# Characterization of *E. coli* levels at 63rd Street Beach

Prepared for

**City of Chicago**

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## INTRODUCTION

The City of Chicago, the United States Geological Survey (USGS), and the Chicago Park District developed a cooperative relationship in early 2000 to provide information on the nearshore waters of the Chicago area. In particular, the City wanted a more thorough understanding of excessive *E. coli* occurrences at 63rd Street Beach, near Jackson Park. Chicago historically complies with monitoring rules on a voluntary basis in the interest of public heath and recreational enjoyment. In fact, Chicago has one of the most intensive monitoring programs in the Great Lakes region. Chicago's hope was that with a thorough understanding of the nature and source of *E. coli* problems, they could begin to address remediation. Further, Chicago desired information on factors that related to these exceedances for purposes of developing real-time prediction models that could eventually augment or even replace traditional monitoring approaches. USGS, in turn, desired the opportunity to gather intensive information on the spatial-temporal distributions and population characteristics of *E. coli* in order to understand how environmental conditions, sources, and bacteria concentrations interact. This, in turn, would help USGS provide information for better management of public swimming areas in the Great Lakes area.

Specifically, Chicago wanted to:

1. Identify suspected sources that may be contributing to the elevated *E. coli* levels at 63rd St. Beach and quantify to what extent these sources are contributing to the *E. coli* levels;
2. Determine the efficacy of proposed mitigation measures by monitoring the post-effects of city-implemented measures;
3. Develop a more efficient testing and analysis protocol using a forecast model in order to alert the public of beach closures in a more timely manner;

To this end, we assembled a team of experts and support staff that could attend to these questions. Five senior scientists (Drs. Haack, Horvath, Olyphant, Whitman and Wolcott) and 9 supporting staff (Ms./Mr. Berlowskii, Goodrich, Gutzman, Laplante, Nevers, Price, Reynolds, Shiu, and Stenftenagel) worked actively on this project. Four institutions were involved: Lake Michigan Ecological Research Station, Great Lakes Science Center, USGS; National Wildlife Heath Center, USGS; Michigan Water Resources Division, USGS; and Indiana University. We believe this report goes far in achieving the goals set above. Some of the methods used to address these objectives included looking at the effects of routine beach raking and the May 24 beach renovation on *E. coli* concentrations. Examining the efficacy of pumping water across Casino Pier lies outside the scope of this report. In this report, we also briefly discuss some of the factors contributing to *E. coli* exceedances and some hypothetical options that might alleviate high *E. coli* concentrations.

The Chicago 63rd Street project has clearly taught us that even a small confined system such as 63rd Street beach is far more complex and varied than imagined. Even when we employ many of the available modern scientific tools (intensive descriptive statistics, modeling, advance biochemistry, genetic fingerprinting, biotyping, antibiotic resistance profiling), it is difficult to determine all the factors affecting *E. coli* on the beach. We now know that there is no single factor, or even set of factors, that can be universally relied on to predict specific concentrations

of *E. coli*. We understand, thanks to this project, that sampling depth and time are critical sampling considerations and will greatly affect results. In short, the Chicago 63rd Street study has shown us that there is much we didn't know; has taught us a lot about how the system works and has clearly laid the foundation for further studies both in the interest of Chicago and the Great Lakes.

## ACKNOWLEDGEMENTS

We especially thank Mayor Richard M. Daley for his support of this project and his commitment to maintaining healthy beaches. We also thank the Chicago Park District for their valuable assistance in carrying out this project. We thank the support staff that made this project possible, including Yvette Shiu and Jessie Stenftenagel, who worked diligently from June through September collecting water samples and transporting them to the Jardine Water Treatment Laboratory. Justin LaPlante and Valerie Price had the challenging job of sampling in April and May. Charles Bowling and Ellen Flanagan provided invaluable data on *E. coli* concentrations in sand and water throughout the study. They worked long stressful hours in the interest of the Chicago public. The field crew and laboratory formed the foundation of the project and we are indebted. Ellen Oberdick helped prepare several of the graphs and arranged data for this report. Naren Prasad and Christine Wolski were truly valued associates whose patience, commitment and energy made this project possible. Pam Thomas and Marcia Jimenez were instrumental in conceptual development of the project, providing administrative support and logistics. The assistance of Jean Adams, Angel Gochee and Evert Ting was greatly appreciated in reviewing this report.

## SUMMARY

### *Introduction*

To characterize the distribution and possible sources of *E. coli* at 63rd Street Beach, Chicago, an intensive study was undertaken between April and September 2000. Swimmability has been affected by high concentrations of *E. coli* in the past several years and in particular during the summer of 1999. Beach closures are enforced to protect the public from possible harmful illness associated with contamination. Most strains of *E. coli* are harmless, but it is typically associated with more harmful bacteria that can cause illness. The City of Chicago wanted to eliminate *E. coli* contamination at the beach in order to increase swimming safety and reduce beach closures. In order to accomplish this, sources of *E. coli* and the movement of *E. coli* within the system had to be determined.

### *Methods*

#### Daily Sampling

Over the course of six months, water samples and sand samples were collected. In April, water samples were collected at two depths (45 cm and 90 cm) along five transects three days each week, and onshore and submerged sand samples were collected in these transects. Additional water samples were collected off the north revetment, at the end of Casino pier, near the mouth of the Jackson Harbor, and from the Jackson Lagoon outflow. One additional sand sample was collected where the density of seagulls on the beach was observed to be the highest. Between May and September, an additional set of water samples was collected in the afternoon at the same locations along the five transects. Field observations were also noted in the morning, including the number of gulls on the beach, wind speed and direction, air and water temperature, and wave height at 45 cm depth.

#### Replication and Hourly Sampling

On ten randomly selected days, replicate water samples were collected. Two water samples were collected at each sampling site, and ten samples were collected at the 90 cm site in one transect. During ten other randomly selected days, samples were collected at the usual ten sites hourly from 7:00 a.m. to 3:00 p.m.

#### Sunlight

In order to test a hypothesis about *E. coli* survival during normal sunlight exposure, an experiment was conducted on-site using clear and dark bags containing lake water. The experiment was conducted on September 18, 2000 between 8:00 a.m. and 3:00 p.m.

#### Groundwater Testing

Seepage meters and piezometers were deployed on two separate days to determine the direction of water flow between the beach and the lake. Seepage meters were placed either in the lake bed or in the beach swash zone. Water movement was recorded, and samples were collected for *E. coli* analysis.

#### Source Testing

Additional tests conducted included DNA analysis of gull droppings to determine potential sources of *E. coli* at the beach. Water, sand, and fecal samples were analyzed for *E.*

*coli* and *Salmonella* spp. using rep-PCR and pulsed gel electrophoresis. Isolates were also analyzed for antibiotic resistance of *E. coli* and Enterococci.

An analysis of harbor water and sediment was conducted once to determine potential *E. coli* sources. Samples were collected in Jackson Harbor and analyzed for *E. coli* concentrations.

Finally, water samples from each transect and the lagoon were tested for wastewater compounds in order to determine potential sources of *E. coli*. The water was analyzed for chemicals that would indicate human influence.

#### Modeling

Weather data were collected throughout the study at a weather station located on Casino Pier. Data collected included wind speed and direction, barometric pressure, temperature, rainfall, and solar radiation.

Ambient water conditions were tested simultaneously throughout the sampling period using a multiprobe water quality monitoring instrument. Temperature, pH, conductivity, dissolved oxygen, turbidity, chlorophyll *a*, nitrate, and ammonium were measured every fifteen minutes at a remote platform located in 1.25-m-deep water. Using these data and *E. coli* results from water and sediment sampling, models were developed to predict elevated *E. coli* concentrations at the beach.

## *Results*

#### Daily Sampling

*E. coli* concentrations in water samples at both depths and times collected were correlated with each other, and similarly, *E. coli* in sand samples at foreshore and submerged sites were correlated. Comparing the two water depths, *E. coli* concentrations were lower in the deeper water (90 cm) than in the shallow water (45 cm), and counts in the offshore water (off the pier) were lower than both shallow (45 cm) and deep (90 cm) water. *E. coli* concentrations were higher in morning water samples than in afternoon samples. Overall, *E. coli* concentrations were considerably higher in the sand samples than in the water samples. *E. coli* concentrations were highest in sand samples collected near the highest density of seagulls on the beach. Time of day and location of collection are clearly important considerations for beach monitoring with the amount of variation found in this study.

#### Replication and Hourly Sampling

Results of replicate sampling indicate that a single sample is not sufficient for accurately estimating *E. coli* concentration in the water—the technique used by most *E. coli* monitoring programs.

Hourly sampling results indicate a dramatic decrease in *E. coli* concentration over the course of the day. *E. coli* concentrations exceeding the safe limit in the morning typically dropped off to concentrations below the safe limit in the afternoon. On days when samples were collected twice, afternoon samples were significantly lower than morning samples. The samples collected in 90 cm of water showed a smoother decrease over the course of the day than water collected from 45 cm depth. Samples collected on the ten instances of hourly sampling extended this result. *E. coli* concentrations decreased exponentially between 8:00 and 15:00.

**Sunlight**

Light readings clearly supported the hourly sampling results. Over the course of the day on September 18 and 25, both visible and UV increased between 7:30 and 13:00 and then appeared to fall off slightly. Submerged probes indicated that water severely impeded UV penetration, more so than visible light. Results of the light/dark bag experiments supported the hourly sampling *E. coli* results. *E. coli* concentrations decreased throughout the day in the bags exposed to sunlight and in ambient lake water while concentrations in bags shielded from light decreased only slightly through the day.

**Groundwater Testing**

Groundwater studies indicated that the general movement of water was downward into the sand except for the swash zone, where the gradient was directed from the sand to the lake. Seepage flux was always limited, but the *E. coli* concentrations in seepage water were highly variable.

**Source Testing**

Bird density and location was also compared with *E. coli* concentration to examine any correlations. In the morning, gull numbers increased from May to peak in July and then began to decrease. In the afternoon, gull numbers increased consistently from May to September. During both time periods, gulls typically occupied the north end of the beach. Lagged bird counts were correlated with sand and water. Number of bathers was not correlated with *E. coli* concentrations in the water.

Jackson Harbor sediments and water apparently were not important sources of *E. coli* to the beach because concentrations were relatively low. Lagoon water concentrations were also low. Seagulls are a source of *E. coli*, but other sources are possible. Fingerprinting of seagull DNA isolates indicated that *E. coli* and Enterococci at the beach were partly derived from the resident seagull population. DNA analysis of *Salmonella* spp. indicated a relatively close match between gull droppings, water, and sand samples, but some *Salmonella* spp. could have been transferred from other birds. *E. coli* and *Salmonella* were both highly susceptible to antibiotics, indicating a non-human source. These results were supported by the chemical analysis of water samples. Although numerous anthropogenic biochemicals were present, they are likely derived from storm and wastewater rather than sewage.

**Modeling**

Efforts to model the occurrence and prevalence of *E. coli* met with some success. There were correlations between elevated *E. coli* levels and storm events and the associated high winds and waves. There was no single factor that could be used to predict accurately the concentration of *E. coli*. The best predictors overall were rainfall, wave height, wind speed (northern component only), air temperature and solar radiation, lake stage (level), water turbidity, and chlorophyll *a* concentration of the lake water.

## *Conclusions*

The goal of this study was to determine the potential sources and distribution of *E. coli* at the 63rd Street Beach. Because of the unique structure of the beach, it is likely that *E. coli* may be moving south into the area of 63rd Street beach, where it becomes trapped due to shallow depths and the presence of a large pier. With such a scenario, *E. coli* levels could originate from any number of sources along the Chicago lakefront. Although all sources were not identified in

the course of this study, it was determined that seagulls and sand *E. coli* are among the largest contributors.

In order to protect beach visitors from high *E. coli* levels, sources ultimately need to be determined and eliminated. Because the problem still persists, personnel must continue to monitor the beaches for excessive concentrations of *E. coli*. Although the Chicago Park District's monitoring plan far exceeds national standards for testing, statistical analysis shows that ten replicate samples are needed to get a reliable indication of the E. coli concentration. Predictive models that were developed over the course of this study may alleviate shortfalls in sampling precision and timely reporting. Using ambient conditions, a model was developed that can predict excessive *E. coli* contamination most of the time. We suggest a more comprehensive validation and calibration of this model in 2001.

The complexity of the 63rd Street Beach system and the interacting factors associated with a beach in a metropolitan area make source determinations difficult. The results of this study illuminate some of the factors involved and eliminate others. With more information about other beaches and influences along the Lake Michigan shoreline, *E. coli* levels may eventually be minimized.

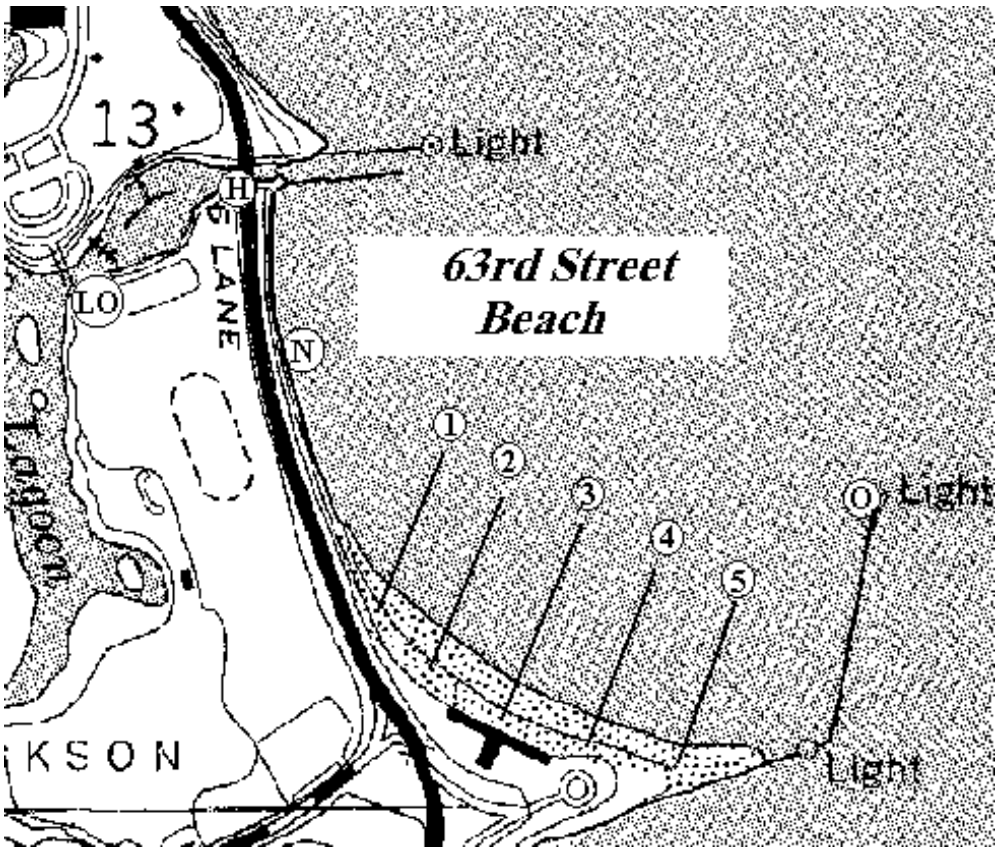



**Figure 1. Sampling locations at 63rd Street Beach: transects 1-5, lagoon outflow (LO), harbor (H), north revetment (N), offshore (O).**

## METHODS

### *Study Area*

The study area was located on the southwest shore of Lake Michigan in Cook County, Illinois, at 63rd Street Beach, Chicago. This public swimming beach is on the south side of Chicago, approximately 8 miles from the downtown area. Breakwaters extend into the lake north and south of the beach and partially enclose the beach basin. Stone revetment covers the north half of the basin shoreline. Figure 1 shows the study area and sample sites. Five transects were established 100 m apart perpendicular to the shore (T1-T5). Three sites were set along each transect. One site was located onshore one meter from the furthest extent of the waves. The other two sites were located at approximately 45 cm and 90 cm water depths (as estimated by field technicians). Other sample sites were located at the Jackson Lagoon outflow (lagoon) and Jackson Harbor (harbor) north of the beach, along the north shore revetment, and offshore at the end of the Casino Pier (offshore). The lagoon outflow is a small cascade from the dam at the west end of 59th Street Harbor. It drains a series of lagoons west of the beach that was suspected as a possible *E. coli* contamination source. Harbor samples were obtained at the mouth of 59th

St. Harbor where it connects to Lake Michigan immediately north of 63rd St. Beach. The north revetment site was located in approximately 45 cm water off the center of the stone revetment in the north part of the 63rd St. Beach basin. Offshore samples were obtained approximately 500 m from shore, from the end of Casino Pier.

***Collection of E. coli***. Water samples were taken in the transects by dipping a sterile 500 ml polyethylene bag below the water surface at the 45 cm and 90 cm sites according to the protocol in Nevers and Whitman (2000). Samples were collected in the same way from the north revetment. An ethanol-sterilized bucket attached to a rope was used to obtain lagoon, harbor, and offshore samples.

Sediment samples were taken by pushing a 2.3 cm x 30 cm AMS slotted soil recovery probe with an ethanol-sterilized butyrate liner at least 20 cm into the sediments at the submerged and foreshore sites. Upon extraction any overlying water was decanted and the liner was capped and removed from the probe. One additional sediment sample was collected onshore from a site with the greatest density of gull droppings, as judged by field technician (gull sand). All water and sediment samples were immediately placed on ice in a cooler and transported to the Jardine Filtration Plant's microbiology laboratory for analysis of *E. coli* concentration.

***Field Observations***. Field crew collected information on wind speed and direction, air and water temperature, wave height at 45 cm, and general weather observations. Distance to the water's edge was measured from two fixed points, one each on the north and south ends of the beach. Bathers were counted in a 5x5 m area around the 45 cm and 90 cm sampling sites. *Larus* spp., mostly Ring-bill gulls, were counted in a 100-m-wide swath centered on each transect. Onshore gull droppings were counted at each transect in 3 randomly placed 1x1m quadrats 1-6 m from the furthest extent of the waves (15 quadrats total).

***Sampling Schedule***. Samples were collected three days per week, generally from Monday through Wednesday or Tuesday through Thursday, from April through September 2000 (Figure 2). During April, sampling took place at 08:00 each day. Sampling occurred twice each day during May, at 08:00 and 13:00. From June through September sampling occurred at 07:00 and 13:00 each day. In the afternoon, water samples were only collected from the transect sites and no sediment samples were collected. Gull droppings were not counted in the afternoon. All other sampling and measuring procedures remained the same for afternoon sampling.

Replicate water sampling to measure sample variability was conducted on ten randomly chosen days. The only modification to the original sampling procedure was that every morning and afternoon water sample was collected in duplicate, except for the 90 cm site on transect 3. This site was sampled 10 times.

Hourly water sampling to measure temporal variability was conducted on another ten randomly chosen days. Samples were taken hourly at all transect sites from 07:00 through 15:00. Wave heights were measured hourly; all other measurements and samples were collected according to the usual sampling schedule.

**April**

| Sun | Mon | Tue | Wed | Thu | Fri | Sat |
|---|---|---|---|---|---|---|
| | | | | | | 1 |
| 2 | 3 | 4 - | 5 - | 6 - | 7 | 8 |
| 9 | 10 | 11 - | 12 - | 13 - | 14 | 15 |
| 16 | 17 | 18 - | 19 - | 20 - | 21 | 22 |
| 23 | 24 | 25 - | 26 - | 27 - | 28 | 29 |
| 30 | | | | | | |

**May**

| Sun | Mon | Tue | Wed | Thu | Fri | Sat |
|---|---|---|---|---|---|---|
| | 1 | 2 - | 3 - | 4 - | 5 | 6 |
| 7 | 8 | 9 - | 10 - | 11 - | 12 | 13 |
| 14 | 15 | 16 - | 17 - | 18 R | 19 | 20 |
| 21 | 22 | 23 | 24 - | 25 H | 26 - | 27 |
| 28 | 29 | 30 - | 31 - | | | |

**June**

| Sun | Mon | Tue | Wed | Thu | Fri | Sat |
|---|---|---|---|---|---|---|
| | | | | 1 R | 2 | 3 |
| 4 | 5 - | 6 R | 7 - | 8 | 9 | 10 |
| 11 | 12 H | 13 - | 14 - | 15 | 16 | 17 |
| 18 | 19 - | 20 - | 21 R | 22 | 23 | 24 |
| 25 | 26 H | 27 - | 28 - | 29 | 30 | |

**July**

| Sun | Mon | Tue | Wed | Thu | Fri | Sat |
|---|---|---|---|---|---|---|
| | | | | | | 1 |
| 2 | 3 | 4 | 5 R | 6 - | 7 - | 8 |
| 9 | 10 - | 11 H | 12 R | 13 | 14 | 15 |
| 16 | 17 - | 18 - | 19 - | 20 | 21 | 22 |
| 23 | 24 H | 25 R | 26 - | 27 | 28 | 29 |
| 30 | 31 - | | | | | |

**August**

| Sun | Mon | Tue | Wed | Thu | Fri | Sat |
|---|---|---|---|---|---|---|
| | | 1 H | 2 - | 3 | 4 | 5 |
| 6 | 7 H | 8 R | 9 - | 10 | 11 | 12 |
| 13 | 14 - | 15 - | 16 H | 17 | 18 | 19 |
| 20 | 21 - | 22 - | 23 R | 24 | 25 | 26 |
| 27 | 28 - | 29 - | 30 - | 31 | | |

**September**

| Sun | Mon | Tue | Wed | Thu | Fri | Sat |
|---|---|---|---|---|---|---|
| | | | | | 1 | 2 |
| 3 | 4 | 5 - | 6 - | 7 - | 8 | 9 |
| 10 | 11 R | 12 - | 13 - | 14 | 15 | 16 |
| 17 | 18 H | 19 - | 20 - | 21 | 22 | 23 |
| 24 | 25 H | 26 - | 27 - | 28 | 29 | 30 |

**Figure 2. Sampling schedule: "-" = regular sampling day, "R"= replicate sampling day, "H"= hourly sampling day.**

***Light/Dark Bag Experiment***. This experiment was conducted to measure the effect of light on *E. coli* in lake water. It took place at 63rd St. Beach and occurred simultaneously with the 9/18/00 hourly sampling. An ethanol-sterilized 20 l carboy was filled with water from 45 cm. From this sample 80 sterile polyethylene bags were filled with 175 ml sub-samples. Half of the bags were covered with opaque silver tape and half remained transparent. Prior experimentation indicated that the transparent polyethylene blocked less than 1% of photosynthetic photon flux (PPF) and ultra violet radiation (UV). Onset StowAway TidbiT Temp Logger temperature sensors were placed in two additional filled sub-sample bags (one each clear and taped). Ten blanks were prepared with 175 ml of sterile buffered water (5 bags taped, 5 clear).

All bags were randomly distributed 20 cm apart on a wire suspended in 45 cm water approximately 15 cm above the sediment surface. Every hour from 08:00 to 15:00 5 taped and 5 clear sub-sample bags were retrieved. These were transported to the laboratory for *E. coli*

analysis with the normal hourly samples. At 15:00 the blanks and temperature sensors were retrieved along with the final sub-sample bags. Blanks were analyzed for *E. coli*.

Readings for PPF and UV were recorded at 5-minute intervals during the experiment using an Apogee Micrologger datalogger and two each of Apogee models QSO and UVS light meters attached to a rebar post. One set of meters was positioned above the water and the other in 45 cm water approximately 20 cm above the sediment surface.

***Additional Harbor Testing***. Additional sampling was conducted at 59$^{th}$ St. Harbor to assess the presence and/or magnitude of *E. coli* storage in the harbor sediments. Sampling took place on 19 July. Water and sediment samples were collected from a total of three points located at the ends of the three easternmost floating piers in the harbor.

The water samples were collected first to avoid contamination from sediments. Water samples were obtained using a plexiglass Wildco vertical Kemmerer water sampler. The sampler was rinsed between samples with 95% ethanol followed by distilled water. Two water samples were taken at each point. The first was taken from just below the water surface; the second was taken from just above the sediment surface. A small amount of water was released to clear the sampler spout, and then 500 ml of sample was collected in a sterile polyethylene bag and placed on ice.

Sediment samples were collected using a Wildco petite Ponar. This sampler was also rinsed between samples with 95% ethanol followed by distilled water. One sediment sample was taken at each point and emptied into a sterilized bucket. The sampler retained a large amount of water, so each sample consisted of approximately 50% sediment and 50% overlying water. This was swirled vigorously for 30 seconds and then 500 ml of the overlying water was emptied into a sterile polyethylene bag and placed on ice.

All samples were analyzed for *E. coli* concentration according to EPA/600/4-85 076 (USEPA, 1985) as described previously (0.1, 1.0, and 4.0 ml dilutions for sediment, 1.0, 10.0, and 50.0 ml dilutions for water). The only exception was that no urea substrate was used prior to counting. Sample analysis took place at the Lake Michigan Ecological Research Station instead of at the Jardine Water Plant.

***Ambient Water Conditions***. Physical and chemical parameters were measured from June through September at a platform in 1.25 m water in the northern portion of the basin. Parameters measured include temperature, pH, oxidation-reduction potential, conductivity, dissolved oxygen, turbidity, chlorophyll *a*, nitrate, and ammonium. A YSI sonde, model 6600, powered by marine battery took these readings every 15 minutes.

The YSI platform was constructed of metal plates and grating attached to an oil drum. Cement blocks and steel cables stabilized the platform. The sonde was suspended in the water column from a post in the center of the platform. Once during the study, the platform was repositioned because it was sinking. By September, submerged parts of the platform were covered with algal growth.

The YSI sonde was initially deployed on June 7. It was removed approximately every two weeks, or when a potential problem developed, for cleaning, calibration, and data transfer. These procedures were performed on site, and the sonde was re-programmed and immediately returned to the platform. The wipers on the turbidity and chlorophyll probes were replaced once during the season.

***Test of E. coli Concentration***. Water samples were tested for *E. coli* according to EPA/600/4-85 076 (USEPA, 1985), with the exception that buffered dilution water was prepared according to APHA, 9050 C (1998). Briefly, samples were tested at three dilutions (generally 1, 10, and 50 ml) by membrane filtration onto mTEC agar (Acumedia, Baltimore, MD, or Difco Laboratories, Detroit, MI). Yellow colonies were confirmed as *E. coli* by transferring membranes to urea substrate following incubation. Counts were taken and results calculated from plates with 20 to 80 colonies; otherwise results were estimated from the plate closest to that range. Results were calculated and reported as colony forming units (CFU) per 100 ml.

Sediment samples required extra preparation prior to *E. coli* analysis. Total sample volume was calculated from measurement of sediment height in the core liner (nearest 1 cm). The liner was then emptied and contents rinsed into a sterile 250 ml polypropylene bottle using 100 ml of sterile buffered dilution water. All sample bottles were simultaneously shaken for 5 minutes at 210 rpm on an Eberbach platform shaker. The supernatant liquid was allowed to settle for a few minutes before sample volumes were removed by pipette. Testing was conducted according to EPA/600/4-85 076 (USEPA, 1985) as summarized above (dilutions of 0.1, 1.0, and 4.0 ml).

***Groundwater Studies***. Seepage meters and mini-piezometers were installed along the five study transects on August 21-22 and September 7-8, 2000 in an effort to evaluate transfers of water that were taking place between the lake, the lake bed sediments, and the foreshore. The mini-piezometers and seepage meters followed the design of Lee and Cherry (1978). Mini-piezometers were placed 34 cm below the sand surface where the lake water was 0.5 m deep. Mini-piezometers were also placed 40 cm below the crest of the berm on the foreshore at each transect. On our first visit (August 21-22), we placed four of the seepage meters in the lake bed below 0.5 meters of water and one in the swash zone at the base of the berm. On our second visit (September 7-8), we placed all of the seepage meters in the swash zone at the base of the berm. On August 21-22, samples were collected from the mini-piezometers and the seepage meters using a vacuum pump that purged each apparatus thoroughly before filling a whirl-pac sample bag for eventual analysis of *E. coli* concentrations. On September 7-8, the vacuum pump again was used to extract the samples from the mini-piezometers, but the seepage meters were allowed to collect samples of water passively. After collection of all seepage meter samples for laboratory analysis, an additional sample was collected from one of the seepage meters using the vacuum pump. This was done to compare samples collected using the two methods. The seepage meters were installed for a third time on September 12-13 in an effort to collect samples of seepage water for *E. coli* concentrations in association with a storm. On that occasion, no mini-piezometers were installed and only 2 of the seepage meters yielded any samples.

***DNA Fingerprinting of E. coli***. Water (45 cm, lagoon, harbor, north and south breakwaters) and sediment (onshore) samples were split from the normal daily samples and collected for DNA analysis on June 26 and August 21. In addition, gull droppings were swabbed for analysis. Coliform bacteria were quantified and *E. coli* isolates were obtained and confirmed following procedures 9222 B, 9222 G, and 9225 D outlined in APHA (1998). Total coliform bacteria were quantified using membrane filtration on mENDO agar medium (Hach Company, Loveland, CO or Difco Laboratories, Detroit, MI) at two dilutions. A blank sample was processed for each environmental sample. Representative positive coliform colonies from filters with optimum counts (20-80 colonies) were transferred to Nutrient Agar (Difco) containing 4-

methylumbelliferyl-ß-D-glucuronide (NA-MUG medium). All blue fluorescent colonies were tentatively identified as *E. coli*. These were confirmed using 3 physiologic tests [cytochrome oxidase, ß-D-galactosidase (ONPG) and indole tests] as well as continued fluorescence on NA-MUG. About 10% were additionally confirmed using multiple physiologic assay test strips (Enterotubes: BBL Becton Dickson or API20E: bioMérieux, Hazelwood, MO).

DNA fingerprints of confirmed *E. coli* isolates were characterized by rep-PCR profiling. Rep-PCR procedures were slightly revised from those described by Versalović et al., 1991. Primers used were REP 1R and REP 2I (Genosys Biotechnologies, The Woodlands, TX) and these were diluted in TE (10 mM Tris, pH 8.0, 1 mM EDTA). The rep-PCR reaction components consisted of: 1 X PCR reaction buffer (100 mM Tris-HCl pH 8.5, 500 mM KCl) (Gibco BRL, Gaithersburg, NY), 3.3 mM $MgCl_2$, 125 $\mu$M of each dNTP (Pharmacia, Piscataway, NJ), 0.25 $\mu$g BSA (Boehringer Mannheim, Indianapolis, IN), 10% DMSO, 2 nM of each primer, 2U Taq DNA Polymerase (Gibco BRL), 1$\mu$l of a 1:10 diluted *E. coli* culture (18-24 hr culture in LB broth), and sterile tissue culture water to bring the volume up to 25 $\mu$l. Cultures used for the PCR were streaked onto EMB (Difco Laboratories, Detroit, MI) and TSA with 5% sheep blood (BBL Becton Dickinson) to be sure the culture was pure. Reactions were carried out in a Perkin Elmer 2400 Gene Amp PCR system (Perkin Elmer-Cetus, Norwalk, CT) with the following conditions: 95° for 7 min; 34 cycles of: 94°C for 3 sec, 92°C for 30 sec, 40°C for 1 min, 65°C for 8 min; a final elongation of 16 min at 65°C; and a final hold at 4°C. PCR products (7 $\mu$l) were electrophoresed on a 2% agarose gel for 100 min at 75V in a Wide Mini-Sub Cell GT system (Bio-Rad Laboratories, Hercules, CA). Gels were visualized by ethidium bromide staining. Isolate DNA banding patterns were grouped based on similarity (UPGMA based on Pearson product moment correlation with global (2.85%) or fine (3.25%) optimization) using GelCompar version 4.0 (Applied Maths, Kortrijk, Belgium). An *E. coli* control sample was run in quadruplicate on one gel and as an internal standard on each subsequent gel to establish the similarity level at which identities can be determined for the library of all isolates.

***Antibiotic Resistance Testing of E. coli***. *Escherichia coli* isolates were grown and maintained on 5% sheep blood agar (Becton Dickinson, Cockeysville, MD) for short duration studies. Stock cultures of each isolate were prepared in TSB with 50% glycerol (NWHC) and maintained at approximately -70 °C for long-term storage. Overnight growth of isolates was identified and antimicrobial susceptibilities determined using the GNI+ and GNS-207 test systems, respectively, of the Vitek (bioMerieux, St. Louis, Missouri, USA) according to manufacturer's instructions.

Antimicrobial minimum inhibitory concentrations (MIC) were determined for 17 antimicrobial agents (Table 1). MIC data was translated into binary data for analysis where breakpoint MIC data was converted into 0 for susceptible and 1 for intermediate or resistant reactions to the antimicrobial agent based on NCCLS M2-A7 Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard —Seventh Edition interpretive data (National Committee for Clinical Laboratory Standards, Inc., 940 West Valley Road, Suite 1400 Wayne, PA 19087-1898). Antimicrobial susceptibilities (antibiotic resistance) similarity patterns were determined by cluster analysis using UPGMA (unweighted pair group methods arithmetic averages) using simple matching of binary data (BioNumerics, Applied Maths, Kortrijk, Belgium).

**Table 1. Antimicrobial Agents Tested**

Amikacin
Amoxicillin
Ampicillin
Carbenicillin
Ceftazidime
Ceftiofur
Cephalothin
Chloramphenicol
Ciprofloxacin
Enrofloxacin
Gentamicin
Nitrofurantoin
Piperacillin
Tetracycline
Ticarcillin
Tobramycin
Trimethoprim-sulfamethoxasol

***Antibiotic Resistance Testing of Enterococci***. Enterococci were quantified using membrane filtration on mEI agar as described in EPA/821/R-97/004 (USEPA, 2000). Enterococci with representative morphologies were isolated and confirmed using multiple physiologic assays (Rapid ID 32Strep, bioMérieux). Selected enterococci isolates from seagulls, sediments and water were tested for resistance to the antibiotics vancomycin, gentamycin, ampicillin, tetracycline, and streptomycin using the Etest® (AB Biodisk, Piscataway, NJ).

***DNA fingerprinting*** *of Salmonella*. Samples analyzed included water, sediment, and fecal material. Portions of the samples were initially enriched using Rappaport-Vassiliadis Medium (RV) and Dulcitol-Selenite Broth (DS) (NWHC, Madison, WI) incubated at 42±0.5 EC for 16 to 18 hours. For the water samples, approximately 500 ml was added to double concentrated enrichment broths. For the sediment samples, approximately 1 ml of the overlying water from a well-mixed sample was added to each enrichment broth. After incubation, a portion of each enrichment was transferred to XLT4 Agar (Difco Laboratories, Detroit MI) and Brilliant Green Agar (Becton Dickinson, Cockeysville, MD). Both media were incubated at 35-37$^{o}$C for 18 to 24 hours. Passage of each enrichment broth into a second enrichment broth set was occasionally done to enhance recovery from samples that failed to yield suspect *Salmonella* isolates on first passage. All bacterial colonies were screened to identify *Salmonella* spp., and those matching morphological and biochemical characters were subcultured on 5% sheep blood agar (Becton Dickinson, Cockeysville, MD). Suspected *Salmonella* isolates were biochemically characterized by either the API-20E or Vitek systems (bioMerieux, St. Louis, MO). Isolates yielding *Salmonella* identification were screened using a polyvalent antisera for *Salmonella* (Becton Dickinson, Cockeysville, MD) before being serotyped for confirmation at USDA National Veterinary Services Laboratory (Ames, IA).

Pulsed field gel electrophoresis (PFGE) of the *Salmonella* isolates was done essentially as previously described (Thong *et al.* 1994, Olsen *et al.* 1997). Briefly, an overnight growth of each *Salmonella* isolate was lysed in InCert agar (BioWhittaker Molecular Applications, Rockland, ME) before being digested with *Xba*I enzyme (Promega, Madison, WI) for 1.5 to 2 hours. The digested plugs were loaded into a SeaKem Gold (BioWhittaker Molecular Applications, Rockland, ME) gel which was electrophoresed for 18 hours at 6 volts with an initial switch time of 2.2 seconds and a final switch time of 63.8 seconds in 0.5X TBE running buffer using a CHEF-DR II system (BioRad, Hercules, CA). The gel was then stained with either ethidium bromide for 30 minutes before being visualized on a Foto/Analyst Investigator system (Fotodyne, Hartland, WI) or with Vistra Green (Amersham Pharmacia Biotech, Piscataway, NJ) for 30 minutes before being visualized on a Flourimager (Molecular Dynamics, Sunnyvale, CA). The electronic images of the gels were analyzed using Dice coefficient on band patterns with an optimization setting of 3% and a position tolerance of 2% (BioNumerics, Applied Maths, Kortrijk, Belgium).

***Antibiotic resistance testing of*** Salmonella. Antimicrobial minimum inhibitory concentrations (MIC) were determined for 17 antimicrobial agents (Table 1). MIC data was translated into binary data for analysis where breakpoint MIC data was converted into 0 for susceptible and 1 for intermediate or resistant reactions to the antimicrobial agent based on NCCLS M2-A7 Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard — Seventh Edition, interpretive data (National Committee for Clinical Laboratory Standards, Inc., 940 West Valley Road, Suite 1400 Wayne, PA 19087-1898). Antimicrobial susceptibilities (antibiotic resistance) similarity patterns were determined by cluster analysis using UPGMA (unweighted pair group methods arithmetic averages) using simple matching of binary data (BioNumerics, Applied Maths, Kortrijk, Belgium).

***Phenotypic analysis of*** Salmonella ***and*** E. coli. Phenotypic data was translated into binary data for analysis based on the presence (1) or absence (0) of the characteristic. The phenotypic characteristics for 30 attributes (Table 2) were then analyzed by cluster analysis using UPGMA (unweighted pair group methods arithmetic averages) based on Pearson product moment correlation (BioNumerics, Applied Maths, Kortrijk, Belgium).

**Table 2 Phenotypic characteristics**

| | |
|---|---|
| DP-300 fermentation | DP3 |
| Glucose, oxidative utilization | OFG |
| Growth control | GC |
| Acetamide utilization | ACE |
| Esculin hydrolysis | ESC |
| Plant indican reaction | PLI |
| Urea utilization | URE |
| Citrate utilization | CIT |
| Malonate utilization | MAL |
| Tryptophan deaminase | TDA |
| Polymixin B growth | PXB |
| Lactose oxidation | LAC |
| Maltose oxidation | MLT |
| Mannitol oxidation | MAN |
| Xylose oxidation | XYL |
| Raffinose utilization | RAF |
| Sorbitol utilization | SOR |
| Sucrose utilization | SUC |
| Inositol utilization | INO |
| Adonitol utilization | ADO |
| p-Coumaric fermentation | COU |
| Hydrogen sulfide production | H2S |
| Ortho-nitophenol galactopyranoside hydrolysis | ONP |
| Rhamnose utilization | RHA |
| L-Arabinose utilization | ARA |
| Glucose fermentation | GLU |
| Arginine dihydrolation | ARG |
| Lysine decarboxylation | LYS |
| Decarboxylation control | NC |
| Ornithine decarboxylation | ORN |

***Chemical Tests for Wastewater Compounds***. Chemical testing was conducted at the lagoon and all five 45 cm transect sites. These locations were analyzed for wastewater chemicals (solvents, pesticides, detergent by-products, cholesterol, caffeine and cleaning agents) that can only result from human influence and may indicate the potential for presence of hormones, pharmaceuticals and other emerging contaminants. These constituents were analyzed by the US Geological Survey's National Water Quality Laboratory in Arvada, CO using gas chromatography mass spectral analysis (GCMS) in the selective ion mode on 1 L samples extracted with methylene chloride (Seiler et al. 1999). This method provides estimates of the concentrations of approximately 40 compounds with detection limits in the range of 10 ng/L to 1 μg/L.

## RESULTS AND DISCUSSION

### *Statistical Data Distribution*.

Normal data distribution is a requirement for many of the statistical analyses we desired to perform. To describe the general distribution of the data, we first separated the data by medium type (i.e., water and sediment), by depth (i.e., foreshore and submerged sand, and 45 and 90 cm water) and then further divided the water into morning and afternoon. Each data set was then checked for outliers using the Stem and Leaf Plot procedure (Wilkinson 1999). The major outliers were removed from the data, and data were plotted on a normal probability plot and checked for normal distribution using the Kolmogorov-Smirnov Lilliefors test. Analyses were run first on raw data, and then on $\log_{10}$-transformed data.

Abundance of *E. coli* in morning water at 45 cm deep fit the normal distribution once it was $\log_{10}$ transformed (Lilliefors p = 0.243). Likewise, the afternoon water dataset at this depth also best fit the normal distribution after $\log_{10}$ transformation (Lilliefors p = 0.038). The morning data at the 90 cm site was not adequately described by typical transformations, although the best probability was achieved after $\log_{10}$ transformation (Lilliefors p = 0.008). The afternoon data fit the distribution well after $\log_{10}$ transformation (Lilliefors p = 0.242). Both of the sand data sets were normalized after $\log_{10}$ transformation (Lilliefors p = 0.096 for foreshore, Lilliefors p = 0.515 for submerged). When water or sand data was partitioned by wave conditions above or below the seasonal log transformed mean normality was notably improved.

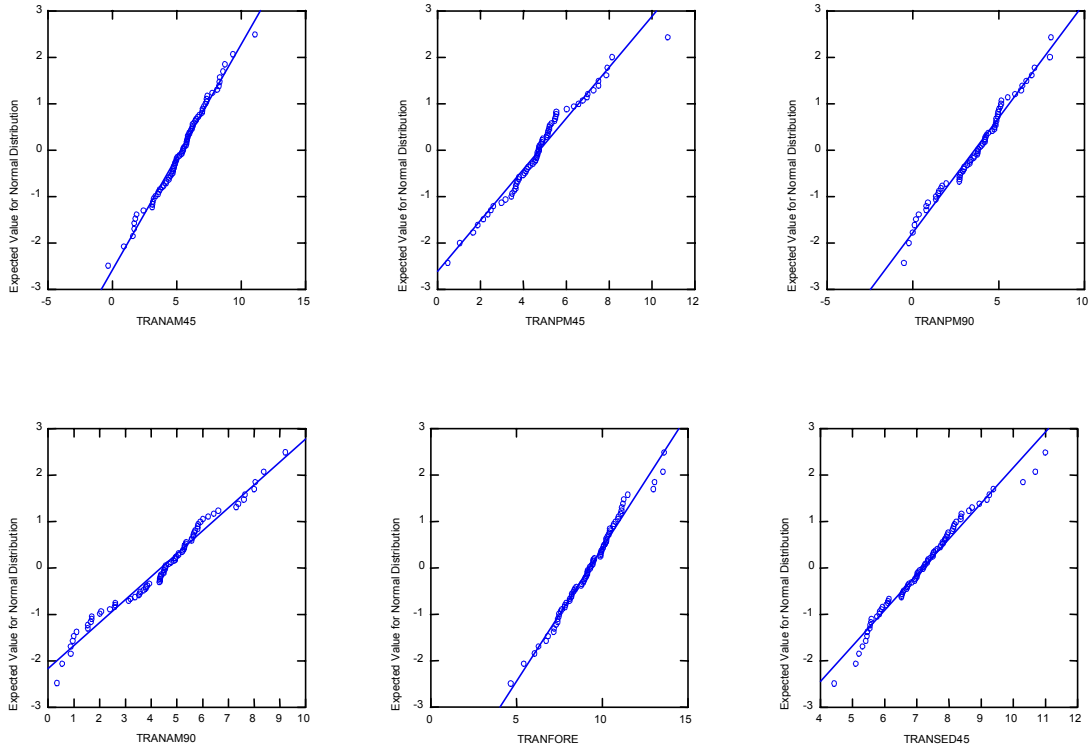



**Figure 3. Normal probability plots for log10-transformed E. coli abundances from water (am, pm and 45, 90 cm deep) and sediments (foreshore and submerged).**

***Sands***

Wilcoxon Signed-Rank Test was used to test for *E. coli* concentration differences in foreshore and submerged sands among transects. The Wilcoxon Signed-Rank Test was used because it is a distribution-free test and does not depend on normally distributed data. Levene test of similarity of variance on log-transformed data indicated that foreshore sands had equal variances ($p = 0.296$) while submerged sands had significantly different variances ($p=0.033$). The gull sand (defined as a sediment samples taken near the physical center of the flock) was added to the data set when analyzing the foreshore transects. $P_{critical}$ was Bonferroni-adjusted to compensate for the increase in procedure-wise error rate due to the multiple comparisons (Sokal and Rohlf 1981).

Repeated-measures ANOVA (rmANOVA) was used to compare *E. coli* abundances in sands between the two depths (i.e., foreshore versus submerged) because the sampling dates were close enough together in time that we felt abundances may not be completely independent among dates. Because of the limitations of the computer and because sampling methods changed between April and May, only the dates from May to September could be run. The data needed to be $\log_{10}$ transformed to achieve normality, but transformation did not completely normalize the data. However, in no cases did the violations exceed 10% of the cells, and because ANOVAs are generally robust to small violations to normality (Underwood 1981), we believe the results of the tests to be valid. *E. coli* abundance was significantly higher in the foreshore sand than in the submerged sand ($F_{65,520} = 1.683$, Huynh-Feldt $p = 0.011$). Paired t-test on log-transformed data confirms that foreshore sands were significantly higher than submerged sands in *E. coli* concentration ($p < 0.001$).

***Water***

Wilcoxon Signed-Rank Test was used to test for differences in water *E. coli* abundance (45 cm and 90 cm deep) among transects (i.e., spatial differences). This test was used because it is a distribution-free test and is not affected when data is not normally distributed. This test compares paired data, and the abundance for each transect/depth couple was paired by date. No transformations were needed for this test (Wilkinson 1999).

No significant differences were detected among transects for *E. coli* abundance from water samples (Table 3).

**Table 3. Two-sided p values from comparisons of E. coli abundances from water samples in morning at 45 cm, morning at 90 cm, afternoon at 45 cm, and afternoon at 90 cm. P values were calculated from Z numbers of the Wilcoxon Signed-Rank Test. Pcritical = 0.005**

| 45 cm AM | Transect1 | Transect2 | Transect3 | Transect4 |
|---|---|---|---|---|
| Transect2 | 0.749 | | | |
| Transect3 | 0.455 | 0.829 | | |
| Transect4 | 0.766 | 0.935 | 0.507 | |
| Transect5 | 0.527 | 0.891 | 0.342 | 0.526 |
| | | | | |
| 90 cm AM | Transect1 | Transect2 | Transect3 | Transect4 |
| Transect2 | 0.577 | | | |
| Transect3 | 0.869 | 0.378 | | |
| Transect4 | 0.538 | 0.121 | 0.524 | |
| Transect5 | 0.886 | 0.819 | 0.779 | 0.852 |
| | | | | |
| 45 cm PM | Transect1 | Transect2 | Transect3 | Transect4 |
| Transect2 | 0.176 | | | |
| Transect3 | **0.**028 | 0.132 | | |
| Transect4 | 0.372 | 0.867 | 0.219 | |
| Transect5 | 0.084 | 0.470 | 0.271 | 0.612 |
| | | | | |
| 90 cm PM | Transect1 | Transect2 | Transect3 | Transect4 |
| Transect2 | 0.557 | | | |
| Transect3 | 0.904 | 0.213 | | |
| Transect4 | 0.674 | 0.747 | 0.235 | |
| Transect5 | 0.331 | **0.**030 | 0.192 | **0.**010 |

We used repeated-measures ANOVA (mANOVA) to compare *E. coli* abundances in water between the two depths (i.e., 45 cm versus 90 cm deep) at each time of day (morning and afternoon) because the sampling dates were close enough together in time that we felt abundances may not be completely independent among dates. Because of the limitation of the computer (it could handle only 66 repeating measures) and because sampling methods changed between April and May, only the dates from May to September could be run. The data needed to be $\log_{10}$ transformed to achieve normality. Normality was tested using the one-sample Kolmogorov-Smirnov Lilliefors test on the residuals of the mANOVA (Wilkinson 1999). Simple $\log_{10}$ transformation did not completely normalize the data. However, because only 6% of the cells violated normality, and because ANOVAs are generally robust to small violations to normality (Underwood 1981), we believe the results of the tests to be valid. Morning *E. coli* abundance was significantly higher in 45 cm than in 90 cm morning waters ($F_{65,520} = 3.075$, Huynh-Feldt, $p = 0.001$). Afternoon *E. coli* abundances also differed significantly ($F_{65,520} = 2.577$, Huynh-Feldt $p = 0.005$). Similar mANOVA analyses were used to compare morning and afternoon *E. coli* abundances at each depth. Morning *E. coli* abundances were higher than afternoon at 45 cm ($F_{65,520} = 14.287$, Huynh-Feldt $p < 0.001$) and at 90 cm ($F_{65,520} = 13.885$,

Huynh-Feldt $p < 0.001$). For both morning and afternoon, paired t-test on transformed data shows that *E. coli* concentration in 45 cm water was significantly higher than *E. coli* concentration in 90 cm water, and both concentrations were correlated with one another ($p < 0.001$).

***Water vs. sediments***

Similar mANOVA analyses were performed to test for differences between water and sand *E. coli* abundances. Comparisons included foreshore and submerged sand vs. morning water at both 45 cm and 90 cm deep, foreshore and submerged sand vs. afternoon water at both 45 cm and 90 cm deep. Differences between sand and water *E. coli* abundances, regardless of the sand or water sample, were always highly significant (Table 4), with sand abundances always higher than water abundances.

**Table 4. Repeated-measures ANOVA for sediment and water *E. coli*. P=normal probability, G-G=Greenhouse-Geisser probability, and H-F=Huynh-Feldt probability. Bonferroni-adjusted Pcritical of 0.0125 used for multiple comparisons (maximum 4) of the same data.**

| Comparison | df | F | P | G-G | H-F |
|---|---|---|---|---|---|
| Foreshore-am, 45 cm | 65,520 | 3.799 | < 0.001 | 0.006 | < 0.001 |
| Foreshore-am, 90 cm | 65,520 | 4.960 | < 0.001 | 0.001 | < 0.001 |
| Foreshore-pm, 45 cm | 65,520 | 4.469 | < 0.001 | 0.002 | < 0.001 |
| Foreshore-pm, 90 cm | 65,520 | 5.426 | < 0.001 | 0.001 | < 0.001 |
| Submerged- am, 45 cm | 65,520 | 3.975 | < 0.001 | 0.003 | < 0.001 |
| Submerged- am, 90 cm | 65,520 | 5.067 | < 0.001 | < 0.001 | < 0.001 |
| Submerged- pm, 45 cm | 65,520 | 3.942 | < 0.001 | 0.003 | < 0.001 |
| Submerged- pm, 90 cm | 65,520 | 5.331 | < 0.001 | < 0.001 | < 0.001 |

The implications for future monitoring are great. For one, the time of day has an important effect on *E. coli* abundance. This point is more thoroughly discussed under the hourly samplings and the light/dark bag experiments. Both analyses indicate that abundances are higher in the morning than in the afternoon. Naturally, if a sampling regime were to consider a single sampling time during the day, the earlier sample will be the most conservative with regards to public safety. In addition, the depth of sampling affects the *E. coli* abundance at this beach. This means the physical location of the sampling may be important in future monitoring at this and other beaches.

***Replicate patterns and sampling confidence***

Confidence intervals (CI, 95%) were calculated for the 10-sample site for each replicate day (total of 20 CIs were calculated). For each 10-sample group, 100 samples were chosen randomly with replacement. The percent of samples falling within the 95% CI was calculated. Only 57% of the samples on average would fall within the 95% CI for that day-depth sampling. This suggests that the variation within a date is high and that the interpretation of any single sample has to be tempered. The range of percentages was 75 (min. = 18%, max. = 93%), so it appears that temporal effects are present.

Wilcoxon Signed-Rank Test was used to test for differences in variance among transects (i.e., spatial differences) because a paired test was necessary since no true replicates existed for each transect. This test was used because it is a distribution-free test. This test compares paired data, and the average variance at each transect/depth couple was paired by date. No transformations were needed for this test (Wilkinson 1999). Wilcoxon Signed-Rank Test also was used to test for differences in variance between morning and afternoon for beach water. ANOVA was used to test for differences in variance between sites (harbor, lagoon, north revetment, offshore, 45-cm water, and 90-cm water) for each time of day (morning and afternoon). Harbor, lagoon, north revetment and offshore sites were only sampled in the morning. We did not expect *a priori* effects of date because the dates of sampling were randomly selected and fortuitously were well-spaced in time. Date was added nevertheless to the model as a covariate to determine if date had a significant effect (i.e., temporal differences in variance). Variance for each replicated sample was calculated and standardized to the mean. The third transect data were deleted to maintain a balanced design in the analyses and variance was log +10 transformed (only for the ANOVA) to help normalize the data. When data were separated by depth and time of day, no spatial differences (i.e., among transects) in variance existed in the data (Wilcoxon Signed-Rank Test, all two-sided $p > 0.1$). Variance was higher in the morning beach-water samples than in the afternoon samples (Wilcoxon Signed-Rank Test, two-sided $p = 0.02$). Variance was not significantly different ($F_{5,112} = 1.907$, $p = 0.099$) among sites in the morning. Using date as a covariate suggested that date did not have a significant effect ($F_{1,112} = 1.012$, $p = 0.317$). However, variance was significantly higher in the 45 cm water than in the 90 cm water ($F_{1,77} = 4.560$, $p = 0.036$) in the afternoon, and again date had no significant effect ($F_{1,77} = 0.060$, $p = 0.807$) in the model.

Based on variance from each 10-sample data set, we calculated the number of samples needed to find a value with a confidence limit ± some % of the mean using $n = 10$ and $\alpha = 0.05$. We used the formula from Elliott (1977),

$$n = \frac{t^2 S^2}{d^2 \overline{Y}^2}$$

where $n$ is the number of replicates required, $t$ is the value from the Student's $t$ distribution with n degrees of freedom (here 9), $S^2$ is sample variance calculated from each 3-sample data set, $d$ is the relative error as percent Confidence Limit (CL) of $\overline{Y}$ and $\overline{Y}$ is the sample mean from each 10-sample data set.

**Table 5. Estimated sample sizes required to achieve 95% Confidence Limits ± d % of the mean. Estimates were calculated using Elliott's (1977) equation for small sample size. Numbers in each column are for AM and PM data sets.**

| Date | *d*=20% | *d*=30% | *d*=40% |
|---|---|---|---|
| 18-May | 526, 91 | 234, 41 | 132, 23 |
| 1-June | 103, 11 | 46, 5 | 26, 3 |
| 6-June | 18, 35 | 8, 16 | 5, 8 |
| 21-June | 17, 46 | 8, 21 | 5, 12 |
| 5-July | 25, 50 | 12, 22 | 7, 13 |
| 12-July | 14, 22 | 7, 10 | 4, 6 |
| 25-July | 12, 19 | 5, 9 | 3, 5 |
| 8-August | 6, 3 | 3, 1 | 2, 1 |
| 23-August | 11, 7 | 5, 3 | 3, 2 |
| 11-September | 63, 14 | 28, 6 | 16, 4 |

Taking 10 samples is adequate to achieve a value within a relative error of 30% of the mean most of the time. To achieve a relative error of 20%, an average of 80 samples would have to be taken in the morning or 30 samples in the afternoon. Much of the error is skewed by the May 18 samples, which were extremely variable. If those data are removed as outliers, 30 samples in the morning and 23 samples in the afternoon would be needed to achieve 20% accuracy. Taking a single sample is not recommended, and any replicate number <5 likely will not give a very precise value.

***Diurnal Patterns***

*E. coli* abundance generally declined exponentially throughout the day. Data were log-transformed to allow construction of linear regression models. Separate time-series regression models were fit on the log-transformed abundance for each day and depth (Table 6).

No significant change in *E. coli* abundance was detected in seven cases: 25 May 45 and 90 cm, 24 July 45 cm, 1 August 45 and 90 cm and 25 September 45 and 90 cm. *E. coli* abundance remained relatively constant on these days. In general, change in *E. coli* abundance over time is reasonably well explained by the model (Table 6). Some dates had relatively low R-values, such as 25 May at 90 cm. Spikes caused by a few individual samples were responsible for breaks in the exponential decline (and thus the low R).

**Table 6. Results from time-series regressions of log-transformed mean hourly E. coli data. The model equation is ln(conc) = ß0 + ß1*time, where conc=number of colony forming units per 100 ml, time=time of day in hours, and ßi are parameters to be estimated**

| date | depth (cm) | R | df | $ß_0$ | $ß_1$ | t-ratio $ß_0$ | t-ratio $ß_1$ | p-value $ß_0$ | p-value $ß_1$ |
|---|---|---|---|---|---|---|---|---|---|
| 25-May | 45 | 0.759 | 5 | 6.51 | -0.38 | 3.20 | -2.18 | <.02 | <.10 |
| | 90 | 0.577 | 5 | 1.31 | -0.08 | 2.45 | -1.78 | <.10 | <.20 |
| 12-Jun | 45 | 0.990 | 6 | 12.37 | -0.48 | 32.83 | -14.31 | <.001 | <.001 |
| | 90 | 0.982 | 6 | 11.18 | -0.44 | 28.00 | -11.84 | <.001 | <.001 |
| 26-Jun | 45 | 0.953 | 6 | 5.66 | -0.21 | 19.69 | -8.11 | <.001 | <.001 |
| | 90 | 0.969 | 6 | 6.28 | -0.36 | 13.97 | -8.61 | <.001 | <.001 |
| 11-Jul | 45 | 0.877 | 6 | 9.22 | -0.23 | 11.07 | -3.16 | <.001 | <.05 |
| | 90 | 0.942 | 6 | 9.60 | -0.30 | 10.21 | -3.48 | <.001 | <.02 |
| 24-Jul | 45 | 0.900 | 6 | 4.43 | -0.02 | 5.62 | -0.23 | <.001 | >.50 |
| | 90 | 0.928 | 6 | 6.13 | -0.29 | 12.19 | -6.10 | <.001 | <.001 |
| 1-Aug | 45 | 0.474 | 6 | 4.72 | -0.01 | 13.88 | -0.34 | <.001 | >.50 |
| | 90 | 0.949 | 6 | 4.73 | -0.03 | 12.78 | -1.00 | <.001 | <.50 |
| 7-Aug | 45 | 0.989 | 6 | 12.86 | -0.59 | 37.67 | -19.43 | <.001 | <.001 |
| | 90 | 0.968 | 6 | 10.60 | -0.52 | 20.09 | -10.48 | <.001 | <.001 |
| 16-Aug | 45 | 0.969 | 6 | 10.93 | -0.46 | 17.29 | -8.21 | <.001 | <.001 |
| | 90 | 1.000 | 6 | 9.96 | -0.39 | 101.75 | -42.86 | <.001 | <.001 |
| 18-Sep | 45 | 0.908 | 6 | 7.16 | -0.25 | 11.92 | -4.78 | <.001 | <.005 |
| | 90 | 0.960 | 6 | 6.53 | -0.26 | 10.48 | -4.47 | <.001 | <.005 |
| 25-Sep | 45 | 0.958 | 6 | 4.01 | 0.02 | 18.09 | 0.93 | <.001 | <.50 |
| | 90 | 0.612 | 6 | 4.09 | -0.03 | 8.49 | -0.75 | <.001 | <.50 |

When log-transformed data were plotted against time, regression lines clearly showed that on most days *E. coli* abundance declined over time (Figures 4 and 5). On four dates (12 June, 11 July, 7 August, and 16 August) *E. coli* abundance was between 1000 and 7000 cfu/100 ml at 7:00, well over the EPA limit of 235 cfu/100ml. These levels declined rapidly on all four days so that at 15:00 *E. coli* abundance was near or below 235 cfu/100 ml. On all other days *E. coli* abundances were moderate at 7:00, falling between 50 and 235 cfu/100 ml (with the exception of very low readings between 0-10 cfu/100 ml for 25 May 90 cm). In about half of these cases, *E. coli* declined from this level over the course of the day. *E. coli* abundance appeared to remain steady for other days. Rates of decline (slope) were usually similar at 45 and 90 cm for each day, as shown by Figures 4 and 5, and the slope ($ß_1$) values in Table 6.

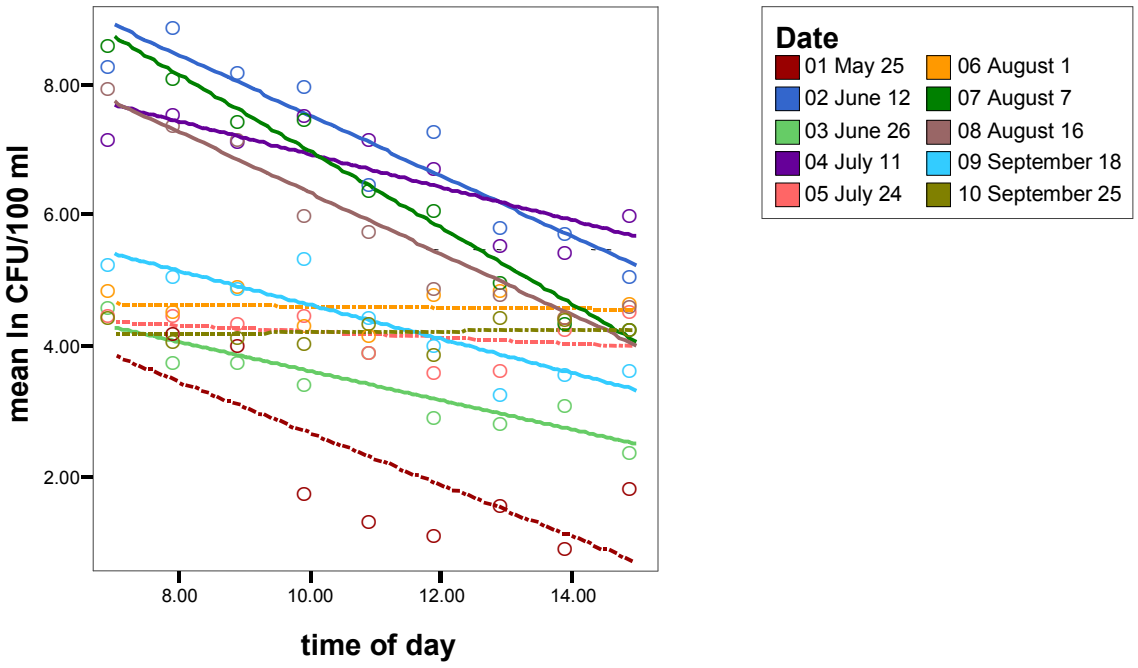



**Figure 4. Mean log-transformed hourly *E. coli* data at 45 cm from 10 different sampling days. Solid lines correspond to time-series regressions with $p < 0.05$ for slope and intercept.**

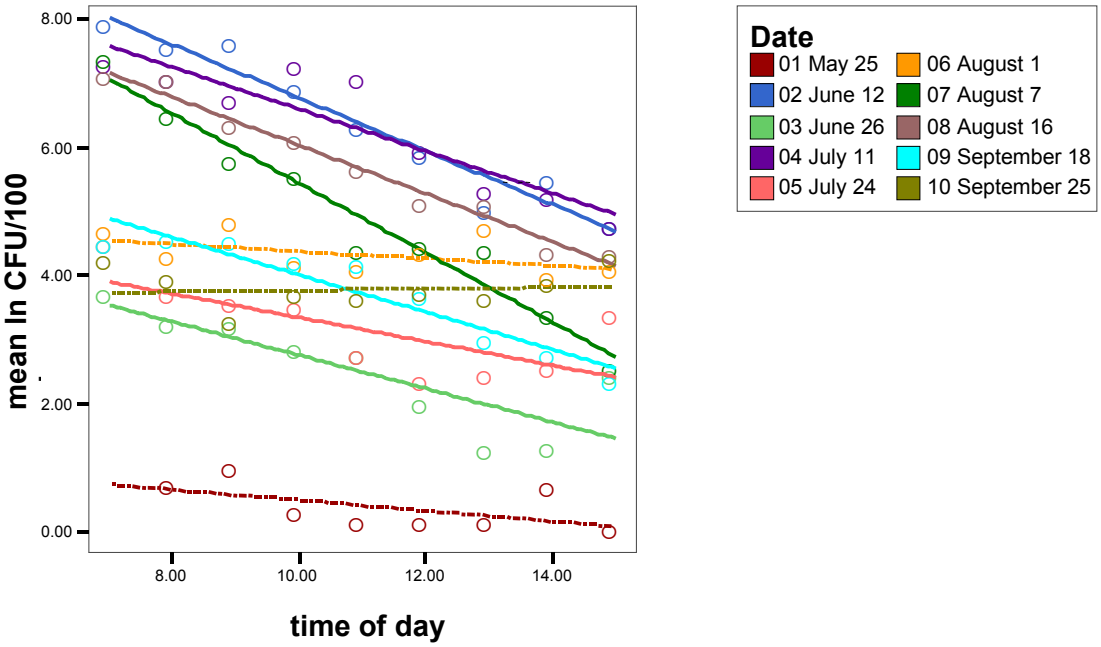



**Figure 5. Mean log-transformed hourly *E. coli* data at 90 cm from 10 different sampling days. Solid lines correspond to time-series regressions with $p < 0.05$ for slope and intercept.**

Average raw *E. coli* abundance data for 90 cm showed a smooth exponential decline, but data at 45 cm appeared to follow this model less closely (Figures 6 and 7). As *E. coli* abundance declined over time, the magnitude of the standard error for the untransformed data also decreased. Regardless of how high morning *E. coli* abundance was, as the day progressed levels appeared to converge to a relatively narrow range. Wide daily variability made it difficult to use one equation to describe the variation for any given day.

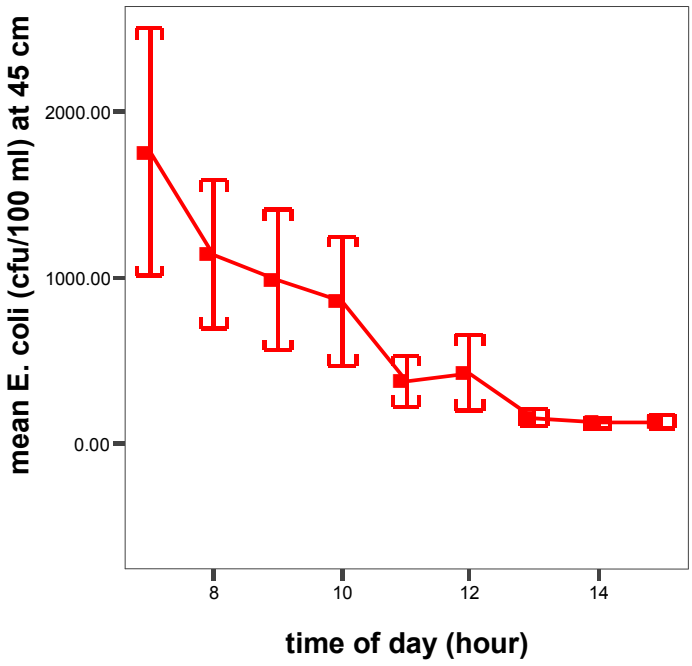



**Figure 6. Hourly E. coli measurements from 45 cm water averaged over 10 sampling days. Error bars show mean + 1 SE**

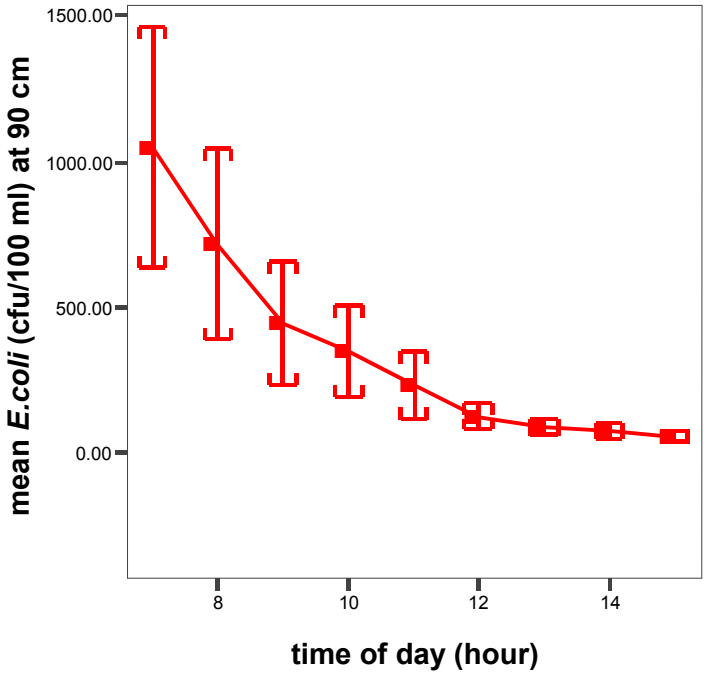



**Figure 7. Hourly *E. coli* measurements from 90 cm water averaged over 10 sampling days. Error bars show mean + 1 SE.**

*E. coli* abundance changed with time of day. Morning abundances were usually much higher than afternoon, with a steady decline throughout the day. Thus samples taken early in the morning will tend to be the most conservative in regards to public safety, but they may not necessarily reflect the conditions experienced by the majority of recreational users.

*Light Readings* Measurements from submerged and atmospheric UV and PPF sensors on 18 September and 25 September showed an overall increase in light intensity over time until approximately 13:00 (Figures 8 and 9). At this point light readings appeared to level off or decrease. Atmospheric UV and PPF behaved very similarly, though UV was generally 10x lower than PPF. Submerged UV was substantially more than 10x lower than submerged PPF, indicating that UV passage through the water column was more severely impeded than PPF. Overall, the disparity between atmospheric and submerged light intensity for UV and PPF appears to increase over the course of the day. This could possibly be affected by an increase in water turbidity as wind and waves increase throughout the day.

Light intensity was generally much higher on 18 September than on 25 September. In particular, UV intensity remained nearly flat all day on 25 September. This may be a result of low incoming light and high water turbidity. Light intensity increased very erratically on 18 September, with a particularly large jump registered on all sensors between 12:30 and 13:00.

This is likely the result of shifting cloud cover. For the most part atmospheric and submerged light readings track each other very well, suggesting that atmospheric light readings are a reasonable surrogate for submerged light readings when assessing the effect of light on *E. coli.*

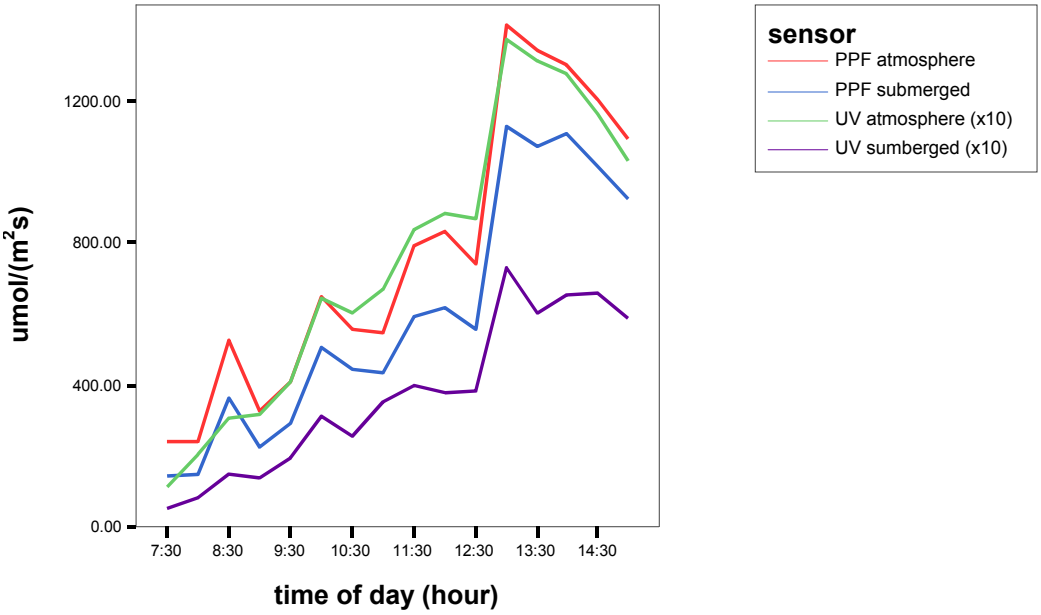



**Figure 8. Measurements of UV and PPF (umol/(m^2)s) over time from above and below the water surface on 18 September. UV measurements are displayed at 10x.**

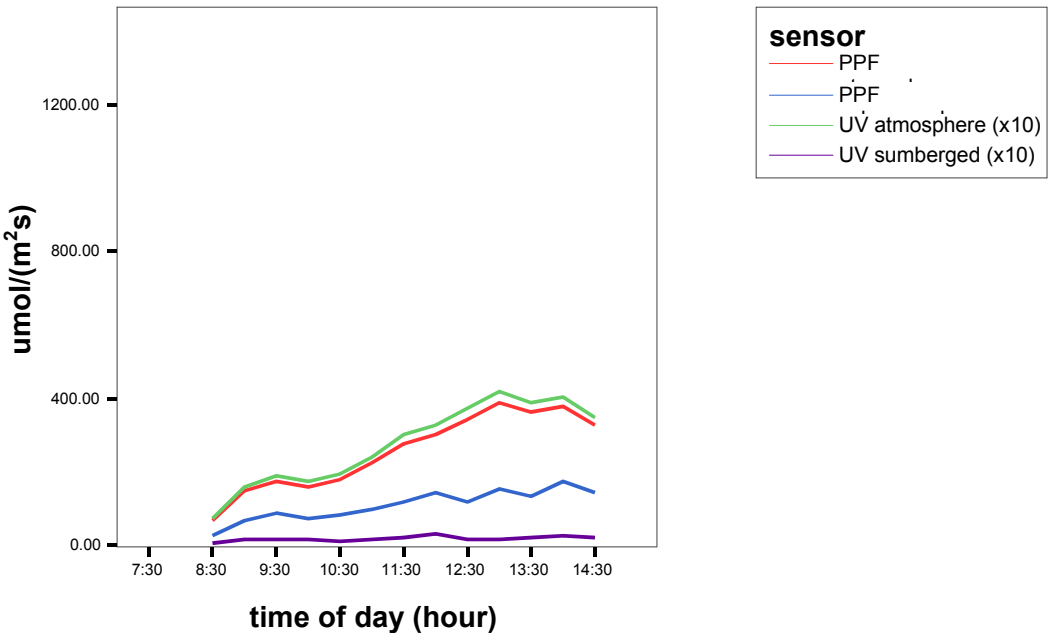



**Figure 9. Measurements of UV and PPF (umol/(m^2)s) over time from above and below the water surface on 25 September. UV measurements are displayed at 10x.**

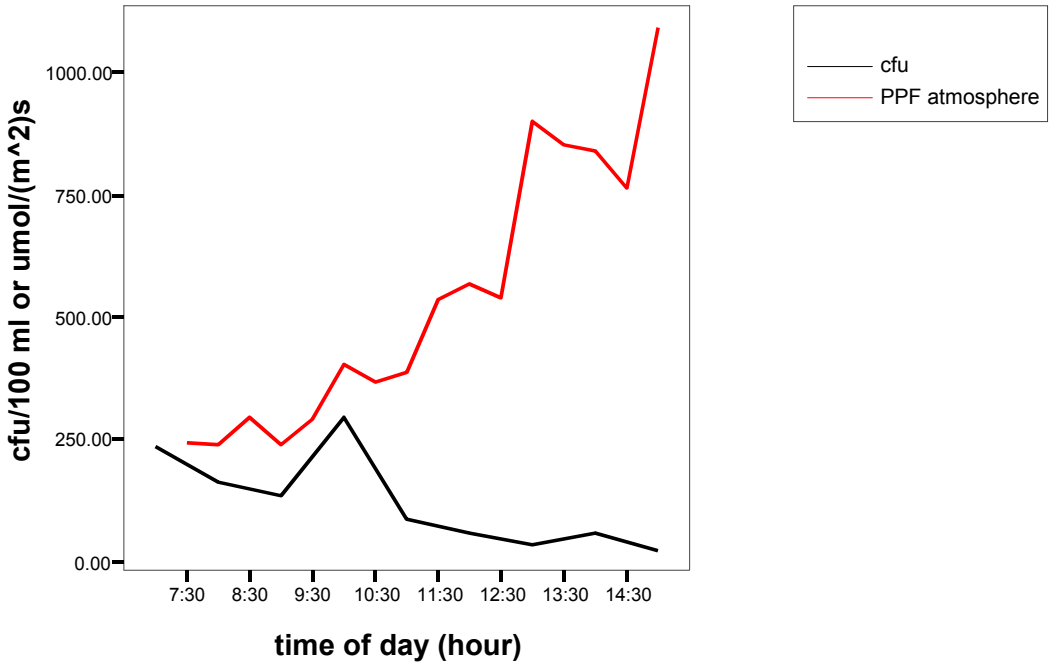



**Figure 10. Above-water PPF measurements and hourly average E. coli on 18 September. E. coli concentrations are averaged from 5 samples at 45 cm and displayed in cfu/100 ml. PPF is displayed in umol/(m2s).**

Increases in light intensity on 18 September were accompanied by a corresponding decline in *E. coli* (Figure 10). Particularly, as atmospheric PPF started to climb rapidly after 10:00, *E. coli* density quickly fell off to near zero. Though this is one isolated example, it does suggest that light has some deleterious effect on the colony-forming ability of *E. coli.*

***Light/Dark Bag experiment***. Mean (n=5) log transformed *E. coli* (CFU/100ml) data were plotted against time of day for light (clear) bags, dark (taped) bags, and the ambient water at 45 cm (Figure 11). *E. coli* concentrations were nearly equal in both light and dark bags at 08:00; after 11:00 *E. coli* concentration in the light bags declines rapidly. The dark bags retained a relatively constant concentration of *E. coli* throughout the course of the experiment. Final *E. coli* concentrations after 8 hours of exposure were greater than 1 log unit different between light and dark bags.

Ambient *E. coli* concentrations at 45 cm started out and remained higher than either bag type until 11:00. The ambient *E. coli* concentration began to decline markedly after 10:00; at 15:00, ambient *E. coli* concentration was between the final concentrations of the light and dark

bags. The manner of decline in *E. coli* concentration appears to be similar for both light bags and ambient water.

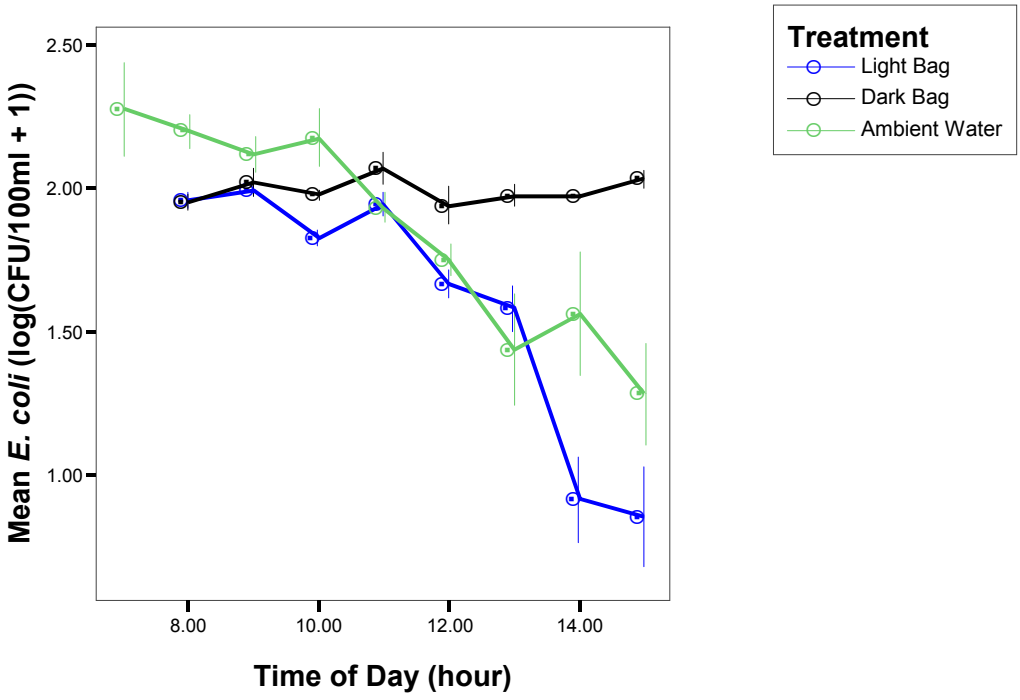



**Figure 11. Mean *E. coli* over time in light bags, dark bags, and ambient conditions. Error bars show mean ± 1 SE**

All statistics were performed on log-transformed data. Two outliers were removed from the ambient data and replaced with the mean of the remaining data for that time and treatment. One value at 10:00 was more than 4X the value of the mean of the remaining raw data; the other value at 15:00 was more than 36X the mean of the remaining data. These data far exceeded all ambient water data for this day, giving rise to the suspicion that contamination during sampling may have caused non-representative values.

Repeated measures testing of the data showed that the within-subjects effect of time of day had a significant effect on *E. coli* concentration ($P<0.01$). Interaction between time of day and treatment was also significant ($P<0.01$). All treatments were significantly different in their effect on *E. coli* concentration (Tukey's HSD, $P<0.01$). However, Pearson correlations demonstrated a 0.84 correlation coefficient for *E. coli* concentration in ambient water and light

bags (P=0.01). *E. coli* concentration in dark bags was not significantly correlated with concentration in either light bags or ambient water.

Temperature was significantly different in the two types of bags (paired t-test, P<0.01). The sensor in the light bag registered an average of 0.32° C higher than that in the dark bag during the course of the experiment. This small difference is likely caused by slight variations in the physical conditions surrounding each bag or a lag in temperature change caused by the tape on the dark bag. The temperature difference is likely not biologically significant.

These results suggest that exposure to light may be a dominant factor in the decline of apparent *E. coli* concentrations over the course of the day. This could suggest that on bright sunny days (when the beach is more likely to be in use) high *E. coli* concentrations may diminish rapidly. A cursory review of weather observations reveals some interesting patterns regarding the highest and lowest *E. coli* counts over the course of the summer. When the highest 20% and lowest 20% of daily *E.coli* concentrations in water are separated, some interesting patterns appear in weather observations (Table 7). Those days during the summer on which the highest counts of *E. coli* were collected are cloudy, overcast, or rainy days. The days on which the highest *E. coli* concentrations were collected were almost all sunny and clear. The data should be subject to some rigorous testing, but some patterns clearly emerge. Because *E. coli* is being used for beach monitoring as an indicator of contamination, it cannot be assumed that other health impacts associated with fecal contamination decline in the same way during exposure to sunlight. Therefore, early morning samples are the most conservative and perhaps the most appropriate when using *E. coli* for water quality monitoring.

**Table 7. Weather observations on days with the highest and lowest E. coli counts.**

Highest 20 percentile *E. coli* in PM, 45 cm

| Date | AM | PM |
|---|---|---|
| **5/9** | rainy/overcast | partly sunny/raining |
| **5/11** | cloudy | cloudy/rainy |
| **5/17** | partly cloudy | cloudy |
| **5/18** | partly cloudy | cloudy,foggy, rainy |
| **6/12** | drizzle | mixed clouds |
| **6/13** | cloudy/fog | rainy |
| **6/20** | rain | rainy |
| **7/6** | hazy | sunny |
| **7/11** | cloudy | partly cloudy |
| **8/23** | cloudy | cloudy/windy |
| **9/12** | rain | sun/windy |
| **9/20** | cloudy/rain | wind/rain |

Lowest 20 percentile *E. coli* in PM, 45 cm

| Date | AM | PM |
|---|---|---|
| **5/2** | sunny, clear | sunny, clear |
| **5/3** | sunny, clear | sunny, clear |
| **5/4** | sunny | sunny |
| **5/25** | sunny, clear | sunny, clear |
| **5/30** | overcast | overcast, thunderstorm |
| **6/1** | sunny and hazy | partly cloudy |
| **6/6** | sunny | sunny |
| **6/7** | sunny, calm | sunny, hazy, breezy |
| **6/14** | mostly sunny | partly cloudy |
| **7/7** | overcast, windy | sunny |
| **9/18** | sunny | sunny |
| **9/19** | sunny | sunny |
| **9/26** | sunny | sunny |

### *Spatial Patterns*

Among site comparisons were made using Wilcoxon Signed-Rank Test to avoid questions of normality of data (Wilkinson 1999). Multiple comparisons were made (45 comparisons) so that a Bonferroni correction of $P_{critical}$ of 0.005 was used for test significance. Data were paired by date with averages from the 5 transects being used for each date for the sediment (by depth) and water (by depth and time of day) samples.

The offshore site had significantly lower *E. coli* abundances compared to all other sites except the 90 cm water samples in the afternoon (Table 8). Submerged sands<foreshore sands<gull sand were all significantly different than one another and from water samples. The 45 cm water sites in the morning were generally higher in *E. coli* than other water sites, except for the lagoon (Table 8). The *E. coli* abundances in the lagoon and the harbor were not significantly different than at the other sites, except the previously mentioned sites (Table 8). $Log_{10}$-transformed means are depicted on the histogram below (Figure 12).

**Table 8. Matrix of two-sided probabilities based on Z numbers calculated from Wilcoxon Signed-Rank Test. Pcritical = 0.005 because of Bonferroni adjustment. Bold p values indicate significance and sign indicates difference between row vs. column heading.**

| | Northshore | Offshore | Lagoon | Harbor | W_am_45 |
|---|---|---|---|---|---|
| Offshore | **- < 0.001** | | | | |
| Lagoon | 0.694 | **+ < 0.001** | | | |
| Harbor | **- 0.002** | **+ < 0.001** | **- 0.004** | | |
| W_am_45 | **+ < 0.001** | **+ < 0.001** | 0.015 | **+ < 0.001** | |
| W_pm_45 | 0.896 | **+ < 0.001** | 0.863 | **+ 0.003** | **- < 0.001** |
| W_am_90 | 0.830 | **+ < 0.001** | 0.790 | **+ 0.002** | **- < 0.001** |
| W_pm_90 | **- 0.003** | 0.091 | **- 0.002** | 0.081 | **- < 0.001** |
| Foreshore | **+ < 0.001** | **+ < 0.001** | **+ < 0.001** | **+ < 0.001** | **+ < 0.001** |
| Submerged | **+ < 0.001** | **+ < 0.001** | **+ < 0.001** | **+ < 0.001** | **+ < 0.001** |
| | | | | | |
| Offshore | W_pm_45 | W_am_90 | W_pm_90 | Sed_Fore | |
| W_am_90 | 0.356 | | | | |
| W_pm_90 | **- < 0.001** | **- < 0.001** | | | |
| Foreshore | **+ < 0.001** | **+ < 0.001** | **+ < 0.001** | | |
| Submerged | **+ < 0.001** | **+ < 0.001** | **+ < 0.001** | **- < 0.001** | |

**Table 9. Mean separation of locations at 63rd Street Beach. Lines which are connected are not significantly different at a=0.033. Overall means are given under each location name.**

| offshore | 90 cm PM | harbor | 90cm AM | lagoon | Nrevetment | 45cm PM | 45cmAM | submerged | foreshore | gull sand |
|---|---|---|---|---|---|---|---|---|---|---|
| 21.26 | 30.81 | 54.98 | 66.16 | 7402 | 78.84 | 84.78 | 158.07 | 735.21 | 4845.19 | 9303.65 |

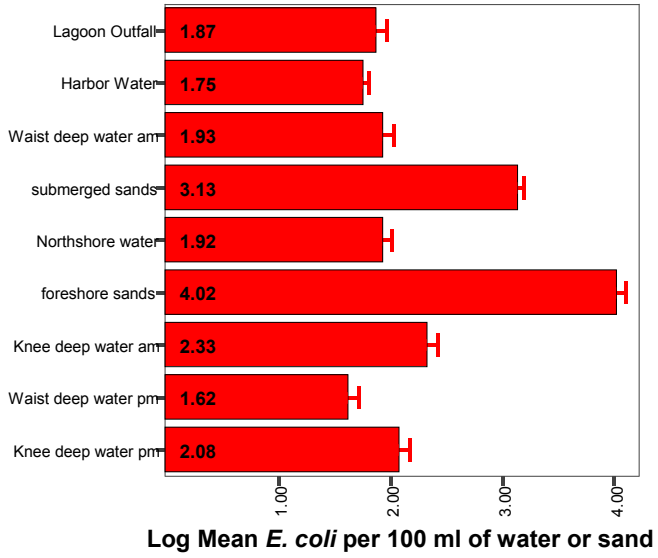



**Figure 12. Mean *E. coli* per 100 ml in water and sand. All numbers are log transformed. Error bars are ± 1 SE.**

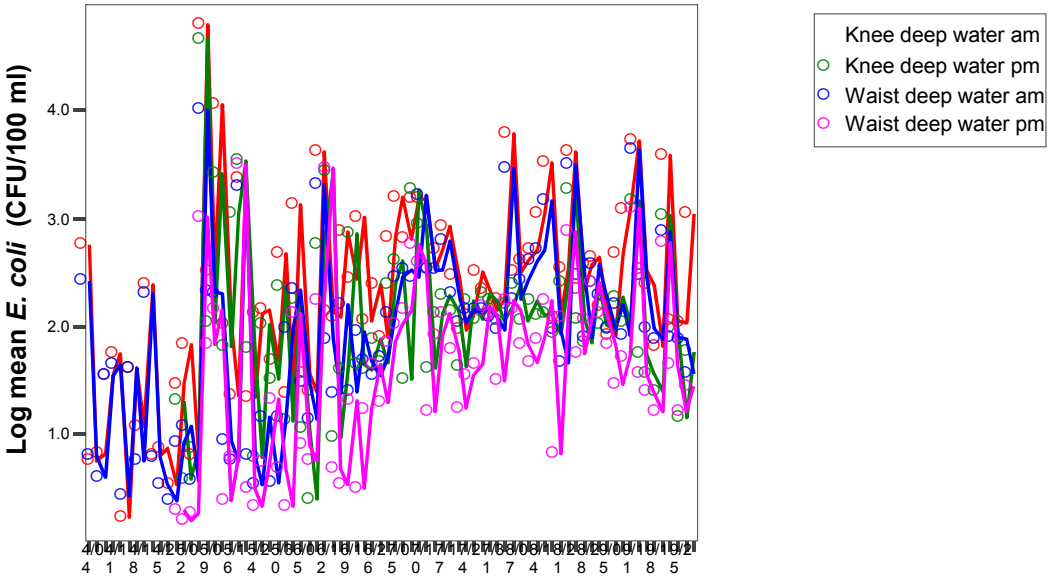



**Figure 13. *E. coli* concentrations over the course of the study at 45 cm and 90 cm water in the morning and afternoon.**

***Groundwater Studies.***

The data in Table 10 indicate that over most of the lake bed, the hydraulic gradient was directed downward into the sand, but in a narrow zone close to the shore face (swash zone), the gradient was directed from the sand into the lake. The measured seepage fluxes were consistently small, but the *E. coli* concentrations in the seepage water were highly variable. The water collected from the seepage meter near the shore face on August 22 had a very high bacterial concentration, and this was consistent with an extremely high concentration (> 50,000 cfu/100 ml) in the sample that was collected that same day from the mini-piezometer on the berm. In contrast, the bacterial concentrations in seepage meter samples collected on September 7 were very low. On that day, samples collected from the mini-piezometers on the berm had concentrations of *E. coli* that were consistently less than 100 cfu/ 100ml. [Note that a vacuum sample collected on September 7 also had a low bacterial concentration of 46 cfu/ 100 ml.]. Finally, the samples of seepage collected in association with the stormy period of August 9 also had elevated bacteria concentrations. Although there is clearly need for more extensive sampling of seepage waters, it appears that the concentration of *E. coli* in such waters can be very high, and that the concentration can vary over orders of magnitude depending on prevailing conditions.

**Table 10. Hydraulic gradients, seepage fluxes, and E. coli concentrations in seepage waters.**

| Date | Δh shore[a] (cm) | Δh lake bed[b] (cm) | Seepage flux Shore[c] (L m-2 h-1) | Seepage flux lake bed[c] (L m-2 h-1) | *E. coli* shore (cfu/100ml) | *E. coli* lake bed (cfu/100ml) |
|---|---|---|---|---|---|---|
| 8/21-22 | 2 – 9 (6) | ~ 0 | 0.19 | -0.01 – [-0.30] (-0.22) | 3,000[d] | 190 – 3,000 (250)[d] |
| 9/7-8 | 4 – 8 (6) | n.d. | 0.04 – 0.96 (0.30) | n.d | 13 – 67 (41) [e] | n.d. |
| 9/12-13 | n.d. | n.d. | 0.03 | n.d. | 3,100 – 4,800 3,900 [e] | n.d. |

Notes: Upper entries in table cells are observed ranges, numbers in parentheses are averages; n.d. indicates no data.
[a]hydraulic gradient existing between the water table in shore sediment and the lake surface.
[b]hydraulic gradient existing between the lake surface and the saturated sand beneath the lake.
[c]positive flux indicates lake is gaining water from sand, negative flux indicates lake is discharging into sand.
[d]water samples were collected using a vacuum pump.
[e]water samples were collected passively by letting hydraulic gradient fill the sample container.

***Spatial Relationship.*** Spearman correlations were used to determine relationships between distinctive sampling sites. This nonparametric test was used because the non-normality of the data does not affect the test (Wilkinson 1999). This Spearman correlation used the average *E. coli* abundance for the 5 transects for the water (by depth and time) and sand (by depth). The data from the others sites (offshore, north revetment, lagoon, and harbor) were the single datum collected per day. Correlation coefficients are shown in Table 11. Although many coefficients are relatively high (e.g., *E. coli* abundance in 45 cm and 90 cm AM water, $R^2 = 0.793$), some increased errors in these comparisons are expected due to the high number of comparisons.

**Table 11. Spearman correlation matrix for tests of relationships between sites.**

| | | | Foreshore | W_45_am | W_45_pm | W_90_am | W_90_pm | SED_SUBM |
|---|---|---|---|---|---|---|---|---|
| Spearman's rho | Foreshore | Correlation Coefficient | 1.000 | .482 | .317 | .450 | .400 | .395 |
| | | Sig. (2-tailed) | . | .000 | .000 | .000 | .000 | .000 |
| | | N | 390 | 389 | 330 | 388 | 330 | 390 |
| | W_45_am | Correlation Coefficient | .482 | 1.000 | .595 | .793 | .620 | .431 |
| | | Sig. (2-tailed) | .000 | . | .000 | .000 | .000 | .000 |
| | | N | 389 | 389 | 329 | 388 | 329 | 389 |
| | W_45_pm | Correlation Coefficient | .317 | .595 | 1.000 | .534 | .687 | .328 |
| | | Sig. (2-tailed) | .000 | .000 | . | .000 | .000 | .000 |
| | | N | 330 | 329 | 330 | 329 | 330 | 330 |
| | W_90_am | Correlation Coefficient | .450 | .793 | .534 | 1.000 | .716 | .363 |
| | | Sig. (2-tailed) | .000 | .000 | .000 | . | .000 | .000 |
| | | N | 388 | 388 | 329 | 388 | 329 | 388 |
| | W_90_pm | Correlation Coefficient | .400 | .620 | .687 | .716 | 1.000 | .339 |
| | | Sig. (2-tailed) | .000 | .000 | .000 | .000 | . | .000 |
| | | N | 330 | 329 | 330 | 329 | 330 | 330 |
| | SED_SUBM | Correlation Coefficient | .395 | .431 | .328 | .363 | .339 | 1.000 |
| | | Sig. (2-tailed) | .000 | .000 | .000 | .000 | .000 | . |
| | | N | 390 | 389 | 330 | 388 | 330 | 390 |

** Correlation is significant at the .01 level (2-tailed).

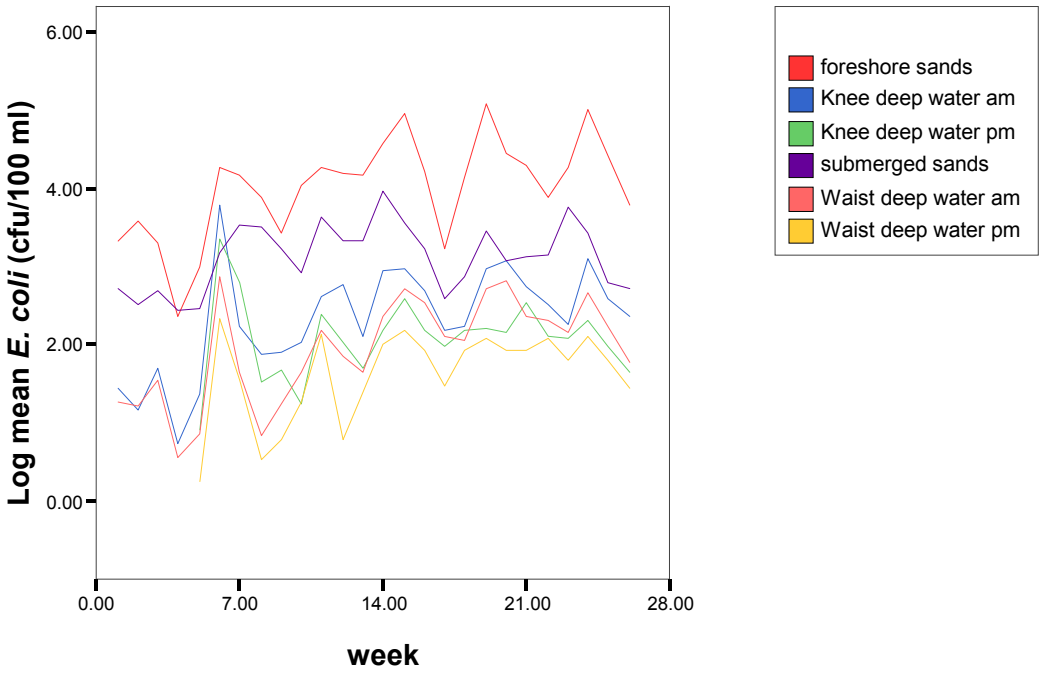



**Figure 14. Weekly average of *E. coli* concentrations in foreshore, submerged sands and swimming waters of 63rd Street Beach.**

The best correlations among sampling sites and times were between water depths sampled at the same time of day (Figure 14). Those, in turn, are correlated to one another and more loosely correlated to foreshore sands. Harbor waters and offshore waters are correlated as expected, considering the similarity of source water for both locations. The lagoon and submerged sands formed a very weak correlation that lacks hydrological justification. North revetment *E. coli* concentrations were not well correlated with other bacteria samples taken.

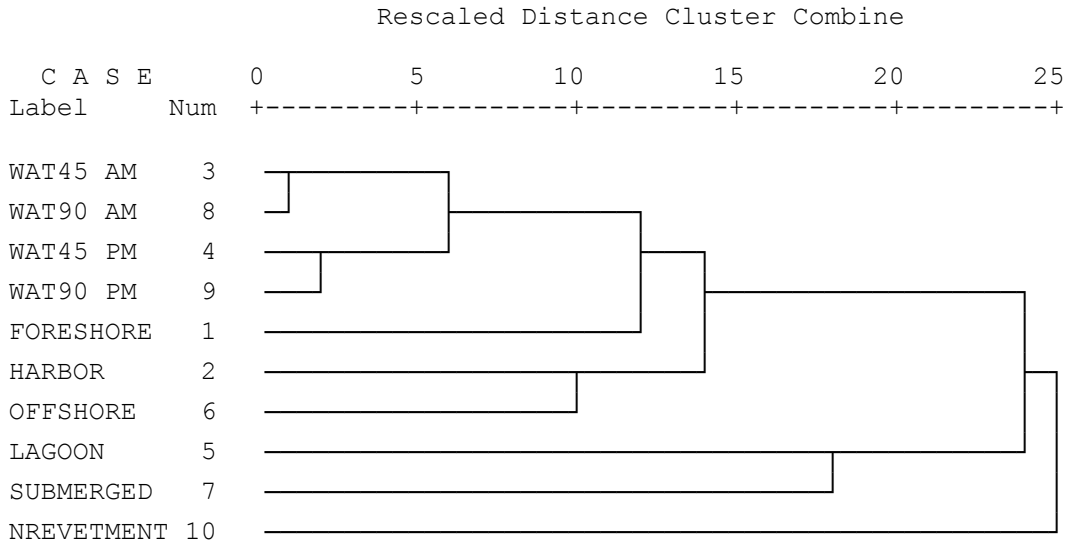



**Figure 15. Dendrogram showing cluster analysis based on Pearson correlation of E. coli concentrations at specific sampling sites**

***Multiple regression of suspected factors.***

One of the most important objectives of this study was to understand the factors that influence *E. coli* concentrations and how these elements might relate to swimming beach results gathered by the Chicago Park District (CPD). It is also important to hypothesize what best explains the nature and sources of *E. coli* fluctuation. Although this is somewhat related to predictive modeling elsewhere presented in this report, the emphasis here is to discover important phenomena affecting *E. coli* occurrence and to explore factors that may be contributing to the source of *E. coli* at tested locations. Statistically, inferences are drawn based on data collected, and hypotheses are rejected (or not rejected) with a certain level of confidence, in this case 95% ($\alpha = 0.05$).

Multiple linear regression was used first to determine what factors best explained variation of *E. coli* concentrations at 45 cm depth in the morning—the same depth at which the CPD takes samples on weekdays. Of the factors considered in this report, those thought to have the greatest potential to affect *E. coli* concentration were:

Density of gull droppings on the beach
Number of Gulls
Morning Wave Height
Morning Width at north revetment and offshore
Morning Wind Speed
Concentration of *E. coli* at the Lagoon
Concentration of *E. coli* at the Harbor

Concentration of *E. coli* within the foreshore sands
Concentration of *E. coli* within the submerged sands
Concentration of *E. coli* at the offshore site

**Table 12. ANOVA analysis of *E. coli* concentrations in 45 cm AM water vs *E. coli* concentrations in foreshore sand and number of gull droppings.**

ANOVA

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 11.580 | 1 | 11.580 | 33.456 | .000 |
| | Residual | 21.461 | 62 | .346 | | |
| | Total | 33.041 | 63 | | | |
| 2 | Regression | 13.753 | 2 | 6.877 | 21.748 | .000 |
| | Residual | 19.288 | 61 | .316 | | |
| | Total | 33.041 | 63 | | | |

a Predictors: (Constant), Foreshore sand
b Predictors: (Constant), Foreshore sand, Gull Droppings
c Dependent Variable: morning 45 cm water

There were significant relationships between *E. coli* concentrations within the foreshore sands and 45 cm AM water ($P < 0.001$) (Table 12). Linear regressions of *E. coli* concentration in 45 cm water and 90 cm water against *E. coli* concentration in foreshore sands showed that *E. coli* in foreshore sands accounts for the same amount of variation at both water depths ($R^2 = 0.43$) (Figures 16 and 17). This means that 43% of the variation shown in either 45 cm or 90 cm water can be accounted for by the concentration of *E. coli* in the sand and 57% of the variation is related to other factors. Only gull droppings contributed significantly to the model. Closer inspection shows that gull droppings only contributed an $R^2$ change of 6.6% to the model and that gull droppings and submerged sands or water were inversely related. The importance of gull droppings alone is minimal without any further considerations such as beach grooming and seasonality.

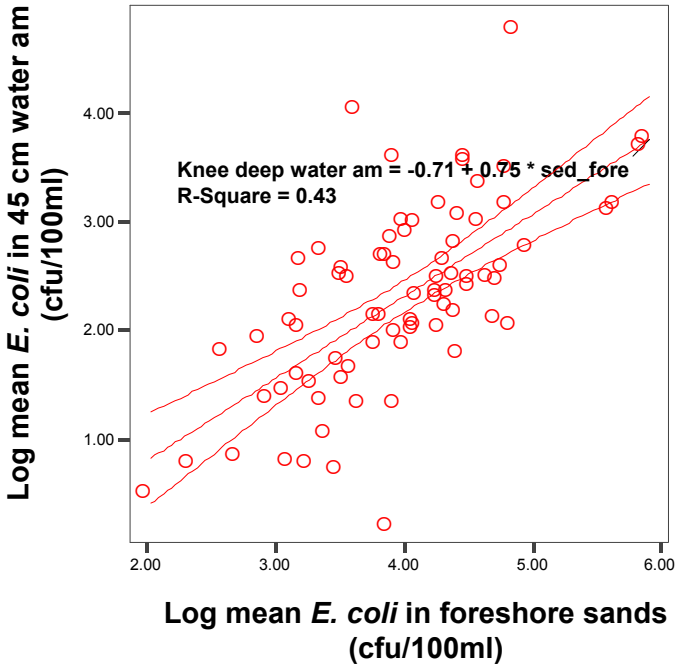



**Figure 16. Linear regression of *E. coli* concentration in 45 cm water AM and foreshore sands. Upper and lower lines represent 95% mean prediction interval.**

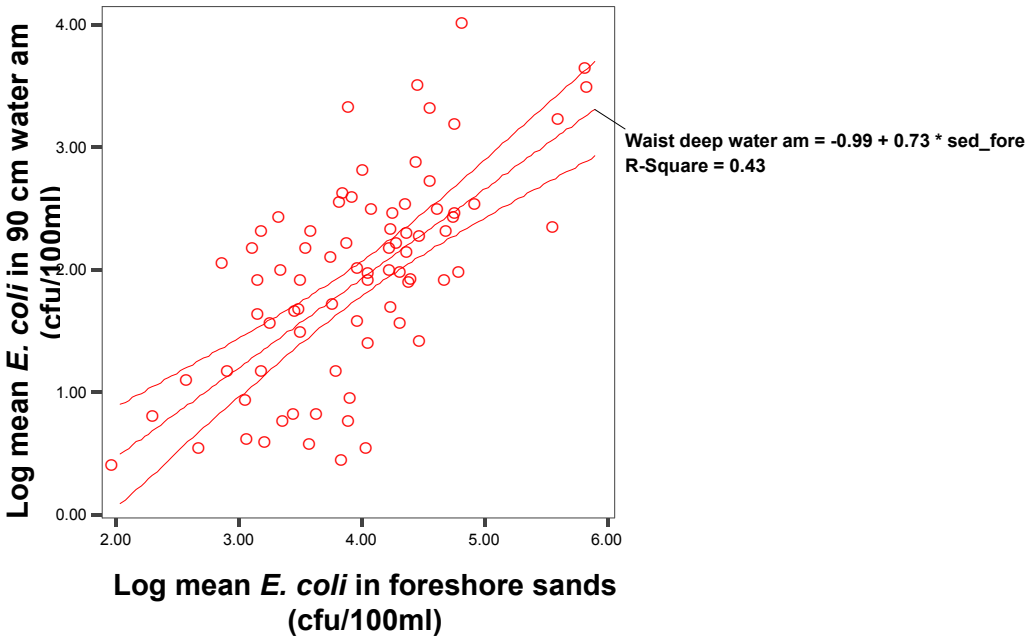



**Figure 17. Linear regression of *E. coli* concentration in 90 cm water AM and foreshore sands. Upper and lower lines represent 95% mean prediction interval.**

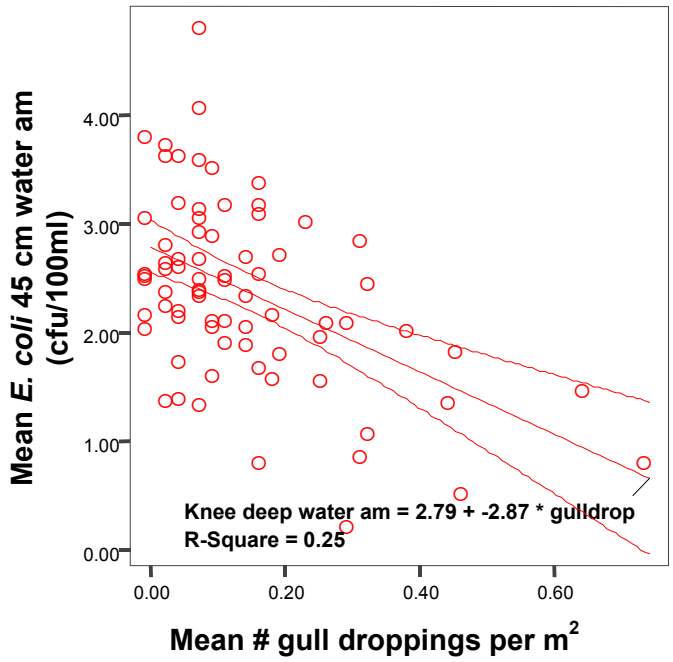



**Figure 18. Regression of gull droppings and E. coli concentration of morning 45 cm water. Upper and lower lines represent 95% mean prediction interval.**

***Effects of raking.***

The inverse relationship of gull droppings and *E. coli* concentration may have more to do with the beach raking (grooming, combing) schedule than original contamination. A student t-test showed that both gull counts and morning *E. coli* counts differed between periods of grooming and non-grooming ($p = 0.017$ and 0.015, when equal variance are not assumed) (Figure 19).

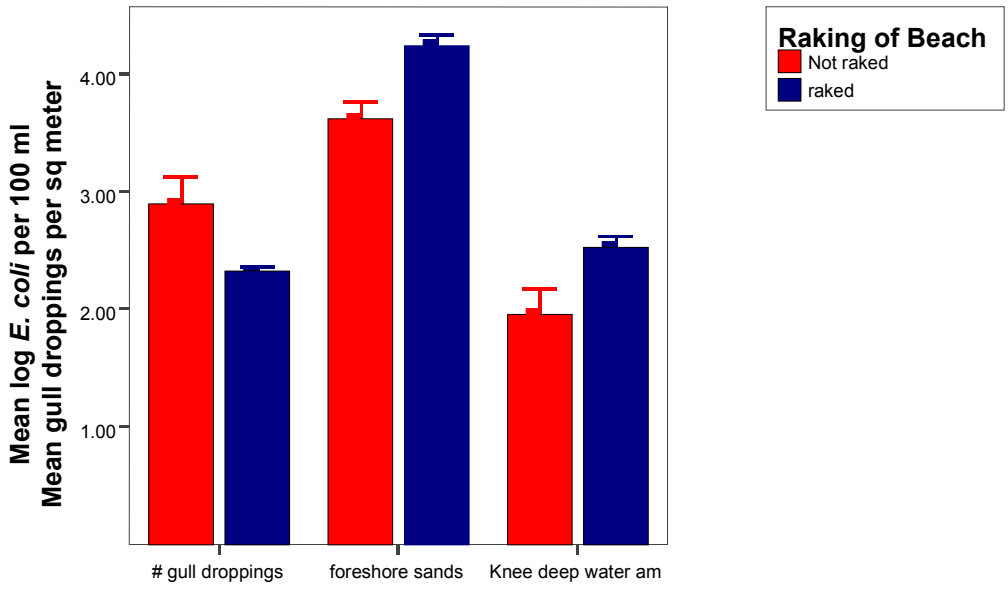



**Figure 19. Number of gull droppings and log transformed *E. coli* in foreshore sands and morning 45 cm water for raked and unraked beaches. Error bars show mean ± 1 SE.**

Thus, it becomes important to consider the number of gull droppings relative to raking treatment. This can be done by partial correlations, which take into account the effect of raking by holding it constant relative to the other parameters evaluated. With this done, the correlation matrix (Table 13) shows that gull droppings are an even more significant factor ($p < 0.001$) although still inverse. The significance of the correlation between *E. coli* concentrations in foreshore sand and 45 cm water also increases ($p < 0.001$).

**Table 13. Partial correlation coefficients for average # gull droppings and *E. coli* concentrations in foreshore sand and 45 cm AM water.**

| Controlling for.. | RAKING | | |
|---|---|---|---|
| | W_45_AM | FORESHORE | GULLDROP |
| W_45_AM | 1.0000 | .6190 | -.4461 |
| | ( 0) | ( 72) | ( 72) |
| | P= . | P= .000 | P= .000 |
| FORESHORE | | 1.0000 | -.2905 |
| | | ( 0) | ( 72) |
| | | | P= .012 |

(Coefficient / (D.F.) / 2-tailed Significance)

Initially, the inverse relationship between raking, gull droppings, and *E. coli* concentrations seem counterintuitive. It is clear that raking breaks up the droppings rendering them uncountable by the field technician. But why would raking increase *E. coli* concentration in the sand and water? Raking, while increasing the aesthetics of the beach, likely does little to reduce the *E. coli* that was associated with the droppings. In fact, raking might enhance *E. coli* survival because the fecal material is now dispersed and buried below the surface sands protecting the bacteria from two of its most lethal threats, desiccation and irradiation Further, raking may make it easier for dispersed bacteria to mobilize in the swash zone. To test this hypothesis, we did a Mann Whitney Test of foreshore and submerged sands and of water on a raked and un-raked beach. We selected a non-parametric test because some of the parameters inspected showed significant difference in variances when raked and un-raked sands were compared (critical value = 0.05, Levene Test). Foreshore sands, submerged sands, 45 cm morning water, 90 cm morning water, north revetment waters and number of gulls were significantly higher during the raking period Table 14). These results further support the idea that *E. coli* concentrations are enhanced in both sands and water during periods of raking. A controlled experiment would be necessary to confirm this phenomenon.

**Table 14. Mann-Whitney Test of *E. coli* concentration during periods with and without beach raking.**

Test Statistics

| | Fore-shore sands | Submerged sands | Offshore water | 45 cm am | 45 cm pm | 90 cm am | 90 cm pm | # of gulls | Gull Droppings | North Revetment |
|---|---|---|---|---|---|---|---|---|---|---|
| Mann-Whitney U | 387.000 | 290.500 | 469.000 | 403.500 | 344.500 | 427.000 | 320.500 | 386.000 | 465.000 | 441.500 |
| Wilcoxon W | 793.000 | 696.500 | 794.000 | 809.500 | 480.500 | 833.000 | 456.500 | 711.000 | 1740.000 | 847.500 |
| Z | -3.261 | -4.266 | -1.755 | -3.089 | -.830 | -2.844 | -1.190 | -2.687 | -1.806 | -2.693 |
| Asymp. Sig. (2-tailed) | .001 | .000 | .079 | .002 | .406 | .004 | .234 | .007 | .071 | .007 |

The confounding problem with this hypothesis is the cofactor correlation of seasonality on *E. coli* abundance. Raking was done during the summer months when *E. coli* would be expected to be higher (Whitman *et al.*1999). Thus, temperature and raking might be covariant factors. Pearson analysis shows no correlation between gull droppings and temperature ($p = 0.218$) and the significance levels between *E. coli* in foreshore sands, submerged sands, and 45 cm water are only slightly improved when partials are adjusted for raking as well as temperature.

***Beach renovation.***

In preparation for the swimming season, 63rd Street Beach foreshore sands were removed to a depth of 8-15 cm and replaced with fresh sands from North Avenue and Montrose beaches on May 24-25, 2000. A few sand samples were taken from the hauling trucks and found to be very low in *E. coli* content. Fortunately for our project, this created a near experimental condition in which we could examine differences between old foreshore and new foreshore sand *E. coli* and possibly clarify influences of foreshore sands on associated aqueous *E. coli*. The statistics demonstrate that foreshore sands, offshore water, 45 cm afternoon water, 90 cm afternoon water were significantly higher before new sand placement (Table 14). There were not significant differences in mean concentration *p>0.05) between number of gulls, gull droppings, and morning *E. coli* concentrations in the water and submerged sands. This suggests that the new

sand may have temporarily reduced the *E. coli* for foreshore sands, and afternoon and offshore waters. Yet, these differences were only statistically significant in the afternoon. Post-treatment morning *E. coli* means at north revetment, 45 cm and 90 cm waters all ranked lower but failed to show a significant difference mainly due to higher statistical variation associated with increased morning *E. coli* concentration (Figure 19). A treatment control would be needed to confirm that sand renewal reduced foreshore and water *E. coli*. Other factors such as weather and antecedent conditions may have explained the pre- and post-treatment effects. Waves were not significantly different during the treatment (p=0.05), but air temperature was significantly warmer for the two weeks following new sand placement (p = 0.001). *E. coli* in sand and water appeared to stabilize after this two week period since the next two weeks were not significantly different ( p > 0.05) even though the first two-week period was relatively warmer.

**Table 15. Mann-Whitney test of mean differences two weeks before and after new beach sand placement on May 24-25, 2000.**

| | foreshore sands | Sub-merged sands | Offshore water | 45 cm am | 45 cm pm | 90 cm am | 90 cm pm | # of gulls | Gull Drop Pings | North revetment |
|---|---|---|---|---|---|---|---|---|---|---|
| Mann-Whitney U | 5.000 | 18.000 | 4.500 | 9.000 | 3.000 | 7.000 | 5.000 | 17.500 | 12.000 | 6.000 |
| Wilcoxon W | 26.000 | 39.000 | 25.500 | 30.000 | 24.000 | 28.000 | 26.000 | 38.500 | 33.000 | 27.000 |
| Z | -2.082 | .000 | -2.169 | -1.441 | -2.402 | -1.764 | -2.082 | -.080 | -.982 | -1.922 |
| Asymp. Sig. (2-tailed) | .037 | 1.000 | .030 | .150 | .016 | .078 | .037 | .936 | .326 | .055 |
| Exact Sig. [2*(1-tailed Sig.)] | .041 | 1.000 | .026 | .180 | .015 | .093 | .041 | .937 | .394 | .065 |

a Not corrected for ties.
b Grouping Variable: Beach Renewal

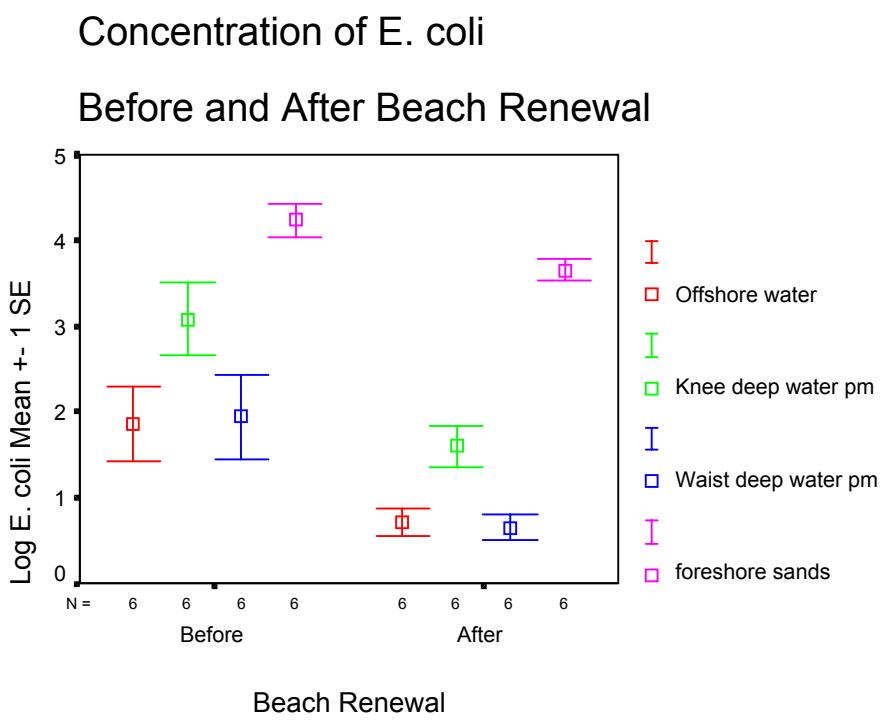



**Figure 20. Concentrations of E. coli before and after beach renewal. Error bars show + 1 SE.**

### *Factors linking E. coli occurrence*

Another way to analyze the relationship between various factors affecting beach *E. coli* is through hierarchical cluster analysis. This procedure shows the relational homogeneity between variables using a selected grouping formula. The distance between variables reflects the relative similarities between the factors being considered. We elected to analyze the average linkage between groups using a centroid algorithm. The cluster (Figure 20) shows clear similarities between foreshore and submerged sediments, 45 cm and 90 cm water *E. coli* for afternoon and morning, morning and afternoon wave heights, gull droppings and north revetment *E. coli* concentration, and harbor *E. coli* and shore width. Secondary linkages are suggested between foreshore and submerged sediments and afternoon 45 cm and 90 cm water, between number of droppings and north revetment *E. coli*, between harbor *E. coli* and shore width and gull numbers, between morning water and offshore *E. coli* concentration. We expect similarities between offshore waters and shallower water samples since all were taken at the same time. The similarity between shore width and harbor *E. coli* can be attributed to lake stage because it drives water in and out of the harbor hydrosystem. It is interesting that north revetment *E. coli* were similar to bird dropping counts even though the two factors were not statistically correlated.

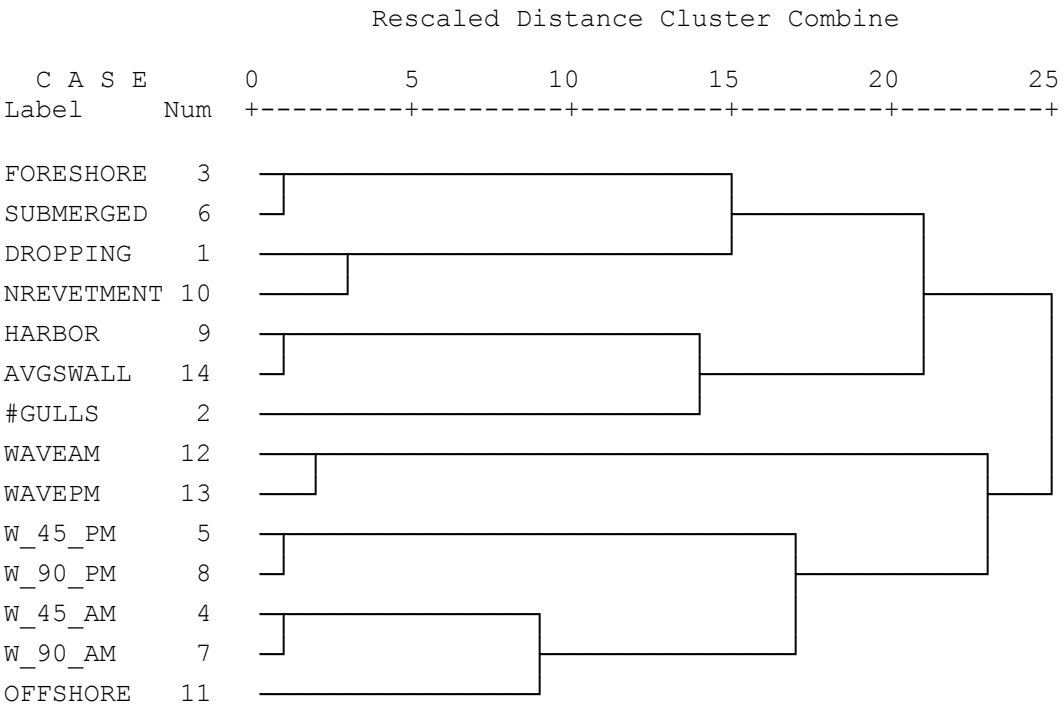



**Figure 21. Centroid cluster analysis of various factors associated with *E. coli* concentrations at 63rd Street Beach.**

For sake of simplicity and for exploratory purposes, the cluster analysis was restricted to potential sources of *E. coli* (Figure 21). The interrelationship between *E. coli* in the water and the foreshore *E. coli* becomes clear as the cluster shows that *E. coli* in the foreshore sand is similar to afternoon waters. This is consistent with the beach renewal relationships discussed earlier under the beach renewal section. In separate clusters using Pearson correlation or cosine method, morning waters were secondarily clustered with afternoon waters as well. The similarity between bird droppings and shore width is interesting, suggesting that rising water either excludes birds or washes away droppings. Correlations between droppings, shore width and number of gulls were not significant ($p>0.10$).

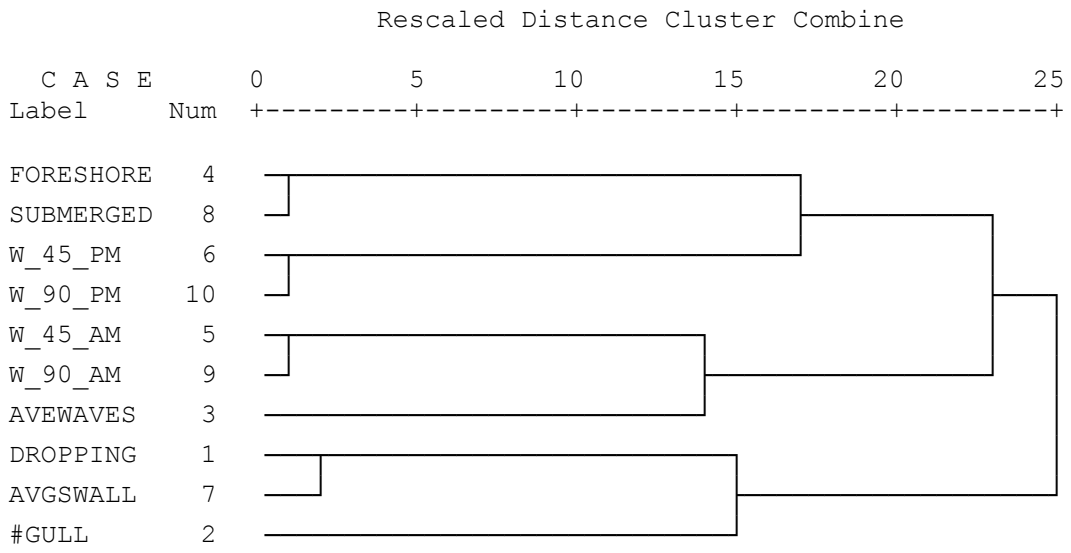



**Figure 22. Centroid dendrogram of major factors associated with *E. coli* concentrations, all data considered.**

Wave height appears to be one of the most important and revealing factors influencing *E. coli* at 63rd Street Beach. It integrates wind speed, wind direction, fetch, and water depth and often translates itself to shore width and swash energy. This, in turn, translates into hydrological energy, which has the potential to mobilize and suspend *E. coli*-laden exposed or submerged sediments. To illustrate this, we compared parameters when waves were above and below the study mean (9 cm). The resulting dendrogram shows very similar results for all clusters except that there is a greater similarity between *E. coli* in submerged and foreshore sands and wave heights during periods of increased wave height (>9 cm). Spearman's statistics show positive correlation between PM waves and 90 cm PM water; foreshore sands and 90 cm AM water; 45 cm and 90 cm AM water; and 45 cm and 90 cm PM water. Submerged sand was not correlated with water or foreshore sand. For waves greater than 9 cm, foreshore sands were positively correlated (critical $p < 0.002$ with Bonferonni adjustment) with submerged sands, 45 cm morning and afternoon water and 90 cm afternoon water. Submerged sands were positively correlated with 45 cm and 90 cm morning water. There was a positive correlation between gull droppings and 45 cm PM water. Negative correlation between gull droppings and *E. coli* concentration is discussed elsewhere. These statistics emphasize the greater importance of resuspended *E. coli*-laden shallow and foreshore sediments during increased wave action.

**Table 16. Correlations when wave heights <9 cm.**

| | | | foreshore sands | Gull Droppings | submerged sands | 90 cm pm | PM wave | 45 cm am | 90 cm water am | 90 cm water pm |
|---|---|---|---|---|---|---|---|---|---|---|
| Spearman's rho | foreshore sands | Correlation Coefficient | 1.000 | -.034 | .176 | .321 | -.059 | .442 | .534 | .443 |
| | | Sig. (2-tailed) | . | .852 | .327 | .069 | .745 | .010 | .001 | .010 |
| | | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| | Gull Droppings | Correlation Coefficient | -.034 | 1.000 | -.035 | -.170 | -.240 | -.205 | -.268 | -.340 |
| | | Sig. (2-tailed) | .852 | . | .845 | .343 | .178 | .253 | .131 | .053 |
| | | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| | submerged sands | Correlation Coefficient | .176 | -.035 | 1.000 | .253 | -.114 | .171 | .061 | .025 |
| | | Sig. (2-tailed) | .327 | .845 | . | .155 | .526 | .341 | .736 | .888 |
| | | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| | Knee deep water pm | Correlation Coefficient | .321 | -.170 | .253 | 1.000 | .148 | .681 | .479 | .709 |
| | | Sig. (2-tailed) | .069 | .343 | .155 | . | .410 | .000 | .005 | .000 |
| | | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| | PMWAVE | Correlation Coefficient | -.059 | -.240 | -.114 | .148 | 1.000 | .422 | .332 | .176 |
| | | Sig. (2-tailed) | .745 | .178 | .526 | .410 | . | .015 | .059 | .326 |
| | | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| | Knee deep water am | Correlation Coefficient | .442 | -.205 | .171 | .681 | .422 | 1.000 | .692 | .676 |
| | | Sig. (2-tailed) | .010 | .253 | .341 | .000 | .015 | . | .000 | .000 |
| | | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| | Waist deep water am | Correlation Coefficient | .534 | -.268 | .061 | .479 | .332 | .692 | 1.000 | .673 |
| | | Sig. (2-tailed) | .001 | .131 | .736 | .005 | .059 | .000 | . | .000 |
| | | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| | Waist deep water pm | Correlation Coefficient | .443 | -.340 | .025 | .709 | .176 | .676 | .673 | 1.000 |
| | | Sig. (2-tailed) | .010 | .053 | .888 | .000 | .326 | .000 | .000 | . |
| | | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |

** Correlation is significant at the .01 level (2-tailed).
* Correlation is significant at the .05 level (2-tailed).

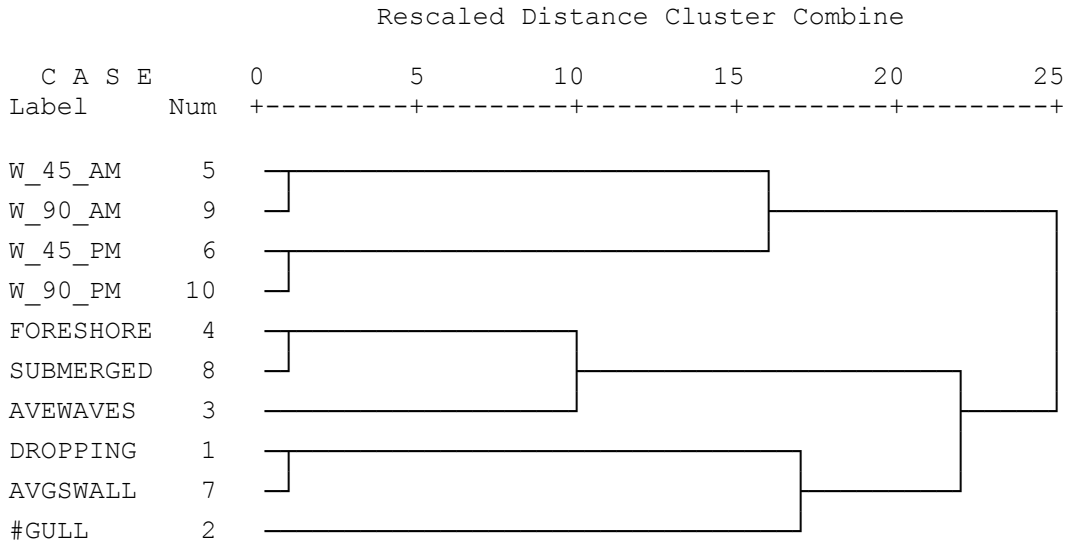



**Figure 23. Centroid cluster analysis of factors associated with *E. coli* concentrations for waves greater than season mean (9 cm).**

**Table 17. Correlations when wave heights >9 cm.**

| | | foreshore sands | Gull Droppings | submerged sands | 45 cm water pm | PM wave | 45 cm water am | 90 cm water am | 90 cm water pm |
|---|---|---|---|---|---|---|---|---|---|
| Spearman's rho | foreshore sands Correlation Coefficient | 1.000 | -.355 | .472 | .344 | .149 | .622 | .518 | .542 |
| | Sig. (2-tailed) | . | .042 | .006 | .050 | .407 | .000 | .002 | .001 |
| | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| | Gull Droppings Correlation Coefficient | -.355 | 1.000 | -.446 | -.272 | -.245 | -.538 | -.483 | -.559 |
| | Sig. (2-tailed) | .042 | . | .009 | .126 | .170 | .001 | .004 | .001 |
| | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| | submerged sands Correlation Coefficient | .472 | -.446 | 1.000 | .460 | .115 | .535 | .447 | .535 |
| | Sig. (2-tailed) | .006 | .009 | . | .007 | .524 | .001 | .009 | .001 |
| | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| | Knee deep water pm Correlation Coefficient | .344 | -.272 | .460 | 1.000 | .039 | .607 | .573 | .762 |
| | Sig. (2-tailed) | .050 | .126 | .007 | . | .828 | .000 | .000 | .000 |
| | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| | PMWAVE Correlation Coefficient | .149 | -.245 | .115 | .039 | 1.000 | .311 | .367 | .295 |
| | Sig. (2-tailed) | .407 | .170 | .524 | .828 | . | .078 | .035 | .095 |
| | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| | Knee deep water am Correlation Coefficient | .622 | -.538 | .535 | .607 | .311 | 1.000 | .906 | .763 |
| | Sig. (2-tailed) | .000 | .001 | .001 | .000 | .078 | . | .000 | .000 |
| | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| | Waist deep water am Correlation Coefficient | .518 | -.483 | .447 | .573 | .367 | .906 | 1.000 | .747 |
| | Sig. (2-tailed) | .002 | .004 | .009 | .000 | .035 | .000 | . | .000 |
| | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| | Waist deep water pm Correlation Coefficient | .542 | -.559 | .535 | .762 | .295 | .763 | .747 | 1.000 |
| | Sig. (2-tailed) | .001 | .001 | .001 | .000 | .095 | .000 | .000 | . |
| | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |

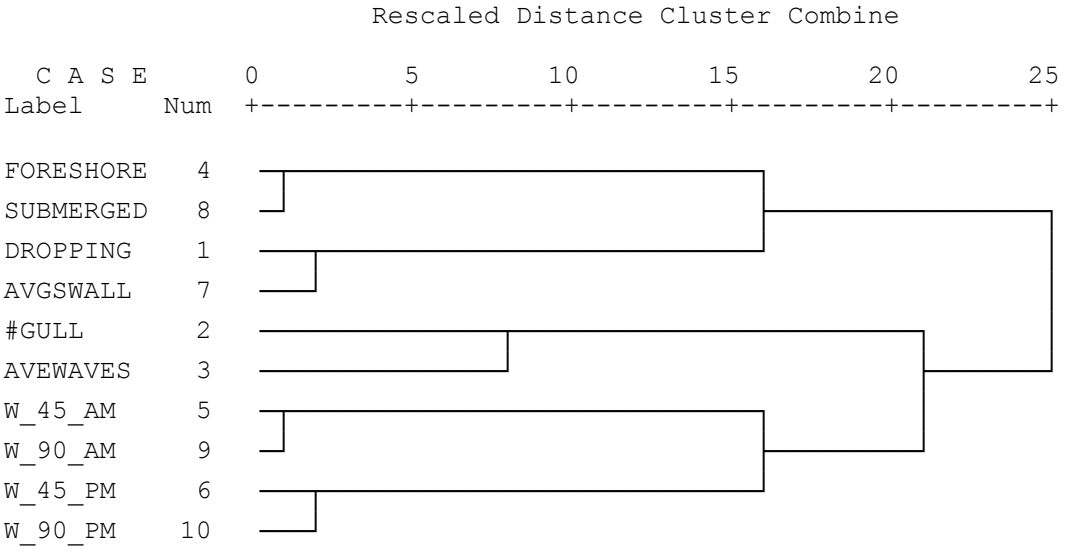



**Figure 24. Centroid cluster analysis of factors associated with *E. coli* concentrations for waves less than season mean (9 cm).**

When non-parametric statistics are used, we discover that number of gulls is positively correlated with *E. coli* concentrations in foreshore sands ($p = 0.012$) but not submerged sands. Foreshore sands are positively correlated to submerged sands. Gulls are not correlated with *E. coli* in either water or sand. We then hypothesize that it takes at least a day for the gulls to influence the sand *E. coli* concentration. To test this theory, gull populations were lagged one day and then tested using a Spearman's rho correlation matrix. Lagged gull populations were correlated with *E. coli* concentrations in foreshore sands, 45 cm water AM, 90 cm water AM, harbor water and offshore sands. *E. coli* concentrations in 90 cm water PM were nearly significant ($p=0.008$, which exceeded Bonferonni critical value of 0.006). Submerged sands and 45 cm water PM were not correlated with lagged gull samples. There may be evidence that gulls and foreshore sand *E. coli* interact with one another and with swimming water.

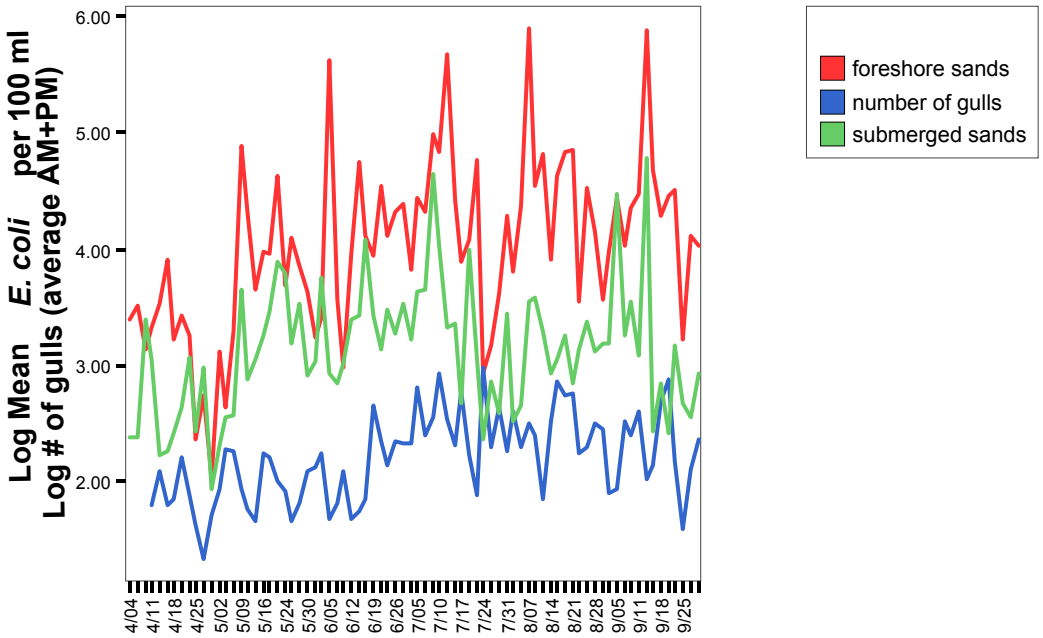



**Figure 25. Number of gulls and concentration *E. coli* in of foreshore sands.**

**Table 18. Correlations of # of gulls against foreshore sand E. coli concentrations before and after lagging foreshore sand by one day.**

| | #gulls Lagged Sig. (2-tailed) | #gulls Unlagged Sig. (2-tailed) |
|---|---|---|
| #gulls Lagged | . | .341 |
| #gulls Unlagged | .341 | . |
| foreshore sands | **.000** | .133 |
| 45 cm water am | **.004** | .224 |
| 45 cm water pm | .167 | .916 |
| 90 cm water am | **.001** | .037 |
| 90 cm water pm | **.008*** | .432 |
| Harbor Water | **.000** | .899 |
| North Rev water | .101 | .381 |
| Offshore water | **.000** | .265 |
| submerged sands | .046 | .972 |

Critical p value Bonferroni corrected =0.006

### *Morphology and Remediation*

Beach and adjacent morphology may be an important aspect of water quality at 63rd Street Beach. Morphology directly affects circulation patterns, waves, sediment deposition, resuspension, and entrainment of contaminants and helps control the export or dilution of pollutants such as *E. coli*. The bounding of 63rd Street Beach by the Jackson Harbor breakwater and the doglegged Casino Pier forms an embayment. To develop a confident understanding of the hydrodynamics of the 63rd Street Beach 'embayment', circulation tracing and hydrodynamic models would have to be employed. Nonetheless, there are some approximate intuitive assumptions that can be made by the morphological setting at the beach. With a northerly longshore current created by high winds, suspended contaminants might be captured by Casino Pier. Because of the geometry of the embayment, wind-generated internal circulation during calmer periods might be favored, thus retarding exportation, dilution, and externally and internally introduced *E. coli*. Sand and fine sediments with associated *E. coli* become trapped in the embayment. Accrual of sediments accounts for the beach's shallowness, the increased silty nature of the sand and the more abundant natural and anthropogenic debris along the beach relative to other observed nearby beaches. The north and south breakwaters also act as protection against wind by reducing fetch. The increased calmness may translate itself into

reduced exportation and dilution of internal or external loadings of bacteria and anthropogenic chemicals that were found in this study. On the other hand, the walls increase the total energy in the area during certain flow conditions because wave energy is reflected rather than absorbed. This energy may act to keep material suspended long enough for some of it to be exported from the beach area. The shallowness of the beach compounds the problem by further decreasing circulation and by allowing less volume of water for dilution of bacteria. Bacteria tend to be associated with detritus and fine sediments (i.e. silts and clays). In deeper water this bacteria-laden material eventually settles to the bottom, and the bacteria eventually dies. This may be why offshore water, and even 90-cm water or harbor water, was lower in *E. coli* content than 45-cm water.

If assumptions concerning the negative effects of morphology and hydrodynamics on bacteria concentration are correct, increasing circulation could be desirable. These hydrodynamic questions lie beyond the scope of the current study but need to be answered before significant funds and effort are made toward remediation. Some of the remediation options that might be considered are: 1) dredging just beyond the swimming area to increase circulation and increase volume, 2) allowing water to move more freely through Casino Pier or 3) creating a sand shunt under the Pier. Evaluating the efficacy of remediation approaches needs to be attended to systematically by hydrologists/engineers working closely with environmental scientists that know this beach system. Remediation approaches will likely have secondary environmental or cultural impacts. These effects need to be clearly identified before any corrective action is undertaken.

***Gull Distribution***

By using the factors of time (AM, PM) and space (transects 1 to 5) for comparison, several similarities and differences may be found in the distribution of gulls on the 63rd Street Beach. In general, there was no significant difference between morning and afternoon bird populations when the entire season was inspected (paired t-test, $p = 0.296$), although morning and afternoon were significantly correlated ($p=0.001$). Graphs with the date versus the number of gulls in each transect for AM and PM allow descriptive comparisons to be made (Figures 25 and 26). The peak in total number of gulls on the beach for AM is in early July, and it is in mid-September for PM. The structures of these peaks differ as well. The AM gull numbers stay low throughout April and May, peak in July, and gradually decrease through September. On the other hand, the PM numbers begin small and increase over time to the maximum in September. The total number of gulls for the sampling period tends to be higher for the PM. A second comparison is between AM/PM and the location on the beach that the gulls occupy. For the AM, the gulls are most often in transects 2 and 3, while in the PM the gulls are in 1 and 2. Overall, the gulls seem to prefer the north end of the beach during June to September. In May, however, the gulls occupy transect 5 almost exclusively in both the AM and PM. This shift in location preference may be due to the presence of bathers beginning in June, and thus, the presence of food litter.

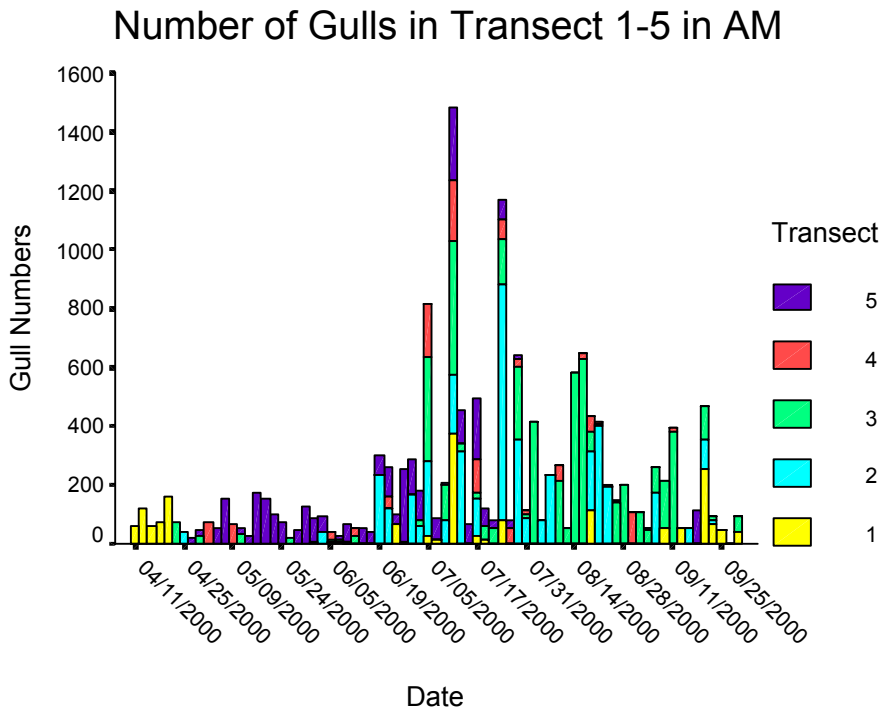



**Figure 26. Number of gulls in transects 1-5 at 63rd St. Beach, AM.**

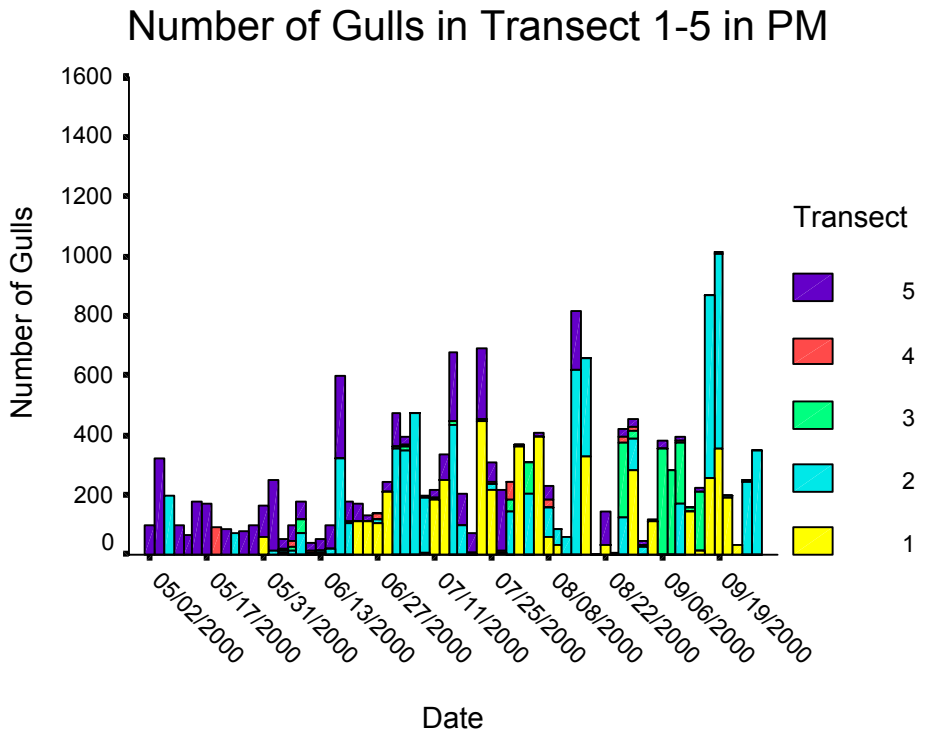



**Figure 27. Number of gulls in transects 1-5 at 63rd St. Beach, PM.**

***Gull and E. coli distribution***

For sake of simplicity and statistical efficiency we separated transects 1 and 2 into the north component and 4 and 5 into the south component, dropping the middle transect (3) from the analysis. For foreshore sands, the northern transects were significantly higher in *E. coli* content than the southern transects (Wilcoxon test, p=0.025). Northern and southern submerged sands were not significantly different using the same non-parametric test (p=0.630). Paired t-test and Wilcoxon failed to show a north or south preference for either morning or afternoon full abundance (p>0.05). Pearson Correlation Analysis shows that although north end and south end sand *E. coli* concentrations were significantly correlated (p=0.002), bird counts were not correlated with sand *E. coli* concentrations for either north or south sand. When sand *E. coli* concentrations were lagged by a day, bird counts for the south transects correlated well with foreshore sand *E. coli* (p=0.008) (Table 18). North and south transect *E. coli* concentrations continued to be correlated, and south transect *E. coli* were weakly correlated with northern bird counts.

**Table 19. Pearson correlation between bird counts in north and south transects (LNBRDPM, LSBRDPM) and *E. coli* in north and south foreshore sand transects (LNSED0, LSED0). Sediments were lagged by one day.**

| | | LSBRDPM | LNBRDPM | LSSED0 | LNSED0 |
|---|---|---|---|---|---|
| LSBRDPM | Pearson Correlation | 1.000 | -.018 | .374 | .303 |
| | Sig. (2-tailed) | . | .904 | .008 | .034 |
| | N | 50 | 50 | 49 | 49 |
| LNBRDPM | Pearson Correlation | -.018 | 1.000 | -.002 | -.053 |
| | Sig. (2-tailed) | .904 | . | .987 | .720 |
| | N | 50 | 50 | 49 | 49 |
| LSSED0 | Pearson Correlation | .374 | -.002 | 1.000 | .425 |
| | Sig. (2-tailed) | .008 | .987 | . | .002 |
| | N | 49 | 49 | 50 | 50 |
| LNSED0 | Pearson Correlation | .303 | -.053 | .425 | 1.000 |
| | Sig. (2-tailed) | .034 | .720 | .002 | . |
| | N | 49 | 49 | 50 | 50 |

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

***Bather Effects.*** Bather numbers and distribution among transects are shown in Figure 27. Total bather numbers were not significantly correlated with *E. coli* concentrations at either 45 cm or 90 cm. It appears from these data that if bathers did have an impact on *E. coli* concentration at 63rd Street Beach, that effect was relatively small and was masked by other factors.

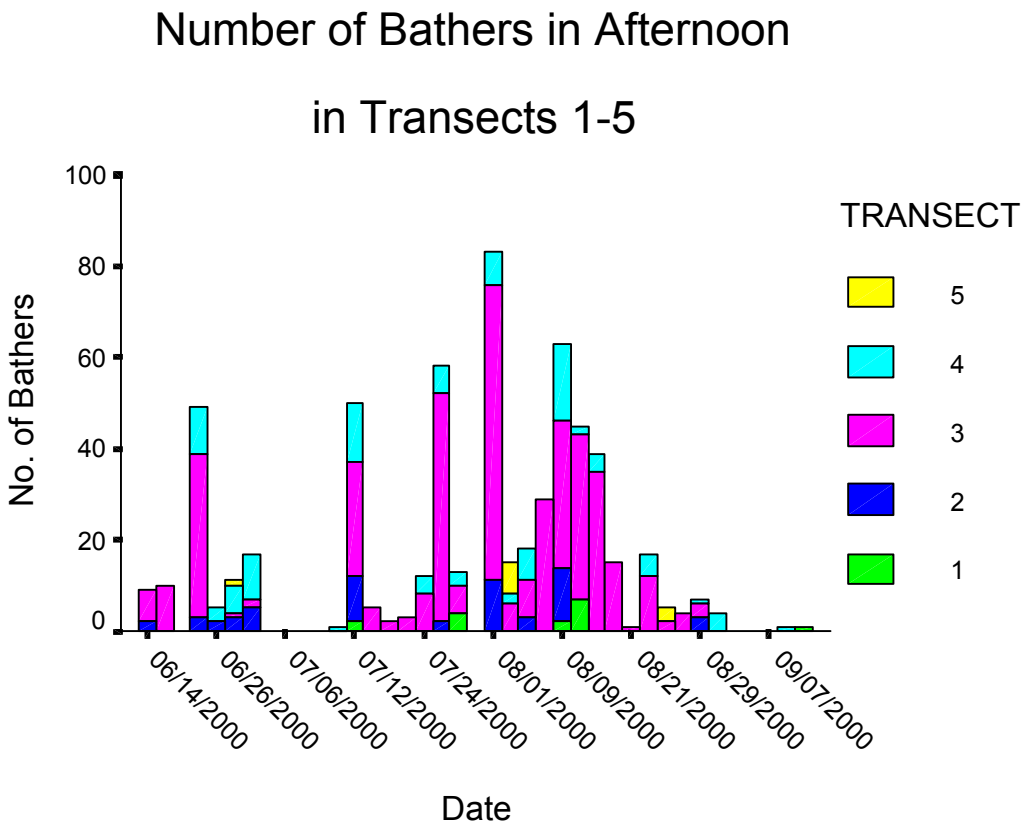



**Figure 28. Number of bathers in transects 1-5 at 63rd St. Beach.**

**Table 20. Pearson Partial Correlation for Bather Frequency versus *E. coli* at 45 and 90 cm depths.**

```
- - -  P A R T I A L   C O R R E L A T I O N   C O E F F I C I E N T S  - - -

Controlling for..    W_90_PM

            BATHERPM    W_45_PM

BATHERPM      1.0000      .0942
             (    0)    (   48)
             P= .       P= .515

W_45_PM         .0942     1.0000
             (   48)    (    0)
             P= .515    P= .

(Coefficient / (D.F.) / 2-tailed Significance)

" . " is printed if a coefficient cannot be computed



- - -  P A R T I A L   C O R R E L A T I O N   C O E F F I C I E N T S  - - -

Controlling for..    W_45_PM

            BATHERPM    W_90_PM

BATHERPM      1.0000     -.1472
             (    0)    (   48)
             P= .       P= .308

W_90_PM       -.1472     1.0000
             (   48)    (    0)
             P= .308    P= .

(Coefficient / (D.F.) / 2-tailed Significance)

" . " is printed if a coefficient cannot be computed
```

### *Results of Harbor Sediment Testing*

Additional sampling was conducted at Jackson Harbor to assess the presence and/or magnitude of *E. coli* storage in the harbor sediments relative to the overlying water. Sampling took place on 19 July. Water and sediment samples were collected from a total of three points located at the ends of the three easternmost floating piers in the harbor. Means of cfu/100 ml for all three sample types were very similar. Mean *E. coli* concentration was highest (before correction) in sediment samples (367 $\pm$ 79 cfu/100 ml), followed by subsurface water (327 $\pm$46 cfu/100 ml)

and then water from just above the sediment surface (287 $\pm$ 54 cfu/100 ml). Sample means failed to show a statistically significant difference ($p>0.05$). The elutriation water for the sediment samples was harbor water, so *E. coli* concentrations from overlying water have to be subtracted to get a true estimation of sediment *E. coli* concentrations (367-287=80 cfu/100 ml). Therefore sediment *E. coli* concentrations actually appear to be lower than concentrations in the water. This makes it unlikely that the harbor sediments are storing large amounts of *E. coli* or contributing significantly to *E. coli* concentrations in the harbor water.

## DNA, MAR, AND CHEMICAL ANALYSIS

Samples for source determination were collected on June 26, 2000 and August 21, 2000. Environmental conditions for these two dates place them in the low to average wave height category. Seagull populations on each date were typical. All samples were collected in the morning. The population numbers for *E. coli* and enterococci varied between these two sets of samples (Table 21). Estimates of population numbers were generally comparable between laboratories. Numbers of *E. coli* and enterococci were generally lower for the August 21 samples. Numbers of enterococci in sediments were dramatically lower for the August 21 samples (Table 21). Numbers of *E. coli* and enterococci in seagull feces varied between samples (Table 22) with no apparent relation to date of collection.

**Table 21. Numbers of total coliform bacteria, *E. coli*, and enterococci for 63rd Street Beach samples on the two sampling dates.**

| | **USGS Total Coliforms** | | **USGS *E. coli*** | | **Chicago *E. coli*** | | **USGS Enterococci** | |
|---|---|---|---|---|---|---|---|---|
| **Sample ID** | **per 100 ml** | | **per 100 ml** | | **per 100 ml** | | **per 100 ml** | |
| | *June 26* | *August 21* | *June 26* | *August 21* | *June 26* | *August 21* | *June 26* | *August 21* |
| T1 W 45cm | 160 | ND | 90 | ND | 100 | 34 | 14 | 0 |
| T2 W 45cm | 190 | ND | 50 | ND | 120 | 84 | 37 | 1 |
| T3 W 45cm | 50 | ND | 17 | ND | 32 | 140 | 11 | 1 |
| T4 W 45cm | 270 | ND | 130 | ND | 140 | 160 | 13 | 0 |
| T5 W 45 cm | 310 | ND | 160 | ND | 160 | 180 | 26 | 1 |
| | | | | | | | | |
| North Revet. | 420 | ND | 130 | ND | 600 | 120 | 5 | 0 |
| Offshore | 2 | ND | 3 | ND | 14 | 4 | 2 | 0 |
| Lagoon | 1000 | ND | 800 | ND | 3100 | 280 | 42 | 2 |
| Harbor | 270 | ND | 90 | ND | 110 | 38 | 41 | 0 |
| | | | | | | | | |
| T1 S foreshore | 1.4 x 105 | TNTC | 6.0 x 104 | TNTC | 2.7 x 104 | TNTC | 4.0 x 103 | 5 |
| | 3369/cm3 * | NA | 1444/cm3 | NA | 650/cm3 | NA | 96/cm3 | <1/cm3 |
| T2 S foreshore | 3.0 x 103 | 3.3 x 104 | 7.0 x 103 | 1.0 x 104 | 7.2 x 103 | 9.0 x 103 | 1.5 x 104 | 0 |
| | 60/cm3 | 456/cm3 | 140/cm3 | 14/cm3 | 144/cm3 | 125/cm3 | 301/cm3 | <1/cm3 |
| T3 S foreshore | 1.0 x 103 | 6.0 x 102 | 4.0 x 103 | 3.8 x 102 | 4.8 x 103 | 1.3 x 103 | 1.7 x 104 | 0 |
| | 15/cm3 | 12/cm3 | 60/cm3 | 8/cm3 | 72/cm3 | 24/cm3 | 256/cm3 | <1/cm3 |
| T4 S foreshore | 1.0 x 104 | 6.9 x 103 | 9.0 x 103 | 4.9 x 103 | 5.7 x 103 | 3.7 x 103 | 2.4 x 104 | 0 |
| | 201/cm3 | 113/cm3 | 181/cm3 | 80/cm3 | 114/cm3 | 61/cm3 | 481/cm3 | <1/cm3 |
| T5 S foreshore | 2.0 x 103 | 4.8 x 103 | 2.0 x 103 | 3.8 x 103 | 1.9 x 103 | 2.0 x 103 | 5.2 x 103 | 0 |
| | 32/cm3 | 110/cm3 | 32/cm3 | 88/cm3 | 46/cm3 | 46/cm3 | 83/cm3 | <1/cm3 |
| Gull Sand | 1.4 x 104 | 1.0 x 103 | 1.3 x 104 | 4.0 x 103 | 9.0 x 103 | NA | 9.0 x 103 | 1 |
| | 198/cm3 | NA | 184/cm3 | NA | 127/cm3 | NA | 127/cm3 | <1/cm3 |

*The volume of sediment suspended in 100 mL buffer was determined from the inner diameter of the core tube and the recorded length of the core. ND, not determined. NA, not available.

**Table 22. Numbers of total coliforms, E. coli and enterococci in seagull feces.**

| | **USGS Total Coliforms** | USGS *E. coli* | **USGS Enterococci** |
|---|---|---|---|
| **Seagull ID** | **per 100 mla** | **per 100 mla** | **per 100 mla** |
| | **June 26** | | |
| SGA | 1.9 x 107 | 3.1 x 106 | 4.5 x 107 |
| SGB | 2.9 x 107 | 6.0 x 106 | 6.0 x 105 |
| SGC | 2.8 x 106 | 2.7 x 106 | TNTC |
| SGD | 1.3 x 109 | 1.0 x 109 | TNTC |
| SGE | 0 | 0 | TNTC |
| SGF | 2.5 x 106 | 0 | 5.0 x 106 |
| | | | |
| | **August 21[b]** | | |
| SGA | 1.9 x 109 (1.9 x 109 per g feces) | 1.9 x 109 (1.9 x 109 per g feces) | 4.1 x 106 (4.0 x 106 per g feces) |
| SGB | 2.3 x 107 (9.2 x 107 per g feces) | 2.3 x 107 (9.2 x 107 per g feces) | 7.0 x 104 (2.8 x 105 per g feces) |
| SGC | 1.9 x 107 (9.3 x 106 per g feces) | 1.9 x 107 (9.3 x 106 per g feces) | 1.3 x 108 (6.5 x 107 per g feces) |
| SGD | 5.0 x 106 (4.0 x 106 per g feces) | 5.0 x 106 (4.0 x 106 per g feces) | 2.5 x 105 (2.0 x 104 per g feces) |
| | | | |

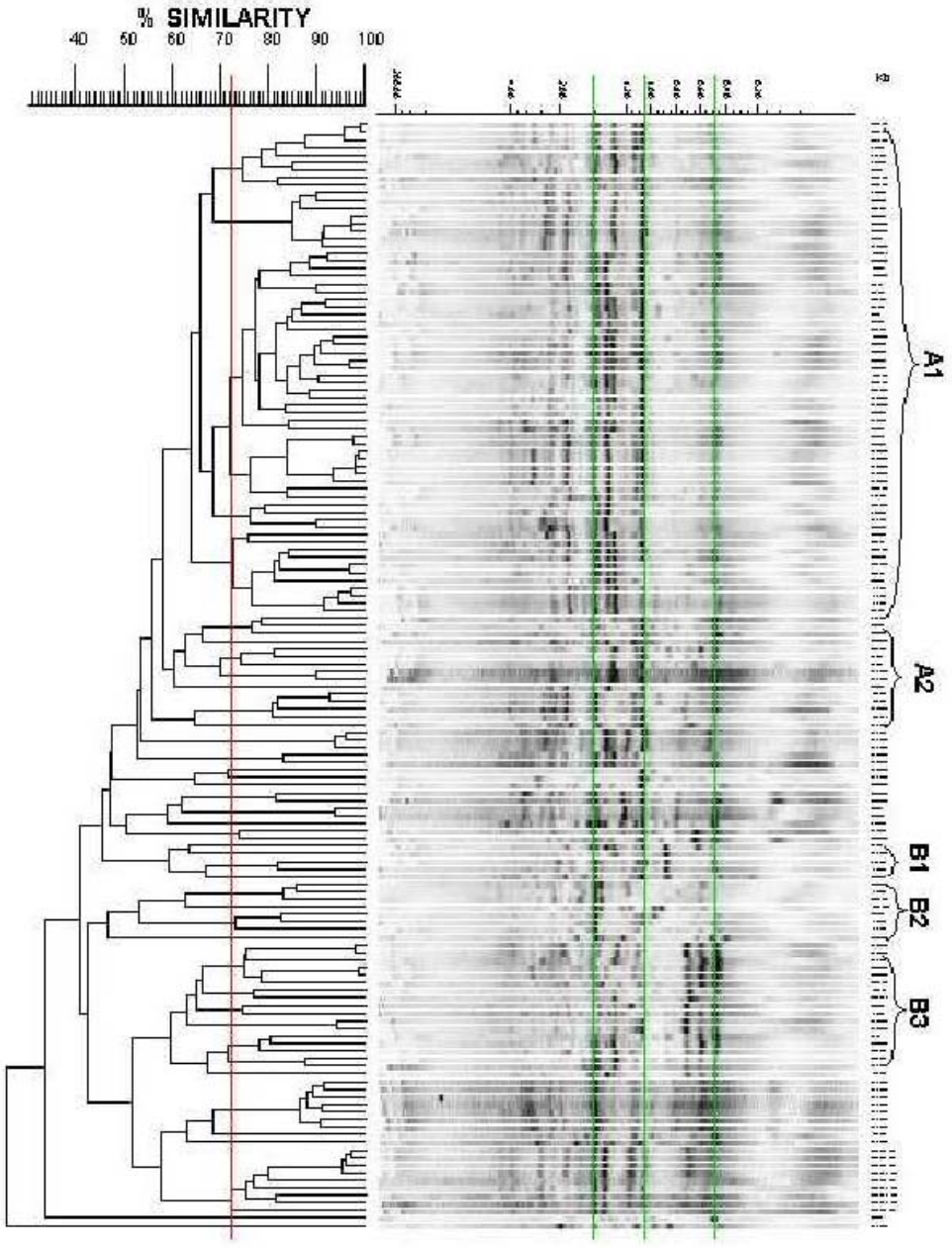
TNTC, too numerous to count; aNumbers are reported per 100 mL of resuspension buffer. Weight of feces was not determined; bThe wet weight of the August feces was determined.

## *E. COLI* DNA FINGERPRINTS

### *Overall analysis*

DNA fingerprints were obtained for 136 isolates on the two dates: June: 26 water, 20 sediment, 20 seagull; and August: 33 water, 20 sediment, 18 seagull. Cluster analysis was conducted on these samples (Figure 29); the vertical red line indicates the minimum similarity of the control *E. coli*. Any isolates that group at a similarity greater than this level are considered indistinguishable given the variability in the method. The green vertical lines drawn at approximately 0.5 kilobases (a measure of the size of DNA; Kb), 1.1 Kb and 1.5 Kb indicate major bands that, together with groups of other bands, result in the clusters depicted. Certain clusters are marked for discussion. The large cluster A1 contains isolates that generally possess all three major bands. In addition, groups of bands (2 around 1.3-1.4 Kb and several > 2 Kb) define subsets of cluster A1. Cluster A2 retains some features of the A1 group but not the entire set of features that would allow members of this group to cluster more closely with A1 members. Anything not in clusters A1 or A2 is designated as "B." The group B members usually lack one of the major bands, and/or have a new feature that is not found in the A1 or A2 clusters. For example, B1 possesses a unique band around 0.8 Kb, B2 lacks two of the major bands and B3 possesses a group of 3 bands between 0.5 and 0.7 Kb that makes a unique feature.

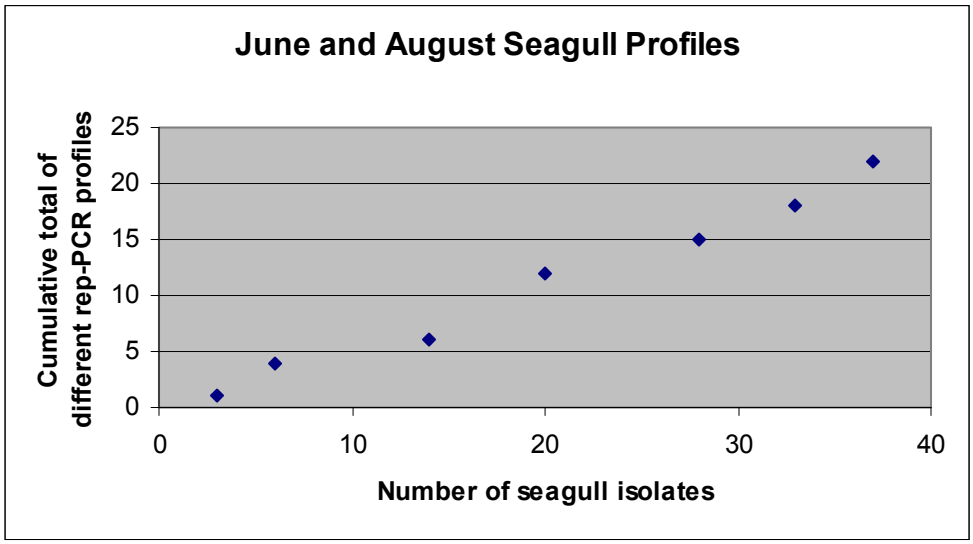
**Figure 29. Cluster diagram for rep-PCR DNA fingerprints for all isolates.**



Some aspects of this large cluster analysis are noteworthy. First, of 40 sediment samples analyzed for both dates, 30 are in the A1-A2 group. On the other hand, of 32 beach water samples, 19 (10 June and 9 August) are in the A1-A2 group and 13 (4 June and 9 August) are in the B group. One interpretation may be that the sediment isolates are a more homogeneous population than the water isolates and are less susceptible to date-to-date variation. Since the cores aggregate material from land surface to the water table, there is a possibility that different populations are aggregated as well. If, as prior studies indicate, the majority of the sediment *E. coli* occur at shallow ground water (near the water table) then these may be a separate population or bacteria earlier derived from groundwater inputs or through-flow over time from materials applied to the surface of beach sand. Seepage tests of through-flow *E. coli* delivery was inconclusive. Core samples of sands which include the groundwater at 1 meter would not necessarily be related to *E. coli* populations in surface waters sampled at the same time. This would be especially true during periods of lake calm when entrainment of *E. coli* laden sands in the swash zone is less.

Seagull isolates (20 tested in June and 18 in August) are about equally distributed between the A(16) and B(22) groups (Figure 29). However, group A contains 12 seagull isolates from August and only 4 from June and vice versa for group B. This has a significant effect on the June and August results (discussed separately below) and suggests temporal population differences in *E. coli* in seagulls. Temporal *E. coli* population variability in seagull feces may be supported by ecological information suggesting a shift in seagull species from mostly adult Ring-bill gulls in the early summer to adult and juvenile Ring-bill gulls, Herring gulls and migrants in early fall. Therefore, we may have sampled different birds in August. Alternatively, they may have been the same birds on both dates, but food sources or other behavioral differences may account for fecal bacterial population shifts. Finally, it is possible that the analysis of only a few fecal samples on each date may have resulted in an under-representation of *E. coli* types from this source on any given date. If so, then there may have been no true difference in *E. coli* populations between dates, and the observed difference would be attributed to sampling error. A plot of cumulative total rep-PCR profiles against isolates for all seagull isolates indicates a linear relationship (Figure 30) with no asymptote, suggesting that more *E. coli* types would have been found in additional samples. Since the total number of *E. coli* rep-PCR fingerprint types is unknown, it is not possible to state how representative the *E. coli* analyzed were of all those possibly found on the beach. On the other hand, enterococci (see below) and *Salmonella* suggest adequate sampling for these particular species.

**Figure 30. Different rep-PCR profiles for seagulls, June and August.**



June *E. coli* DNA fingerprints.

DNA fingerprints were obtained for 26 water *E. coli* isolates (14 from the beach transects), 20 sediment isolates and 20 seagull isolates (Figure 31). Only two samples, both sediments (4SA and 5SA), can be construed to match a seagull (Figure 29). The red line again marks the similarity criterion for the control *E. coli*. All but one June seagull has the "B" fingerprint pattern while most water samples have the "A" fingerprint pattern. Seagull fingerprints were only identical within a seagull. There were no identities among seagulls. Within-site identical fingerprints occurred at several locations: H, L, N, 4SA, and 1SA. There is no apparent spatial pattern to isolates designated as identical using our criterion. For example, five clusters representing identical isolates are marked (Figure 31). Each cluster includes isolates from very different parts of the beach and from both sediments and water. Using our identity criterion, six foreshore beach sediment isolates are identical to an isolate from beach water. On the other hand, six foreshore beach sediment isolates are identical to an isolate from the harbor, lagoon, offshore or north revetment areas. Likewise, eight water isolates from 45 cm can be related to an isolate from the harbor, lagoon, or offshore or north revetment areas. The June results suggest little influence of seagulls on the *E. coli* found in foreshore sediments or knee-deep waters. Interestingly there was some limited overlap between beach sand strains and genotypes recovered from lagoon, harbor, or north revetment samples.

***August E. coli DNA fingerprints.***

DNA fingerprints were obtained for 33 water *E. coli* isolates (18 from the beach transects), 20 sediment isolates, and 18 seagull isolates (Figure 32). There was a seagull match, using our identity criterion, for 26 samples (14 water and 12 sediment). There was no seagull match for any water sample from site 5 or LO and only one of four north revetment samples matched a seagull. For water from the beach transects, 10 of 18 isolates could be matched to a seagull. Of the remaining eight samples, five presented unique profiles: one matched an LO isolate and two matched each other. There were no identities among seagulls. There were several within-site identities at 2SA, 4SA, and 2WA. In addition, there was a very close match between 1SA and 4WA and between 2WA and 4WA. Again, there is no apparent spatial pattern to isolates that cluster within identity groups.

**Figure 31. Cluster diagram of rep-PCR DNA fingerprints for all June isolates.**

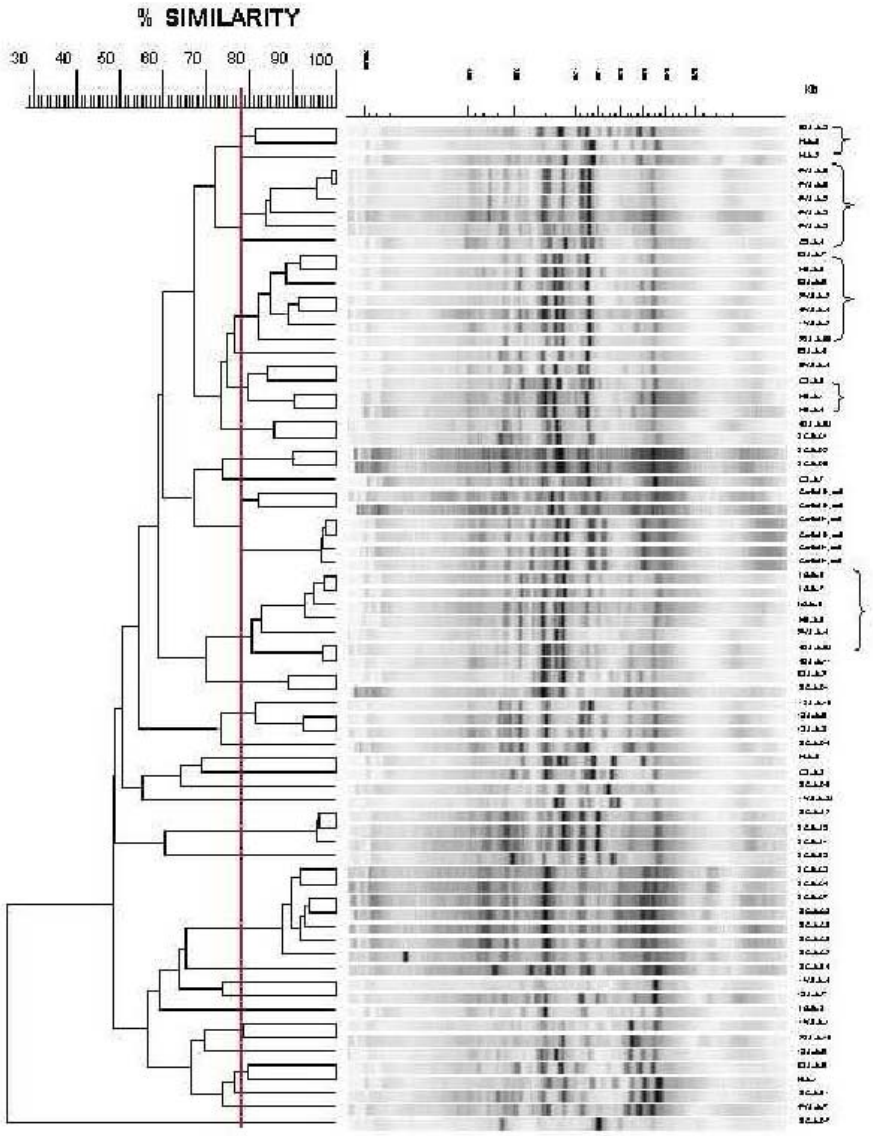
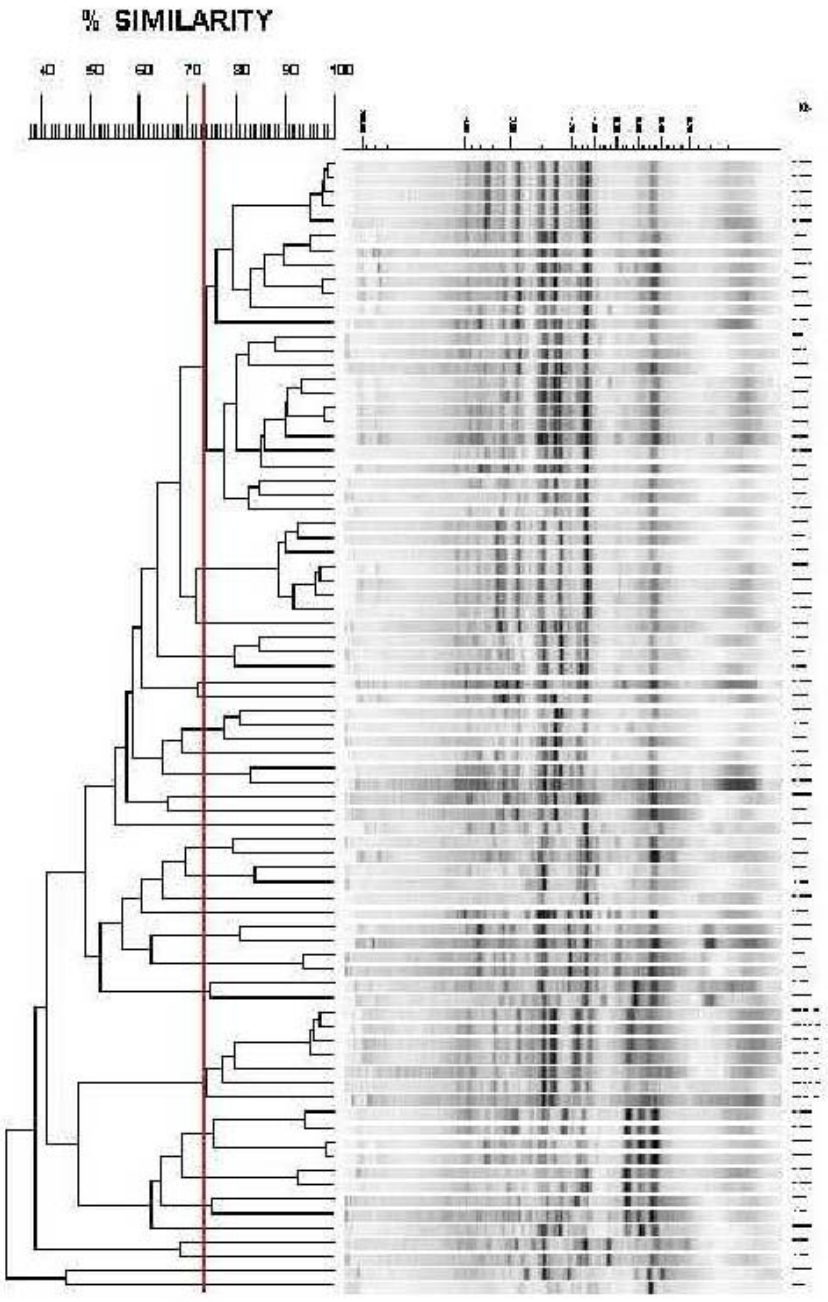


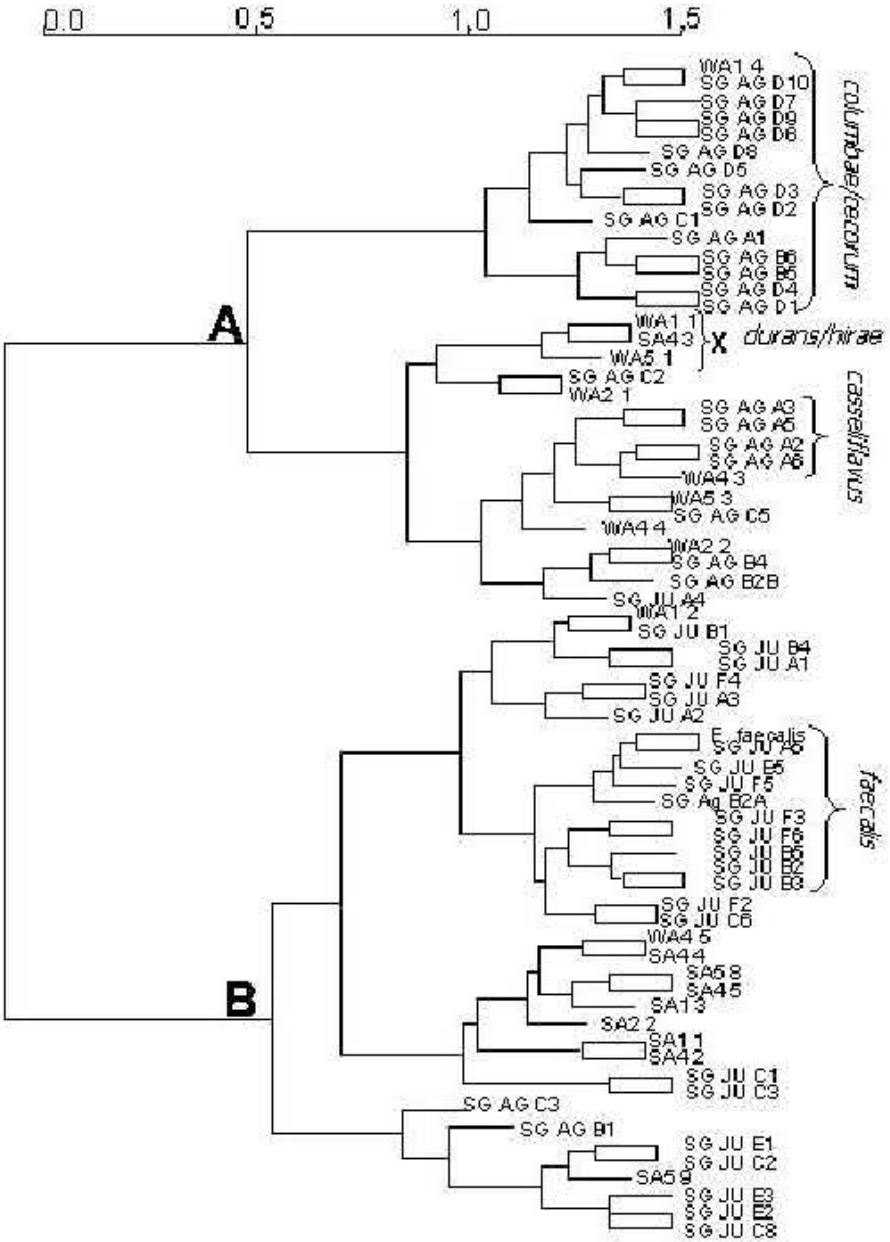
**Figure 32. Cluster diagram of rep-PCR DNA fingerprints for all August isolates.**



## ENTEROCOCCI PHENOTYPES

Enterococci were isolated from seagull feces in June and August and from water and sediment (June only) at the 63rd Street Beach (Figure 33). Enterococci numbers in water and sediment in August were very low, which precluded their isolation from the August samples. The enterococci grouped in two major clusters (A and B, Figure 33). Cluster A contains 22 (of 25) August seagull isolates, while Cluster B contains the majority of the June seagull isolates (23 of 24). Nine sediment isolates and 10 water isolates from June were also analyzed. The sediment isolates fall primarily (8 of 9) in Cluster B, and the water isolates fall primarily (9 of 10) into group A. Within their respective clusters, most of the June water and sediment isolates do not group closely with isolates from seagulls (see sub-group X Figure 33). The identities of selected sub-clusters are indicated on Figure 33. The most likely species for the June water and sediment group X suggests that they are indeed different from the closest related seagull isolate. Other groups equally separated in these clusters are also likely different species, however the test method employed does not permit a definitive naming of all enterococci species.

**Figure 33. Cluster diagram of enterococci from seagulls (June and August) and water and sediments (June).**



These results indicate that populations of enterococci in seagull feces were different for the June and August samplings. This result is consistent with the *E. coli* DNA-fingerprinting results for seagull isolates as discussed above. This would suggest that the differences in rep-PCR DNA fingerprints for seagull isolates on the two dates were most likely the result of true fecal bacterial population differences, and not an artifact of sampling. Another important finding from the enterococci tests is that June sediment and water isolates may arise largely from a source other than seagulls. This result is again consistent with the *E. coli* DNA-fingerprinting results from June, which showed very little correlation between *E. coli* in seagulls and those in beach water and sand on that date.

The numbers of enterococci in seagull feces did not vary substantially between the two dates (Table 22). However, the absence of enterococci in sediments and the lower numbers in water for August, as compared to June (Table 21), suggests that hydrologic or environmental conditions were different on the two dates and affected the enterococci differently than the *E. coli*. One possibility is that the enterococci are delivered to the sediments and water by a process that was present during or prior to the June sampling but not the August sampling. Since the enterococci in water and sediment in June were not similar to those in seagulls, we must assume there is another source of these bacteria to the system. That source may have been present (or had lingering effects) in June, but not in August.

*Evidence for other sources*

Water samples were analyzed for a variety of constituents associated with human wastewater (Table 23). These constituents include a variety of chemicals used (detergents, fumigants, solvents, polycyclic aromatic hydrocarbons, an insect repellent, fire-retardants); consumed (caffeine, cotinine—a by-product of nicotine); or produced (cholesterol, coprostanol) by humans. These constituents commonly occur in water influenced by human activities through the addition of storm water or sanitary waste. Results indicate that beach water contained some of these target chemicals on September 11, 2000. This sampling date followed a significant rainfall event. The results suggest that storm water runoff affects the beach water under such conditions. The chemicals detected are not especially suggestive of human sanitary waste since the detergents and human metabolites such as cholesterol and coprostanol were not detected. Rather, the combination of chemicals detected could conceivably arise from water washing off parking lots or picnic areas.

**Table 23. Concentrations of detected wastewater constituents in mg/L.**

| Sample ID | Caffeine | N,N,-diethyl-toluamide | Tri (2-chloroethyl) phosphate | Ethanol, 2-butoxy-, phosphate | 5-methyl-1H-benzotriazole | Triclosan | Cotinine |
|---|---|---|---|---|---|---|---|
| | (μg/L) | | | | | | |
| **LO** | nd | 0.138 | 0.060 | nd | 0.147 | nd | 0.052 |
| **1WA** | 0.618 | Nd | 0.052 | nd | nd | nd | nd |
| **2WA** | 0.588 | 0.135 | 0.052 | nd | nd | 0.070 | nd |
| **3WA** | nd | Nd | 0.052 | 1.6 | nd | nd | nd |
| **4WA** | 0.096 | Nd | 0.050 | nd | nd | 0.070 | nd |
| **5WA** | nd | Nd | nd | nd | nd | nd | nd |

Nd, no detection; N,N, diethyltoluamide, DEET (an insect repellant); tri (2-chloroethyl) phosphate (a fire retardant); ethanol, 2-butoxy-, phosphate (a plasticizer), 5-methyl-1H-benzotraizole (antioxidant used in antifreeze), triclosan (major ingredient of antibacterial soaps), cotinine (a by-product of nicotine metabolism).

These results suggest that *E. coli* isolates identical or very similar to those found in seagulls occur in beach water and beach sediment at the 63rd Street Beach. However, the impact of seagulls is apparently different on different dates, and seagulls did not account for the majority of beach water or sediment isolates on either of the two dates. Instead, approximately one-half the August beach water and sediment isolates and virtually all June beach water and sediment isolates appear to originate from other sources. This conclusion is also supported by the enterococci results. Finally, the presence of human-generated chemicals in beach water indicates that sources other than seagulls affect the system. Other sources may include:

- Storm-water runoff
- Bacteria associated with aquatic plants or detritus brought to the beach by long-shore drift
- Bacteria associated with septic or sewage waste brought to the site by long-shore currents that may originate from gray water waste from boats or domestic effluents
- Groundwater or through-flow inputs (e.g., seepage from the lagoon, or harbors or washing-in of bacteria on the "hill" separating the lagoon and beach which would then be transported to the shallow water table).

The hydrologic connection between the lagoon and/or harbor and specific sites on the beach through human infrastructure and geologic heterogeneities also may present potential contamination channels. In addition, there may be currents that affect the movement of water along the beach and may provide unanticipated connections between sites. Since the chemicals found are generally persistent, they may have been in the system for quite some time with origins that are relatively distant to Chicago.

Source determination sampling was conducted on two dates that represent average to low wave height for the 63rd Street Beach. Under such conditions, numbers of *E. coli* in foreshore beach sands may not be as strongly correlated with numbers in knee-deep water. In addition, seagull droppings are not correlated with the numbers of *E. coli* in foreshore sands or water. Finally, as demonstrated earlier, there appears to be a statistically significant lag time in the influence of seagulls on beach waters and sands. These factors and correlations were not known at the outset of the study, so could not have been included in the present sampling design. However, future studies should make use of the environmental information and should attempt to conduct source determination studies under more varying environmental conditions.

A number of scientific issues are also raised by this research. The difference in fecal bacterial populations on the two sampling dates was not anticipated, and indeed this research represents the first report of this phenomenon. This is an important finding that will allow better design of future source-determination studies. However, further information will be needed on the temporal scale over which fecal bacterial population shifts take place.

Secondly, the source determination studies have identified some *E. coli* and enterococci at 63rd Street Beach not related to those found in seagull feces. As discussed, these results are inconclusive and in need of more rigorous sampling. Further testing is warranted. The presence of chemicals indicative of human-influenced storm or wastewater in beach waters is significant. This is the first report of such detections in ambient lake waters and may indeed be common at many urban beaches. However, this influence will have to be taken into consideration as mitigation strategies to improve recreational water quality at the 63rd Street Beach are undertaken. Further investigation of the frequency of such detections and the source(s) of these chemicals may be warranted.

*E. coli* MAR

Antibiotic testing results (n=274) were grouped into four clusters. The vast majority of the isolates were susceptible to all antibiotic agents tested (n=254; ~93%). One group of isolates (n=8; ~3%) was susceptible to only one antibiotic (tetracycline) while another group (n=8; ~3%) was resistant only to Cephalothin. Four isolates (~1%) were resistant to multiple antibiotics. Of the isolates resistant to a single antibiotic agent, three were from seagulls (~1%), nine were from sediments (3.3%), and four were from water (1.5%). Of the isolates resistant to multiple antibiotic agents (MAR), two were from water (0.7%), and two were from sediment (0.7%); no isolates from seagulls were resistant to multiple antibiotic agents. No significant temporal trends were noted. Of the MAR isolates from water, both were from sources distant from the actual beach area (sample sites NB and LO). Of the MAR from sediment, both isolates, one from June and one from August, were from the sample site 2893 (5 Sediment A). There was some agreement between MAR results of the north revetment and lagoon outfall suggesting a linkage. We do not know if this implies a hydrological connection or reflects *E. coli* that is ubiquitous to the general area.

Multiple antibiotic resistance has been used to determine or speculate on host populations (human vs. animal) in an effort to determine sources of *E. coli* (Parveen *et al.* 1997, Harwood *et al.* 2000). Human resistance to single antibiotics varies by antibiotic and population sampled but there are typical expected resistances (Table 24). Multiple antibiotic resistance of *E. coli* from humans is typically considered to be more prevalent than multiple antibiotic resistance of *E. coli* from animal sources. The low percentage of ambient MAR *E. coli* coupled with the complete absence of seagull MAR supports the use of this parameter for source determination. It follows that the MAR results imply reduced frequency of human derived *E. coli*, which would intrinsically have higher MAR values.

**Table 24. Resistance to antibiotics.**

| Antibiotic agent | % *E. coli* susceptible |
|---|---|
| Amikacin | 99 |
| Amoxicillin-Clav. Acid | 82 |
| Ampicillin | 66 |
| Carbenicillin | 68 |
| Cefonicid | 94 |
| Ceftriaxone | 100 |
| Cephalothin | 88 |
| Ciprofloxacin | 97 |
| Gentamicin | 89 |
| Nalidixic Acid | 95 |
| Nitrofuntoin | 99 |
| Pipercillin | 91 |
| Tetracycline | 87 |
| Trimethoprim-sulfamethoxasole | 90 |

*E. coli* Biotyping

The biotyping cluster analysis results of the *E. coli* isolates shows that overall there are eleven distinct groups of isolates (with 2 isolates not grouping with other isolates) based on the significance of the Dice correlation coefficient for the phenotypic data. Generalizations on these data suggest that the *E. coli* isolates from this study are phenotypically well distributed. Within the two main clusters there is fairly equal distribution between the three sources of isolates

(water, sediment, and seagull). One cluster is composed entirely of isolates from seagulls (n=6; ~2%). Three primary phenotypic clusters form when examining the seagull isolates alone: two of which do not cluster with any other isolates. The clusters support the suggestion that a temporal distribution of isolates exists because one cluster is predominately from the August samples (~74%), another is primarily from June samples (~63%), and the other cluster is solely from June samples.
Applying similar analysis to June sampling, there are four distinct clusters of *E. coli*. The first cluster is solely composed of isolates from seagulls (the same isolates that were clustered in the cluster of the seagull isolates) whereas other clusters were heterogeneous with all three sources of isolates represented.
In the cluster analysis of the isolates from the August sampling, there were five distinct clusters of *E. coli*. Within the two predominant clusters, representation of all three sources of isolates was again seen.

There were 38 distinct biotyping patterns based on the phenotypic data from these isolates. While the overall phenotypic data permutations that would result in an identity of *E. coli* is not known, it is suspected that these biotypes represent only a fraction of the possible *E. coli* biotypes possible. The biotyping data show that *E. coli* from the seagulls constitutes a significant portion (typically one-third) of each of the groups of *E. coli* isolates, consistent with their overall contribution to the data. Only one group (n=2) did not have a phenotypic pattern consistent with an *E. coli* isolated from a seagull. This suggests that other sources of *E. coli*, such as human sources, do not seem to be uniquely represented in these data. This lends strong support to the hypothesis that seagulls are a significant source of *E. coli* in this location.

Antibiotic resistance testing resulted in the determination that all *Salmonella* isolated were susceptible to all antibiotics tested.

*Salmonella* was isolated from samples representative of each sample type collected. Of the approximately 80 fecal samples collected, ten isolates of *Salmonella* were recovered. From the five samples of water, four yielded isolates of *Salmonella*. From ten samples of sediment, five isolates of *Salmonella* were obtained. On serotyping the isolates, only one serotype was obtained: *Salmonella enterica* subsp. *enterica* ser. Typhimurium. *S.* Typhimurium is the second most predominant isolate associated with human gastroenteritis, accounting for typically 20% of all reported salmonellosis outbreaks (Koneman et al. 1997). In wildlife, *S.* Typhimurium has typically accounted for over 40% of the wildlife mortality due to salmonellosis (NWHC unpublished data). With over 2000 serotypes of *Salmonella* and *S.* Typhimurium typically accounting for only about 20% of the human cases, the isolation of only *S.* Typhimurium from all the samples (although probably not statistically significant) probably indicates contributing sources.

The cluster analysis results of the *Salmonella* isolates from the 63rd Street Beach shows that there are three distinct groups of isolates at the $85% coefficient (Figure 34). Typically, a Dice coefficient of $90% is considered to have good genetic relatedness, however, this analysis suggested a broader latitude in determining relatedness. All the *Salmonella* isolated from sediment samples (n=5) grouped well within the first group. The *Salmonella* isolated from the water (n=4), however, was split equally between two groups. The *Salmonella* isolated from

seagulls (n=10) were represented with isolates in all three groups and included one isolate that was distinct enough to not group with any other isolates. Based on the diversity of *S.* Typhimurium isolated from seagulls, the similarity of seagull isolates to isolates obtained from the water and sediment strongly suggest that seagulls are potentially the source of *Salmonella* isolated from the water and sediment samples. Although *Salmonella* spp. have been reported to remain viable for long periods of time in water, it is most likely, given the similarity of isolates within each group, that the sediment and water *Salmonella* isolates originated from the seagulls frequenting the beach.

The cluster analysis of the Chicago derived *Salmonella* isolates suggests three groups of genetically related *S.* Typhimurium, but the strength of those relationships must be determined. Water samples from the area in and around Indiana Dunes were also tested for the presence of *Salmonella* spp. From the samples submitted, seven isolates of *S.* Typhimurium were obtained, including a repeat sample from the 63rd Street area. In addition, *S.* Typhimurium isolates from diverse human sources (Wisconsin State Laboratory of Hygiene, Madison, WI) and other sources including wildlife (NWHC) were also included in a cluster analysis. Using a Dice coefficient of $85%, five related clusters were evident with five isolates that were not closely related to other isolates (Figure 35). One cluster was not previously seen, based on the Chicago isolates alone, and includes only wildlife isolates not obtained as part of the study. One sediment and one water isolate from the Chicago isolates that previously grouped with the sediment and seagull (Figure 34, group I) were shifted into a cluster that was strong enough to stand alone (Figure 35, group IV) and was composed of isolates obtained from both wildlife and Indiana Dunes. Interestingly, however, a water sample obtained from the 63rd Street site included with the Indiana Dunes samples also clustered in this group, significantly away from the other water and sediment samples. The inclusion of *S.* Typhimurium from human cases of salmonellosis, however, leaves open the possibility of human source contamination of the Chicago beach, either through beach users or through sewage contamination. Of six distinct human isolates included, four clustered with the isolates from water and seagulls (Figure 35, group IV). While contamination of the Chicago beach with human sources of *S.* Typhimurium is a possibility, it is not considered the most likely because: 1) humans are not typically considered reservoirs on *Salmonella* (except *S. typhi*) (Bopp et al. 1999, Koneman et al. 1997) 2) human salmonellosis is not restricted to a single serotype such as *S.* Typhimurium 3) salmonellosis from seagulls has been previously well established (Levesque et al. 2000, Literak et al. 1992).

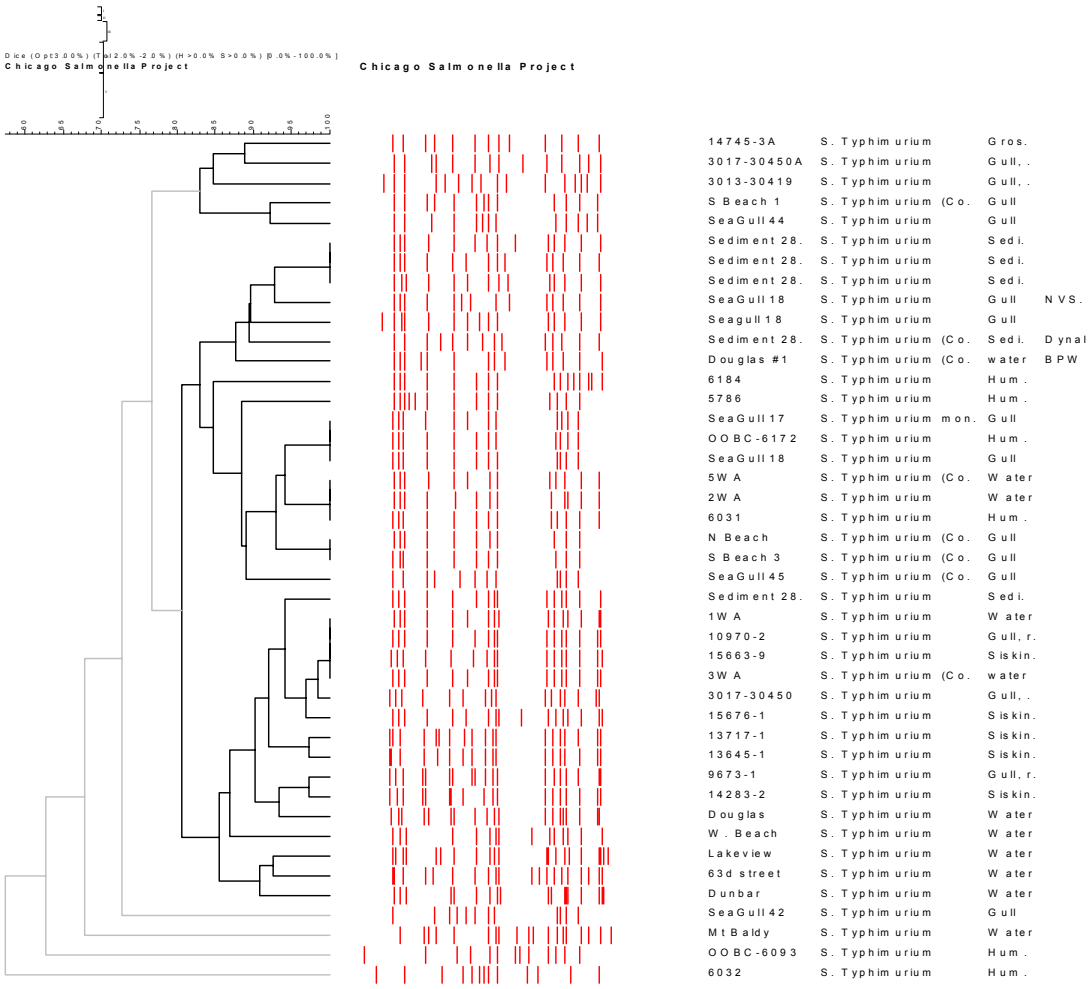
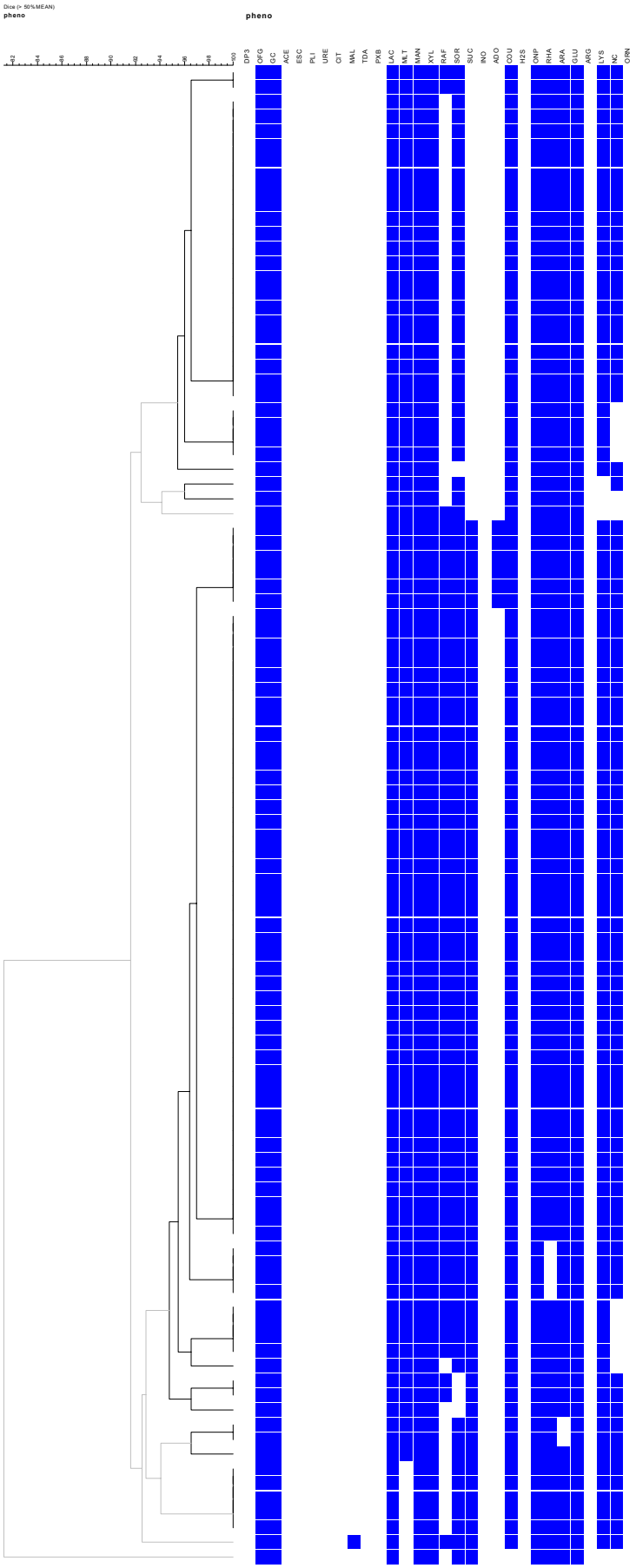



**Figure 34. *Salmonella* isolates from 63rd Street Beach.**




| | | | | | |
|---|---|---|---|---|---|
| . 1WA | . . . E. coli | sorb. - (. | . water | Aug | Yes |
| . 4WA | . . . E. coli | | . water | Aug | Yes |
| . SGD-2 | . . E. coli | | . gull | June | Yes |
| . SGD-5 | . . E. coli | | . gull | June | Yes |
| . SGD-4 | . . E. coli | | . gull | June | Yes |
| . SGD-6 | . . E. coli | | . gull | June | Yes |
| . SGD-7 | . . E. coli | | . gull | June | Yes |
| . 4SA | . . . E. coli | 2892 | . sediment | Aug | Yes |
| . 4SA | . . . E. coli | 2892 | . sediment | Aug | Yes |
| . 5WA | . . . E. coli | | . water | Aug | Yes |
| . 5WA | . . . E. coli | | . water | Aug | Yes |
| . 2SA-10 | . . . E. coli | | . sediment | June | Yes |
| . 1SA-9 | . . . E. coli | | . sediment | June | Yes |
| . 1SA-8 | . . . E. coli | | . sediment | June | Yes |
| . SGC-AG-2 | . . . E. coli | | . gull | Aug | Yes |
| . SGC-AG-5 | . . . E. coli | | . gull | Aug | Yes |
| . SGC-AG-4 | . . . E. coli | | . gull | Aug | Yes |
| . 1SA-10 | . . E. coli | | . sediment | June | Yes |
| . 1SA-6 | . . E. coli | | . sediment | June | Yes |
| . 1WA-3 | . . E. coli | | . water | June | Yes |
| . 4SA-5 | . . E. coli | | . sediment | June | Yes |
| . SGB-1 | . . E. coli | | . gull | June | Yes |
| . SGC-1 | . . E. coli | | . gull | June | Yes |
| . 2WA | . . . E. coli | | . water | Aug | Yes |
| . 5WA | . . . E. coli | no resis. | . water | Aug | Yes |
| . 4WA-8 | . . E. coli | | . water | June | Yes |
| . 5SA-9 | . . E. coli | | . sediment | June | Yes |
| . 2WA | . . . E. coli | sorb. - (. | . water | Aug | Yes |
| . 5SA | . . . E. coli | 2893 | . sediment | Aug | Yes |
| . 5SA-8 | . . E. coli | | . sediment | June | Yes |
| . SGB-3 | . . E. coli | | . gull | June | Yes |
| . SGB-AG-5 | . . . E. coli | | . gull | Aug | Yes |
| . SGC-AG-1 | . . . E. coli | | . gull | Aug | Yes |
| . SGB-AG-3 | . . . E. coli | | . gull | Aug | Yes |
| . SGC-AG-8 | . . . E. coli | | . gull | Aug | Yes |
| . 2SA-9 | . . E. coli | | . sediment | June | Yes |
| . 4WA-7 | . . E. coli | no resis. | . water | June | Yes |
| . 2SA | . . . E. coli | 2890 | . sediment | Aug | Yes |
| . 1SA | . . . E. coli | 2889 | . sediment | Aug | Yes |
| . 4SA | . . . E. coli | 2892 | . sediment | Aug | Yes |
| . 4SA | . . . E. coli | 2892 | . sediment | Aug | Yes |
| . 4SA | . . . E. coli | 2892 | . sediment | Aug | Yes |
| . 4SA | . . . E. coli | | . sediment | Aug | Yes |
| . 5SA | . . . E. coli | 2893 | . sediment | Aug | Yes |
| . 2SA | . . . E. coli | 2890 | . sediment | Aug | Yes |
| . 1WA | . . . E. coli | | . water | Aug | Yes |
| . 1WA | . . . E. coli | | . water | Aug | Yes |
| . 2WA | . . . E. coli | | . water | Aug | Yes |
| . 2WA | . . . E. coli | | . water | Aug | Yes |
| . 4WA | . . . E. coli | | . water | Aug | Yes |
| . 4WA | . . . E. coli | | . water | Aug | Yes |
| . 5WA | . . . E. coli | | . water | Aug | Yes |
| . 5WA | . . . E. coli | | . water | Aug | Yes |
| . 5SA-6 | . . . E. coli | | . sediment | June | Yes |
| . SGA-AG-1 | . . . E. coli | | . gull | Aug | Yes |
| . SGA-AG-5 | . . . E. coli | | . gull | Aug | Yes |
| . SGA-AG-4 | . . . E. coli | | . gull | Aug | Yes |
| . SGA-AG-7 | . . . E. coli | | . gull | Aug | Yes |
| . SGA-AG-8 | . . . E. coli | | . gull | Aug | Yes |
| . SGA-AG-6 | . . . E. coli | | . gull | Aug | Yes |
| . SGB-AG-2 | . . . E. coli | | . gull | Aug | Yes |
| . SGB-AG-4 | . . . E. coli | | . gull | Aug | Yes |
| . SGB-AG-6 | . . . E. coli | | . gull | Aug | Yes |
| . SGA-AG-2 | . . . E. coli | | . gull | Aug | Yes |
| . 1SA-7 | . . E. coli | | . sediment | June | Yes |
| . 1WA-1 | . . E. coli | no resis. | . water | June | Yes |
| . 2WA-4 | . . E. coli | | . water | June | Yes |
| . 4SA-11 | . . E. coli | | . sediment | June | Yes |
| . 2WA-2 | . . E. coli | | . water | June | Yes |
| . 4SA-9 | . . E. coli | | . sediment | June | Yes |
| . 4WA-4 | . . E. coli | | . water | June | Yes |
| . 5SA-4 | . . E. coli | | . sediment | June | Yes |
| . 5WA-4 | . . E. coli | | . water | June | Yes |
| . Sediment, Gull. | . . E. coli | | . sediment | June | Yes |
| . Sediment, Gull. | . . E. coli | | . sediment | June | Yes |
| . SGA-3 | . . E. coli | | . gull | June | Yes |
| . SGA-2 | . . E. coli | | . gull | June | Yes |
| . SGA-1 | . . E. coli | | . gull | June | Yes |
| . SGB-4 | . . E. coli | | . gull | June | Yes |
| . Sediment, Gull. | . . E. coli | | . sediment | June | Yes |
| . 4WA-3 | . . E. coli | | . water | June | Yes |
| . 4WA-2 | . . E. coli | | . water | June | Yes |
| . 4WA-6 | . . E. coli | | . water | June | Yes |
| . 4WA-5 | . . E. coli | | . water | June | Yes |
| . SGD-1 | . . E. coli | | . gull | June | Yes |
| . 1WA-4 | . . E. coli | | . water | June | Yes |
| . 5SA-2 | . . E. coli | | . sediment | June | Yes |
| . Sediment, Gull. | . . E. coli | | . sediment | June | Yes |
| . 5SA | . . . E. coli | 2893 | . sediment | Aug | Yes |
| . 5SA-7 | . . E. coli | O157:H. | . sediment | June | Yes |
| . 1WA-2 | . . E. coli | possible. | . water | June | Yes |
| . 4WA | . . . E. coli | | . water | Aug | Yes |
| . 2WA | . . . E. coli | | . water | Aug | Yes |
| . 4WA | . . . E. coli | | . water | Aug | Yes |
| . SGA-AG-3 | . . . E. coli | | . gull | Aug | Yes |
| . SGC-4 | . . E. coli | | . gull | June | Yes |
| . SGC-6 | . . E. coli | | . gull | June | Yes |
| . SGC-7 | . . E. coli | | . gull | June | Yes |
| . SGC-5 | . . E. coli | | . gull | June | Yes |
| . SGC-2 | . . E. coli | | . gull | June | Yes |
| . SGC-3 | . . E. coli | | . gull | June | Yes |
| . 4SA | . . . E. coli | 2892 | . sediment | Aug | Yes |

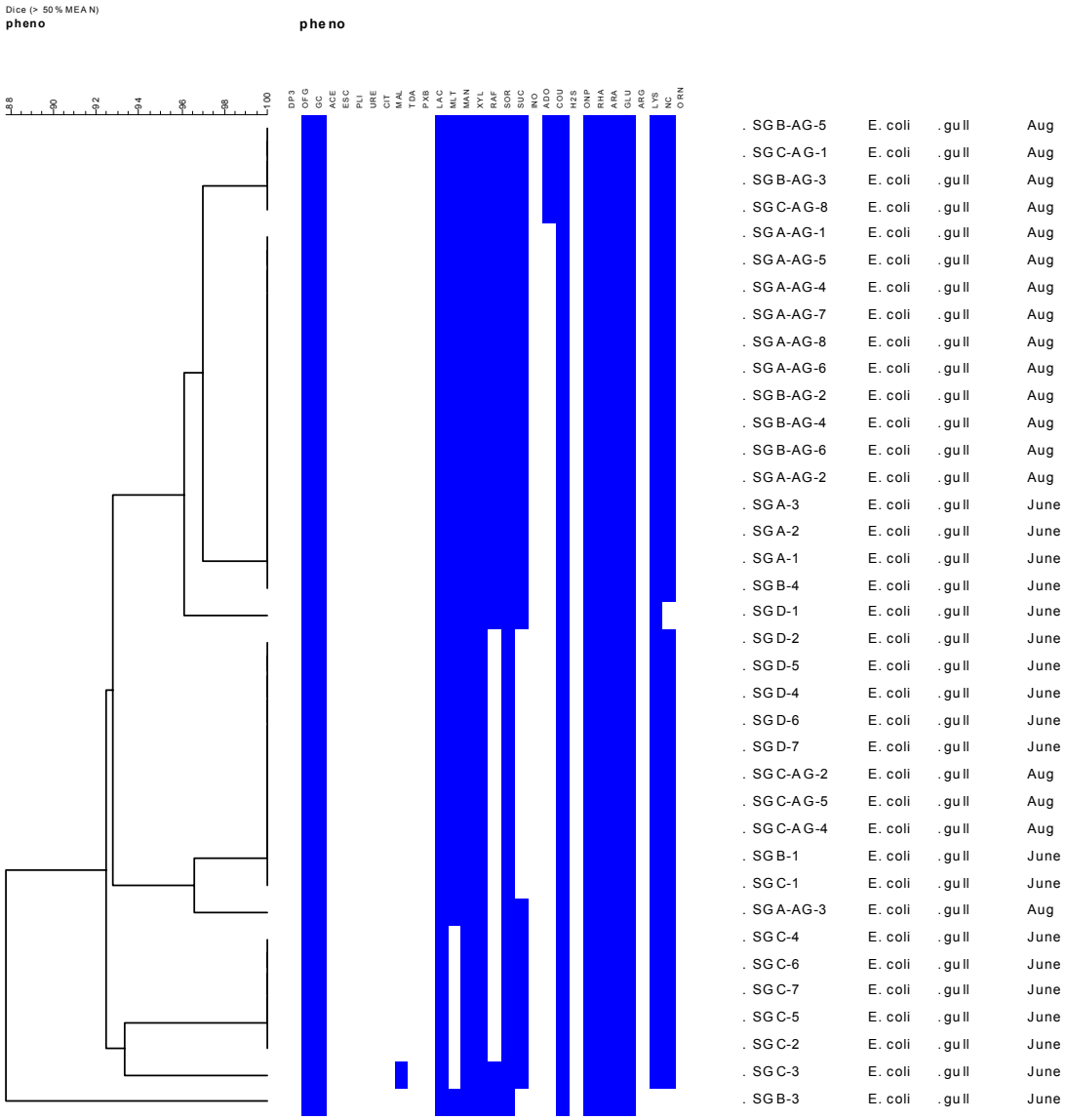



**Figure 35. Cluster analysis of water samples from near the Indiana Dunes.**

## CONCLUSIONS

1. The statistical distribution of *E. coli* concentration does not tend to be normal. Normalization was improved when data were partitioned by high and low wave conditions.
2. *E. coli* concentrations in foreshore sands were higher than in submerged sands that in turn were higher than in water samples. Sands taken near gull flocks were highest in *E. coli*.
3. Shallow water (45 cm depth) had higher *E. coli* than deeper water (90 cm). Offshore water generally had lower *E. coli* concentrations than nearshore water.
4. Replicate sampling shows that a high degree of variation can be expected in *E. coli* samples, especially during periods of increased concentrations. In general, taking ten samples is adequate to achieve a value within a relative error of 30% of the mean most of the time.
5. In general, populations of *E. coli* rapidly decrease during the morning and early afternoon in an exponential pattern. There is a significant difference in morning and afternoon *E. coli* levels. The decrease appears to be caused by exposure to sunlight.
6. Morning, afternoon, 45 cm, and 90 cm water *E. coli* concentrations were generally correlated with one another. Foreshore and submerged sands were correlated with each other.
7. Stepwise regression shows that foreshore sand was the best prediction factor for 45 or 90 cm AM water *E. coli* concentrations, accounting for about 43% of the variation.
8. Water moves into the lake bed a few meters offshore but wells up along the narrow band in the swash zone at 63rd Street Beach
9. The *E. coli* content of the upwelling water varies considerably depending on wave conditions and retrieval technique. More information is needed to offer generalities about the contribution of upwelling water to beach *E. coli* levels.
10. *E. coli* concentrations in submerged and foreshore sands are more closely associated with water *E. coli* concentrations during periods of increased wave height (>9 cm).
11. When number of gulls is lagged by one day it correlates significantly with *E. coli* in 45 and 90 cm water.
12. Morphology of 63rd Street Beach has embayment conditions that may affect *E. coli* loading and retention.
13. Seagulls showed no spatial preference on the beach, although there were some seasonal tendencies. When seagull counts were lagged by a day, their densities were correlated with water and foreshore sand *E. coli* concentration.
14. No significant relationships existed between number of bathers and *E. coli* concentrations for the weekdays inspected.
15. *E. coli* concentrations in Jackson Harbor sands were not particularly high, suggesting that this bottom material is not an important reservoir of *E. coli*.
16. DNA fingerprinting of *Salmonella* spp. isolates from sand and water show a reasonably good match with gull feces isolates, but other birds also could act as *Salmonella* vectors.
17. *E. coli* and *Salmonella* isolates were highly susceptible to all antibiotics tested. This reduces the discrimination capacity of the tests but also increases the possibility that the *E. coli* isolates originate generally from non-human sources.

18. *E. coli* fingerprinting suggests that seagulls contribute to bacteria in the water and sand but that *E. coli* also comes from other sources. *E. coli* populations varied substantially between the two times tested. Enterococci fingerprinting results were consistent with conclusions reached with *E. coli* observations.
19. Anthropogenic biochemicals found in 63rd Street beach water may implicate storm or wastewater inputs, but not necessarily direct sewage introduction.
20. A statistical model was developed that successfully predicted regulatory monitoring exceedances for *E. coli* concentrations 79% of the time.
21. Remediation options need to be evaluated carefully by hydrologists and engineers working closely with environmental scientists.
22. 63rd Street Beach *E. coli* problem needs to be examined within the context of the entire Chicago lakefront in order to understand its idiosyncrasies.

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