix N, Table 17). Anclam had a total of 39 *E. coli* isolates recovered post-rainfall with 48.7% (19 rain isolates) above the method limit of discrimination, with the highest number of these isolates (10) identified as total avian isolates (Appendix N, Tables 18 & 19). Additionally, six of Anclam's *E. coli* isolates (post-rainfall) were identified as from human sources, five from cow, and two were identified as coming from a dog. No *E. coli* isolates (post-rainfall) matched duck or cat sources (Appendix N, Table 19).

Jackknife analysis revealed Anclam's post-rainfall *E. coli* isolates had a similarity value of 100, when compared to known gull *E. coli* isolates. No other sources showed similarity. Known *E. coli* isolates from human, goose, cow, dog, and cat produced similarity values 97, 96, 97, 94, and 96, respectively, when compared sole to isolates within each source (Appendix A, Table F).

Principal Component Analysis (PCA) was used to show relationships between waste isolate sources and rain isolates. Isolates can be discriminated from one another by their color. Known *E. coli* isolate sources were clustered in the lower left quadrant of the

figure. Post-rainfall *E. coli* isolates (unknown) from Anclam's beach water, also appeared within this cluster (Appendix A, Figure E).

Bailey's Harbor Beach (Appendix B, Figure G).

In 2005 and 2006, most maximum *E. coli* concentrations from beach water were reached within the first four hours after a significant rainfall. Four out of the six sampling events showed *E. coli* concentrations exceeding the advisory threshold (235 MPN/100 mL) and closure threshold (1000 MPN/100 mL) (Appendix B, Figures H & I). The exceptions were seen in the summer of 2005, when a maximum *E. coli* concentration of 2,282 MPN/100 mL was enumerated during the twelfth hour post-rainfall (Figure H) and in the summer of 2006 when a maximum *E. coli* concentration of 5,822 MPN/100 mL was reached after the fourth hour of sampling, five times above the *E. coli* closure threshold (Appendix B, Figure I).

Bailey's Harbor has two pipes and a stream flowing into the beach area. Pipe 1's water samples reached a maximum *E. coli* concentration of 1,333 MPN/100 mL, exceeding the closure threshold (1000 MPN/100 mL) after the first hour. Additional water samples could not be obtained due to lack of water flow (Appendix B, Figure J). Pipe 2's water samples contained *E. coli* concentrations of 645 MPN/100 mL and 903 MPN/100 mL, within the first and third hour post-rainfall, respectively. Both samples exceed the advisory threshold (235 MPN/100 mL) (Appendix B, Figure K). Water collected from Bailey's Harbor Stream 1 (hour 2 post-rainfall) reached an *E. coli* concentration of 1,386 MPN/100 mL, which exceeds the closure threshold (1000

MPN/100 mL). An additional water sample from Stream 1 taken during the third hour post-rainfall exceeded the advisory threshold (235 MPN/100 mL), with an *E. coli* concentration of 898 MPN/100 mL (Appendix B, Figure L).

In both 2005 and 2006, the seasonal *E. coli* concentrations in beach water (no rainfall) were significantly less than post-rainfall data at Bailey's Harbor ($\alpha=0.05$) with $p=0.000$ (Appendix N, Tables 15 & 16). Due to significant differences between seasonal *E. coli* concentrations and *E. coli* concentrations after a rainfall, a Scheffe Matrix analysis was performed on all of Bailey's data to show if and where significance had occurred. Significant differences occurred during hours 1-4 ($p=0.000$) and hour 12 ($p=0.000$) post-rainfall, when compared with the seasonal mean. No significance was seen post-rainfall during the 8th and 24th hour; when data was compared to the non-rainfall seasonal mean (Appendix B, Table 2).

A total of 451 *E. coli* isolates were collected post-rainfall from Bailey's Harbor beach water. The following shows the number of *E. coli* isolates collected from Bailey's Harbor beach water (post-rainfall) above the method limit of discrimination, for known sources. The majority of unknown *E. coli* isolates (113) were identified as total avian, followed by human and gull totaling 53 and 61 isolates, respectively (Appendix N, Table 17).

Bailey's Harbor had a total of 46 *E. coli* isolates recovered post-rainfall with 69.6% (32 rain isolates) above the method limit of discrimination. The highest number of isolates (21) were identified as total avian isolates (Appendix N, Tables 18 & 19).

Additionally, of Bailey's *E. coli* isolates (post-rainfall) 12 were identified as human, five cow, seven dog, seven goose, nine gull, and five were identified as duck. No *E. coli* isolates (post-rainfall) matched cat isolates (Appendix N, Table 19).

Jackknife analysis revealed Bailey's post-rainfall *E. coli* isolates, when compared amongst each other had a similarity value of 92. Bailey's post-rainfall *E. coli* isolates had a similarity value of 4, for gull and cat isolates when compared to a library of known source isolates. Bailey's post-rainfall *E. coli* isolates were not similar to other sources. Known *E. coli* isolates from human, gull, and cat had similarity values (87, 97, and 97, respectively) when compared to isolates sole within each source (Appendix B, Figure N).

Principal Component Analysis (PCA) was used to show relationships between waste isolate sources and rain isolates. Isolates can be discriminated from one another by their color. Known *E. coli* isolates were clustered in the lower half of the figure with most grouping in the lower left quadrant. This grouping showed post-rainfall *E. coli* isolates from Bailey's Harbor beach water and *E. coli* isolates from human and gull (Appendix B, Figure M).

Egg Harbor Beach (Appendix C, Figure O).

In 2005 and 2006, one post-rainfall beach water sample had an *E. coli* concentration exceeding the closure threshold (1000 MPN/100 mL). This sample was taken during the eighth hour post-rainfall in 2005 and had an *E. coli* concentration of 5,172 MPN/100 mL, five times the closure threshold (Appendix C, Figure P). Five beach water samples in 2005 had *E. coli* concentrations exceeding the advisory threshold (235

MPN/100 mL), during the first twelve hours post-rainfall. The MPN's/100 mL were 292, 269, 576, 987, and 464, respectively (Appendix C, Figure P).

Two post-rainfall sampling events in 2006 showed beach water *E. coli* concentrations above 235 MPN/100 mL, the beach advisory threshold. Both of these samples were enumerated during the first two hours post-rainfall with *E. coli* concentrations of 248.1 MPN/100 mL and 307.6 MPN/100 mL, respectively (Appendix C, Figure Q).

In 2005, the seasonal *E. coli* concentration in beach water (no rainfall), was significantly less than post-rainfall *E. coli* concentrations at Egg Harbor ($\alpha=0.05$) with $p=0.000$. In 2006 there was no significant difference between seasonal *E. coli* concentrations in beach water (no rainfall) and post-rainfall *E. coli* concentrations ($\alpha=0.05$) with $p=0.909$ (Appendix N, Tables 15 & 16). Due to significance present between seasonal *E. coli* concentrations and *E. coli* concentrations post-rainfall from Egg Harbor's beach water, a Scheffe Matrix analysis was performed (2005 data) to show if and where significance had occurred. According to the Scheffe Matrix, significance did not occur between post-rainfall sampling times 1-4, 8, 12, or 24 hours, versus the non-rainfall seasonal mean (Appendix C, Table 3).

A total of 421 *E. coli* isolates were collected post-rainfall from Egg Harbor's beach water. A total of 15 *E. coli* isolates from Egg Harbor's beach water were identified as from an avian source. 59 of Egg Harbor's *E. coli* isolates were identified as from gull sources, 51 from human, and 51 were identified as coming from a goose source

(Appendix N, Table 17). Egg Harbor had a total of 16 *E. coli* isolates recovered post-rainfall with 12.5% (2 rain isolates) above the method limit of discrimination, with the highest number isolates (4) from both human and total avian sources (Appendix N, Tables 18 & 19). Egg Harbor's *E. coli* isolates (post-rainfall) identified three gull isolates and one goose isolate. No *E. coli* isolates (post-rainfall) matched cow, dog, duck, or cat isolates (Appendix N, Table 19).

Jackknife analysis showed Egg Harbor's post-rainfall *E. coli* isolates, had a similarity value of 25, when compared amongst them. Egg Harbor's post-rainfall *E. coli* isolates had a similarity value of 75 when compared to known gull isolates. Egg Harbor's post-rainfall *E. coli* isolates were not similar to other known sources. Known *E. coli* isolates from gull, human, goose, dog, and cat had similarity values of 100, when compared amongst them (Appendix C, Figure S).

Principal Component Analysis (PCA) was used to show relationships between waste isolate sources and rain isolates. Isolates can be discriminated from one another by their color. Known *E. coli* isolates were clustered mostly in the lower left quadrant and through the lower middle part of the figure. Post-rainfall *E. coli* isolates from Egg Harbor's beach water also appeared within this cluster (Appendix C, Figure R).

Ellison Bay Beach (Appendix D, Figure T).

Data collected from Ellison Bay's beach water in 2006 showed one water sample (of five) had an *E. coli* concentration greater than the closure threshold (1000 MPN/100 mL), with 1300.5 MPN/100 mL. Few water samples post-rainfall had sampling results

after 24 hours, with the highest *E. coli* concentration at 41 MPN/100 mL (Appendix D, Figure U). Pipe 1, located to the right of the beach had low concentrations of *E. coli* post-rainfall. *E. coli* concentrations from Pipe 1 never exceeded the advisory threshold, with *E. coli* concentrations of 99 MPN/100 mL and 86 MPN/100 mL taken one and two hours post-rainfall, respectively (Appendix D, Figure V).

Data was not collected in 2005 due to low precipitation. In 2006, the seasonal *E. coli* concentration mean in beach water (no rainfall) was significantly less than post-rainfall *E. coli* concentrations at Ellison Bay ($\alpha=0.05$) with $p=0.001$ (Appendix N, Table 16). Due to significance between the seasonal *E. coli* concentration mean and *E. coli* concentrations post-rainfall, a Scheffe Matrix analysis was performed on all of Ellison Bay's data, to show if and where significance had occurred. Significance occurred during the 1-4 hour sampling time post-rainfall ($p=0.000$) versus the seasonal mean. *E. coli* concentrations were not significant during the 8th, 12th, or 24th hour sampling times, versus the non-rainfall seasonal mean (Appendix D, Table 4).

A total of 424 *E. coli* isolates were collected post-rainfall from Ellison Bay's beach water. A total of 138 *E. coli* isolates from Ellison Bay's beach water were identified as from an avian source. 72 *E. coli* isolates were identified as from gull sources, 64 from human, 63 from goose, and 52 were identified as coming from a cow (Appendix N, Table 17). Ellison Bay had a total of 20 *E. coli* isolates recovered post-rainfall, with 60% (12 rain isolates) above the method limit of discrimination with the highest number of isolates (10) identified as total avian isolates (Appendix N, Tables 18

& 19). Of Ellison Bay's post-rainfall *E. coli* isolates nine matched goose, four human, one cow, one dog, and one matched the gull source. No *E. coli* isolates (post-rainfall) matched duck or cat sources (Appendix N, Table 19).

Jackknife analysis showed Ellison Bay's post-rainfall *E. coli* isolates compared amongst each other, had a similarity value of 63. Rain isolates had a similarity value of 37 compared to gull isolates. Ellison Bay's post-rainfall *E. coli* isolates showed no similarity to other sources. Known *E. coli* isolates from gull, human, goose, and cat had high similarity values 93, 96, 99, and 92, respectively, when compared sole to isolates amongst each source (Appendix D, Figure X).

Principal Component Analysis (PCA) was used to show relationships between waste isolate sources and rain isolates. Isolates can be discriminated from one another by their color. *E. coli* isolates were clustered mostly in the lower left quadrant and through the lower middle part of the figure. Post-rainfall *E. coli* isolates also appeared within this cluster (Appendix D, Figure W).

Ephraim Beach (Appendix E, Figure Y).

In 2005 and 2006 ten samples showed a higher *E. coli* concentration than the advisory threshold (235 MPN/100 mL) with *E. coli* concentrations generally peaking within the first eight hours after a rain event (Appendix E, Figures Z & A-1). Only one sample, from six rain events in 2005 and 2006, had an *E. coli* concentration higher than the closure threshold (1000 MPN/100 mL). This sample was taken during the eighth hour

after a significant rainfall in 2005, and had an *E. coli* concentration of 3,873 MPN/100 mL (Appendix E, Figure Z).

Stream 2 at Ephraim Beach leads directly into the beach water, where water samples (taken post-rainfall in 2006) exceeded the closure threshold for *E. coli* concentrations (1000 MPN/100 mL). Samples taken one hour post-rainfall from Stream 2 exceeded the *E. coli* concentration advisory threshold (235 MPN/100 mL) at 816.4 MPN/100 mL. The second and third hours after a significant rainfall have *E. coli* concentrations of 1299.7 MPN/100 mL and 1203.3 MPN/100 mL, both exceeding the closure threshold (1000 MPN/100 mL) (Appendix E, Figure B-1).

In 2005 and 2006, the seasonal *E. coli* concentrations in beach water (no rainfall) were significantly less than post-rainfall ($\alpha=0.05$) with $p=0.000$ (Tables 15 & 16). Due to significance between Ephraim's seasonal *E. coli* concentration and *E. coli* concentrations after a rainfall, a Scheffe Matrix analysis was performed on all of Ephraim's data to show if and where significance occurred. Significant differences were seen between the 8th hour ($p=0.000$) post-rainfall and the seasonal mean. *E. coli* concentrations were not significantly different between post-rainfall sampling times between hours 1-4, 12th, and 24th hours versus the non-rainfall seasonal mean (Appendix E, Table 5).

A total of 428 *E. coli* isolates were collected post-rainfall from Ephraim's beach water. A total of 131 *E. coli* isolates, from Ephraim's beach water were identified as from an avian source. 68 *E. coli* isolates were identified as from a gull source and 63 were

identified as being from a human source (Appendix N, Table 17). Ephraim had a total of 23 *E. coli* isolates recovered post-rainfall with 17.4% (4 rain isolates) above the method limit of discrimination. The highest number of isolates (5) were identified as avian (Appendix N, Tables 18 & 19). One of Ephraim's *E. coli* isolates (post-rainfall) was identified as from human, one from goose, one from gull, and three were identified as coming from a duck source. No *E. coli* isolates (post-rainfall) matched cow, dog, or cat sources (Appendix N, Table 19).

Jackknife analysis revealed Ephraim's post-rainfall *E. coli* isolates had a similarity value of 86, when compared amongst them. Ephraim's post-rainfall *E. coli* isolates had a similarity value of 13 for gull isolates, when compared to a library of known gull *E. coli* isolates. Known *E. coli* isolates from gull, human, goose, cow, and cat all had high similarity values (95, 98, 97, 95, and 92, respectively) when compared amongst them (Appendix E, Figure D-1).

Principal Component Analysis (PCA) was used to show relationships between waste isolate sources and rain isolates. Isolates can be discriminated from one another by their color. *E. coli* isolates were clustered mainly in the lower half of the figure, including numerous post-rainfall *E. coli* isolates (Appendix E, Figure C-1).

Fish Creek Beach (Appendix F, Figure E-1).

In 2005 and 2006, all rain post-rainfall beach water samples showed *E. coli* concentrations above the advisory threshold (235 MPN/100 mL). Four of five events resulted in *E. coli* concentrations above the closure threshold (1000 MPN/100 mL)

(Appendix F, Figures F-1 & G-1). During 2005, *E. coli* concentrations spiked at hours two and three with concentrations of 3,255 MPN/100 mL and 3,076 MPN/100 mL; three times higher than the closure threshold (Appendix F, Figure F-1).

A single sample collected directly from runoff at Fish Creek in 2006 had a low concentration of *E. coli* (100 MPN/100 mL) during the first hour (Appendix F, Figure H-1). Pipe 1's water was sampled during a single rain event in the summer of 2006; which both samples exceeded the advisory threshold for *E. coli* concentration. Pipe 1's water had an *E. coli* concentration of 423 MPN/100 mL after the first hour and 250.5 MPN/100 mL after the second hour (Appendix F, Figure I-1).

In both 2005 and 2006, the seasonal *E. coli* concentrations in beach water (no rainfall) were significantly less than post-rainfall ($\alpha=0.05$) with $p=0.000$ (Appendix N, Tables 15 & 16). Due to significant differences between Fish Creek's seasonal *E. coli* concentration and *E. coli* concentrations after a rainfall, a Scheffe Matrix analysis was performed on all of Fish Creek's data to show if and where significance had occurred. Significant differences were shown during the 1-4 hour ($p=0.000$) and 8th hour ($p=0.011$) after a rain event when compared with the seasonal mean. There were no significant differences between post-rainfall sampling times during the 12th and 24th hour versus the non-rainfall season mean (Appendix F, Table 6).

A total of 436 *E. coli* isolates were collected post-rainfall from Fish Creek's beach water. A total of 119 *E. coli* isolates from Fish Creek's beach water were identified as from an avian source. 51 isolates were identified as being from a gull source and 53

isolates from a goose source (Appendix N, Table 17). Fish Creek had a total of 30 *E. coli* isolates recovered post-rainfall with 16.6% (5 rain isolates) above the method limit of discrimination. The the highest number of these isolates (6) were identified as avian isolates (Appendix N, Tables 18 & 19). Two *E. coli* isolates post-rainfall at Fish Creek were identified as from human, two from cow, three from goose, two from gull, and one was identified as coming from duck. No *E. coli* isolates (post-rainfall) matched dog or cat sources (Appendix N, Table 19).

Jackknife analysis showed Fish Creek's post-rainfall *E. coli* isolates had a similarity value of 88, when compared amongst them. Fish Creek's post-rainfall *E. coli* isolates had a similarity value of 12 for dog isolates, when compared to a library of known dog *E. coli* isolates. Fish Creek's post-rainfall *E. coli* isolates were not similar with other sources. Known *E. coli* isolates from human, dog, and duck had similarity values of 100, when compared amongst them (Appendix F, Table K-1).

Principal Component Analysis (PCA) was used to show relationships between waste isolate sources and rain isolates. Isolates can be discriminated from one another by their color. *E. coli* isolates are clustered mainly in the lower half of the figure. Post-rainfall *E. coli* isolates appear to be scattered throughout the figure (Appendix F, Figure J-1).

Lakeside Park Beach (Appendix G, Figure L-1).

In 2005 and 2006, five out of six rainfall events, sampled within the first four hours after a significant rainfall had *E. coli* concentrations above the advisory threshold

(235 MPN/100 mL). Four out of six events had *E. coli* concentrations exceeding the closure threshold (1000 MPN/100 mL) (Appendix G, Figures M-1 & N-1).

In 2005 during each sampling event, *E. coli* concentrations peaked during a different time. The first sampling event peaked during the first four hours after a significant rainfall with an *E. coli* concentration of 1211 MPN/100 mL. The next two sampling events had *E. coli* concentrations peak during the 8th and 24th hours with *E. coli* concentrations of 2,755 MPN/100 mL and 413 MPN/100 mL, respectively. The final sampling event in 2005 had the highest *E. coli* concentration of 24,196 MPN/100 mL, which peaked during the 12th hour. This is 24 times greater than the *E. coli* closure threshold (1000 MPN/100 mL) (Appendix G, Figure M-1). In 2006, the first sampling event reached an *E. coli* concentration of 17,432 MPN/100 mL in the fourth hour, exceeding the closure threshold by seventeen fold. During the second sampling event an *E. coli* concentration peaked during the 8th hour at a value of 255 MPN/100 mL, exceeding the advisory threshold (235 MPN/100 mL) (Appendix G, Figure N-1).

Pipe 1, at Lakeside Park was sampled twice during the summer swimming season of 2006. In both events all samples significantly exceeding the *E. coli* closure threshold (1000 MPN/100 mL). One sample taken during the first hour reached an *E. coli* concentration of 11,472 MPN/100 mL (Appendix G, Figure O-1).

In 2005 and 2006, the seasonal *E. coli* concentrations in beach water (no rainfall) were significantly less than post-rainfall ($\alpha=0.05$) with $p=0.000$ (Appendix N, Tables 15 & 16). Due to significant differences between Lakeside's seasonal *E. coli*

concentration and *E. coli* concentrations after a rainfall, a Scheffe Matrix analysis was performed on all of Lakeside's data to show if and where significance had occurred. Significant differences were seen during the 12th hour ($p=0.000$) after a rain event when compared to the seasonal mean. There were no significant differences between post-rainfall sampling times during the 1-4, 8th, and 24th hours, versus the non-rainfall season mean (Appendix G, Table 7).

A total of 439 *E. coli* isolates were collected post-rainfall from Lakeside's beach water. A total of 93 *E. coli* isolates from Lakeside's beach water were identified as from an avian source. 49 *E. coli* isolates were identified as from a cow source, 48 from gull and 46 were identified as human (Appendix N, Table 17). Lakeside had a total of 28 *E. coli* isolates recovered post-rainfall with of 35.7% (10 rain isolates) above the method limit of discrimination, with the highest number (9) isolates from the total avian source (Appendix N, Tables 18 & 19). Additionally, five of Lakeside's *E. coli* isolates five isolates (post-rainfall) were identified as from a human source, five from cow, four from goose, four from gull, and one isolate was identified as coming from a duck. No *E. coli* isolates (post-rainfall) matched dog or cat (Appendix N, Table 19).

Jackknife analysis showed Lakeside's post-rainfall *E. coli* isolates compared amongst each other and a library of known cat *E. coli* isolates, had similarity values of 92 and 7, respectively. Lakeside's post-rainfall *E. coli* isolates were not similar to other known sources. Known *E. coli* isolates from gull, goose, dog, and cat had high similarity

values (94, 94, 97, and 97, respectively) when compared to isolates sole within each source (Appendix G, Table Q-1).

Principal Component Analysis (PCA) was used to show relationships between waste isolate sources and rain isolates. Isolates can be discriminated from one another by their color. *E. coli* isolates were clustered mainly in the lower half of the figure. Numerous post-rainfall *E. coli* isolates also appeared within this cluster (Appendix G, Figure P-1).

Murphy Park Beach (Appendix H, Figure R-1).

In 2005 and 2006, six out of nine sampling events had *E. coli* concentrations (from beach water) exceeding the advisory threshold (235 MPN/100 mL) (Appendix H, Figures S-1 & T-1). Of these samples all three rain events in 2005 had *E. coli* concentrations exceeding the closure threshold (1000 MPN/100 mL) (Appendix H, Figure S-1). Four out of nine events had the highest *E. coli* concentrations (from beach water) within the first four hours after a significant rainfall.

The highest beach water *E. coli* concentrations were collected in 2005. Two out of three sampling events showed a spike in *E. coli* concentrations after 24 hours with 3,784 MPN/100 mL and 6,294 MPN/100 mL, respectively (Appendix H, Figure S-1). Some data collected from 2006 is incomplete due to severe weather and inability to collect water samples. Of the samples collected those that exceeded the advisory *E. coli* threshold (235 MPN/100 mL) spiked during the 1st, 8th, and 12th hours with 548 MPN/100 mL, 560 MPN/100 mL, and 306 MPN/100 mL, respectively. A single

sampling event from 2006 had no *E. coli* present during collection times of 2, 3, 4, 8, 12, and 24 hours (Appendix H, Figure T-1). There was not significant runoff to collect for analysis.

In 2005 the seasonal *E. coli* concentrations in beach water (no rainfall) were significantly less than post-rainfall ($\alpha=0.05$) with $p=0.000$. In 2006, there were no significant differences between seasonal *E. coli* concentrations in beach water (no rainfall) and post-rainfall *E. coli* concentrations with $p=0.838$ (Appendix N, Tables 15 & 16). Due to significance found in 2005 between seasonal *E. coli* concentration and *E. coli* concentrations after a rainfall, a Scheffe Matrix analysis was performed to show if and where significance had occurred. Significant differences were seen during the 8th hour ($p=0.001$) after a rain event, when compared with the seasonal mean. No significant differences were seen between 1-4, 12th, and 24th hours verses the non-rainfall season mean (Appendix H, Table 8).

A total of 413 *E. coli* isolates were collected post-rainfall from Murphy Park's beach water. A total of 137 *E. coli* isolates from Murphy Park's beach water were identified as from an avian source. 71 *E. coli* isolates were identified as from gull source, 64 from human, and 60 were identified as goose (Appendix N, Table 17). Murphy Park had a total of 8 *E. coli* isolates recovered post-rainfall with 37.5% (3 rain isolates) above the method limit of discrimination. The highest numbers of these isolates were from cow (2), with (1) isolate similar to the cat source (Appendix N, Tables 18 & 19). No *E. coli*

isolates (post-rainfall) matched human, dog, goose, gull, or duck sources (Appendix N, Table 19).

Jackknife analysis showed post-rainfall *E. coli* isolates compared amongst each other and a library of known gull *E. coli* isolates had similarity values of 73 and 27, respectively. Murphy Park's post-rainfall *E. coli* isolates were not similar to other known sources. Known *E. coli* isolates from gull, human, goose, dog, and cat had similarity values (97, 96, 94, 94, and 96, respectively) when compared to isolates sole within each source (Appendix H, Table V-1).

Principal Component Analysis (PCA) was used to show relationships between waste isolate sources and rain isolates. Isolates can be discriminated from each other by their color. *E. coli* isolates were clustered mostly in the lower middle and right of the figure, including post-rainfall *E. coli* isolates (Appendix H, Figure U-1).

Nicolet Beach (Appendix I, Figure W-1).

This beach was only sampled during three rainfall events in the 2006 swim season due to insignificant rainfall amounts and difficulty entering the state park in 2005. Data from two of three events were impacted by severe weather causing inability to collect water samples. The highest *E. coli* concentration was reached during the fourth hour of sampling with 63 MPN/100 mL. The second highest *E. coli* concentration, reached during the 24th hour had an *E. coli* concentration of 61 MPN/100 mL (Appendix I, Figure X-1). Both concentrations are below the *E. coli* advisory threshold (235 MPN/100 mL).

No significant differences were found between seasonal *E. coli* concentrations in beach water (no rainfall) and *E. coli* concentrations post-rainfall ($p=0.979$) (Appendix N, Table 16). A Scheffe Matrix analysis was performed on all of Nicolet's data (no data was collected in 2005), even though significance was not seen in an ANOVA. There were no significant differences seen between sampling times after a rain event and the regular season mean (Appendix I, Table 9).

A total of 412 *E. coli* isolates were collected post-rainfall from Nicolet's beach water. The following shows the number of *E. coli* isolates collected from Nicolet's beach water (post-rainfall) above the method limit of discrimination for known sources. A total of 140 *E. coli* isolates from Nicolet's beach water were similar to the total avian source, followed by gull and goose isolates at 77 and 60, respectively (Appendix N, Table 17). Nicolet had a total of 7 *E. coli* isolates recovered post-rainfall with 0% of the isolates above the method limit of discrimination (Appendix N, Table 18). Nicolet had a total of 2 beach water *E. coli* isolates (post-rainfall) matching gull. No *E. coli* isolates (post-rainfall) matched human, dog, goose, duck, or cat sources (Appendix N, Table 19).

Jackknife analysis showed Nicolet's post-rainfall *E. coli* isolates compared amongst each other and a library of known gull *E. coli* isolates, had similarity values of 82 and 18, respectively. Nicolet's post-rainfall *E. coli* isolates were not similar to other known sources. Known *E. coli* isolates from gull, human, goose, dog, and cat had similarity values of 97, 95, 97, 97, and 92, respectively, when compared to isolates between each known source (Appendix I, Table Z-1).

Principal Component Analysis (PCA) was used to show relationships between waste isolate sources and rain isolates. Isolates can be discriminated from one another by their color. *E. coli* isolates were clustered mainly in the lower left quadrant and lower center of the figure. Many post-rainfall *E. coli* isolates appeared within the clusters (Appendix I, Figure Y-1).

Otumba Park Beach (Appendix J, Figure A-2).

In 2005 and 2006, all rain events (10) had beach water concentrations of *E. coli* higher than the advisory threshold (235 MPN/100 mL). Six of ten beach water samples post-rainfall had *E. coli* concentrations higher than the closure threshold (1000 MPN/100 mL) (Appendix J, Figures B-2 & C-2). A single beach water sample in 2006 had an *E. coli* concentration of 121,128 MPN/100 mL post-rainfall. This is 120 times the closure threshold (1000 MPN/100 mL) (Appendix J, Figure C-2).

E. coli concentrations generally peaked within the first four hours after a rain event. There were two occasions, one in 2005 and 2006 where *E. coli* concentrations peaked during the 12th hour with concentrations of 3,255 MPN/100 mL and 935 MPN/100 mL, respectively. On two occasions, one in 2005 and 2006, *E. coli* concentrations were above the advisory threshold (235 MPN/100 mL) after a 24 hour period (Appendix J, Figures B-2 & C-2).

Pipe 2 at Otumba Park, was sampled three times during the summer swimming season in 2006. All samples except one, from this location, exceeded the *E. coli* closure threshold (1000 MPN/100 mL). Three samples taken during the second and third hours

exceeded the *E. coli* closure threshold with 241,960 MPN/100 mL, 104,620 MPN/100 mL, and 241,960 MPN/100 mL, respectively. All samples collected from Pipe 2 (except one) had *E. coli* concentrations between 2,801 MPN/100 mL and 34,480 MPN/100 mL. This is up to 34 times the closure threshold of 1000 MPN/100 ml (Appendix J, Figure D-2).

In 2005 and 2006, the seasonal *E. coli* concentrations in beach water (no rainfall) were significantly less than post-rainfall ($\alpha=0.05$) with $p=0.000$ (Appendix N, Tables 15 & 16). Due to significant differences between Otumba's seasonal *E. coli* concentration and *E. coli* concentrations after a rainfall, a Scheffe Matrix analysis was performed on all of Otumba's data to show if and where significance had occurred. Significant differences were shown during the 1-4 hour ($p=0.000$) sampling time after a rain event, when compared to the seasonal mean. There were no significant differences between post-rainfall sampling during the 8th, 12th, and 24th hours versus the non-rainfall seasonal mean (Appendix J, Table 10).

A total of 430 *E. coli* isolates were collected post-rainfall from Otumba's beach water. A total of 138 *E. coli* isolates from Otumba's beach water were identified as from an avian source. 74 *E. coli* isolates were identified as from gull sources, 65 from human, and 59 from goose (Appendix N, Table 17). Otumba had a total of 25 *E. coli* isolates recovered post-rainfall with 88% (22 rain isolates) of these isolates above the method limit of discrimination. The highest numbers of these isolates (8) were from total avian (Appendix N, Tables 18 & 19). Two of Otumba's post-rainfall *E. coli* isolates were

identified as from human sources, eleven from cow, two from dog, two from goose and six from a gull source. No *E. coli* isolates matched duck or cat (Appendix N, Table 19).

Jackknife analysis showed Otumba's post-rainfall *E. coli* isolates compared amongst each other and a library of known gull *E. coli* isolates, had similarity values of 88 and 12, respectively. Otumba's post-rainfall *E. coli* isolates were not similar to other sources. Known *E. coli* isolates from gull, human, goose, dog, and duck had similarity values of 97, 97, 93, 91, and 91, respectively, when compared to isolates within each source (Appendix J, Table G-2).

Principal Component Analysis (PCA) was used to show relationships between waste isolate sources and rain isolates. Isolates can be discriminated from one another by their color. *E. coli* isolates were grouped in the lower left and lower middle part of the figure. Post-rainfall *E. coli* isolates appeared within these clusters (Appendix J, Figure F-2).

Sister Bay Beach (Appendix K, Figure H-2).

During 2005 and 2006, five out of seven beach water samples post-rainfall had *E. coli* concentrations exceeding the advisory threshold (235 MPN/100 mL). Three of seven events resulted in concentrations of *E. coli* higher than the closure threshold (1000 MPN/100 mL) (Appendix K, Figures I-2 & J-2). In 2006 some data is incomplete, due to severe weather and inability to collect water samples. In 2006, *E. coli* concentrations generally peaked within the first four hours after a rainfall event. In all but one case,

concentrations of *E. coli* were below the advisory threshold after the 12th hour. All samples had declined by the 24th hour (Appendix K, Figure I-2).

Pipe's 1 and 2 at Sister Bay beach were sampled during three separate rain events during 2006. All samples exceeded the *E. coli* advisory threshold (235 MPN/100 mL) (Appendix K, J-2 & K-2). Two out of three sampling events from Pipe 1 exceeded the *E. coli* closure threshold (1000 MPN/100 mL) with *E. coli* concentrations spiking during hours one and three at 2,723 MPN/100 mL and 1,520 MPN/100 mL, respectively (Appendix K, Figure J-2). Pipe 2 had no samples exceed the *E. coli* closure threshold (1000 MPN/100 mL). *E. coli* concentrations generally spiked within the first two hours of sampling (Appendix K, Figure K-2).

In 2005 and 2006, the seasonal *E. coli* concentrations in beach water (no rainfall) were significantly less than post-rainfall ($\alpha=0.05$) with $p=0.000$ (Appendix N, Tables 15 & 16). Due to significant differences between Sister Bay's seasonal *E. coli* concentration and *E. coli* concentrations after a rainfall, a Scheffe Matrix analysis was performed on all of Sister Bay's data to show if and where significance had occurred. Significant differences were shown during the 1-4 hour sampling time ($p=0.000$) after a rain event compared to the seasonal mean. *E. coli* concentrations were not significantly different in post-rainfall sampling times during the 8th, 12th, and 24th hours' versus the non-rainfall seasonal mean (Appendix K, Table 11).

A total of 434 *E. coli* isolates were collected post-rainfall from Sister Bay's beach water. A total of 139 *E. coli* isolates collected from Sister Bay's beach water (post-

rainfall) were identified as avian isolates. 74 *E. coli* isolates were identified as from gull sources, 73 from human and 60 from goose (Appendix N, Table 17). Sister Bay had a total of 30 *E. coli* isolates recovered post-rainfall with 36.6% (11 rain isolates) above the method limit of discrimination. The highest numbers of isolates (11) were from gull (Appendix N, Tables 18 & 19). Of Sister Bay's post-rainfall *E. coli* isolates nine were identified as from human sources, one from cow, two from dog, three from goose, and eleven were identified as coming from avian. No *E. coli* isolates matched duck or cat (Appendix N, Table 19).

Jackknife analysis showed post-rainfall *E. coli* isolates compared amongst each other and a library of known human *E. coli* isolates, had similarity values of 96 and 4, respectively. Sister Bay's post rainfall *E. coli* isolates were not similar to other known sources. Known *E. coli* isolates from gull, human, cow, dog, and duck had similarity values of 95, 98, 98, 91, and 91, respectively, when compared to isolates within each source (Appendix K, Table M-2).

Principal Component Analysis (PCA) was used to show relationships between waste isolate sources and rain isolates. Isolates can be discriminated from one another by their color. *E. coli* isolates were grouped in the lower right and lower middle part of the figure. Post-rainfall *E. coli* isolates appeared throughout the figure, including clusters (Appendix K, Figure L-2).

Sunset Park Beach (Appendix L, Figure N-2).

In 2005 and 2006, three of five beaches sampled after a significant rainfall had *E. coli* concentrations exceeding the advisory threshold (235 MPN/100 mL). The two that did not were taken late during the 2006 swim season. One out of five rainfall events resulted in *E. coli* concentrations higher than the closure threshold (1000 MPN/100 mL) (Appendix L, Figures O-2 & P-2). Beach water samples taken during the summer of 2005 peaked with *E. coli* concentrations during the 8th and 12th hours at 309 MPN/100 mL and 473 MPN/100 mL. No samples taken in 2005 had *E. coli* concentrations exceeding the closure threshold 24 hours after a rainfall (Appendix L, Figure O-2). In 2006, one sample taken after 24 hours (post-rainfall) had *E. coli* concentrations exceeding the closure threshold with a concentration of 1,990 MPN/100 mL. All other samples taken in 2006 did not exceed the *E. coli* advisory limit (Appendix L, Figure P-2).

In both 2005 and 2006, Sunset's seasonal beach water *E. coli* concentration (no rainfall) and *E. coli* concentrations post-rainfall were not significant ($\alpha=0.05$) with p-values of 0.955 and 0.206, respectively (Appendix N, Tables 15 & 16). A Scheffe Matrix analysis was performed on all of Sunset's data from 2005 and 2006, even though significance was not found in an ANOVA. There was no significance between sampling time after a rain event and the regular season mean (Appendix L, Table 12).

Whitefish Dunes Beach (Appendix M, Figure Q-2).

In 2005 and 2006, all four rain events had *E. coli* concentrations higher than the advisory threshold (235 MPN/100 mL) with one sample exceeding the *E. coli* closure

threshold (1000 MPN/100 mL) (Appendix M, Figures R-2 & S-2). Water samples collected from the summer of 2005 spiked in *E. coli* concentrations during the 2nd and 8th hours at 744 MPN/100 mL and 275 MPN/100 mL, respectfully (Appendix M, Figure R-2). In 2006, one water sample had *E. coli* concentrations exceeding the closure threshold in the 8th hour, by two and a half times the closure threshold, with a concentration of 2460 MPN/100 mL. Another single beach water sample, collected during this summer had *E. coli* concentrations exceeding the advisory threshold 24 hours after a significant rainfall with 275 MPN/100 mL (Appendix M, Figure S-2).

In 2005, Whitefish Dunes seasonal beach water *E. coli* concentration (no rainfall) and *E. coli* concentrations post-rainfall were not significant ($\alpha=0.05$) with $p=0.994$. In 2006, significant differences were found between Whitefish Dunes' seasonal beach water *E. coli* concentration (no rainfall) and *E. coli* concentrations post-rainfall with $p=0.001$ (Appendix N, Tables 15 & 16).

A Scheffe Matrix analysis was performed on all of Whitefish Dune's data to show if and where significance occurred. There was a significant difference during the 8th hour ($p=0.011$) after a rain event, when compared with the seasonal mean. No significant differences were found between post-rainfall sampling during the 1-4, 12th and 24th hours versus the non-rainfall season mean (Appendix M, Table 13).

A total of 428 *E. coli* isolates were collected post-rainfall from Whitefish Dunes' beach water. A total of 136 *E. coli* isolates from Whitefish Dunes' beach water were identified as avian isolates. 73 *E. coli* isolates were identified as from gull sources, 67

from human, and 58 from goose (Appendix N, Table 17). Whitefish Dunes had a total of 26 *E. coli* isolates recovered post-rainfall with 73.1% (14 rain isolates) above the method limit of discrimination. The highest number of isolates (17) were from the total avian source (Appendix N, Tables 18 & 19). Five *E. coli* isolates from Whitefish Dunes' post-rainfall samples were identified as from human sources, one from cow, one from dog, five from goose, eleven from gull, and one isolate was identified as duck (Appendix N, Table 19).

Jackknife analysis showed post-rainfall *E. coli* isolates compared amongst each other and a library of known gull and human isolates, with similarity values of 87, 9 and 4, respectively. Whitefish Dunes' post-rainfall *E. coli* isolates were not similar to other known sources. Known *E. coli* isolates from gull and goose had similarity values of 98 and 96, when compared to isolates sole within each source (Appendix M, Figure U-2).

Principal Component Analysis (PCA) was used to show relationships between waste isolate sources and rain isolates. Isolates can be discriminated from one another by their color. *E. coli* isolates were grouped mainly in the lower left and lower center of the figure. Post-rainfall *E. coli* isolates appeared throughout the clusters (Appendix M, Figure T-2).

Results Summary.

Based on these results, rainfall increases *E. coli* concentrations in beach water. There tends to be higher concentrations of *E. coli* detected between the first and eighth hour after a significant rainfall versus the 12th to 24th hours, where concentrations usually

fall below the advisory threshold. Many pipes and storm sewer basins found at beaches contain water with concentrations of *E. coli* significantly exceeding both advisory and closure thresholds. This could contribute to increased *E. coli* concentrations in the swimming area, since many of these pipes and streams flow directly into the beach water.

Overall, *E. coli* isolates from beach water collected post-rainfall most frequently match *E. coli* isolates from avian feces (total avian group). The principal component analysis further supports this information, by showing similar relationships between waste isolates and post-rainfall isolates.

Discussion

According to data summarized in the results section, the overarching hypothesis that rainfall directly correlates to *E. coli* concentrations in beach water is generally true. The impacts of rainfall on *E. coli* concentrations allow the beaches studied to be grouped into categories. Not only did these data demonstrate that rainfall commonly increased *E. coli* concentrations in beach water, it also revealed the degree to which rainfall affected certain beaches. Of the 13 beaches sampled for this study, four different beach “categories” were developed with respect to rainfall and *E. coli* concentrations in beach water.

The first observed beach category (category 1) includes Egg Harbor, Nicolet Bay, and Sunset Park. These beaches can be classified as non-storm drain impacted beaches (not adjacent to storm drain pipes) and were not significantly impacted by rainfall. The first location, Nicolet Bay Beach is located in Peninsula State Park and is surrounded by a large wooded park, where its remote location and park hours made accessing and sampling this location difficult. As a result, inadequate data were collected from this site to make robust statistical analyses or predictions. Egg Harbor Beach, the second location, is located within a small village and close to other more heavily impacted sites (Murphy Park Beach and Sister Bay Beach). The beach is adjacent to riprap and a pier, and oftentimes had birds present. One would expect to see high levels of *E. coli* at this beach based on the downward slope of the beach, poor circulation (due to embayment, riprap

and pier) and birds. Despite these conditions Egg Harbor Beach remained minimally impacted by rainfall. The third location minimally impacted by rainfall is Sunset Park Beach. This beach, located in a relatively large park area, does not appear to be heavily influenced by urban surroundings although it is located adjacent to a shipping yard. Overall, based on these data stormwater seemed to have little impact on *E. coli* concentrations at these three beach locations.

Anclam, Bailey's Harbor and Fish Creek beaches make up the second category (category 2) of beaches. These beaches are located adjacent to storm drain pipes and were significantly impacted by rainfall (stormwater) during more than one time period (1-4, 8, 12, and 24 hours post-rainfall), when compared to the seasonal mean *E. coli* concentration. All three locations were adjacent to more than one pipe, stream, or overland flow area, where intermittent flows may have led to fluctuating *E. coli* concentrations in the beach water.

Anclam Beach showed a significant difference between seasonal beach water *E. coli* concentrations and *E. coli* concentrations eight hours and 24 hours post-rainfall ($P < .023$ and $P < .001$, respectively). Bailey's Harbor results were opposite of Anclam's, where seasonal beach water *E. coli* concentrations and *E. coli* concentrations during the first four hours and 12 hours post-rainfall were statistically significant ($P = .001$ and $P < .001$, respectively). The third beach, Fish Creek, showed a significant increase in *E. coli* concentration due to rainfall during the first four hours ($P < .001$) and eight hours ($P < .011$) post-rainfall. There were no significance differences between mean seasonal *E.*

coli concentration and the *E. coli* concentration at 12 hours ($P = .916$) and at 24 hours ($P = .998$) post-rainfall.

Beach topography, specifically at Anclam and Fish Creek beaches, which are partially enclosed, may have reduced *E. coli* mixing rates in beach water post-rainfall and allowed *E. coli* concentrations to remain elevated for longer periods. Intermittent discharges from multiple stormwater discharge pipes and/or overland flow during a rainfall may have attributed to the increased concentrations of *E. coli* (observed at different times) in the beach water.

The third beach category (category 3) is also made up of stormdrain impacted beaches, however, elevated *E. coli* concentrations are observed in the beach water only within the first four hours of a rain event and then fall below closure/advisory thresholds. The beaches that fall in this category are: Ellison Bay, Otumba Park and Sister Bay beaches. Ellison Bay and Sister Bay beaches are partially enclosed by adjacent piers, which may inhibit the circulation of stormwater discharge, leading to elevated *E. coli* concentrations. Otumba Park Beach is not enclosed, but is located in a canal. However, there is no likely reason for the cause of Otumba's elevated *E. coli* concentrations post-rainfall. All three locations were significantly impacted by rainfall, however, only during the first four hours after a rainfall ($P < .001$). By the time the eight hour samples were collected, the difference was no longer significant (Ellison Bay, $P = .566$; Otumba, $P = .996$; and Sister Bay, $P = .672$).

These results are likely the result of initial stormwater discharges post-rainfall and inadequate beach water mixing. As time progresses the initial stormwater discharges are diluted into the larger body of water, causing *E. coli* concentrations to fall below the closure and advisory thresholds.

Lakeside Park, Ephraim, Murphy Park and Whitefish Dunes beaches are the fourth observed beach category (category 4) where the effects of rainfall on beach water *E. coli* concentrations are delayed. Statistically significant results between seasonal *E. coli* concentrations and *E. coli* concentrations post-rainfall were not observed until between eight and 12 hours post-rainfall at these beaches. Ephraim Beach was located adjacent to one pipe and two streams however, only a single rain event produced enough flow to collect samples. The slow stormwater flow may have delayed the impact of elevated *E. coli* concentrations in the beach water, as mean *E. coli* concentrations were significantly greater than the seasonal mean eight hours post-rainfall ($P < .001$). Lakeside Park Beach appears to be highly influenced by stormwater runoff, as *E. coli* concentrations (collected from Pipe 1) exceeded the beach closure threshold by more than six fold, on average (Appendix G, Figure O-1). Pipe 1 is at a distance (~100 yards) from the center of the beach, and stormwater would have taken longer to directly mix with and increase *E. coli* concentrations in the beach water. Therefore, it is plausible that stormwater discharges may have taken up to 12 hours to mix with Lakeside's beach water before seeing significantly greater *E. coli* concentrations than the seasonal mean ($P < .001$). Murphy Park Beach does not have an adjacent stormwater outfall pipe, although

it may be impacted by overland runoff located adjacent to the swimming area. There was never enough flow to collect a sample however, a slow trickle of overland runoff flowing into the partially enclosed swim area, may have led to the statistically significant *E. coli* concentrations observed eight hours post-rainfall compared to the seasonal mean ($P < .001$). The last location, Whitefish Dunes Beach, has a large sandy beach area with sand dunes, and oftentimes has many birds present. Water quality results were similar to Murphy Park's, as mean *E. coli* concentrations eight hours post-rainfall were statistically greater than the seasonal mean ($P < .011$). This may also be the result of delayed overland runoff mixing with beach water. All four beaches show delayed effects of rainfall on beach water *E. coli* concentrations due to delayed runoff and/or delayed mixing as a result of stormwater discharge location (far) or flow rate (slow).

Overall, ten of the 13 beach locations studied showed significant post-rainfall impacts on beach water *E. coli* concentrations as a result of rain events greater than 0.5 cm within a 24-hour period. This study clearly demonstrates the impacts rainfall had on several Door County, WI beaches. Although the magnitude and duration of the adverse impacts were somewhat variable, beaches can be grouped based on the effects of beach type, location and topography on *E. coli* concentrations following rainfall. Though the study only included water quality results for 13 beaches over two beach seasons, the rainfall-beach interaction data could still pose value for beach managers who are developing preemptive beach closures related to rainfall. For example, based on these data, beach managers may want to implement preemptive beach closures at storm drain impacted

beaches post-rainfall, in order to protect beach-goers. However, post-rainfall beach water quality should still be monitored in order to justify the issuance of predictive public health closures. Additionally, these data should be used to evaluate *E. coli* concentration fluctuations and patterns, so better post-rainfall beach water quality “predictions” (and perhaps new beach category types) can be developed to aid in beach management decisions.

Future Research

Numerous factors should be considered for having an impact on *E. coli* concentrations after a significant rainfall. This study was a small piece of a much greater mystery addressing rainfall amounts and *E. coli* concentrations. Future research that could contribute to better understanding rainfall and its influence on beaches in Door County, WI, would include researching a larger number of beaches in Door County, WI, both on the bay and lakeside. Increasing the number of rain gauges throughout the peninsula could present more accurate rainfall amounts, better indicating when a beach should be sampled as well as the amount of water samples collected from each location to increase percentage of isolates recovered. Other biological factors that could be considered are wind and water temperature, including their possible influence on *E. coli* concentrations, as well as their effect at specific locations. Additional research to be considered include spatial sampling at variable depths, monitoring at storm drains verses up and downstream (50 ft, 100ft etc. from storm drain), and sampling known point sources verses adjacent beach water.

Only a small number of sources were examined from the genetic library at UW Oshkosh, but creating a larger library could increase possible source identification. Other animals to be considered could be horses, deer, or raccoons. All results from previous years' of rainfall sampling should be investigated for change, as well as the use of other indicator organisms to fully assess fecal contamination.

APPENDIX A

Rainfall Effects on *E. coli* and Source Identification at Anclam Beach



Figure A. Aerial view of Anclam beach's sampling sites. This view shows Pipe 1 and Pipe 2 along with the hours each site was sampled.

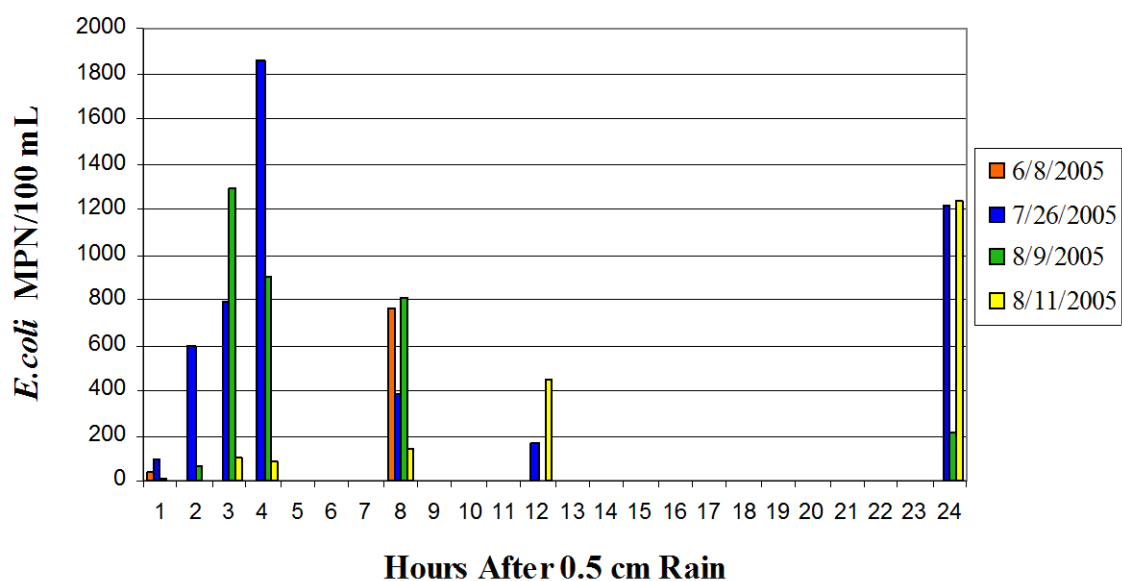


Figure B. *E. coli* MPN/100 mL of water sampled at Anclam Park each hour, up to 24 hours, after a rain accumulation ≥ 0.5 cm in a 24 hour period. Samples were collected during the summer swimming season of 2005.

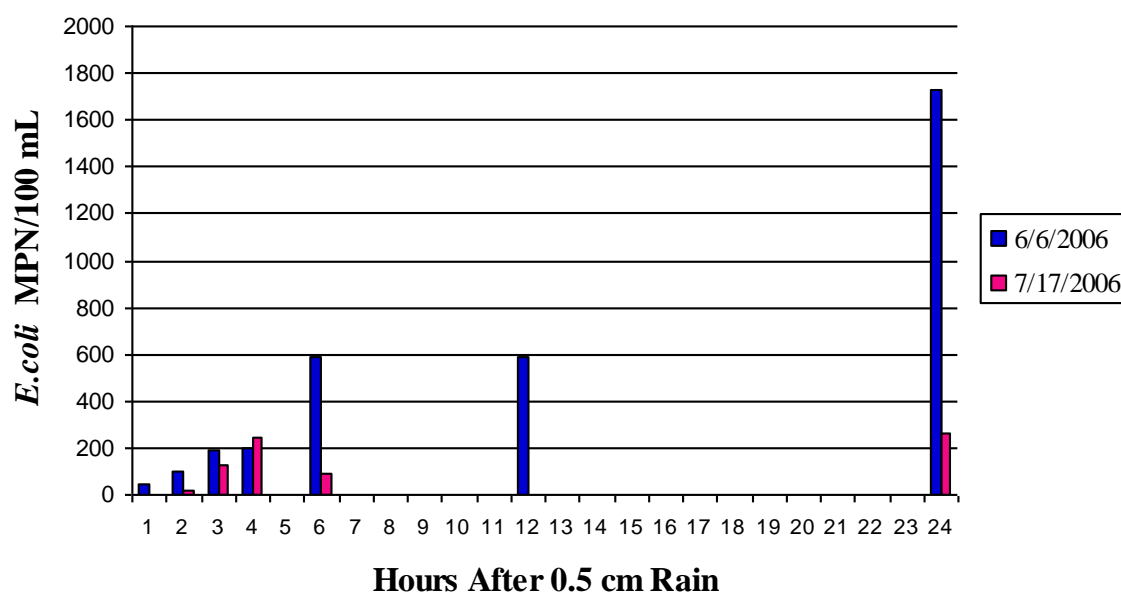


Figure C. *E. coli* MPN/100 mL of water sampled at Anclam Park each hour, up to 24 hours, after a rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were collected during the summer swimming season of 2006.

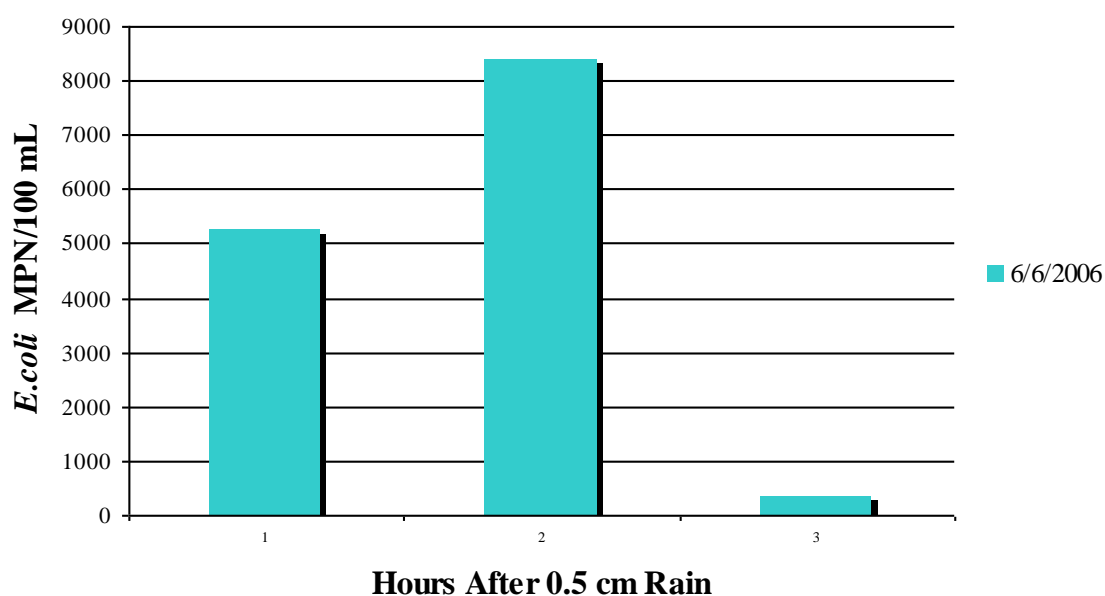


Figure D. *E. coli* MPN/100 mL of water sampled directly out of Pipe 1 at Anclam Park each hour, up to 3 hours, after a rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

Table 1. Scheffe matrix showing the relationship between various sampling times and their *E. coli* concentrations compared with seasonal *E. coli* means, at Anclam, during the 2005 and 2006, summer swimming season. This matrix shows significance between the 8 and 24 hour sampling times, after a rain event, compared with the seasonal mean.

	Seasonal Mean	1-4 hour mean	8 hour mean	12 hour mean	24 hour mean
Seasonal Mean	1.000				
1-4 hour mean	0.475	1.000			
8 hour mean	0.023	0.849	1.000		
12 hour mean	0.881	0.986	0.543	1.000	
24 hour mean	0.000	0.038	0.362	0.008	1.000

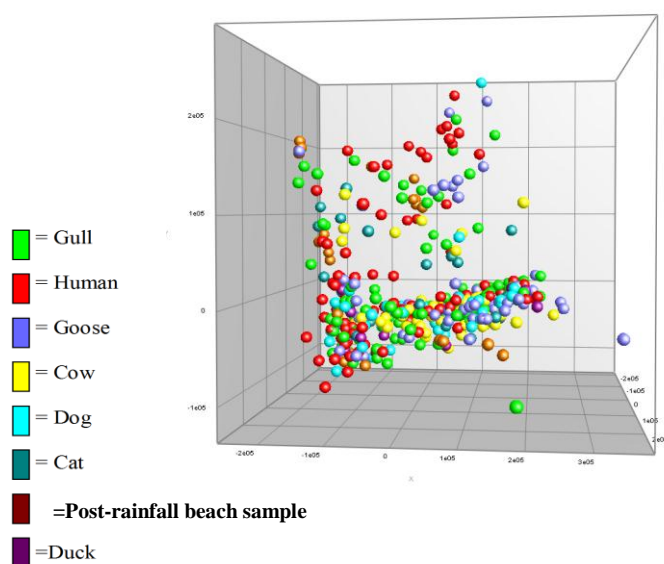


Figure E. PCA of Anclam's waste isolates.

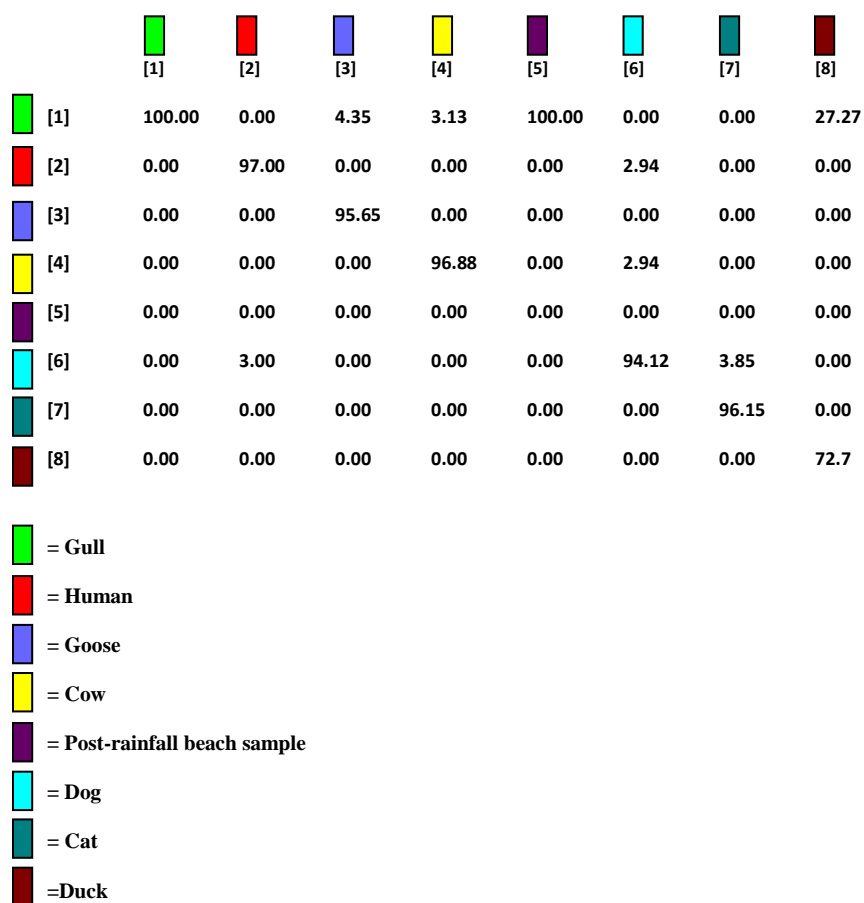


Figure F. Jackknife analysis of Anclam's rain vs. gull, human, goose, cow, dog, cat, and duck waste isolates.

APPENDIX B

Rainfall Effects on *E. coli* and Source Identification at Bailey's Harbor Beach



Figure G. Aerial view of Bailey's Harbor's sampling sites. This view shows the center of the beach, Pipe 1, Pipe 2, Stream 1, as well as the hours each site was sampled.

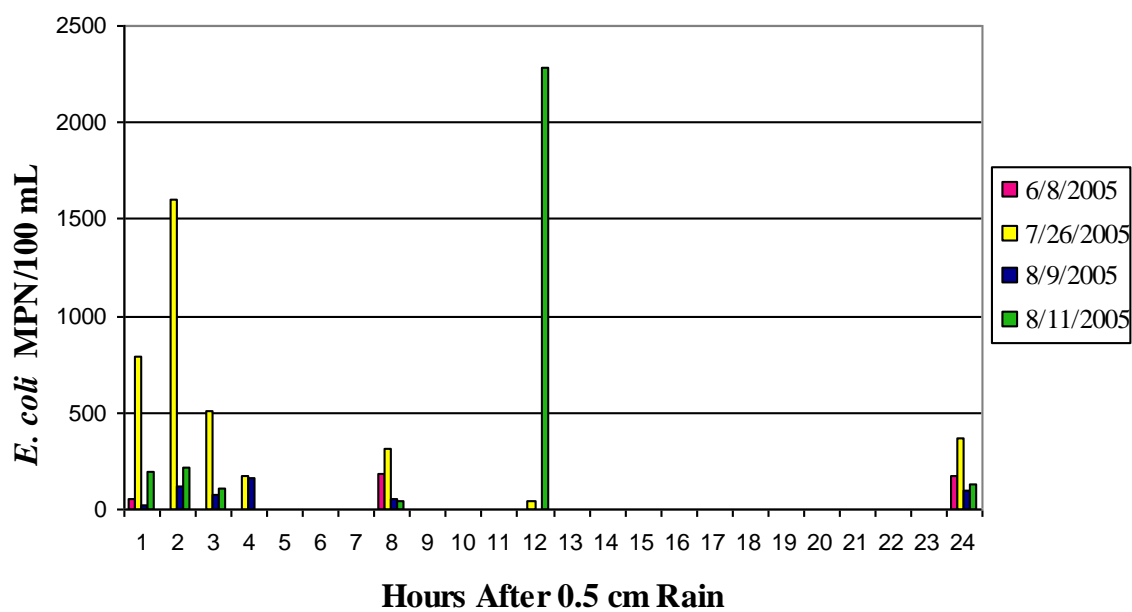


Figure H. *E. coli* MPN/100 mL of water sampled at Bailey's Harbor each hour, up to 24 hours, after a rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2005.

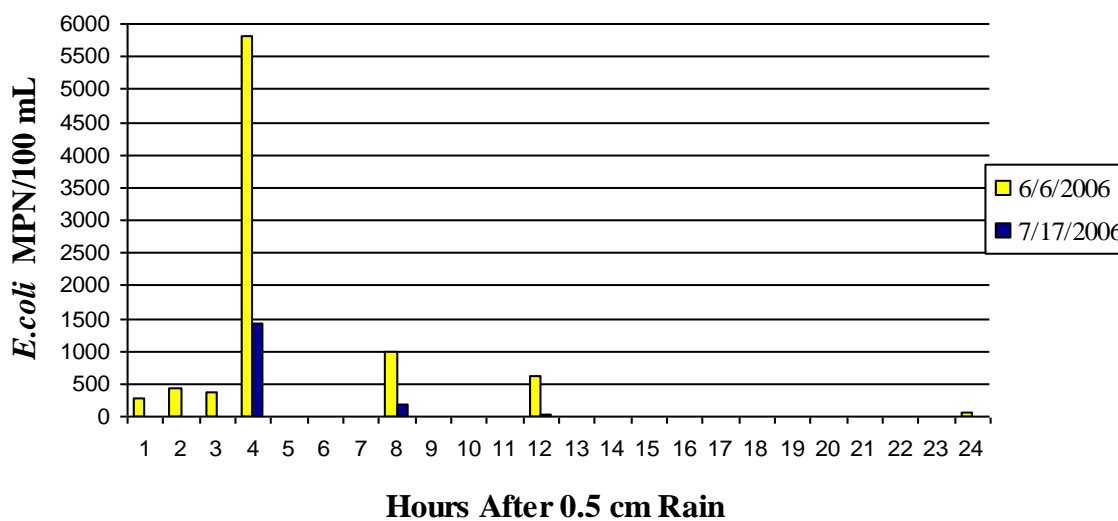


Figure I. *E. coli* MPN/100 mL of water sampled at Bailey's Harbor each hour, up to 24 hours, after a rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were collected during the summer swimming season of 2006.

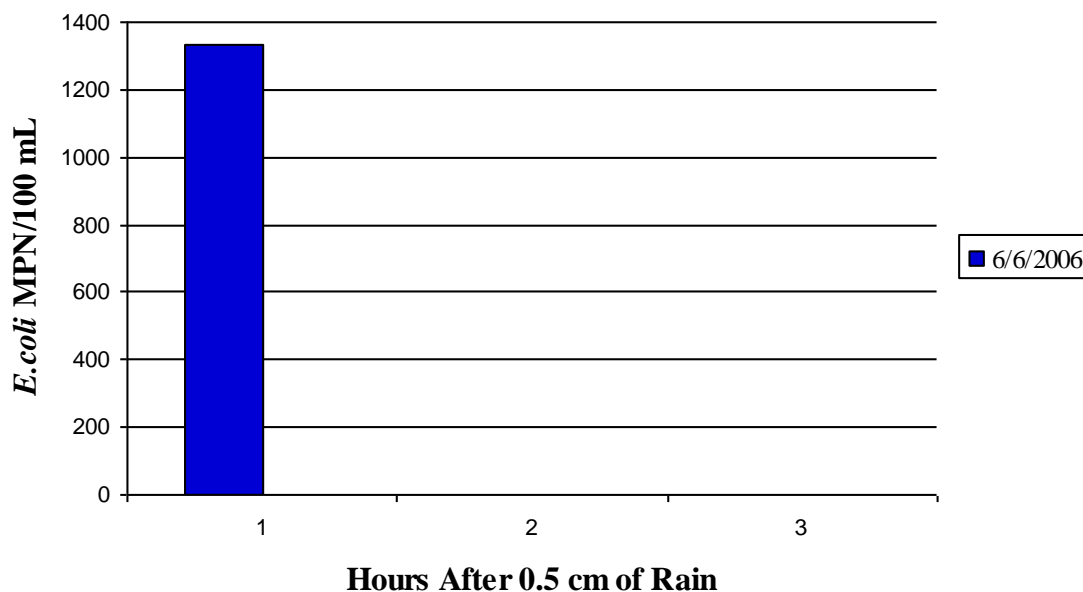


Figure J. *E. coli* MPN/100 mL of water sampled directly out of Pipe 1 at Bailey's Harbor, each hour, up to 3 hours, after a rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer

swimming season of 2006

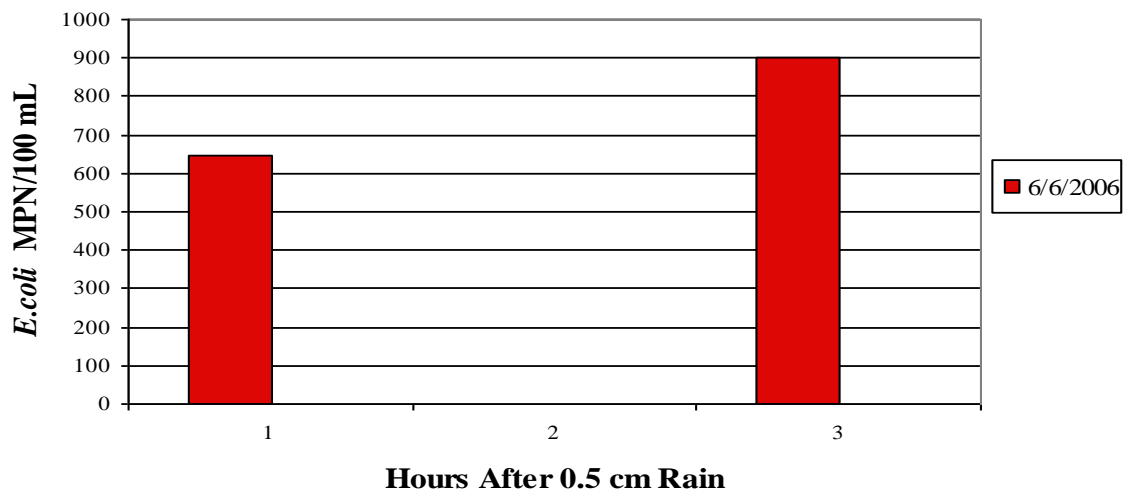


Figure K. *E. coli* MPN/100 mL of water sampled directed out of Pipe 2 at Bailey's Harbor, each hour, up to 3 hours, after a rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

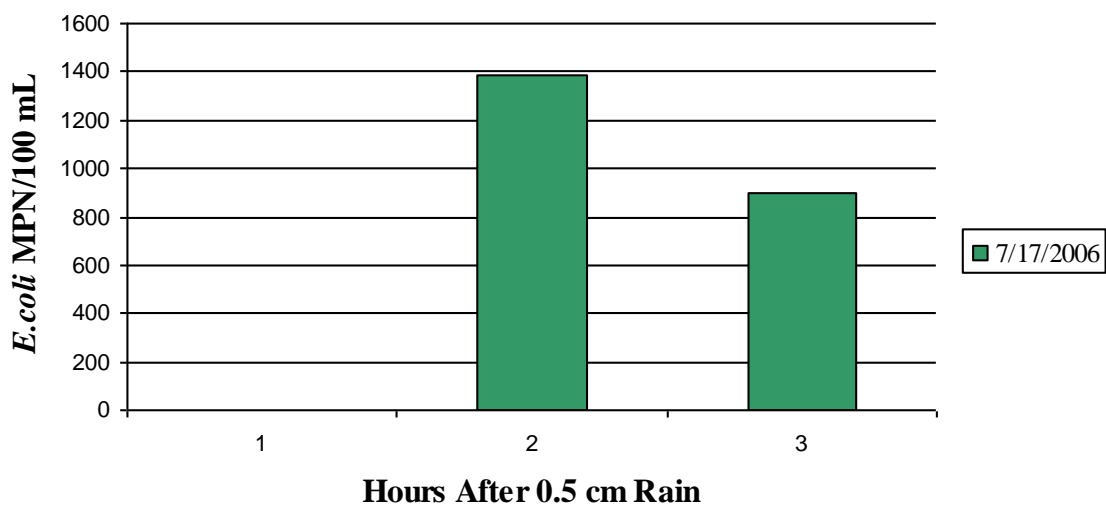


Figure L. *E. coli* MPN/100 mL of water sampled from Bailey's Harbor Stream 1 every hour, up to 3 hours, after a rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

Table 2. Scheffe matrix showing the relationship between various sampling times and their *E. coli* concentrations compared with seasonal *E. coli* means, at Bailey's Harbor, during the 2005 and 2006, summer swimming season. This matrix shows significance between 1-4 and 12 hour sampling times, after a rain event, compared with the seasonal mean.

	Seasonal Mean	1-4 hour mean	8 hour mean	12 hour mean	24 hour mean
Seasonal Mean	1.000				
1-4 hour mean	0.000	1.000			
8 hour mean	0.204	0.541	1.000		
12 hour mean	0.000	0.998	0.384	1.000	
24 hour mean	0.940	0.083	0.861	0.049	1.000

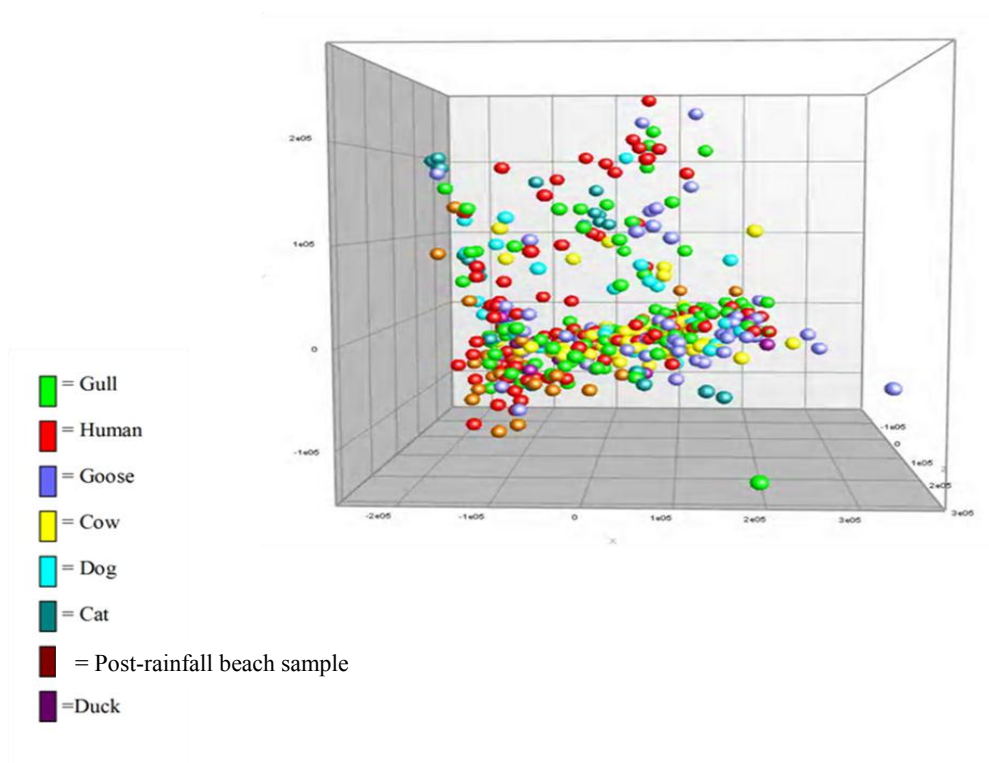


Figure M. PCA of Bailey's Harbor waste isolates.

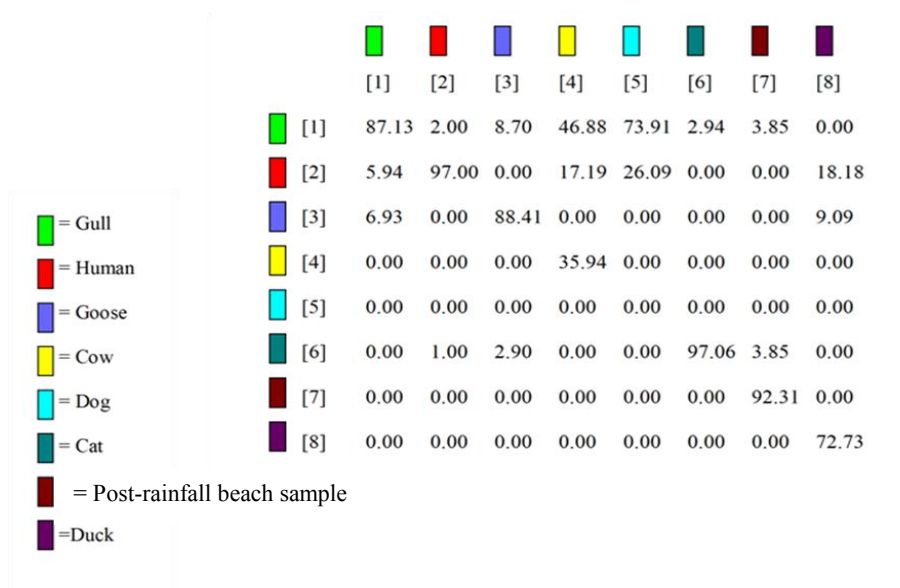


Figure N. Jackknife analysis of Bailey's Harbor rain vs. gull, human, goose, cow, dog, cat, and duck waste isolates.

APPENDIX C

Rainfall Effects on *E. coli* and Source Identification at Egg Harbor Beach



Figure O. Aerial view of Egg Harbor's sampling sites. This view shows the center of the beach and Runoff #1, as well as the hours each site was sampled.

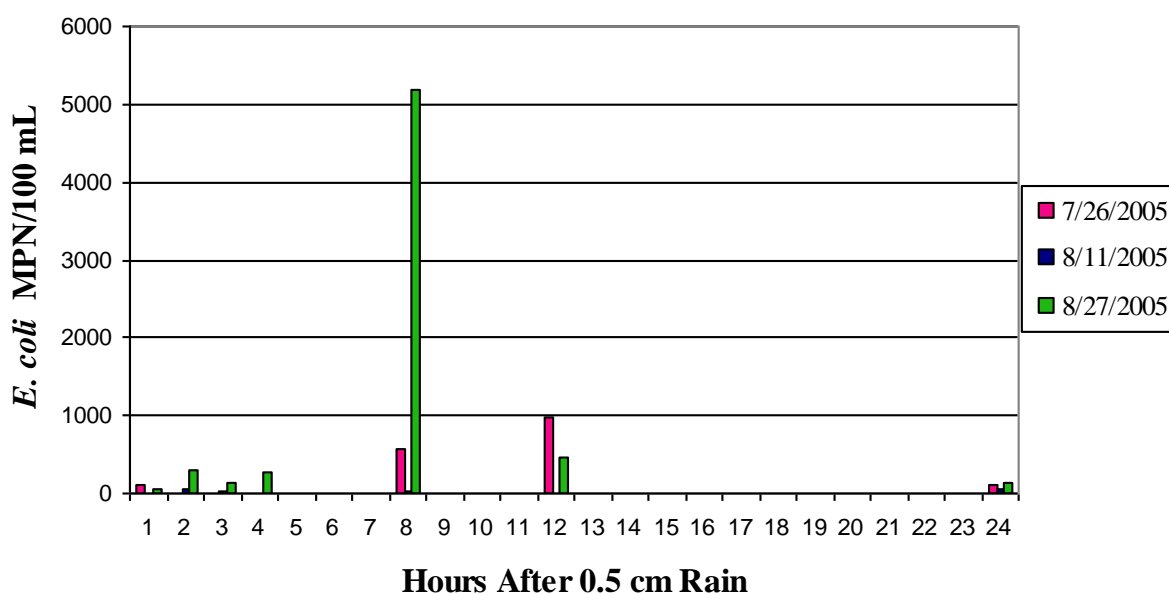


Figure P. *E. coli* MPN/100 mL of water sampled from Egg Harbor every hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2005.

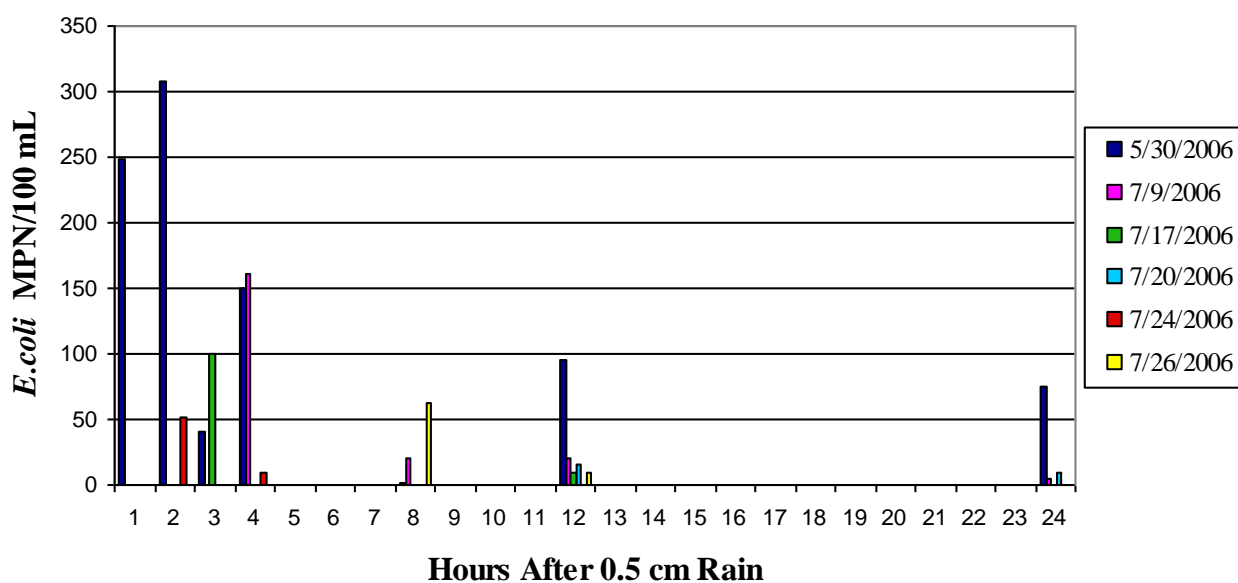


Figure Q. *E. coli* MPN/100 mL of water sampled from Egg Harbor every hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

Table 3. Scheffe matrix showing the relationship between various sampling times and their *E. coli* concentrations compared with seasonal *E. coli* means, at Egg Harbor, during the 2005 and 2006, summer swimming season. This matrix shows no significance between sampling times after a rain event and the seasonal mean.

	Seasonal Mean	1-4 hour mean	8 hour mean	12 hour mean	24 hour mean
Seasonal Mean	1.000				
1-4 hour mean	1.000	1.000			
8 hour mean	0.092	0.308	1.000		
12 hour mean	1.000	1.000	0.400	1.000	
24 hour mean	1.000	1.000	0.271	1.000	1.000

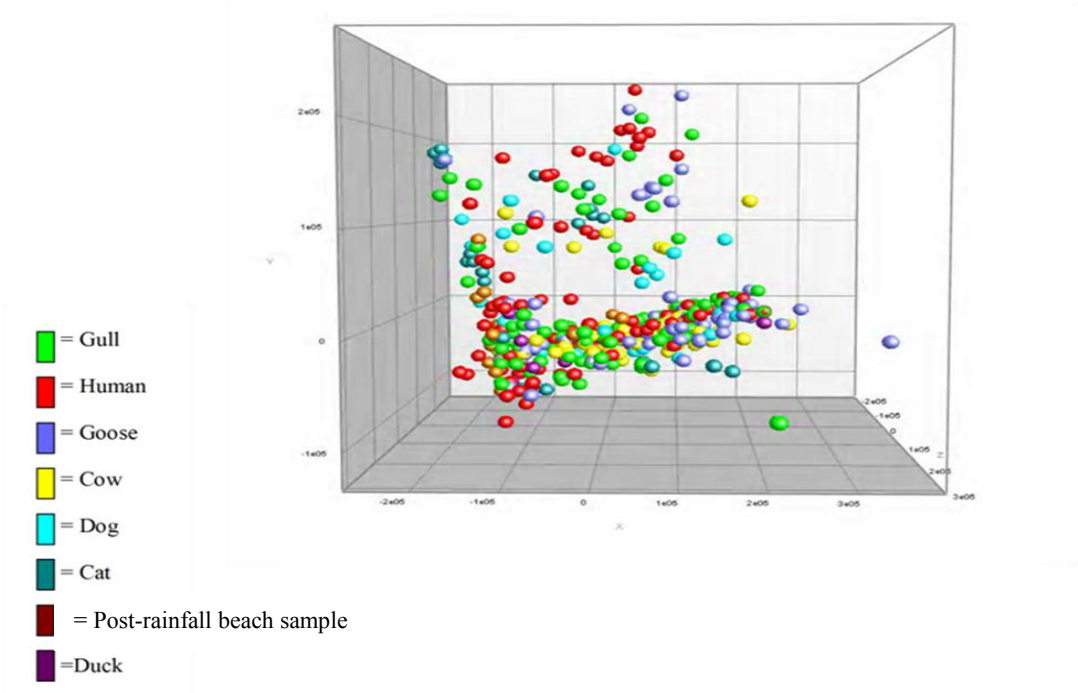


Figure R. PCA of Egg Harbor waste isolates.

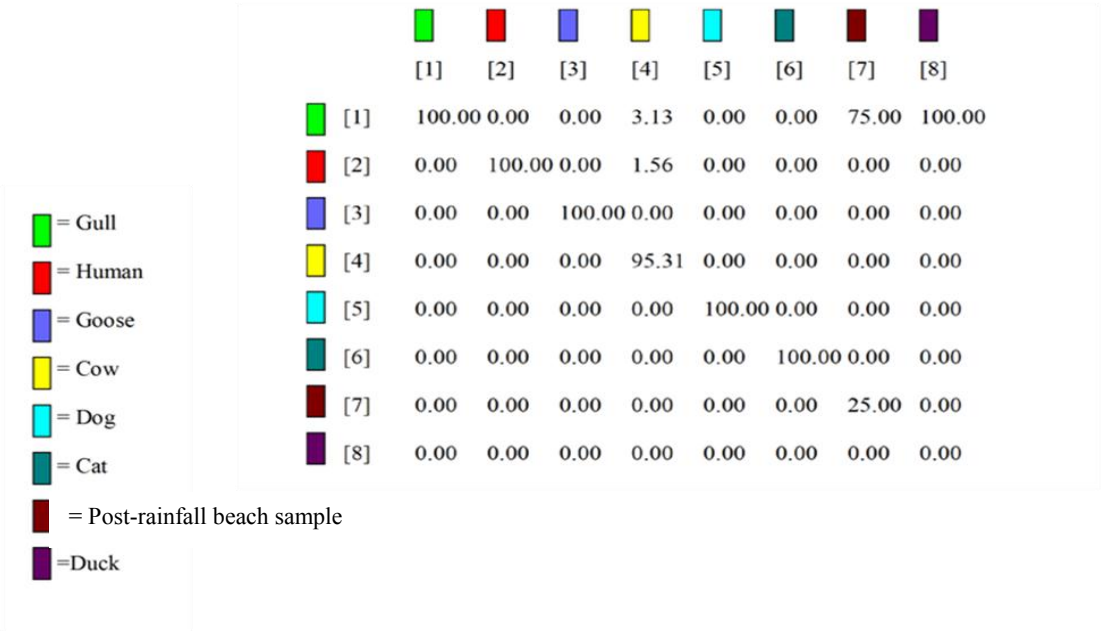


Figure S. Jackknife analysis of Egg Harbor rain vs. gull, human, goose, cow, dog, cat, and duck waste isolates.

APPENDIX D

Rainfall Effects on *E. coli* and Source Identification at Ellison Bay Beach



Figure T. Aerial view of Ellison Bay's sampling sites. This view shows the center of the beach, Pipe 1, Pipe 2, Runoff 1, as well as the hours each site was sampled.

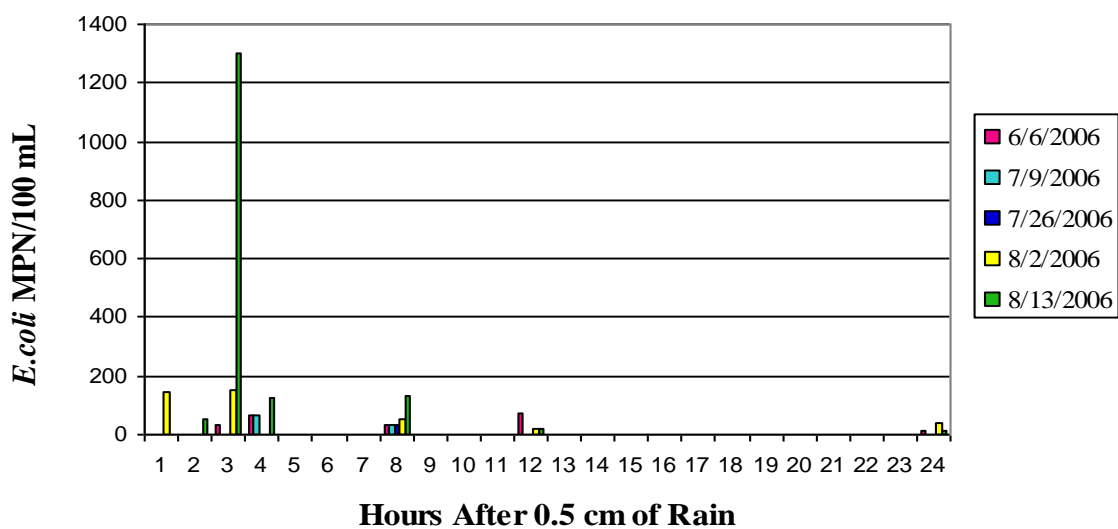


Figure U. *E. coli* MPN/100 mL of water sampled from Ellison Bay each hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

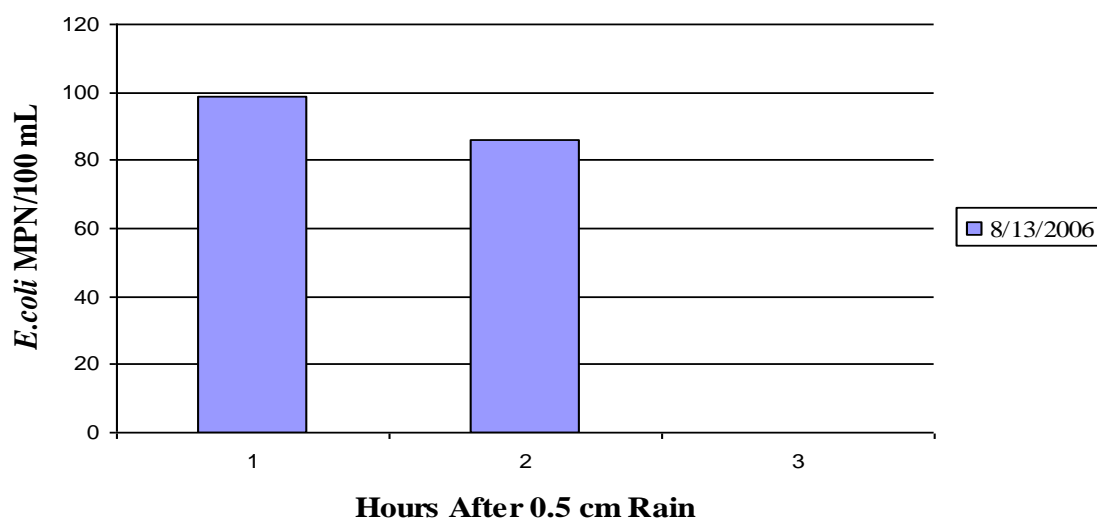


















Figure V. *E. coli* MPN/100 mL of water sampled directly from Ellison Bay Pipe 1 every hour, up to 3 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

Table 4. Scheffe matrix showing the relationship between various sampling times and their *E. coli* concentrations compared with seasonal *E. coli* means, at Ellison Bay, during the 2006 summer swimming season. This matrix shows significance at the 1-4 hour sampling time, after a rain event, compared with the seasonal mean.

	Seasonal Mean	1-4 hour mean	8 hour mean	12 hour mean	24 hour mean
Seasonal Mean	1.000				
1-4 hour mean	0.000	1.000			
8 hour mean	0.566	0.022	1.000		
12 hour mean	0.997	0.003	0.935	1.000	
24 hour mean	1.000	1.001	0.766	0.996	1.000

									
		[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]
	[1]	93.07	4.00	1.45	42.19	0.00	0.00	36.84	27.27
	[2]	1.98	96.00	0.00	0.00	0.00	0.00	0.00	0.00
	[3]	4.95	0.00	98.55	0.00	2.94	0.00	0.00	0.00
	[4]	0.00	0.00	0.00	57.81	5.88	0.00	0.00	0.00
	[5]	0.00	0.00	0.00	0.00	88.24	7.69	0.00	0.00
	[6]	0.00	0.00	0.00	0.00	2.94	92.31	0.00	0.00
	[7]	0.00	0.00	0.00	0.00	0.00	0.00	63.16	0.00
	[8]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	72.73









 = Gull
 = Human
 = Goose
 = Cow
 = Dog
 = Cat
 = Post-rainfall beach sample
 = Duck

Figure X. Jackknife analysis of Ellison Bay rain vs. gull, human, goose, cow, dog, cat, and duck waste isolates.

APPENDIX E

Rainfall Effects on *E. coli* and Source Identification at Ephraim Beach



Figure Y. Aerial view of Ephraim's sampling sites. This view shows the center of the beach, Pipe 1, Stream 1, Stream 2, as well as the hours each site was sampled.

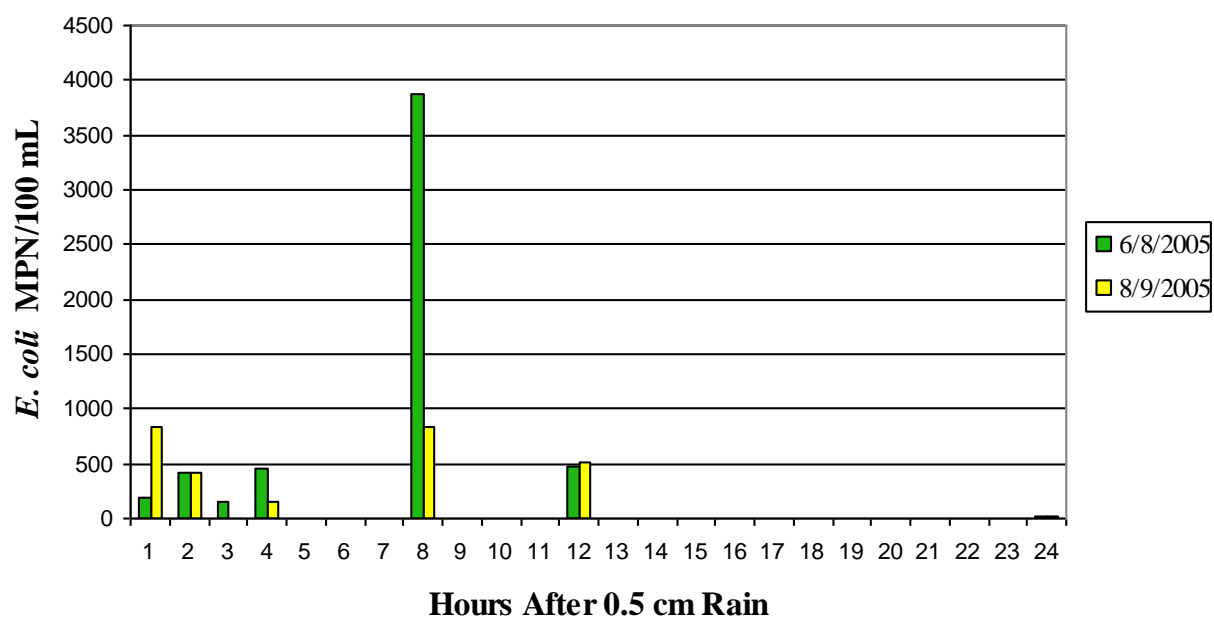


Figure Z. *E. coli* MPN/100 mL of water sampled from Ephraim each hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2005.

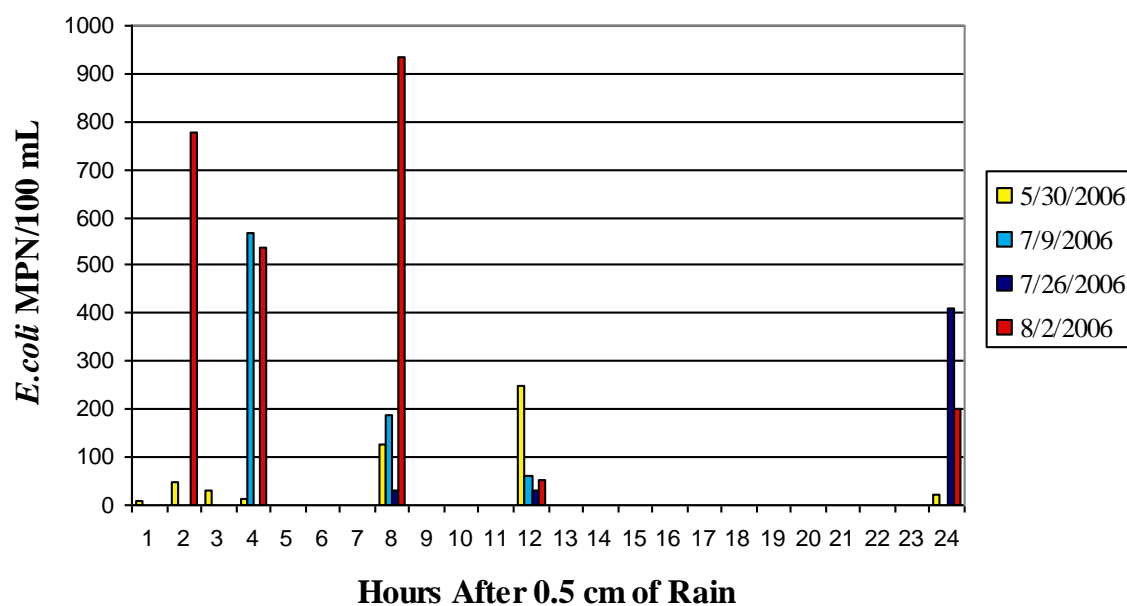


Figure A-1. *E. coli* MPN/100 mL of water sampled from Ephraim each hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

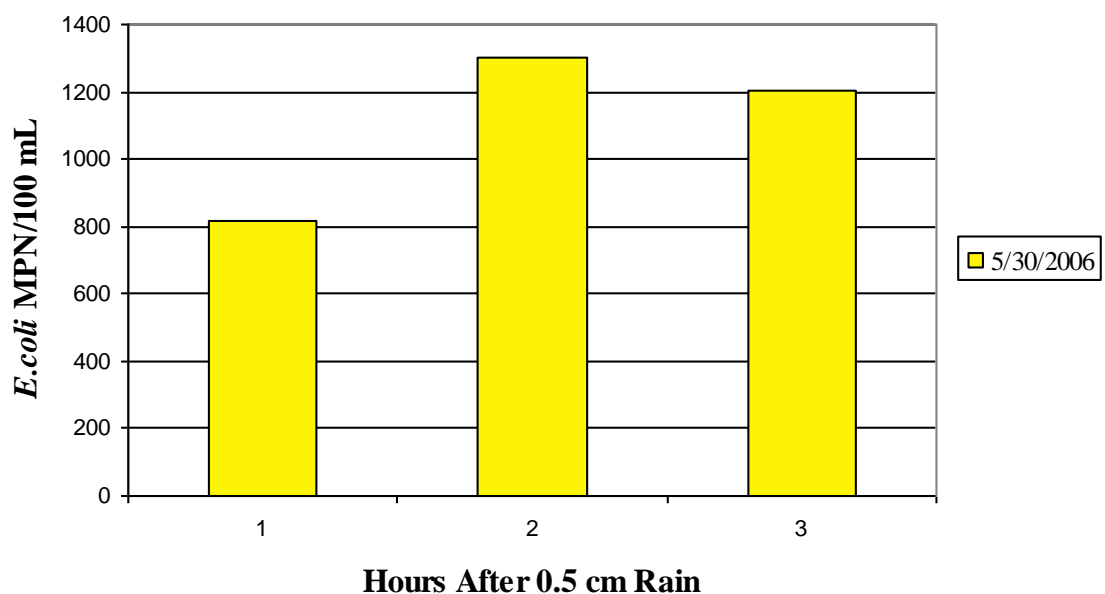


Figure B-1. *E. coli* MPN/100 mL of water sampled from Ephraim Stream 2 each hour, up to 3 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

Table 5. Scheffe matrix showing the relationship between various sampling times and their *E. coli* concentrations compared with seasonal *E. coli* means, at Ephraim, during the 2005 and 2006, summer swimming season. This matrix shows significance at the 8 hour sampling time, after a rain event, compared with the seasonal mean.

	Seasonal Mean	1-4 hour mean	8 hour mean	12 hour mean	24 hour mean
Seasonal Mean	1.000				
1-4 hour mean	0.410	1.000			
8 hour mean	0.000	0.201	1.000		
12 hour mean	0.854	0.970	0.031	1.000	
24 hour mean	0.996	0.818	0.007	0.990	1.000

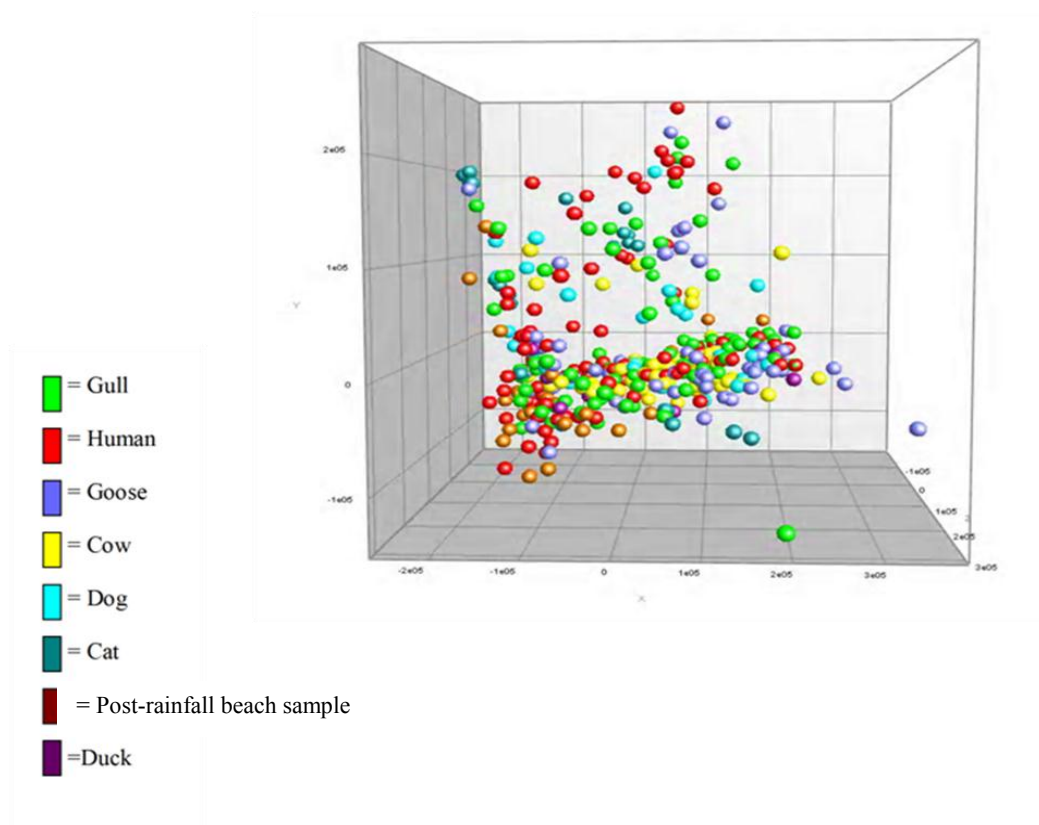


Figure C-1. PCA of Ephraim waste isolates.

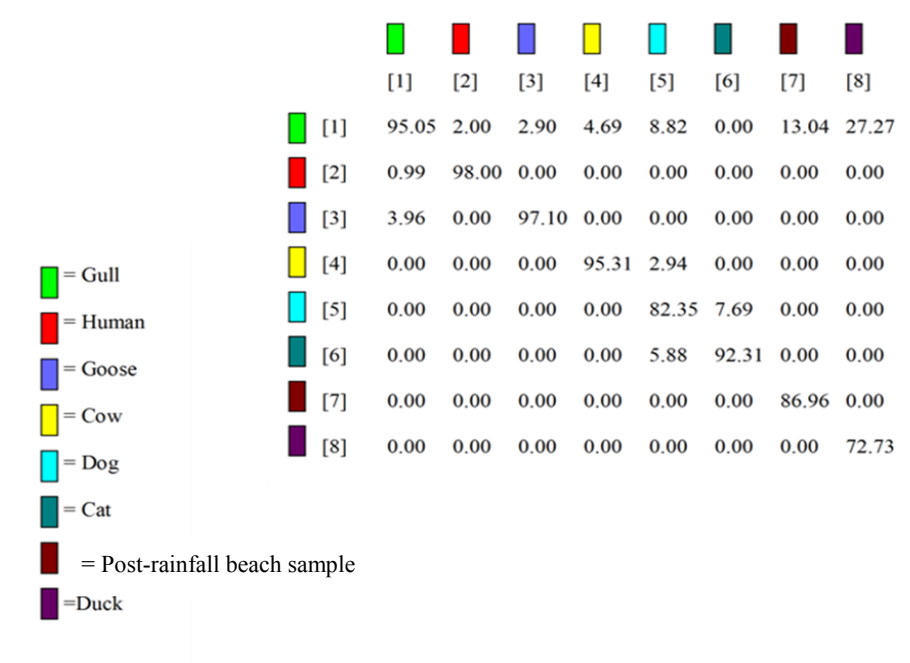


Figure D-1. Jackknife analysis of Ephraim rain vs. gull, human, goose, cow, dog, cat, and duck waste isolates.

APPENDIX F

Rainfall Effects on *E. coli* and Source Identification at Fish Creek Beach



Figure E-1. Aerial view of Fish Creek's sampling sites. This view shows the center of the beach, Pipe 1, Stream 1, Runoff 1, as well as the hours each site was sampled.

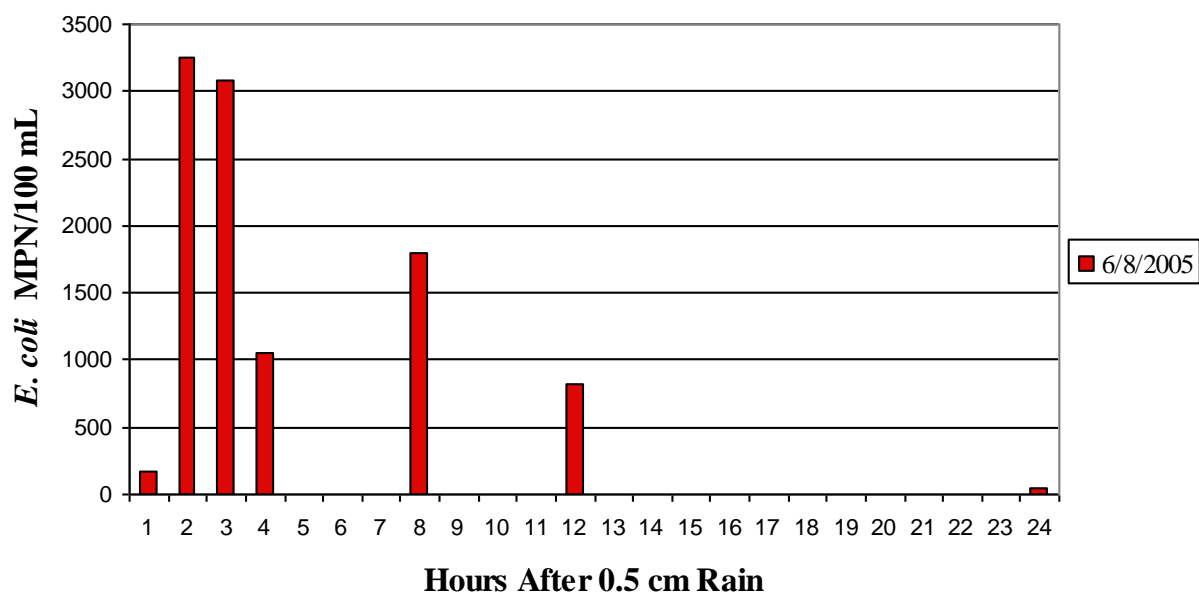


Figure F-1. *E. coli* MPN/100 mL of water sampled from Fish Creek each hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2005.

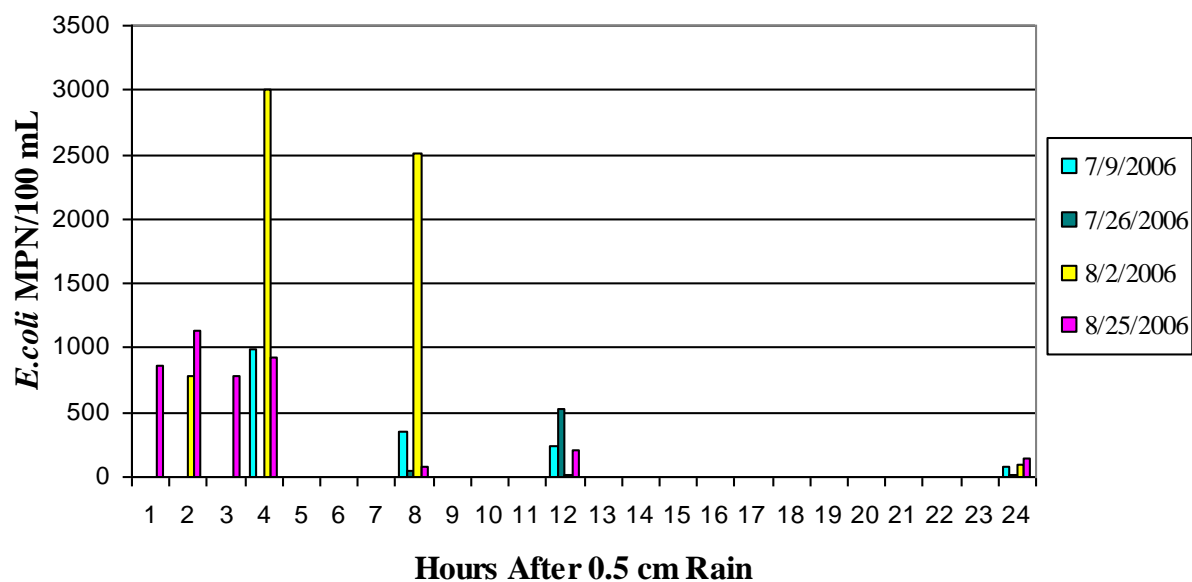


Figure G-1. *E. coli* MPN/100 mL of water sampled from Fish Creek each hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

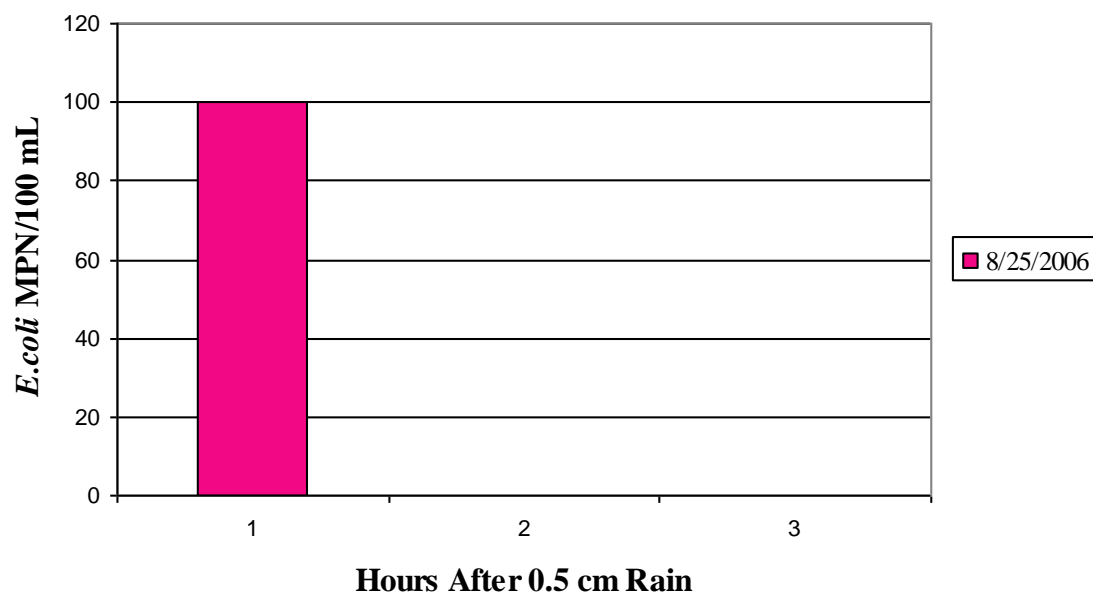


Figure H-1. *E. coli* MPN/100 mL of water sampled from Fish Creek runoff each hour, up to 3 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

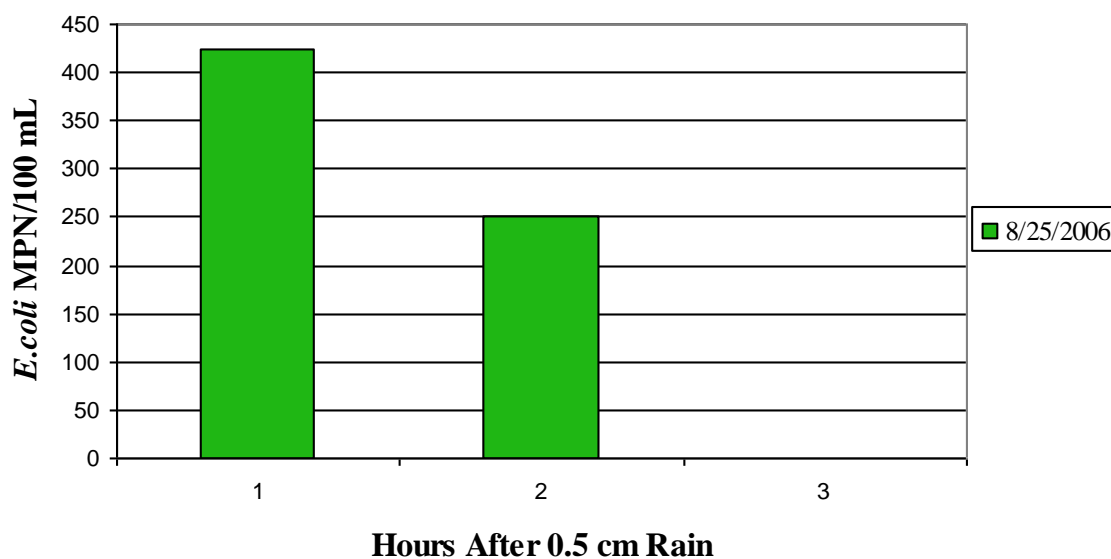


Figure I-1. *E. coli* MPN/100 mL of sampled taken directly from Fish Creek Pipe 1 each hour, up to 3 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

Table 6. Scheffe matrix showing the relationship between various sampling times and their *E. coli* concentrations compared with the seasonal *E. coli* means, at Fish Creek, during the 2005 and 2006, summer swimming season. This matrix shows significance between 1-4 and 8 hour sampling times, after a rain event, compared with the seasonal mean.

	Seasonal Mean	1-4 hour mean	8 hour mean	12 hour mean	24 hour mean
Seasonal Mean	1.000				
1-4 hour mean	0.000	1.000			
8 hour mean	0.011	0.679	1.000		
12 hour mean	0.916	0.024	0.383	1.000	
24 hour mean	0.998	0.002	0.011	0.917	1.000

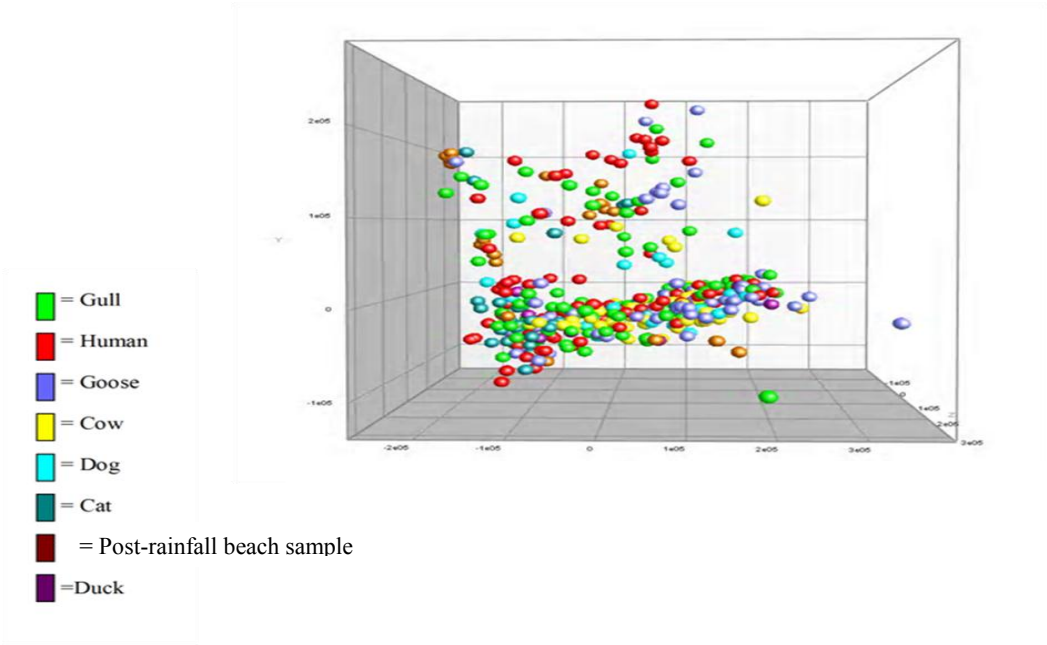


Figure J-1. PCA of Fish Creek’s waste isolates.

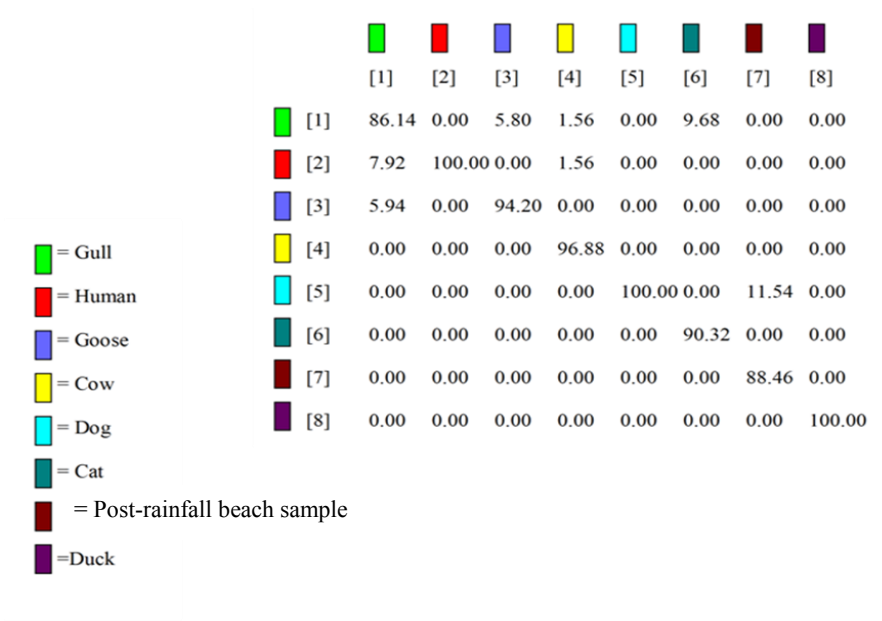


Figure K-1. Jackknife analysis of Fish Creek rain vs. gull, human, goose, cow, dog, cat, and duck waste isolates.

APPENDIX G

Rainfall Effects on *E. coli* and Source Identification at Lakeside Park Beach



Figure L-1. Aerial view of Lakeside's sampling sites. This view shows the center of the beach, Pipe 1, Pipe 2, as well as the hours each site was sampled.

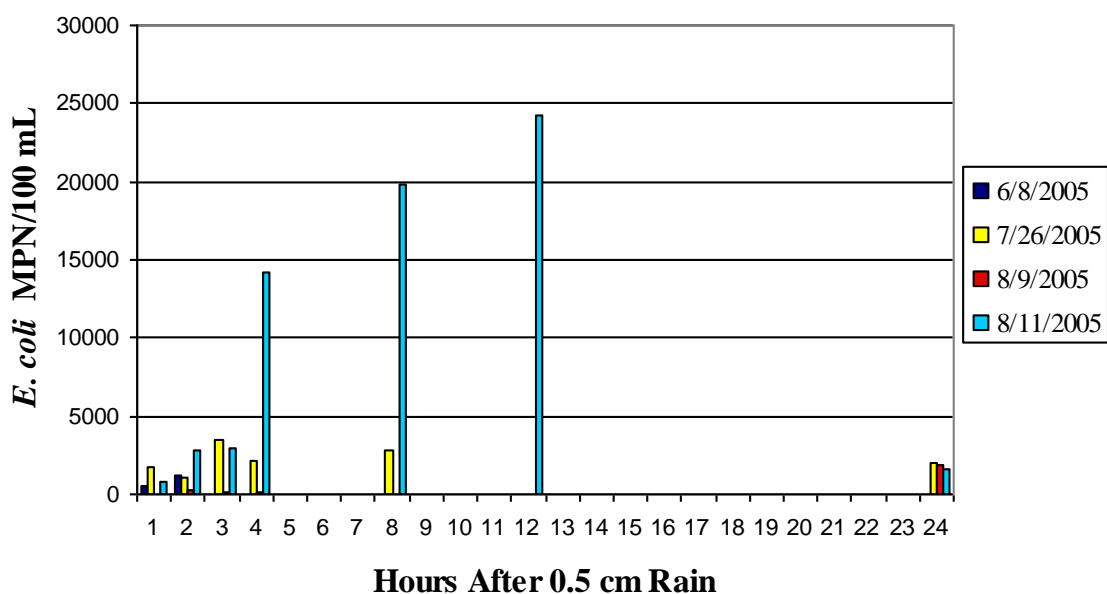


Figure M-1. *E. coli* MPN/100 mL of sample from Lakeside Park each hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2005.

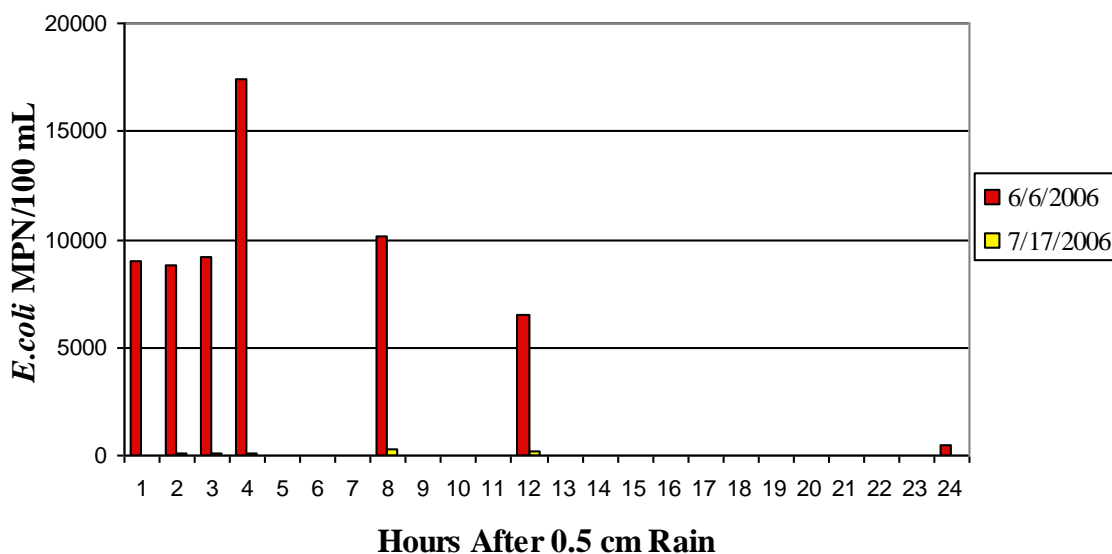


Figure N-1. *E. coli* MPN/100 mL of water sampled from Lakeside Park each hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

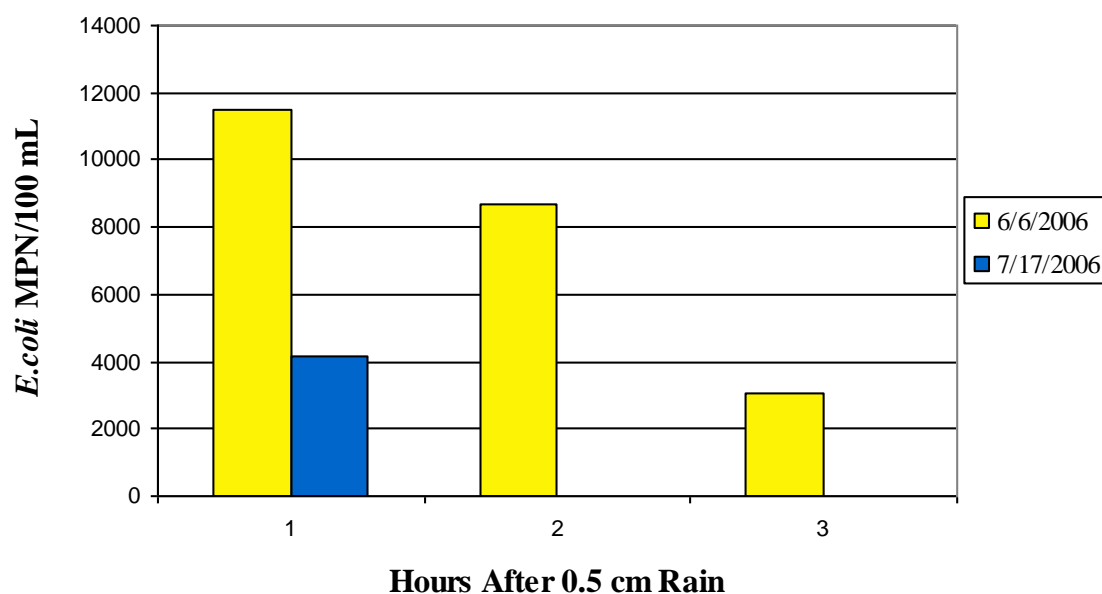


Figure O-1. *E. coli* MPN/100 mL of water sampled directly from Lakeside Park Pipe 1 each hour, up to 3 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

Table 7. Scheffe matrix showing the relationship between various sampling times and their *E. coli* concentrations compared with the seasonal *E. coli* means, at Lakeside, during the 2005 and 2006, summer swimming season. This matrix shows significance at the 12 hour sampling time, after a rain event, compared with the seasonal mean.

	Seasonal Mean	1-4 hour mean	8 hour mean	12 hour mean	24 hour mean
Seasonal Mean	1.000				
1-4 hour mean	0.160	1.000			
8 hour mean	0.185	1.000	1.000		
12 hour mean	0.000	0.185	0.168	1.000	
24 hour mean	0.975	0.735	0.766	0.010	1.000

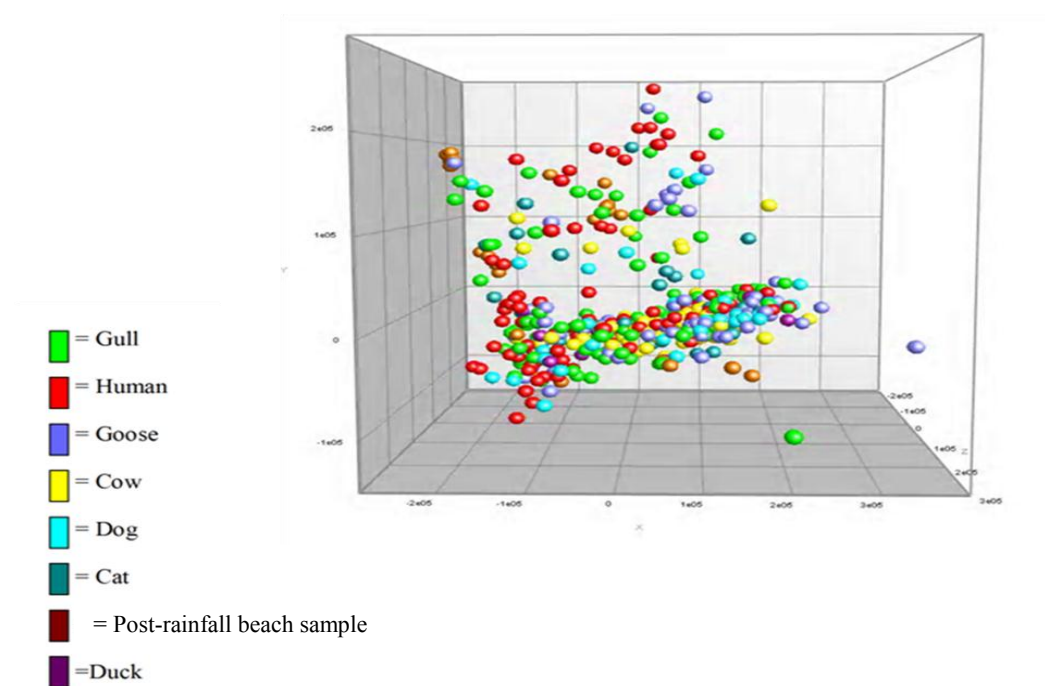


Figure P-1. PCA of Lakeside's waste isolates.

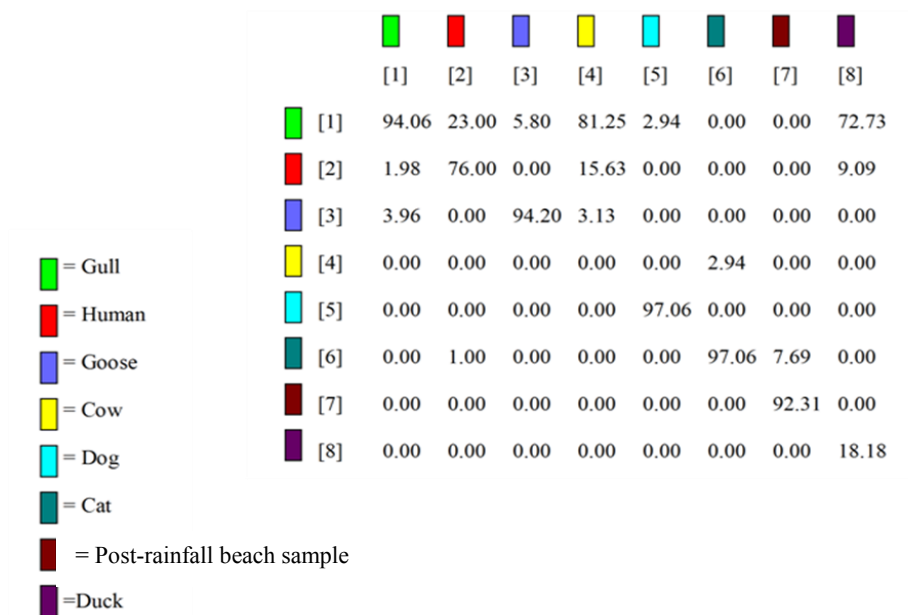


Figure Q-1. Jackknife analysis of Lakeside rain vs. gull, human, goose, cow, dog, cat, and duck waste isolates.

APPENDIX H

Rainfall Effects on *E. coli* and Source Identification at Murphy Park Beach



Figure R-1. Aerial view of Murphy Park's sampling sites. This view shows the center of the beach and Stream 1, as well as the hours each site was sampled.

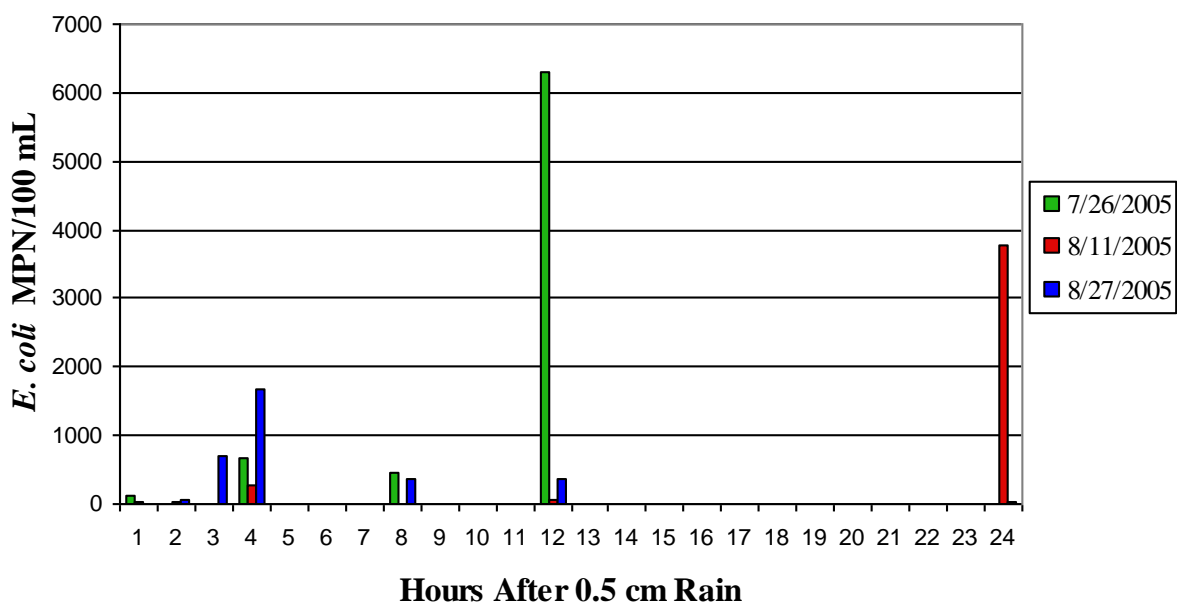


Figure S-1. *E. coli* MPN/100 mL of sample from Murphy Park each hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2005.

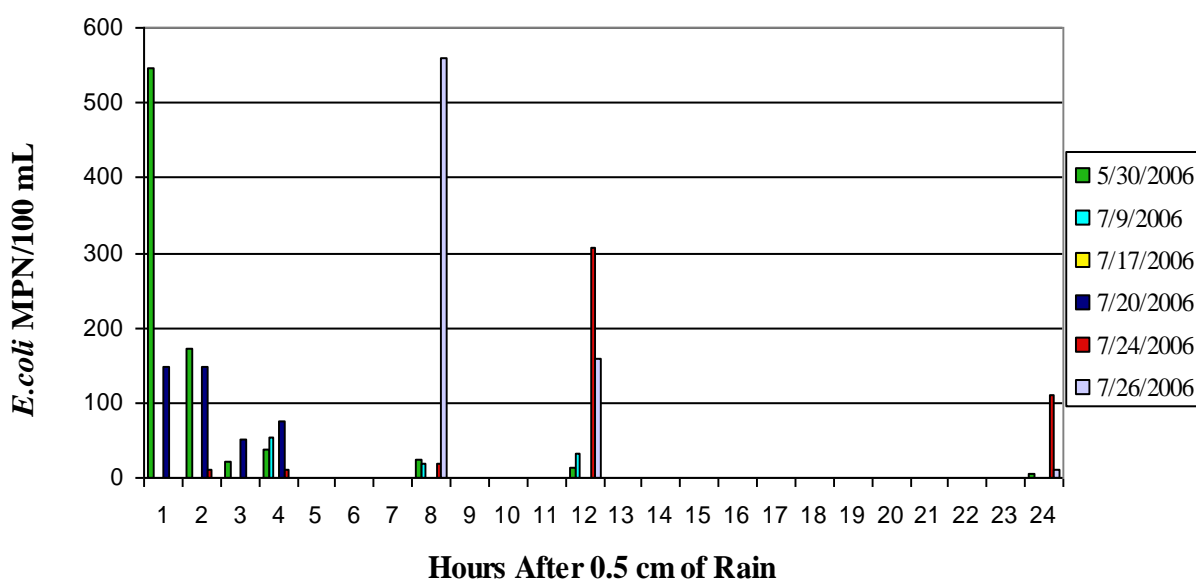


Figure T-1. *E. coli* MPN/100 mL of water sampled from Murphy Park each hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

Table 8. Scheffe matrix showing the relationship between various sampling times and their *E. coli* concentrations compared with the seasonal *E. coli* means, at Murphy Park, during the 2005 and 2006, summer swimming season. This matrix shows significance at the 8 hours sampling time, after a rain event, compared with the seasonal mean.

	Seasonal Mean	1-4 hour mean	8 hour mean	12 hour mean	24 hour mean
Seasonal Mean	1.000				
1-4 hour mean	1.000	1.000			
8 hour mean	0.001	0.048	1.000		
12 hour mean	0.955	0.989	0.134	1.000	
24 hour mean	0.999	1.000	0.021	0.956	1.000

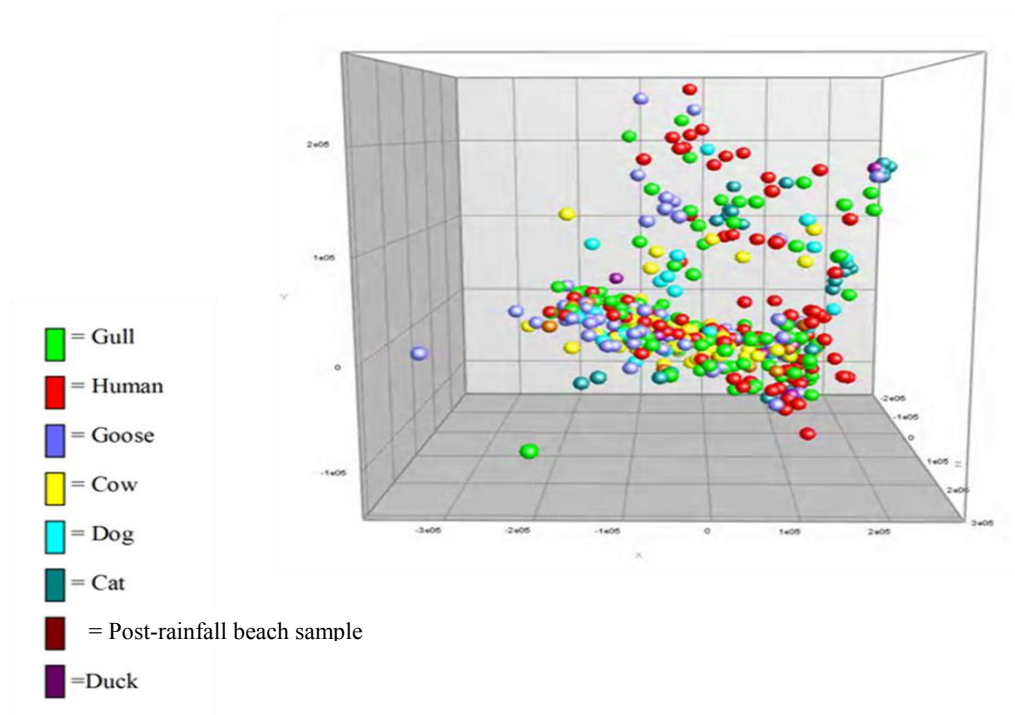


Figure U-1. PCA of Murphy Park's waste isolates.

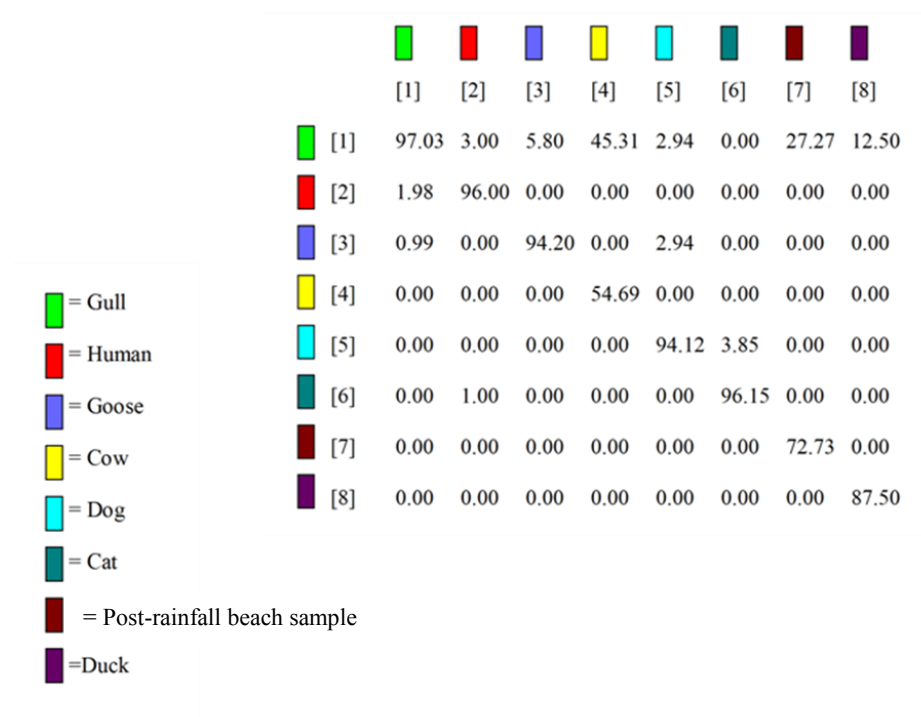


Figure V-1. Jackknife analysis of Murphy Park's rain vs. gull, human, goose, cow, dog, cat, and duck waste isolates.

APPENDIX I

Rainfall Effects on *E. coli* and Source Identification at Nicolet Park Beach



Figure W-1. Aerial view of Nicolet's sampling sites. This view shows the center of the beach and Runoff #1, as well as the hours each site was sampled.

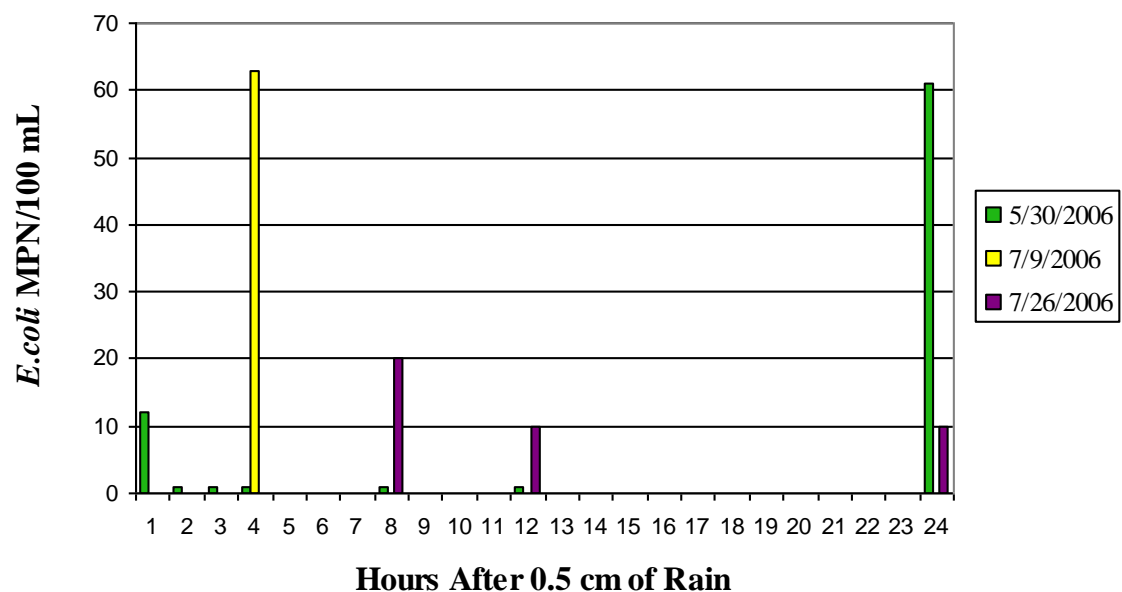


Figure X-1. *E. coli* MPN/100 mL of water sampled from Nicolet each hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

Table 9. Scheffe matrix showing the relationship between various sampling times and their *E. coli* concentrations compared with the seasonal *E. coli* means, at Nicolet, during the 2006 summer swimming season. This matrix shows no significance between sampling times, after a rain event, compared with the seasonal mean.

	Seasonal Mean	1-4 hour mean	8 hour mean	12 hour mean	24 hour mean
Seasonal Mean	1.000				
1-4 hour mean	1.000	1.000			
8 hour mean	0.997	0.999	1.000		
12 hour mean	0.993	0.998	1.000	1.000	
24 hour mean	1.000	1.000	1.000	1.000	1.000

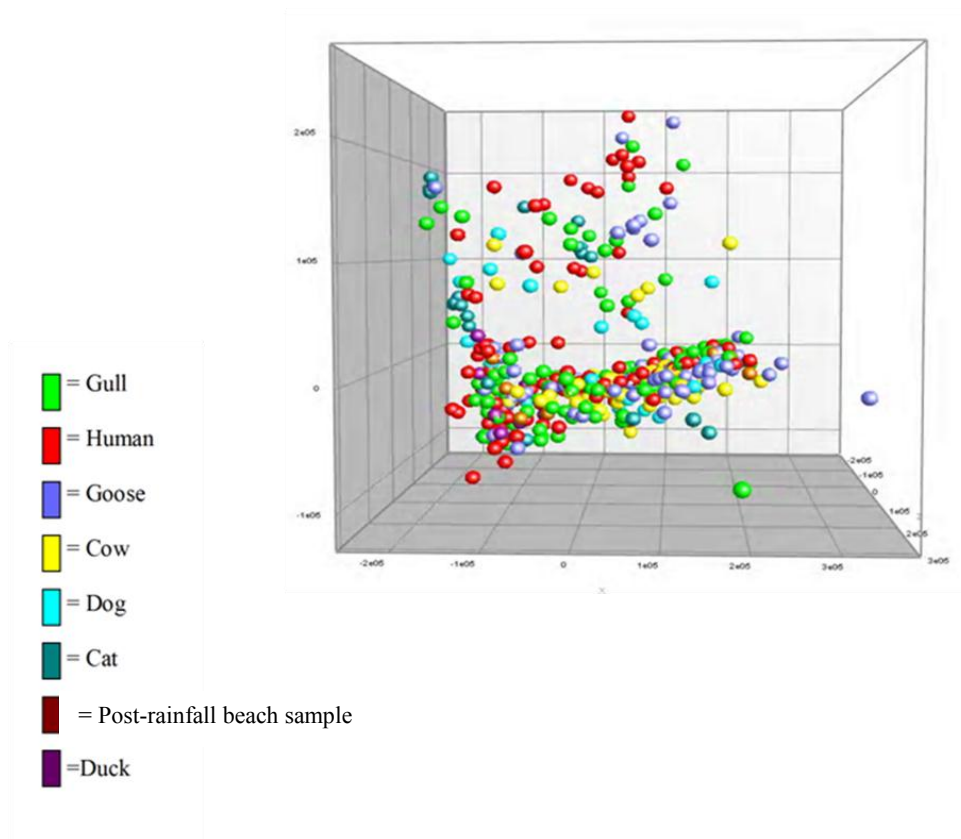


Figure Y-1. PCA of Nicolet waste isolates.

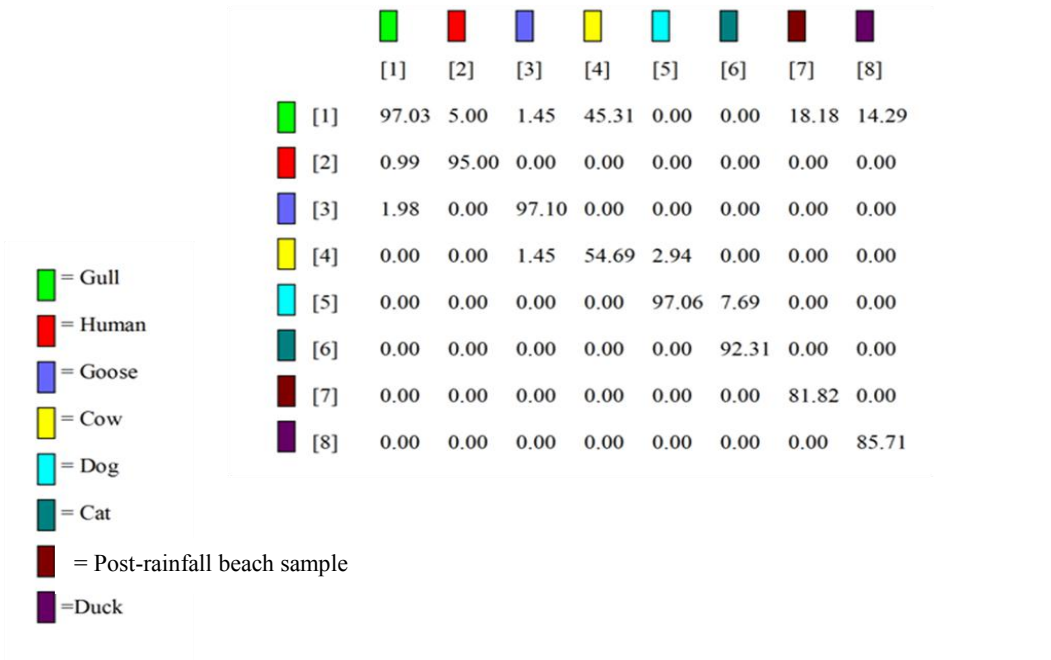


Figure Z-1. Jackknife analysis of Nicolet rain vs. gull, human, goose, cow, dog, cat, and duck waste isolates.

APPENDIX J

Rainfall Effects on *E. coli* and Source Identification at Otumba Park Beach



Figure A-2. Aerial view of Otumba's sampling sites. This view shows the center of the beach, Pipe 1, Pipe 2, as well as the hours each site was sampled.

**Pipe 1 was extended in 2006 and therefore not sampled that year.

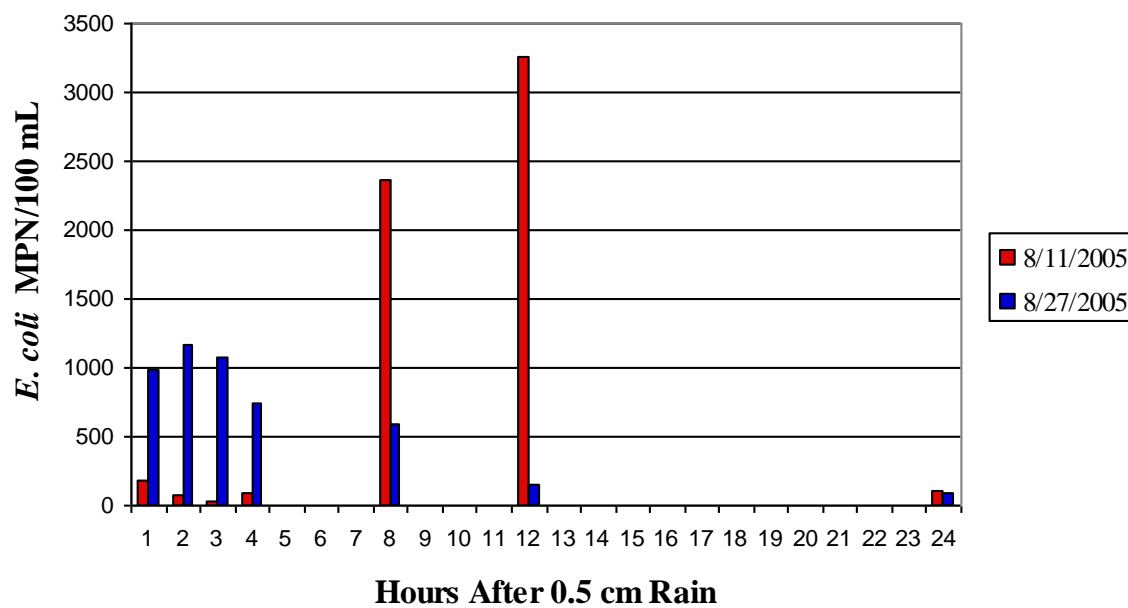


Figure B-2. *E. coli* MPN/100 mL of water sampled from Otumba each hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2005.

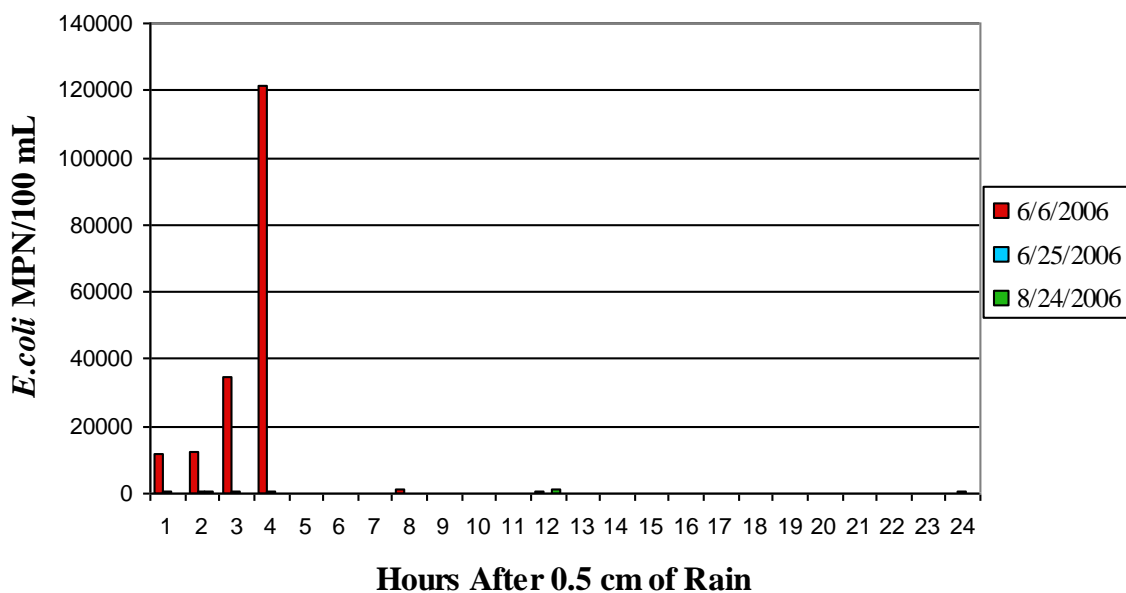


Figure C-2. *E. coli* MPN/100 mL of water sampled from Otumba each hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

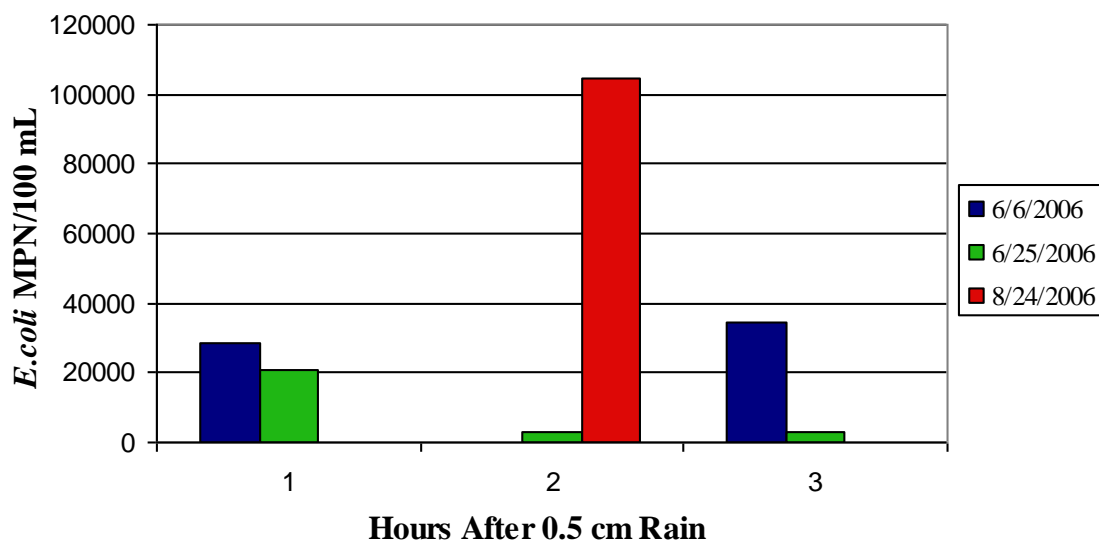


Figure D-2. *E. coli* MPN/100 mL of water sampled directly from Otumba Pipe 2 each hour, up to 3 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

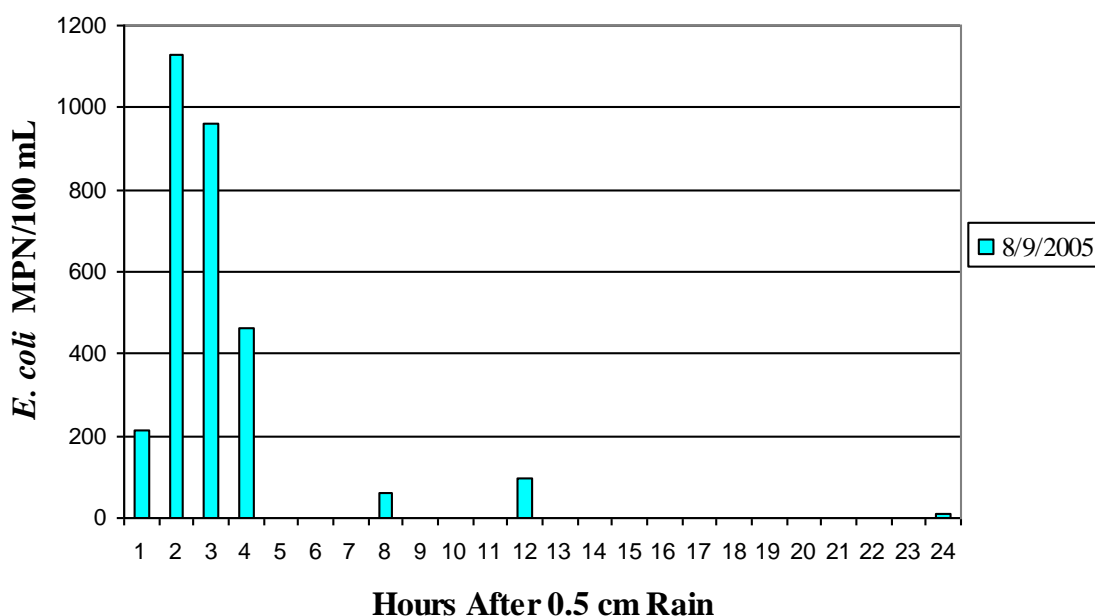


Figure E-2. *E. coli* MPN/100 mL of water sampled from Sister Bay each hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2005.

Table 10. Scheffe matrix showing the relationship between various sampling times and their *E. coli* concentrations compared with the seasonal *E. coli* means, at Otumba, during the 2005 and 2006, summer swimming season. This matrix shows significance at the 1-4 hour sampling time, after a rain event, compared with the seasonal mean.

	Seasonal Mean	1-4 hour mean	8 hour mean	12 hour mean	24 hour mean
Seasonal Mean	1.000				
1-4 hour mean	0.000	1.000			
8 hour mean	0.996	0.008	1.000		
12 hour mean	0.990	0.010	1.000	1.000	
24 hour mean	1.000	0.003	0.999	0.998	1.000

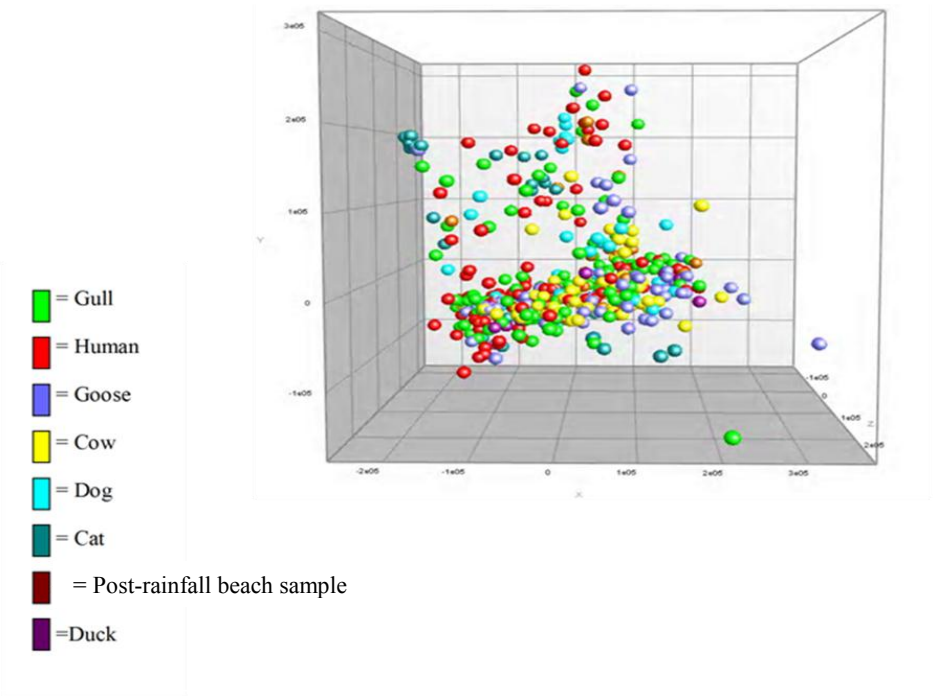


Figure F-2. PCA of Otumba’s waste isolates.

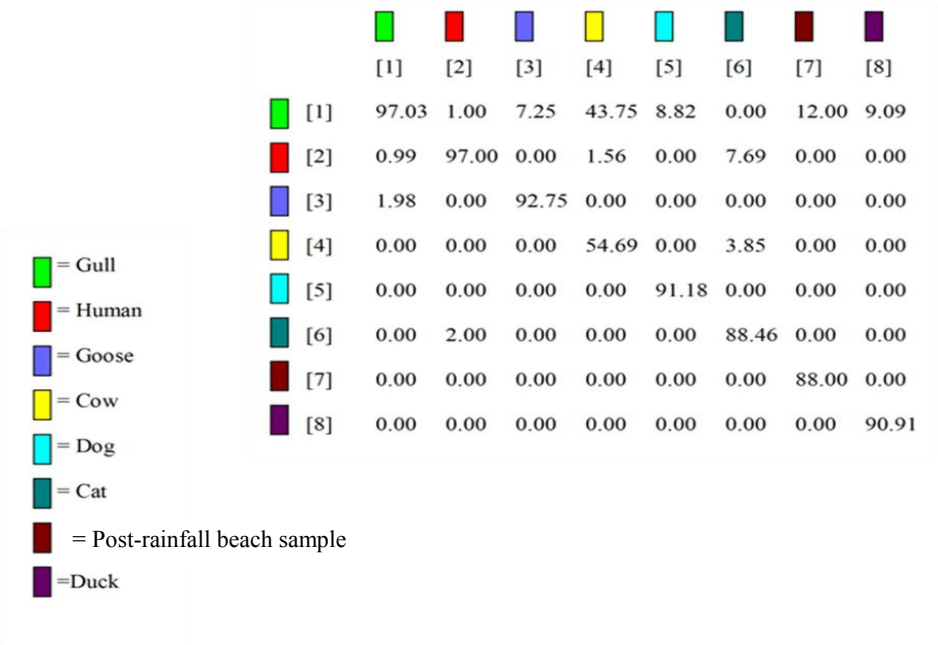


Figure G-2. Jackknife analysis of Otumba’s rain vs. gull, human, goose, cow, dog, cat, and duck waste isolates.

APPENDIX K

Rainfall Effects on *E. coli* and Source Identification at Sister Bay Beach



Figure H-2. Aerial view of Sister Bay's sampling sites. This view shows the center of the beach, Pipe 1, Pipe 2, as well as the hours each site was sampled.

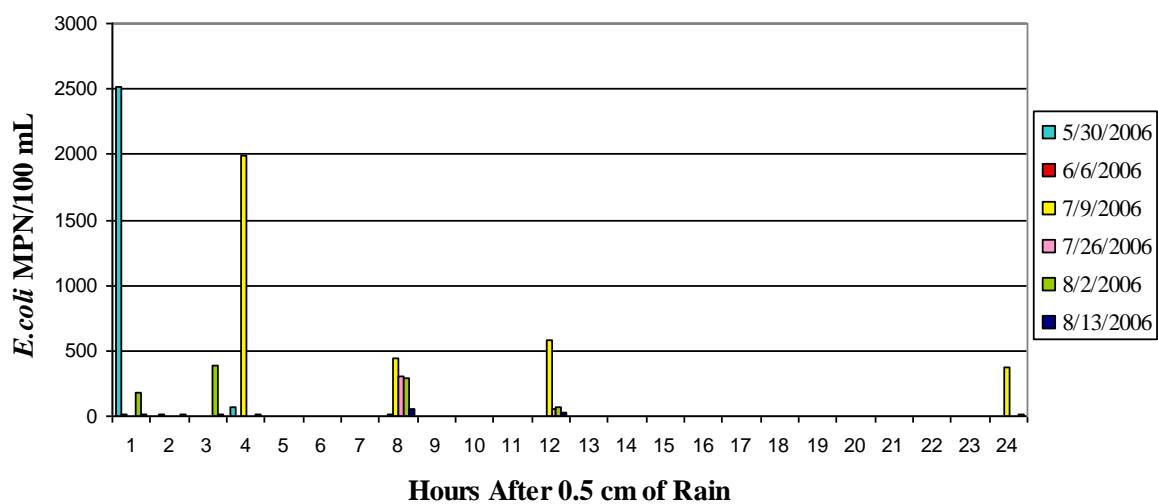


Figure I-2. *E. coli* MPN/100 mL of water sampled from Sister Bay each hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

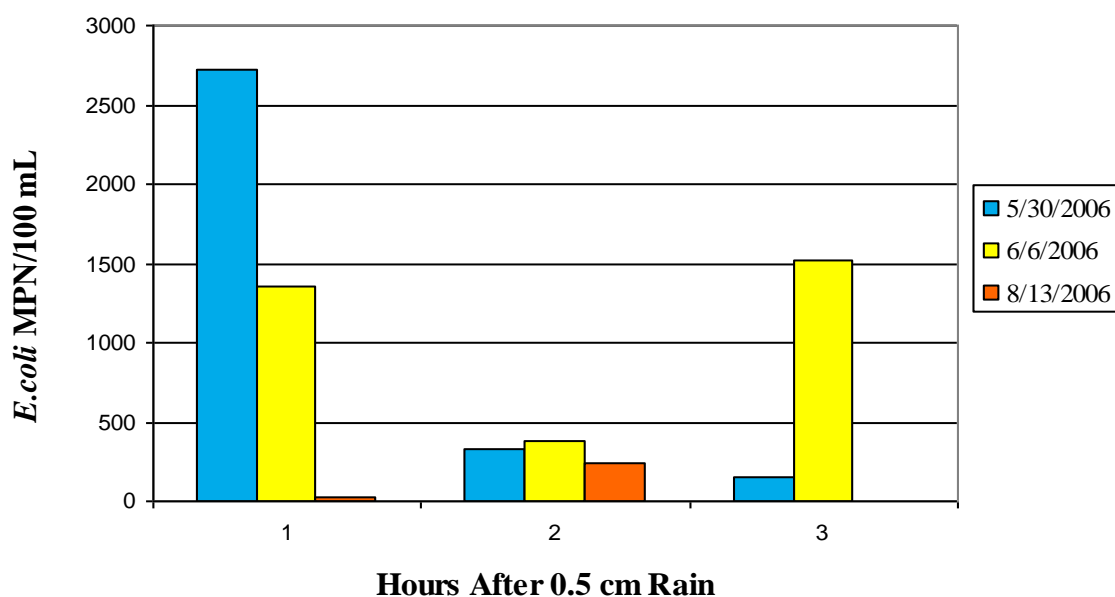


Figure J-2. *E. coli* MPN/100 mL of water sampled directly from Sister Bay Pipe 1 each hour, up to 3 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

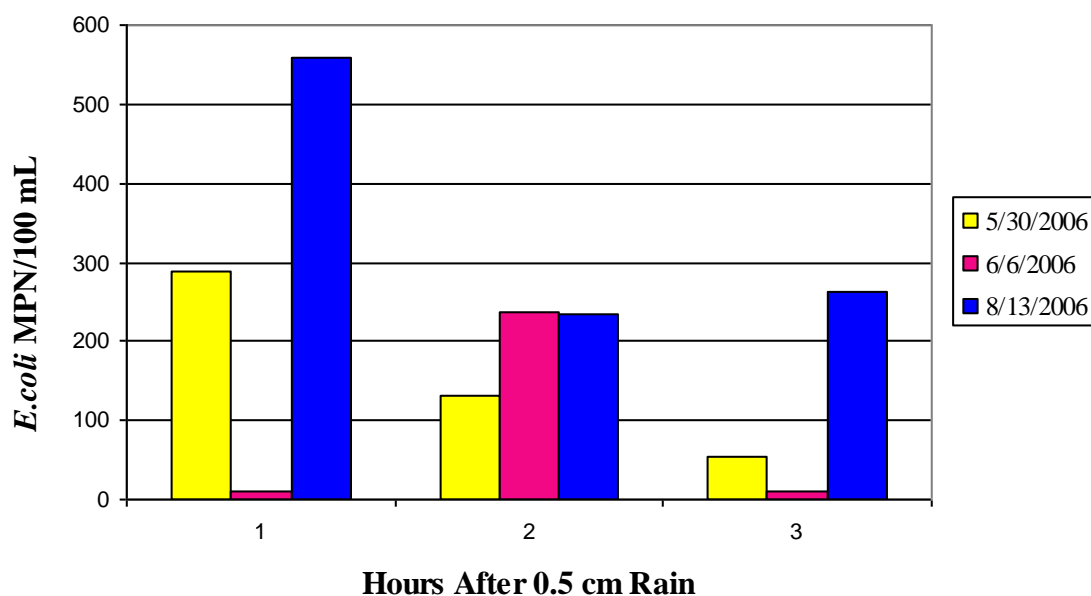


Figure K-2. *E. coli* MPN/100 mL of water sample taken directly from Sister Bay Pipe 2 each hour, up to 3 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

Table 11. Scheffe matrix showing the relationship between various sampling times and their *E. coli* concentrations compared with the seasonal *E. coli* means, at Sister Bay, during the 2005 and 2006, summer swimming season. This matrix shows significance at the 1-4 hour sampling time, after a rain event, compared with the seasonal mean.

	Seasonal Mean	1-4 hour mean	8 hour mean	12 hour mean	24 hour mean
Seasonal Mean	1.000				
1-4 hour mean	0.000	1.000			
8 hour mean	0.672	0.000	1.000		
12 hour mean	0.817	0.000	0.999	1.000	
24 hour mean	0.999	0.000	0.925	0.976	1.000

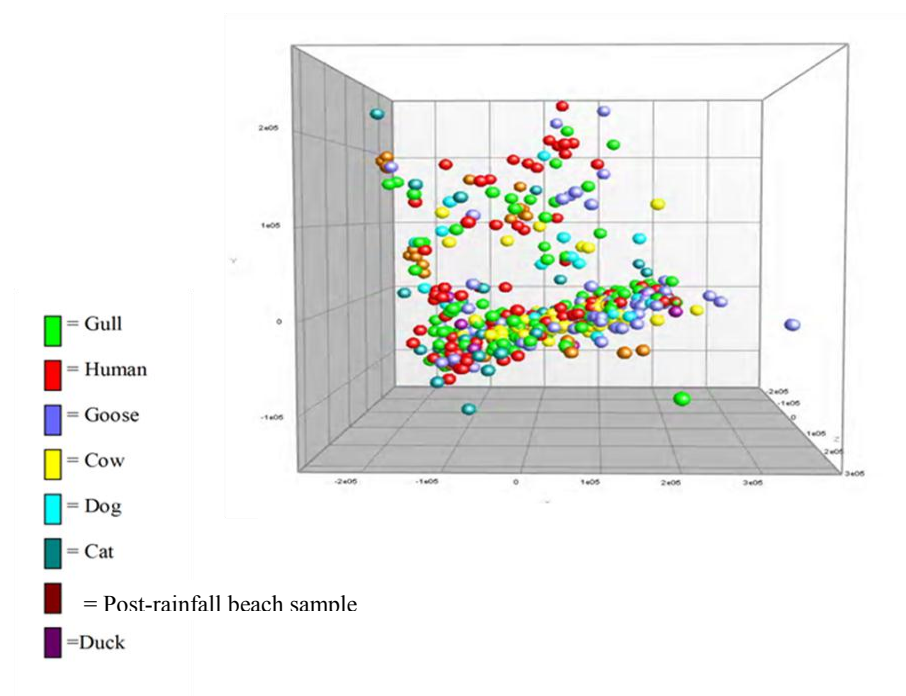


Figure L-2. PCA of Sister Bay's waste isolates.

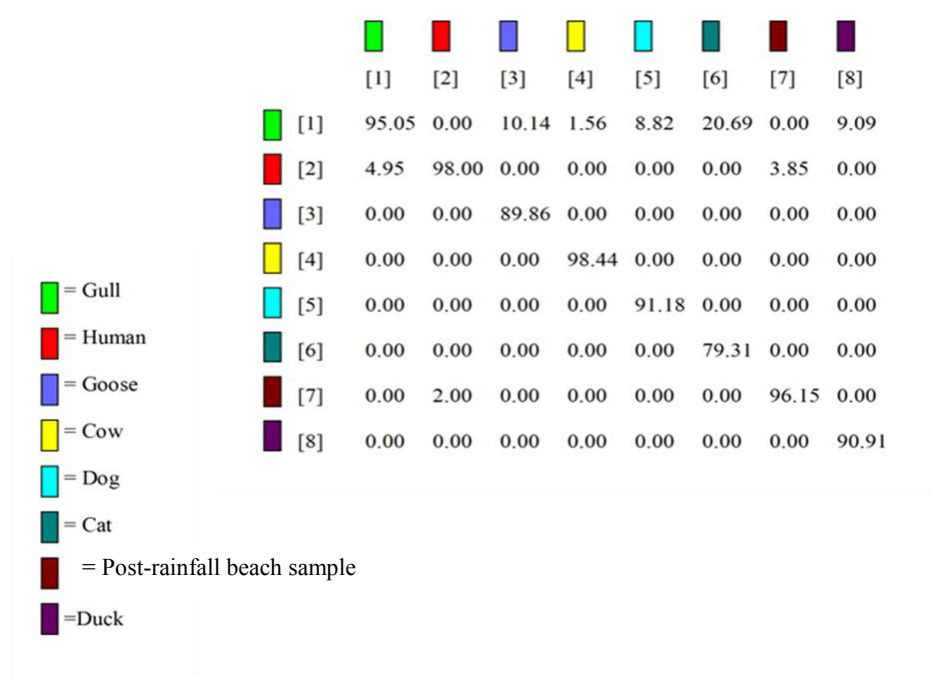


Figure M-2. Jackknife analysis of Sister Bay's rain vs. gull, human, goose, cow, dog, cat, and duck waste isolates.

APPENDIX L

Rainfall Effects on *E. coli* and Source Identification at Sunset Park Beach



Figure N-2. Aerial view of Sunset Park's sampling sites. This view shows the center of the beach and Runoff 1, as well as the hours each site was sampled.

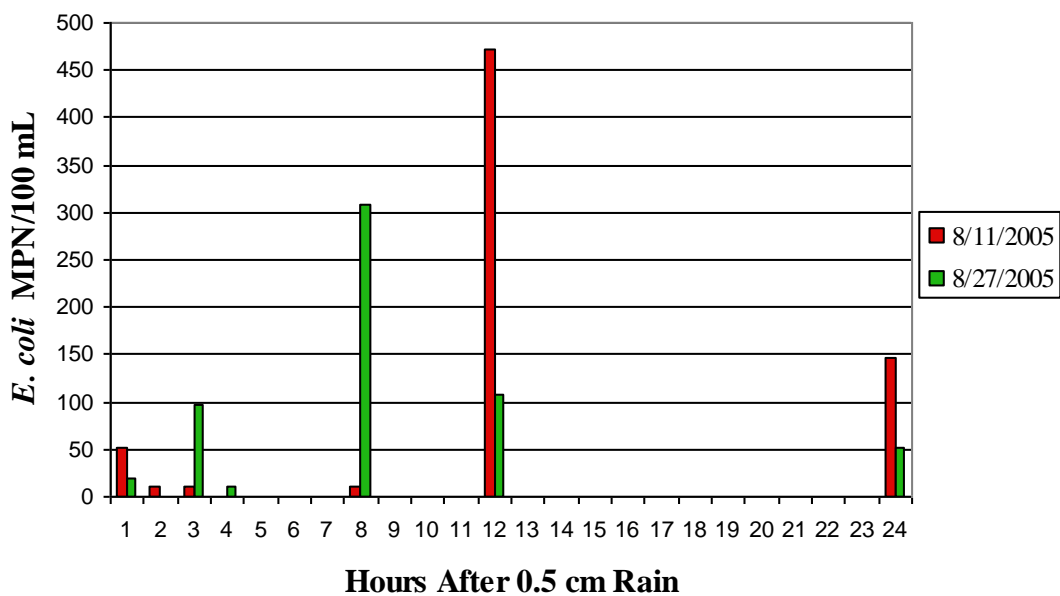


Figure O-2. *E. coli* MPN/100 mL of water sampled from Sunset Park each hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2005.

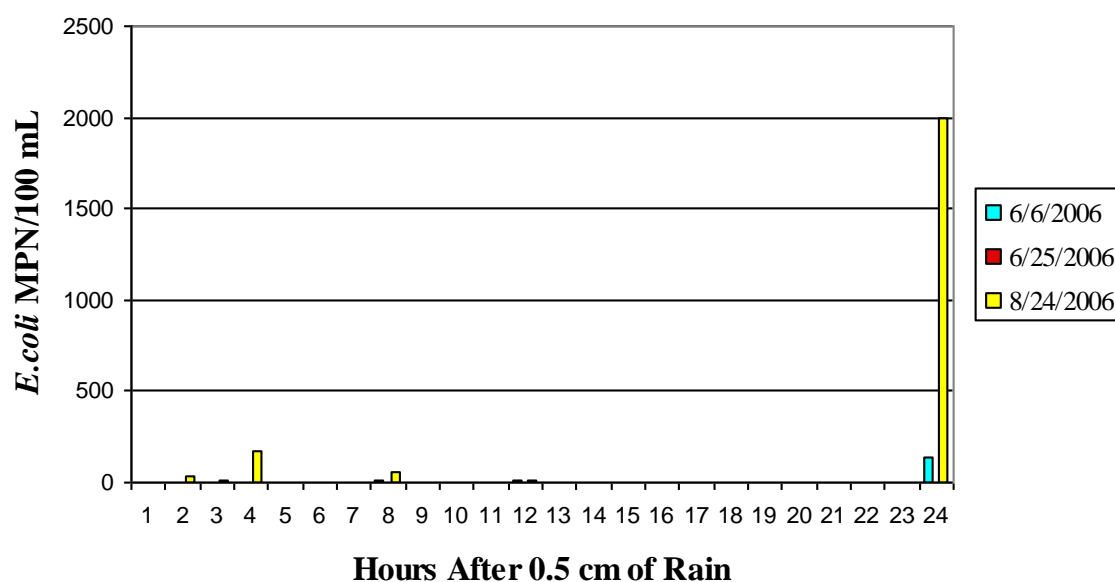


Figure P-2. *E. coli* MPN/100 mL of water sampled from Sunset Park each hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

Table 12. Scheffe matrix showing the relationship between various sampling times and their *E. coli* concentrations compared with the seasonal *E. coli* means, at Sunset, during the 2005 and 2006, summer swimming season. This matrix shows no significance between sampling times after a rain event and the seasonal mean.

	Seasonal Mean	1-4 hour mean	8 hour mean	12 hour mean	24 hour mean
Seasonal Mean	1.000				
1-4 hour mean	0.910	1.000			
8 hour mean	0.971	1.000	1.000		
12 hour mean	0.992	0.998	1.000	1.000	
24 hour mean	0.843	0.649	0.755	0.827	1.000

APPENDIX M

Rainfall Effects on *E. coli* and Source Identification at Whitefish Dunes Beach

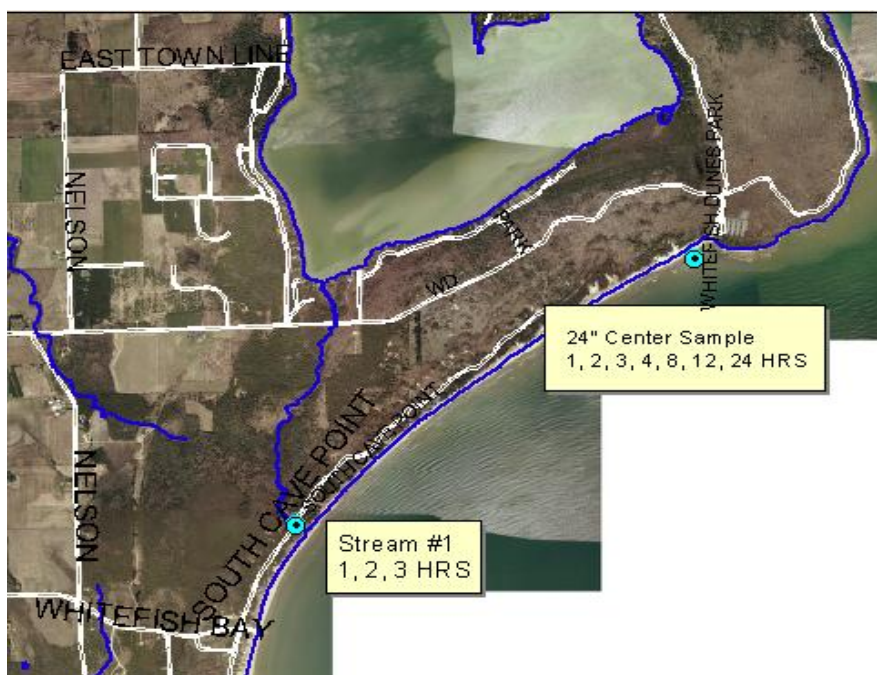


Figure Q-2. Aerial view of Whitefish Dune's sampling sites. This view shows the center of the beach and Stream 1, as well as the hours each site was sampled.

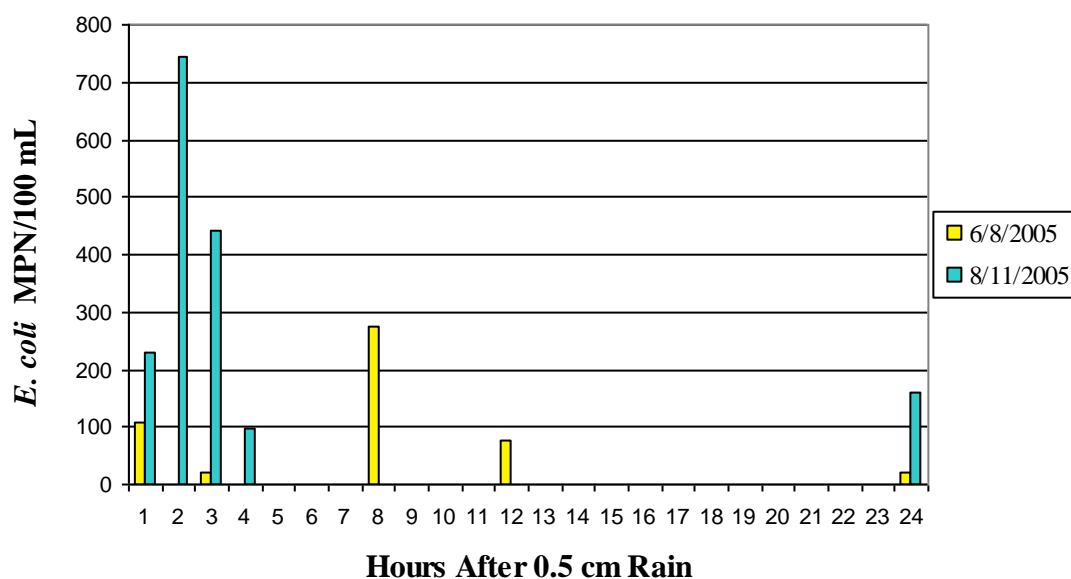


Figure R-2. *E. coli* MPN/100 mL of water sampled from Whitefish Dunes each hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2005.

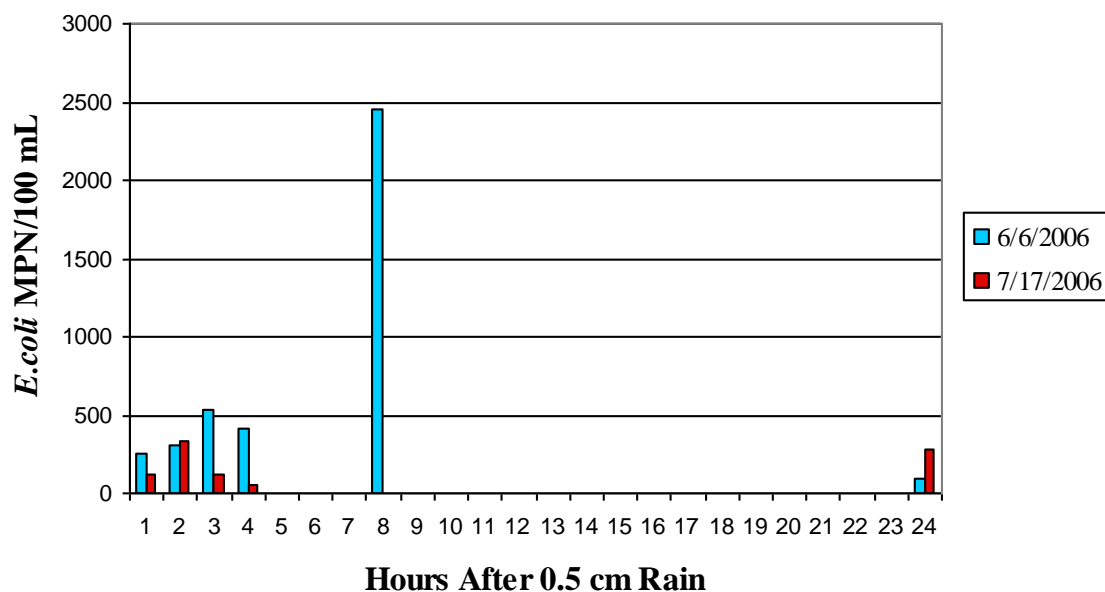


Figure S-2. *E. coli* MPN/100 mL of water sampled of Whitefish Dunes each hour, up to 24 hours, after a total rain accumulation of ≥ 0.5 cm in a 24 hour period. Samples were taken during the summer swimming season of 2006.

Table 13. Scheffe matrix showing the relationship between various sampling times and their *E. coli* concentrations compared with the seasonal *E. coli* means, at Whitefish Dunes, during the 2005 and 2006, summer swimming season. This matrix shows significance at the 8 hour sampling time, after a rain event, compared with the seasonal mean.
 Note there is no 12 hour sampling time

	Seasonal Mean	1-4 hour mean	8 hour mean	24 hour mean
Seasonal Mean	1.000			
1-4 hour mean	0.997	1.000		
8 hour mean	0.011	0.074	1.000	
24 hour mean	0.998	0.992	0.041	1.000

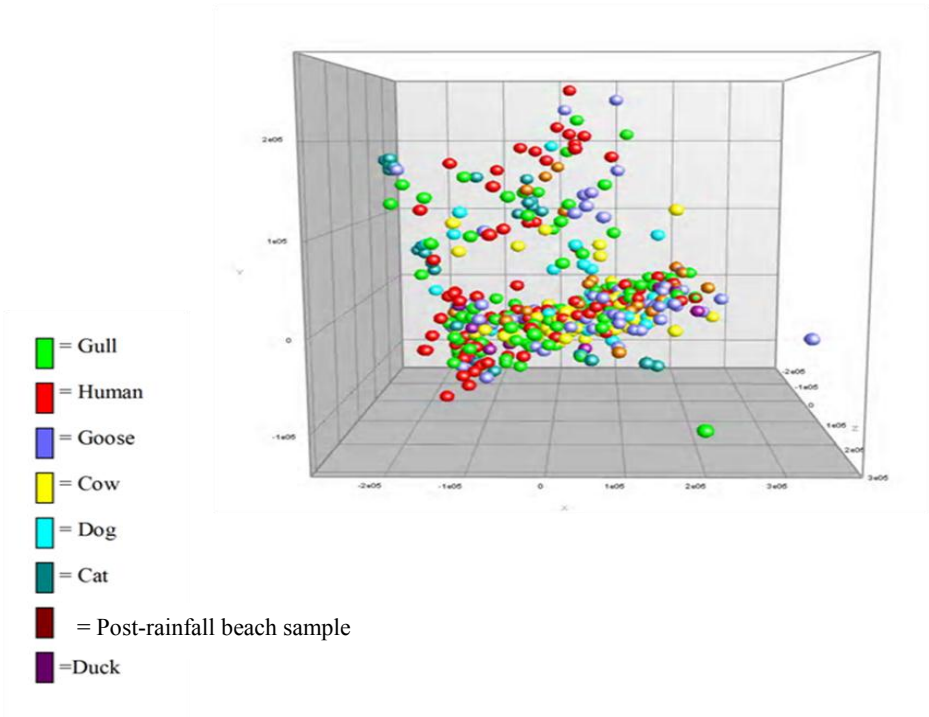


Figure T-2. PCA of Whitefish Dunes waste isolates.

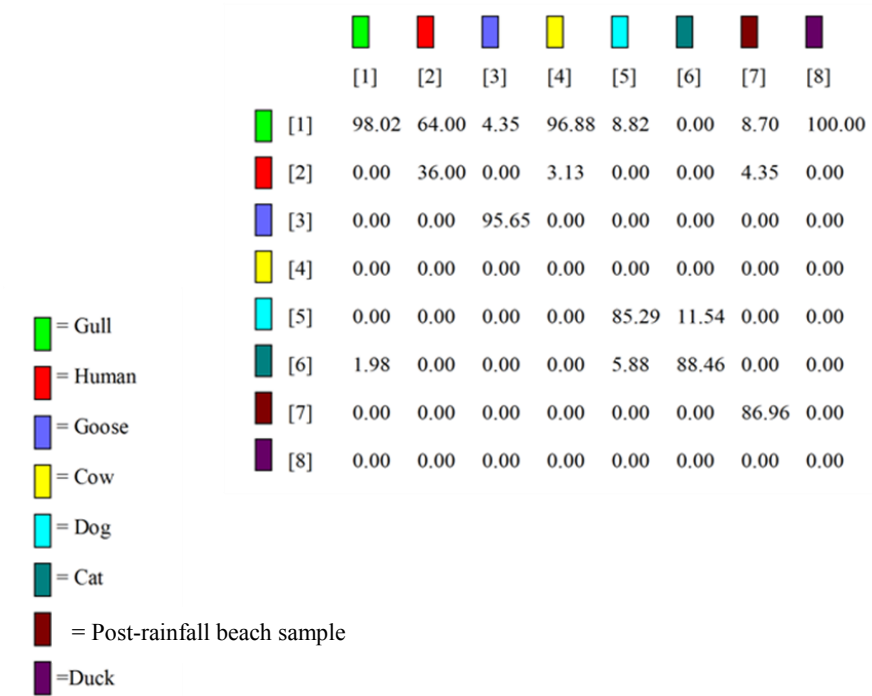


Figure U-2. Jackknife analysis of Whitefish Dunes rain vs. gull, human, goose, cow, dog, cat, and duck waste isolates.

APPENDIX N

Statistical and Source Identification Comparison and Summary

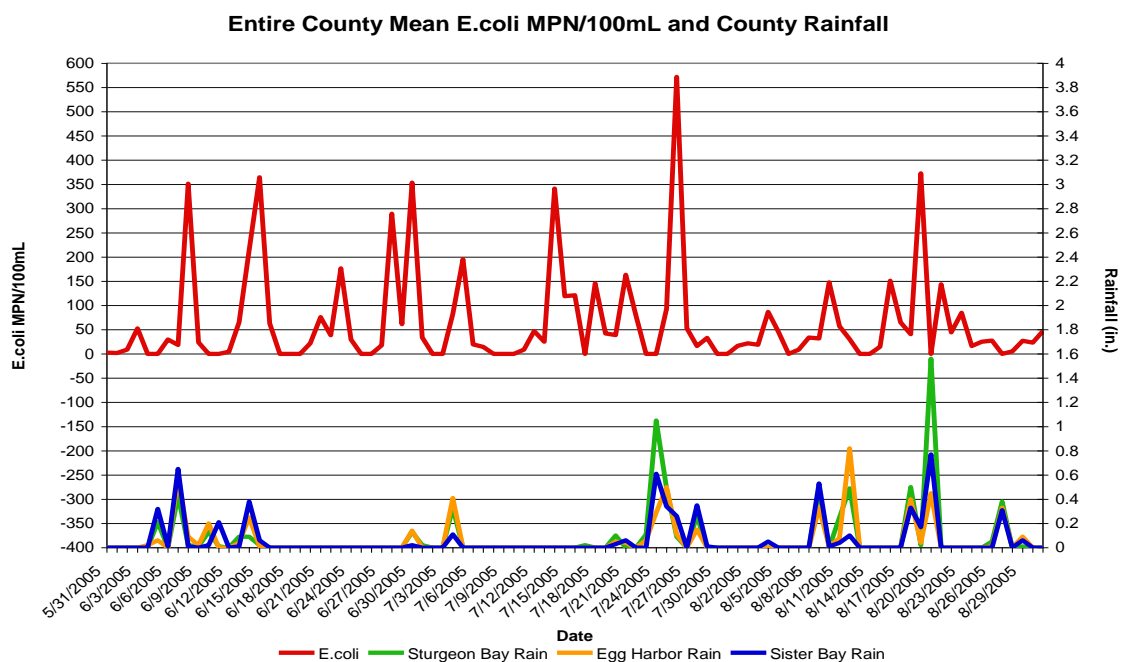


Figure V-2. County mean *E. coli* MPN/100 mL and county rainfall, 2005.

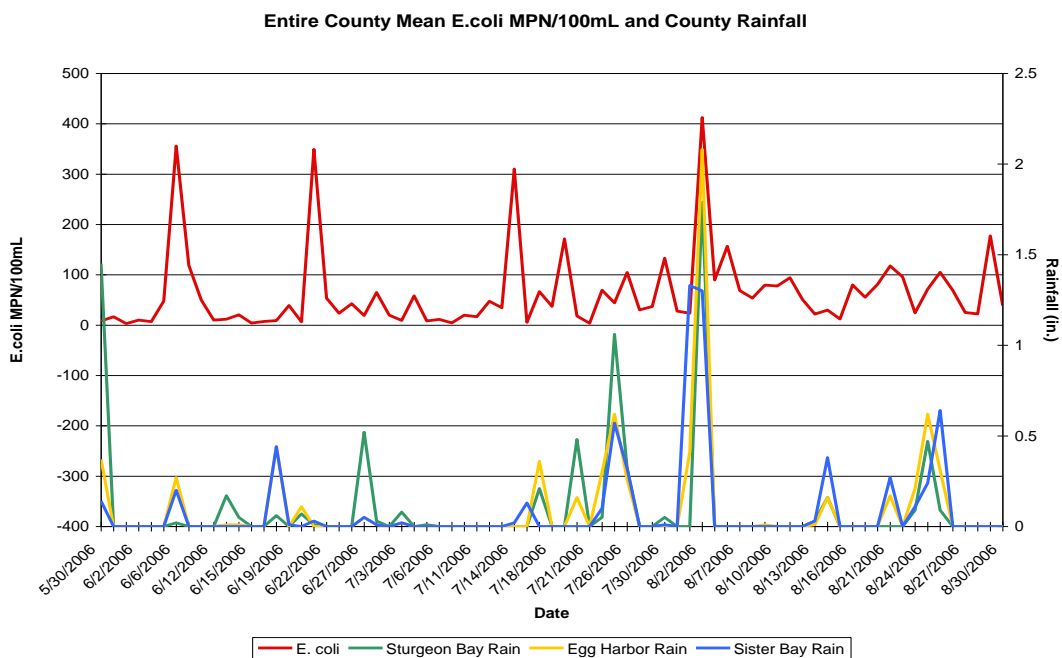


Figure W-2. County mean *E. coli* MPN/100 mL and county rainfall 2006.

Table 14. Statistical analysis between *E. coli* concentrations post-rainfall (at least 0.5 centimeters) verses 24, 48 and 72 prior to a rain event. Pearson correlations (alpha=0.05). Ellison Bay was the only beach to show significance (p=0.000) at 72 hours prior to a rain event.

Beach Name	Rainfall Hours Prior to High <i>E. coli</i> Sample	Correlation Coefficient	p-value
Anclam	24	0.06	0.744
	48	-0.083	0.652
	72	-0.101	0.582
Baileys Harbor	24	-0.072	0.596
	48	-0.116	0.396
	72	-0.133	0.330
Egg Harbor	24	-0.073	0.588
	48	-0.065	0.631
	72	-0.064	0.635
Ellison Bay	24	-0.041	0.765
	48	0.246	0.068
	72	0.465	0.000
Ephraim	24	-0.114	0.401
	48	-0.151	0.265
	72	-0.145	0.287
Fish Creek	24	-0.084	0.533
	48	-0.09	0.502
	72	-0.09	0.500
Lakeside Park	24	-0.112	0.549
	48	-0.072	0.700

	72	-0.094	0.616
Murphy Park	24	0.035	0.794
	48	0.138	0.307
	72	0.074	0.586
Otumba Park	24	-0.067	0.623
	48	-0.102	0.452
	72	-0.134	0.320
Sister Bay	24	0.006	0.965
	48	-0.018	0.897
	72	-0.044	0.745
Sunset Park	24	-0.045	0.735
	48	-0.091	0.498
	72	-0.124	0.352
Whitefish Dunes	24	-0.007	0.957
	48	0.006	0.963
	72	0.043	0.752

Table 15. Analysis of Variance (ANOVA) between all beach locations in 2005, showing overall impact of rainfall on selected beaches. Bolded p-values show where significance occurred ($p \geq 0.000$) ($\alpha = 0.5$) between rainfall and elevated *E. coli* concentrations in beach water. All beaches except Sunset and Whitefish Dunes show significance between rainfall and *E. coli* concentrations.

Beach Name	p-value
Anclam	0.000
Bailey's Harbor	0.000
Egg Harbor	0.000
Ephraim	0.000
Fish Creek	0.000
Lakeside	0.000
Murphy	0.000
Otumba	0.000
Sister Bay	0.000
Sunset	0.955
Whitefish Dunes	0.994

Table 16. Analysis of Variance (ANOVA) of all beach locations in 2006, showing overall impact of rainfall on selected beaches. Bolded p-values show where significance occurs ($p \geq 0.000$) ($\alpha = 0.05$) between rainfall and elevated *E. coli* concentrations in beach water. All beaches except Egg Harbor, Murphy, and Sunset show significance between rainfall, and elevated *E. coli* concentrations in beach water.

Beach Name	p-value
Anclam	0.002
Bailey's Harbor	0.000
Egg Harbor	0.909
Ephraim	0.000
Fish Creek	0.000
Lakeside	0.000
Murphy	0.838
Nicolet	0.979
Otumba	0.000
Sister Bay	0.000
Sunset	0.206
Whitefish Dunes	0.001

	Human > 86	Human (S.D. +/- 7)	Cow > 86	Cow (S.D. +/- 7)	Goose > 86	Goose (S.D. +/- 7)	Gull > 86	Gull (S.D. +/- 7)	Duck > 86	Duck (S.D. +/- 7)	Total Avian > 86	Avian (S.D. +/- 7)	Dog > 86	Dog (S.D. +/- 7)	Cat > 86	Cat (S.D. +/- 7)	Rain > 86	Rain (S.D. +/- 7)	Total # of isolates
Anclam	45	21	47	17	48	5	54	21	4	5	106	31	29	6	23	2	19	4	433
Bailey's	53	27	49	14	46	16	61	22	6	5	113	43	23	3	26	1	32	11	451
Egg Harbor	51	30	18	15	51	13	59	20	5	4	115	37	32	4	24	1	2	4	421
Ellison Bay	64	19	53	8	63	7	72	18	3	4	138	29	31	3	28	0	12	1	424
Ephraim	63	20	50	8	60	6	68	16	3	4	131	26	34	3	24	1	4	2	428
Fish Creek	49	32	49	11	53	12	61	18	5	4	119	34	34	2	25	2	5	4	436
Lakeside	46	30	49	10	42	12	48	18	3	5	93	35	16	3	24	1	10	6	439
Murphy	64	20	51	9	60	7	71	16	6	2	137	25	34	3	25	0	3	0	413
Nicolet	71	14	51	10	60	7	77	15	3	3	140	25	36	2	25	0	0	2	412
Otumba	65	20	52	10	59	7	74	15	5	2	138	24	31	3	25	3	22	4	430
Sister Bay	73	11	55	8	60	6	74	15	5	2	139	23	36	1	25	0	11	4	434
Whitefish Dunes	67	17	53	9	58	8	73	14	5	2	136	24	31	3	27	0	19	3	428

Table 17. Total number of human, cow, goose, gull, duck, total avian, dog, cat, and rain isolates exceeding the correlation coefficient of 86, along with the number of isolates with a standard deviation (+/- 7). The total isolate count, for each beach, is listed from the summer swimming season of 2005 and 2006.

Table 18. All isolates from human, cow, goose, gull, duck, total avian, cat and rain from the summer swimming season of 2005 and 2006 for each beach. Percentage of total isolates from human, cow, goose, gull, duck, total avian, cat, and rain collected during the 2005 and 2006 summer swimming seasons at individual beaches.

	% of Human	% of cow	% of Dog	% of Goose	% of Gull	% of Duck	% of Avian Total	% of Cat	Rain Total	% of Rain
Anclam	44.5	73.4	80.6	69.6	56.3	36.4	60.2	82.2	39	48.7
Bailey's	52.4	76.6	63.8	66.7	63.5	54.5	64.2	92.9	46	69.6
Egg Harbor	50.4	28.1	88.9	73.9	61.5	45.5	65.3	85.7	16	12.5
Ellison Bay	63.3	82.8	86.1	91.3	75	27.3	78.4	100	20	60
Ephraim	62.3	78.1	94.4	87	70.8	27.3	74.4	85.7	23	17.4
Fish Creek	48.5	76.6	94.4	76.8	63.5	45.5	67.6	89.3	30	16.6
Lakeside	45.5	76.6	44.4	60.9	50	27.3	52.8	85.7	28	35.7
Murphy	63.3	79.7	94.4	87	74	54.5	77.8	89.3	8	37.5
Nicolet	70.3	79.7	100	87	80.2	27.3	79.5	89.3	7	0
Otumba	64.4	81.3	86.1	85.5	77.1	45.5	78.4	89.3	25	88
Sister Bay	72.2	85.9	100	87	77.1	45.5	79	89.3	30	36.6
Whitefish Dunes	66.3	82.8	86.1	84.1	76.1	45.5	77.3	96.4	26	73.1

Human % of total Cow % of Total Dog % of Total Goose % of Total Gull % of Total Duck % of Total Avian % of Total Cat

Total # of Isolates: Human Isolates: 101 Cow: 64 Dog: 36 Goose: 69 Gull: 96 Duck: 11 Avian Total: 176 Cat: 28

Table 19. Total number of rainfall (unknown) isolates matching human, cow, dog, goose, gull, duck, total avian, and cat from individual beaches.

	Human	Cow	Dog	Goose	Gull	Duck	Total Avian	Cat
Anclam	6 (2)	5 (1)	2 (1)	5	5 (2)	0	10 (2)	0
Bailey's	12 (1)	5 (2)	7 (2)	7 (3)	9 (5)	5	21 (8)	0
Egg Harbor	4 (1)	0	0	1	3 (1)	0	4 (1)	0
Ellison Bay	4	1 (1)	1	9 (1)	1	0	10 (1)	0
Ephraim	1 (1)	0	0	1	1 (1)	3	5 (1)	0
Fish Creek	2 (1)	2 (1)	0	3	2	1	6	0
Lakeside	5 (3)	5 (4)	0	4 (1)	4	1	9 (1)	0
Murphy	0	2	0	0	0	0	0	1
Nicolet	0	0	0	0	2	0	2	0
Otumba	2	11	2	2	6	0	8	0
Sister Bay	9 (6)	1 (1)	2 (2)	3 (1)	8 (3)	0	11 (4)	0
Whitefish Dunes	5 (1)	1 (1)	1	5 (2)	11 (1)	1	17 (3)	1 (1)

The following isolates were counted twice due to the same value between isolates

Table 20. General Beach Summary (2005, 2006)

Beach	Peak hours of Microbial Contamination	Genetic Findings
Anclam	<p>The general trend showed a steady incline the first 4 hours after a rainfall, declining by hour 12. Microbial contamination peaked at hour 24 consecutively.</p> <p>A pipe, going directly into the swim area, was sampled directly revealing microbial contaminants more than 8 times the closure limit, within the first 3 hours of sampling.</p>	<p>The highest number of rain isolates belonged to total avian, even though no duck isolates were found. Human isolates were identified as the second leading source of collected rain isolates.</p>
Bailey's Harbor	<p>Most microbial contamination happens within the first 4 hours of sampling. All events, except for one, show a significant decrease in bacteria by the 12th and 24th hour. Pipes sampled directly showed bacterial contamination equivalent to advisory and closure limits.</p>	<p>The highest number of rain isolates identified, were from total avian. Human isolates were the second leading source, above cow, dog, and cat.</p>

Egg Harbor	Most individual sampling events, after a rainfall, showed minimal bacterial contamination at this beach. The elevation that does occur happens within the first 12 hours. There was only a single sample that exceeded the closure limit from both seasons.	Genetic findings reveal only gull, goose, and human present from collected rain isolates. The total avian and human isolates are found in equal amounts.
Ellison Bay	This beach was only sampled in 2006 due to minimal sampling events in 2005. Microbial activity at this location typically is very low (under the advisory level) throughout all sampling times. There was only a single occasion during the 3 rd hour of sampling that levels exceeded the closure limit. Water sampled directed from Pipe #1 contained levels lower than 100 CFU/100mL.	Rain isolates from this location had the most similarity to goose isolates. Human and other isolates were found but in a lower concentration.
Ephraim	Bacterial contamination occurs mostly during the first 12 hours after a rainfall. There was only one instance in 2005 where the closure limit was exceeded. There is usually minimal contamination by the 24 th hour, but in 2006 a sample spiked up above the advisory limit at hour 24. A stream, flowing into the beach area, shows elevated bacterial levels within the first 3 hours after a rainfall.	Ephraim's rain isolates had the highest genetic similarity within the total avian category, with duck isolates containing the most genetic matches.
Fish Creek	The highest bacterial contamination occurs within the first 8 hours after a rainfall, and continues to drop significantly through the 12 th and 24 th hour. Runoff was collected directly and did not show elevated bacterial levels. A pipe was also sampled directly from this location and showed elevated levels (above advisory level) the first two hours after a rainfall event.	Rain isolates were most similar to the total avian category. Rain isolates at this location also showed similarity with human and cow isolates.
Lakeside Park	This location continuously had extremely high levels of bacterial contamination, up to over 20 times higher than the closure limit. High levels were found mostly within the first 12 hours of sampling, with bacterial levels significantly falling after 24 hours. A pipe sampled directly from Lakeside showed levels over ten times the closure limit with levels still exceeding more than twice the closure limit after the 3 rd hour of sampling.	Genetic findings at this location showed rain isolates matching human, cow, gull, and goose, relatively even. Only one duck isolate was recovered and no dog or cat isolates.
Murphy Park	Most bacterial contamination occurs within the first 12 hours of sampling. 2005 samples showed a higher amount of contamination when compared with 2006. In 2006 there was not a single sample that exceeded the closure limit.	Rain isolates matching examined sources were limited at this beach, only recovering

Nicolet	This location was only sampled during the swim season of 2006, due to lack of rain and state park limitations. All three events that were sampled in 2006 showed very minimal bacterial activity from all time points.	one isolate matching cat and two from cow. Only two isolates from this location were identified. Rain isolates collected only revealed similarity to gull isolates.
Otumba Park	This location consistently showed extreme levels of microbial contamination, frequently much higher than the closure limit. There is heightened microbial contamination seen both years within the first 4 hours, and in 2005 this increases up to the 12 hour. This beach had the highest bacterial level out of all testing from 2005 and 2006, with levels exceeding more than 120 times the closure limit within the first 4 hours. Samples collected directly from Pipe #1 also show extreme levels of contamination within the first 3 hours. At hour 2 bacterial levels increase to over 100,000 CFU/100mL.	Genetic testing showed a mixture of sources, excluding duck and cat, with the most isolates identified from cow. This was followed by a total of gull and goose.
Sister Bay	The highest levels of bacterial contamination happen within the first 4 hours of sampling, with most levels significantly lower after a 24 hour period. There are two pipes, running directly into the beach area that showed increased bacterial contamination. Pipe #2 had lower bacterial levels than Pipe #1, reaching only advisory limits. Pipe #1 had three occasions within the first three hours that that exceeded the closure limit.	Rain isolates were mostly similar to human and gull, with small amounts of cow, dog, and goose.
Sunset Park	In 2005 this location showed increased bacterial levels, in the advisory range, during the 8 th and 12 th hour of sampling. By the 24 hour samples decreased to under advisory levels. Sampling from 2006 showed minimal bacterial contamination throughout the 24 hour sampling period. There was one sample during this year that spiked, at hour 24, to over the closure limit.	Of the isolates collected from this location, none were reproducible.
Whitefish Dunes	Most bacterial contamination occurs within the first 8 hours of sampling. There was only one sample in 2006 that exceeded the closure limit, happening during the 8 th hour.	This location had the highest number of rain isolates matching gull isolates, out of all 13 locations. There were lesser but equal numbers of human and gull isolates.

Table 21. *E. coli* Values after a Rainfall Compared with Beach Monitoring Data 2005

	1-4 h	8 h	12 h	24 h	<i>E. coli</i> Low	<i>E. coli</i> High	Regular Season <i>E. coli</i> Average
Anclam							
Average	417	524	204	668	1	488.7	55.5
Standard Deviation	590.1	319.2	222.8	654.2			
Bailey's Harbor							
Average	310	147	774	193	0	360.9	29.9
Standard Deviation	446.1	126.4	1306	121.2			
Egg Harbor							
Average	81	1926	484	95	0	1046.2	65.7
Standard Deviation	103	2824	494	49			
Ephraim							
Average	379	2353	500	10	0	1444.07	57.9
Standard Deviation	243	2150	30	0			
Fish Creek							
Average	1887	1789	816	41	0	2419.6	57.9
Standard Deviation	1520.3	0	0	0			
Lakeside							
Average	2247	5670	12103	1396	0	1697.5	122.8
Standard Deviation	3608	9548.9	17102.1	943.3			
Murphy							
Average	297	272	2242	1278	0	>2419.6	153.9
Standard Deviation	500	241.8	3512.5	2170.8			
Otumba							
Average	548	1477	1707	98	0	1986.3	147.8
Standard Deviation	497.5	1248	2190	18			
Sister Bay							
Average	693	63	97	10	0	547.5	41.9
Standard Deviation	425	0	0	0			
Sunset							
Average	25	160	291	99	0	2419.6	246.8
Standard Deviation	34	211.4	258.1	67			
Whitefish Dunes							
Average	273	275	75	90	1	2419.6	160.7
Standard Deviation	273.9	0	0	99			

Table 22. *E. coli* Values after a Rainfall Compared with Beach Monitoring Data 2006

	1-4 h	8 h	12 h	24 h	<i>E. coli</i> Low	<i>E. coli</i> High	Regular Season <i>E. coli</i> Average
Anclam							
Average	132	341	295	993	0	727	102.8
Standard Deviation	84.9	350.4	416.5	1037			
Bailey's Harbor							
Average	1190	598	335	26	0	700	65.3
Standard Deviation	2097	566	415.8	36.8			
Egg Harbor							
Average	71	17	25	15	0	320.8	37.1
Standard Deviation	100.9	27.2	35.3	29.8			
Ellison Bay							
Average	177	55	29	15	0	200	19.8
Standard Deviation	377.1	43.9	31.7	17.8			
Ephraim							
Average	282	318	98	159	0	218.7	36.5
Standard Deviation	331.1	414.6	100.3	190.3			
Fish Creek							
Average	1213	749	244	86	1	2419.6	112.9
Standard Deviation	802.3	1178.6	210	57.4			
Lakeside							
Average	5592	5211	3345	216	0	2419.6	240
Standard Deviation	6501.8	7008.2	4414.5	305.5			
Murphy							
Average	85	125	85	21	0	320.8	68.8
Standard Deviation	141	243.2	123.8	43.9			
Nicolet							
Average	16	11	6	24	0	579.4	34.5
Standard Deviation	26.9	13.4	6.4	32.7			
Otumba							
Average	15242	441	585	256	1	2419.6	160.1
Standard Deviation	34865	522.8	460.4	180.1			
Sister Bay							
Average	349	163	124	66	0	218.7	33.8
Standard Deviation	786.3	194.7	225.3	150.8			
Sunset							
Average	18	21	7	711	0	2419.6	201.7
Standard Deviation	49.1	27	5.8	1110			
Whitefish Dunes							
Average	266	2459.5	xxx	184	0	2419.6	219.7
Standard Deviation	162.2	0	xxx	128.7			

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