Use of Spatial Sampling and Microbial Source-Tracking Tools for Understanding Fecal Contamination at Two Lake Erie Beaches

By Donna S. Francy, Erin E. Bertke, Dennis P. Finnegan, Christopher M. Kephart, Rodney A. Sheets, John Rhoades, and Lester Stumpe

In Cooperation with the Northeast Ohio Regional Sewer District and Ohio Water Development Authority

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Conversion Factors and Abbreviations

Multiply	By	To obtain
	Length	
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
inch (in.)	25.4	millimeter (mm)
foot (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)
	Volume	
milliliter (mL)	0.06102	cubic inch (in^3)
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

°F=1.8(°C) + 32

Additional abbreviations

In addition to grams, masses are given in micrograms (µg) and nanograms (ng), which are 10^{-6} and 10^{-9} gram, respectively.

In addition to milliliters, volumes are given in microliters (µL), which are 10^{-6} liter (10^{-3} milliliter).

Concentrations for certain polymerase-chain-reaction reagents are given as millimolar (m*M*) and micromolar (µ*M*).

Concentrations of bacteria are given in colony-forming units per 100 milliliters (CFU/100 mL), Most Probable Number per 100 milliliters (MPN/100 mL), colony-forming units per gram dry weight of sediment (CFU/g_{DW}), and colony-forming units per gram (CFU/g).

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Use of Spatial Sampling and Microbial Source-Tracking Tools for Understanding Fecal Contamination at Two Lake Erie Beaches

By Donna S. Francy, Erin E. Bertke, Dennis P. Finnegan, Christopher M. Kephart, Rodney A. Sheets, John Rhoades[1], and Lester Stumpe[1]

Abstract

Source-tracking tools were used to identify potential sources of fecal contamination at two Lake Erie bathing beaches: an urban beach (Edgewater in Cleveland, Ohio) and a beach in a small city (Lakeshore in Ashtabula, Ohio). These tools included identifying spatial patterns of *Escherichia coli* (*E. coli*) concentrations in each area, determining weather patterns that caused elevated *E. coli*, and applying microbial source tracking (MST) techniques to specific sites. Three MST methods were used during this study: multiple antibiotic resistance (MAR) indexing of *E. coli* isolates and the presence of human-specific genetic markers within two types of bacteria, the genus *Bacteroides* and the species *Enterococcus faecium*.

At Edgewater, sampling for *E. coli* was done during 2003–05 at bathing-area sites, at nearshore lake sites, and in shallow ground water in foreshore and backshore areas. Spatial sampling at nearshore lake sites showed that fecal contamination was most likely of local origin; *E. coli* concentrations near the mouths of rivers and outfalls remote to the beach were elevated (greater than 235 colony-forming units per 100 milliliters (CFU/100 mL)) but decreased along transport pathways to the beach. In addition, *E. coli* concentrations were generally highest in bathing-area samples collected at 1- and 2-foot water depths, midrange at 3-foot depths, and lowest in nearshore lake samples typically collected 150 feet from the shoreline. Elevated *E. coli* concentrations at bathing-area sites were generally associated with increased wave heights and rainfall, but not always. *E. coli* concentrations were often elevated in shallow ground-water samples, especially in samples collected less than 10 feet from the edge of water (near foreshore area). The interaction of shallow ground water and waves may be a mechanism of *E. coli* storage and accumulation in foreshore sands. Infiltration of bird feces through sand with surface water from rainfall and high waves may be concentrating *E. coli* in shallow ground water in foreshore and backshore sands.

At Lakeshore, sampling for *E. coli* was done at bathing-area, nearshore lake, and parking-lot sites during 2004–05. Low concentrations of *E. coli* at nearshore lake sites furthest from the shoreline indicated that fecal contamination was most likely of local origin. High concentrations of *E. coli* in water and bed sediments at several nearshore lake sites showed that contamination was emanating from several points along the shoreline during wet and dry weather, including the boat ramp, an area near the pond drainage, and parking-lot sediments. Physical evidence confirmed that runoff from the parking lot leads to degradation of water quality at the beach.

MST samples were collected to help interpret spatial findings and determine whether sources of fecal contamination were from wastewater or bird feces and if a human-specific marker was present. MAR indices were useful in distinguishing between bird feces and wastewater sources because they were about 10 times higher in the latter. The results from MAR indices agreed with results from the two human-specific markers in some but not all of the samples tested. *Bacteroides* and enterococci human-specific markers were found on one day at Edgewater and two days at Lakeshore. On three days at Edgewater and two days at Lakeshore, the MAR index indicated a mixed source, but neither marker was found in bathing-water samples; this may be because bacterial indicator concentrations were too low to detect a marker.

Multiple tools are needed to help identify sources of fecal contamination at coastal beaches. Spatial sampling identified patterns in *E. coli* concentrations and yielded information on the physical pathways of contamination. MST methods provided information on whether the source was likely of human or nonhuman origin only; however, MST did not provide information on the pathways of contamination.

Introduction

To protect the health of swimmers, managers of beach recreational areas issue advisories or closings on the basis

[1]Northeast Ohio Regional Sewer District.

of standards for concentrations of bacterial indicator organisms. For freshwater beaches, *Escherichia coli* (*E. coli*) is the indicator most commonly used to assess recreational water quality. Collecting one or two daily samples for the enumeration of *E. coli* provides information on the occurrence but not the sources of fecal contamination; therefore, water-resource managers lack information on identified targets for effective mitigation measures.

Sources of fecal contamination that trigger most beach closings and advisories in the United States remain unknown (Natural Resources Defense Council, 2005). Frequently suspected sources include combined- and sanitary-sewer overflows; treated wastewater effluents; effluents from private sewage-treatment systems, including septic tanks; fecal pollution from birds, swimmers, or boaters; and stormwater runoff. Identifying and mitigating the source of fecal contamination to a particular beach is often complicated by the spatial and temporal variability of bacterial-indicator concentrations and the dynamic lake currents, weather patterns, and natural processes that affect these concentrations. In addition, many of the sources are of nonpoint origin and not easily identified.

Because of the complexity of coastal environments, a multiple-method approach seems practical at many beaches. At a California beach, for example, investigators used a three-tiered approach to discover that multiple sources of fecal contamination, including human sewage, affected recreational water quality (Boehm and others, 2003). The first two tiers documented the spatial and temporal variability of fecal-indicator bacteria pollution throughout the study area and in suspected sources. The third tier used microbial source tracking (MST) techniques and identified human-specific chemical and biological fecal indicators that corroborated earlier findings. At a bathing beach in northwestern Ohio, spatial patterns of *E. coli* and environmental factors were used in combination to identify sources of fecal contamination (Francy, Struffolino, and others, 2005). The results of the study implicated a ditch that discharges 250 ft east of the beach as a principal source of *E. coli*, and not sources remote to the beach.

A wide range of techniques are available in the emerging field of MST to help identify sources of fecal contamination. Because all MST methods have distinct advantages and disadvantages (Meays and others, 2004), a single approach may not be adequate (Bower and others, 2005). Which methods are chosen for a study depend on the study objectives, the number of potential contamination sources, and the funds available (Meays and others, 2004). At coastal beaches along Lake Erie and elsewhere, the question is often whether the source is human or nonhuman, the nonhuman source being predominantly waterfowl.

Three MST methods were used during this study: multiple antibiotic resistance (MAR) indexing of *E. coli* isolates and the presence of human-specific genetic markers within two types of bacteria, the genus *Bacteroides* and the species *Enterococcus faecium*. MAR is a phenotypic method that has been used to differentiate bacteria from different sources by use of antibiotics commonly associated with human and animal therapy. MAR indexing is based on the percentage of bacteria resistant to a panel of antibiotics. Because humans are exposed to antibiotics, the *E. coli* they harbor will likely be more resistant to antibiotics than those *E. coli* found in the gastrointestinal tracts of wildlife. MAR indexing of *E. coli* isolates has been shown to discriminate among nonhuman and human sources in the food industry (Krumperman, 1983) and in agriculture (Guan and others, 2002) and to characterize surface waters (Parveen and others, 1997; Kaspar and others, 1990; Webster and others, 2004), but it has not been used extensively at beaches. The other two methods detect genetic markers of organisms found in specific hosts. Detection of host-specific genetic markers circumvents the need for culturing bacteria and establishing an extensive host database and seems practical and cost effective for identifying sources at beaches. Bernhard and Field (2000a, b) identified host-specific genetic markers for *Bacteroides*, a genus of bacteria that are strict anaerobes (do not grow in the presence of oxygen) and make up a significant proportion of fecal bacteria in warmblooded animals. Molecular methods are used to target portions of the *Bacteroides* genetic material that are specifically found in *Bacteroides* from human sources. In other research, *Bacteroides* markers were found in association with suspected sources of contamination in Lake Michigan waters, including detections of the human marker at three out of seven bathing beaches (Bower and others, 2005). Another method targets a genetic marker that is a suspected virulence factor in *Enterococcus faecium*—the enterococcal surface protein (*esp*)—found in enterococci isolated from human sources. In a study of composited fecal and wastewater samples from Florida, Michigan, and Arizona, the *Enterococcus faecium esp* gene was detected in 97 percent of sewage and septic-system samples but was not detected in bird or other nonhuman fecal samples (Scott and others, 2005). It was detected in freshwater and simulated seawater samples when culturable enterococci were above 70 CFU/100 mL (Scott and others, 2005).

This report describes results of a study by the U.S. Geological Survey, Ohio Water Science Center (OWSC) in cooperation with the Northeast Ohio Regional Sewer District (NEORSD) and the Ohio Water Development Authority, investigating the use of various tools to identify potential sources of fecal contamination at two Lake Erie bathing beaches. Data on spatial patterns of *E. coli* at nearshore lake sites, in the beach area, at inland sites, and in shallow ground water led to identifying possible sources of fecal contamination. The spatial data were then used to select and target MST methods to specific areas, thereby making the best use of available time and resources. The source-tracking techniques used at Lake Erie beaches can be applied by managers in other coastal areas to address water-quality issues for local beaches.

Study Areas

Studies were done at two Lake Erie beaches in northeast Ohio: Edgewater, in Cleveland, Ohio; and Lakeshore, in Ashtabula, Ohio.

Edgewater

Edgewater is an urban recreational area in Cleveland, Ohio (fig. 1*A*). Edgewater is between the Rocky and Cuyahoga Rivers, both of which receive effluents from wastewater-treatment plants and septic systems, and inputs from combined-sewer overflows (CSOs) and separate storm sewers. Water quality at Edgewater is also potentially influenced by local sources (the park and the immediate area shown on fig. 1*B*) including the Edgewater outfall, stormwater runoff from the park, pets and wildlife (waterfowl and raccoons), and discharges from long-abandoned storm sewers. Discharge from the Edgewater outfall consists of stormwater runoff and CSOs; it flowed once during the recreational season of 2004 (July 31) and twice during 2005 (June 10 and July 21). Other mechanisms of contamination may be release of accumulated *E. coli* from foreshore sands (sands infiltrated by lake water at some time during the season) and (or) resuspension of *E. coli* from bed sediments underlying bathing waters (Francy and Darner, 1998). Previous studies at Edgewater showed that *E. coli* concentrations in foreshore sands and lakebed sediments were as high as 3,000 and 300 CFU/g_{dw}, respectively (Francy and others, 2003). The Main Beach is a popular swimming area that includes 800 ft of guarded beach (fig. 1*B*). The Middle Beach is unguarded, less often used by swimmers, and used by boaters and dog owners with their pets. A groin to the west and breakwater to the east of the Main Beach impede the flow of longshore currents. During 2004 and 2005, water-quality advisories were posted at Edgewater on 9 and 5 days, respectively (Ohio Department of Health, 2006).

Lakeshore

Lakeshore (fig. 2) is a popular bathing beach and recreational area in a small municipality. The bathing beach is adjacent to a paved parking lot and downgradient from a gravel parking lot. Potential sources of fecal contamination include a wastewater-treatment plant to the west of Pinney Dock and local sources. Local sources in the park include wildlife—mainly waterfowl, which frequent the beach and inland ponds—drainage from inland ponds, and runoff from parking lots and grassy areas. Other potential local sources include septic tanks and sanitary sewerlines that serve the small community uphill approximately 500 ft south of the beach, as well as illegal discharges from boats. An overflow line drains a catchment that accumulates stormwater from this community and discharges into the lake near the west groin. Like that at Edgewater, the beach at Lakeshore is surrounded by groins and breakwaters that impede the flow of currents. During 2004 and 2005, water-quality advisories were posted at Lakeshore on 42 and 5 days, respectively (Ohio Department of Health, 2006).

Methods

Data were collected during the recreational seasons (May through September) of 2003, 2004, and 2005 to include a range of conditions: during dry, calm weather; after a light or heavy rainfall; and during increased wave heights.

E. coli concentrations were used to monitor recreational water quality. The Ohio bathing-water single-sample maximum of 235 CFU/100 mL was used as a benchmark to evaluate water quality in this report. For water-quality standards attainment, this standard cannot be exceeded in more than 10 percent of the samples collected in a 30-day period (Ohio Environmental Protection Agency, 2003). This standard has been used as a single-sample maximum for beach-notification decisions in Ohio, effective December 2005 (U.S. Environmental Protection Agency, 2004). Before December 2005, the geometric mean of 126 CFU/100 mL was used for beach-notification decisions in Ohio.

Sampling Frequencies and Locations

At Edgewater, NEORSD collected samples for spatial studies at nearshore lake sites in 2003 and 2004 and at bathing-area sites in 2004 and 2005; the USGS assisted with spatial studies at bathing-area sites and collected samples for shallow-ground-water studies in 2004 and 2005. At Lakeshore, the USGS conducted spatial sampling at bathing-area and nearshore lake sites in 2004 and 2005; parking-lot sites were included in 2005. Samples for MST were collected by the USGS in 2005.

Spatial Sampling at Edgewater

Water samples were collected at nearshore lake (fig. 1*A*) and bathing-area (fig. 1*B*) sites as follows:

- Eight nearshore lake sites (J–Q, about 150 ft from the shoreline)—13 days in 2003 and 6 days in 2004 by use of a boat
- Two bathing-area sites at Main Beach (C and F, 3-ft water depths)—Monday through Friday in 2003–05 as part of the daily beach monitoring program
- Four additional bathing-area sites at Main Beach (A, B, D, and E, 1- and 2-ft water depths)—14 days in 2005
- Three bathing-area sites at Middle Beach (G, H, and I, 3-ft water depths)—48 days during 2005

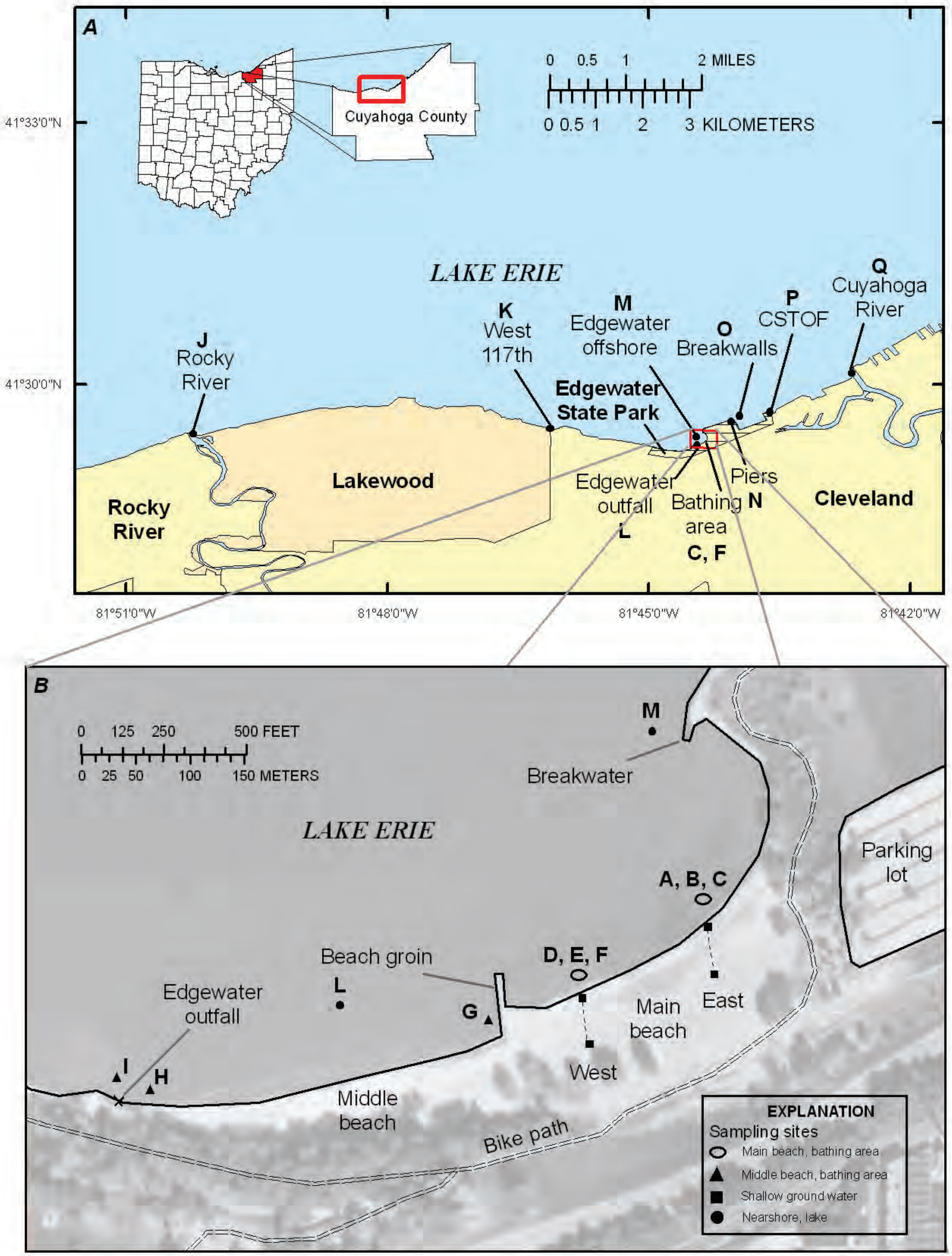

Figure 1. Location of Edgewater, Cleveland, Ohio. *A*, Nearshore lake sampling sites. *B*, Bathing-area and shallow ground-water sampling sites, 2003–2005. (CSTOF is Combined Sewer Treatment Overflow Facility. Bathing-area sites were defined by water depth (3 feet) and not by physical location.)

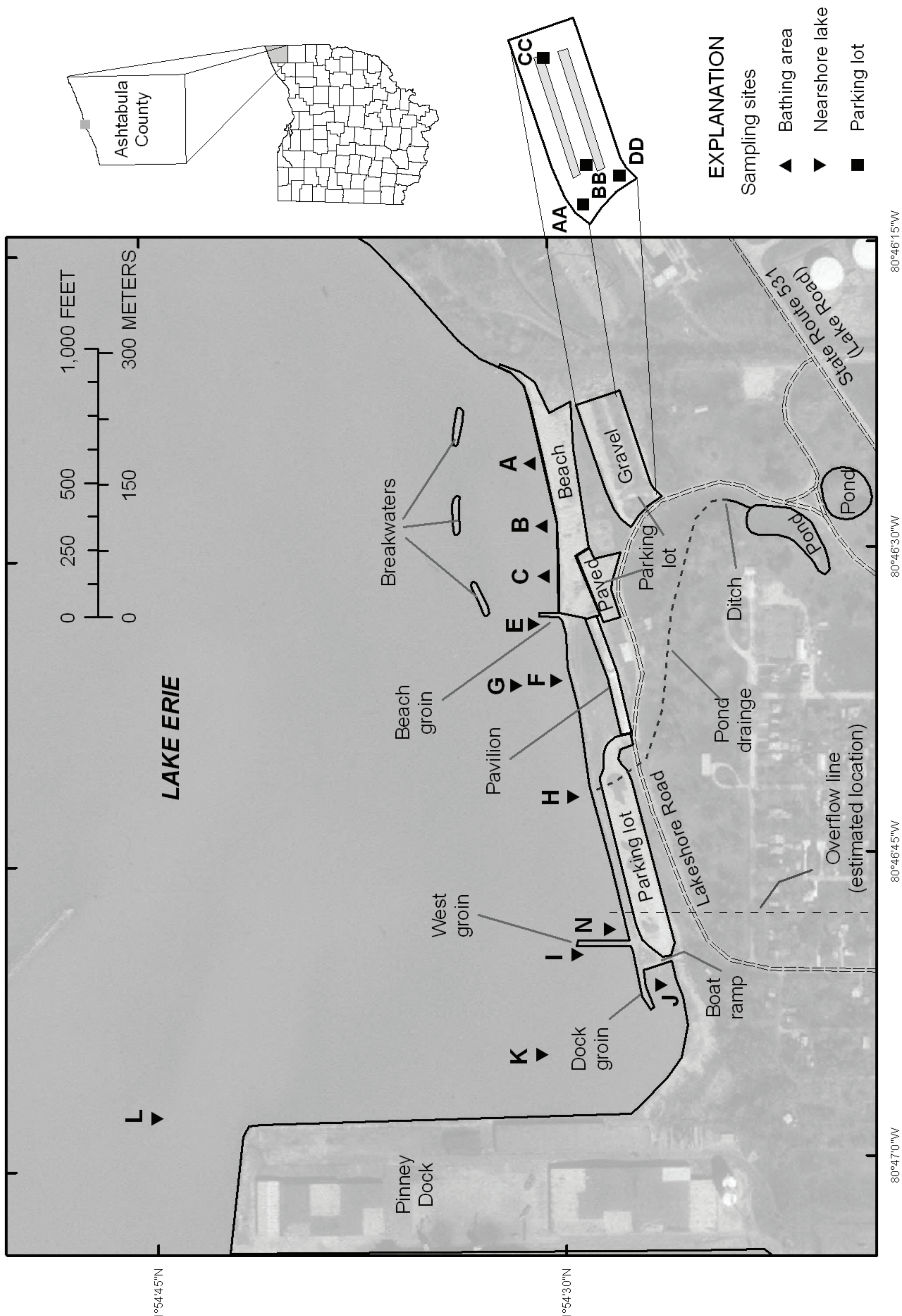

Figure 2. Location of Lakeshore, Ashtabula, Ohio, and bathing-area, nearshore lake, and parking-lot sampling sites, 2004 and 2005.

Short-term (one- or two-day) shallow-ground-water studies were done at the Main Beach at Edgewater during 2004 and 2005. A monthlong ground-water study was done in late summer 2005. For ground-water studies, temporary piezometers (fig. 3) were installed with 0.5-ft-long screens at depths ranging from approximately 0.5 to 3.0 ft. Piezometers were hand driven at the following intervals from the edge of water (the edge of water is the maximum wave run-up point) (in feet) (fig. 1*B*):

- June 30, 2004, east location—0, 25, 50, 75, 100
- July 28–29, 2004, east location—0, 3, 6, 9, 20, 40, 60, 80, 100
- June 23, 2005, west location—0, 3, 6 (shallow), 6 (deep), 9, 20, 40, 60, 80, 100
- July 20–21, 2005, west location—0, 3, 6 (shallow), 6 (deep), 9, 20
- Aug. 16–Sept. 12, 2005, west location—6, 9

The 2-in.-internal-diameter piezometers were sterilized, driven to the desired depth by hand, and developed by mechanical surging (with a sterile surge pipe). To determine lake water level in the bathing area, a temporary stilling well also was installed at 2–3 ft water depths for all studies; the stilling well consisted of a pipe driven into the lake bottom with a screen open to lake waters. The relative elevation of the piezometers and stilling well were determined by conventional surveying techniques. Water levels in piezometers and the stilling well were measured with an electric tape.

Spatial Sampling at Lakeshore

Water or sediment samples were collected as follows (fig. 2):

- Three bathing-area sites (A–C, 3-ft water depths), water samples—Monday through Thursday in 2004 and 2005 as part of the beach monitoring program
- Three bathing-area sites (A–C), bed-sediment samples—7 to 11 days in 2004, depending on the site
- Eight nearshore lake sites (E–L), water and bed-sediment samples—3 days in 2004 (some sites were not sampled on all 3 days)
- Three nearshore lake sites (F, H, and J), water samples—12 days in 2005
- Four parking lot sites (AA–DD), sediment—3 days in 2005

Samples for Microbial Source Tracking

Samples were collected for MST testing on four days during 2005: June 29, July 13, August 21, and September 1 at Edgewater; and June 28, July 14, August 15, and September 1 at Lakeshore. At both beaches, bird fecal samples and wastewater from the local treatment plant served as source samples. Secondary-treated wastewater samples were collected from the final clarifier before chlorination at the Southerly Wastewater Treatment Plant for Edgewater and at the Ashtabula

Figure 3. Temporary piezometers with 0.5-foot-long screens, installed at depths ranging from approximately 0.5 to 3.0 feet, at various intervals from the edge of water.

Wastewater Treatment Plant for Lakeshore. Bathing-water samples were collected for MST testing at both beaches on all four days. At Edgewater, samples for MST testing were also collected from shallow ground water on September 1. At Lakeshore, MST tests were also done on August 15 and September 1 on sediment samples from the gravel parking lot and water samples from additional sites that showed physical evidence of fecal contamination (sites I and N, fig. 2).

Sample-Collection Methods

Water samples from nearshore lake and bathing-area sites and from piezometers were collected into sterile polypropylene bottles. Water samples were collected at nearshore lake and bathing-area sites as grab samples from a boat, from the shore, or by wading to the sampling point (Myers and Wilde, 2003); samples were collected approximately 1 ft below the water surface. Water samples from piezometers were collected by use of a peristaltic pump with sterile tubing. Before sampling, water levels in piezometers were measured with an electric tape that was sterilized with dilute bleach, neutralized with sodium thiosulfate, and rinsed with sterile water. Wave heights were measured by placing a marked survey rod in the water at the 3-ft water-depth sampling location for 1 minute, during which field crews noted the minimum and maximum heights. Lake currents in the bathing area were estimated visually by field crews.

Bed-sediment, parking-lot-sediment, and bird fecal samples were collected by use of sterile techniques. Bed and parking-lot sediments were collected into sterile, wide-mouth, 250-mL polypropylene jars. To collect bed sediments, field personnel reached to the lake bottom, opened the lid of the jar, and scooped the bottom sediments to obtain a sample. The lid of the jar was closed before surfacing. If the water was too deep to reach for a sample, the field crew lowered a clean and sterile Petite Ponar Grab sampler (Wildlife Supply Company, Buffalo, N.Y.) from a small boat and collected a bed-sediment sample per the manufacturer's instructions. Detailed sampling and decontamination procedures using the Petite Ponar Grab sampler are described elsewhere (Francy and others, 2003). Parking-lot-sediment samples were collected by scooping wet sediments into a jar. Three jars were collected from each sediment sampling point and composited before analysis. Bird fecal samples were collected from the sands at the bathing beaches. A fresh mass of bird fecal material was identified, and the top layer was removed with a sterile swab. From the center of the mass, a small piece of fecal material was removed with a second sterile swab and placed into a vial containing 100 mL of phosphate-buffered dilution water (U.S. Environmental Protection Agency, 2002a). These steps were repeated until materials were collected from 16 bird fecal samples into one vial.

Laboratory Methods

Water samples for *E. coli* were analyzed in local laboratories within 6 hours of collection. The NEORSD Analytical Services Laboratory analyzed nearshore lake and bathing-water samples from Edgewater for *E. coli*; the USGS assisted with the analysis of bathing-water samples and analyzed ground-water samples. At Lakeshore, all samples for *E. coli* were analyzed by the USGS. Samples for MST and sediments were sent to the USGS Ohio Water Microbiology Laboratory (OWML) in Columbus, Ohio, and processed within 24 hours of collection. All samples were kept on ice before analysis.

Escherichia coli

Lake-water samples, except for those in piezometer studies, were analyzed for *E. coli* by use of the modified mTEC membrane-filtration method (U.S. Environmental Protection Agency, 2002a). Because of high amounts of suspended materials, ground-water and sediment samples were analyzed for *E. coli* using Colilert Quantitray-2000 (Idexx Laboratories, Westbrook, Maine). Lake-water samples collected specifically for comparisons to piezometer samples were also analyzed using the Colilert Quantitray-2000 to expedite processing. For sediment samples, 20 g were aseptically removed from the composite jar and placed into a bottle containing 200 mL of phosphate-buffered dilution water; a second aliquot of sediment was removed to determine percent dry weight. The analyst placed the bottle on a shaker for 45 minutes, removed the bottle, allowed suspended materials to settle for 30 seconds, and decanted the liquid phase for analysis. Calculations were made as described in Francy and Darner (1998) to convert results to most-probable number per gram of dry weight sediment (MPN/g_{DW}).

Multiple Antibiotic Resistance Indices of *Escherichia coli* Isolates

Samples of bird feces, bathing water, wastewater, ground water, and parking-lot sediments were plated for *E. coli* on modified mTEC agar. Eighty isolated colonies were picked from the ideal count plates (20–80 colonies) from each source and environmental sample, whenever possible; otherwise non-ideal count plates (those plates with <20 or >80 colonies) were used. Each colony was streaked for *E. coli* confirmation and isolation onto eosin methylene blue (EMB) agar and incubated at 36°C for 24 hours. From each EMB plate, one isolated colony was transferred to a well on a 96-well microtiter plate containing Luria-Bertani (LB) broth; separate microtiter plates were used for each source. Controls on each plate included blank wells inoculated with LB broth only (negative control) and one well inoculated with *E. coli* ATCC 25922 (American Type Culture Collection, Manassas, Va.) as a susceptible organism. Every other column of wells on the microtiter plate was left as a blank column to prevent cross contamination. After overnight incubation at 36°C, the microtiter plates were further processed for antibiotic resistance testing. A pin replicator was dipped into the microtiter wells and stamped onto a series of LB agar plates, each LB plate containing a different antibiotic (or different concentration of an antibiotic) and one plate containing no antibiotic. The antibiotics were those used in other studies (Parveen and others, 1997) and in previous tests of Lake Erie samples that showed the most discriminatory power between human and bird sources (data on file at the USGS OWSC). Final antibiotic concentrations were ampicillin, 10 and 20 µg/mL; streptomycin, 25 µg/mL; tetracycline, 25 and 50 µg/mL; nalidixic acid, 25 µg/mL; and sulfathiazole 1,000 µg/mL. Two concentrations were used for some antibiotics to increase the likelihood that data were obtained even if spreading colonies grew on one of the plates. If growth on the antibiotic plate was approximately 50 percent or more of the growth on the control plate containing no antibiotics, the isolate was recorded as resistant (fig. 4). If there was no growth on the control plate, the isolate was removed from the dataset. Only one concentration from each antibiotic was used in the calculations, the concentration that had the smallest number of spreading colonies and the largest number of countable wells. MAR indices were calculated for isolates from each of the sources by the method of Kaspar and others (1990), as follows:

$$\text{MAR index} = \text{number of resistant isolates} / (\text{number of antibiotics tested} \times \text{total isolates tested})$$

Bacteroides Marker

Water samples were tested for the presence of a human marker from enteric anaerobic bacteria of the genus *Bacteroides,* as described in Dick and others (2005), according to the protocol originally described by Bernhard and Field (2000a). Water samples were filtered through a 0.22-µm-pore-size, 47-mm-diameter Durapore filter (Millipore, Billerica, Mass.). Parking-lot samples were not included because of the aerobic nature of the environment. Filters were loaded with measured volumes of sample to capacity; normally a total of 10–100 mL was filtered within 30 minutes. For each sample, a duplicate filter was processed. Immediately after filtration, each filter was aseptically folded to fit into a 15-mL sterile plastic centrifuge tube and was stored at -70°C until further analysis.

To prepare the filter for DNA extraction, a sterile razor blade was used to cut the filter into 1- to 3-mm strips. The filter strips were then processed by the PowerSoil DNA

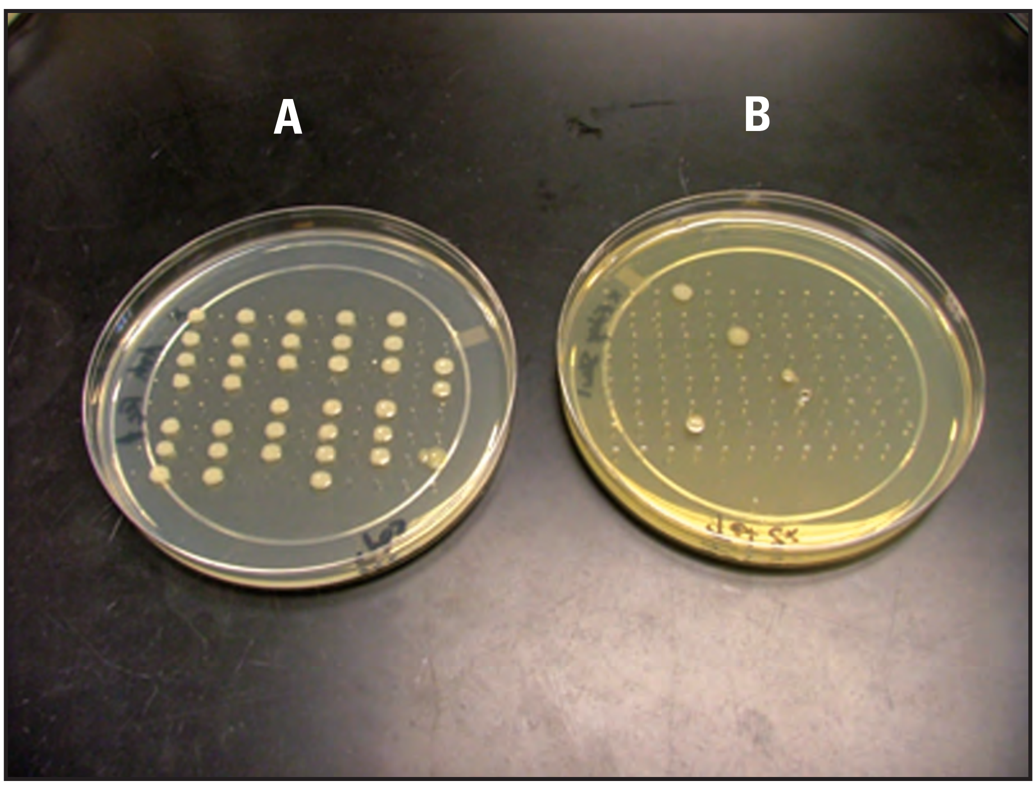

Figure 4. Multiple antibiotic resistance testing on Luria-Bertani agar plates containing *A*, no antibiotic (control plate), and *B*, one antibiotic. (The four colonies on the antibiotic plate that had at least 50 percent of the growth as compared to the control plate were recorded as resistant.)

extraction and purification kit (MO BIO, Carlsbad, Calif.) per manufacturer's instructions. DNA extracts were quantified by use of a PicoGreen-based protocol (Molecular Probes, Eugene, Oreg.) and normalized to a consistent concentration of 2 ng/μL.

The PCR was done in 25-μL volumes containing 1X PCR buffer, 0.2 mM of each of the four deoxyribonucleotides, 0.06 percent bovine serum albumin, 0.2 μM of each primer, 1 unit of HotMaster *Taq* DNA polymerase (Eppendorf, New York, N.Y.), and 2 ng of template DNA. Forward primers used for these analyses were Bac32 (5'-AAC-GCT-AGC-TAC-AGG-CTT-3') for general *Bacteroides* and HF134 (5'-GCC-GTC-TAC-TCT-TGG-CC-3') for the human-associated marker. Bac708 (5'-CAA-TCG-GAG-TTC-TTC-GTG-3') was used as the reverse primer in all reactions. Cycle conditions for the PCR were as follows: initial denaturation at 95°C for 3 minutes followed by 35 cycles of denaturation at 94°C for 60 seconds, annealing for 60 seconds, and extension at 72°C for 90 seconds. A final extension at 72°C for 90 seconds was used to terminate the reaction. The annealing temperatures used were 63°C for HF134 and 62°C for Bac32. PCR products were loaded onto a 7500 DNA chip and visualized by use of a BioAnalyzer (Agilent, Palo Alto, Calif.).

Enterococcus faecium Marker

Water and parking-lot sediment samples were tested for the presence of *Enterococcus faecium* human-specific *esp* marker (enterococci marker) according to the protocol originally described by Scott and others (2005). Samples were analyzed for concentrations of *Enterococcus spp*. by USEPA method 1600, membrane filtration with mEI agar (U.S. Environmental Protection Agency, 2002b). The filter containing the most discernible enterococci colonies from each sample (typically 100–300 colonies) was lifted, suspended in a centrifuge tube containing tryptic soy broth, vortexed, and incubated for 3 hours at 41ºC. This step was done to wash the bacteria from the filters and partially enrich the culture. After incubation, the broth culture was vortexed, and duplicate 1-mL aliquots were dispensed into separate microcentrifuge tubes. Each aliquot was then centrifuged for 5 minutes at 5,000 × *g*, the supernatant was decanted, and the remaining cell pellet was resuspended in 1 mL of tris-EDTA buffer. The cell suspensions were stored at -70ºC until further analysis.

DNA extraction was performed on sample cell suspensions by means of a slightly modified protocol of the UltraClean microbial DNA isolation kit (MO BIO, Carlsbad, Calif). Each cell suspension was centrifuged for 5 minutes at 5,000 × *g*, and the supernatant was decanted. The remaining cell pellet was resuspended in 300 μL of MicroBead solution, and manufacturer's instructions were then followed for DNA extraction and purification. DNA extracts were stored at -20ºC until further analysis.

The PCR was done in 25-μL volumes containing 1X PCR buffer, 0.2 mM of each of the four deoxyribonucleotides, 0.3 μM of each primer, and 1.25 U of HotMaster *Taq* DNA polymerase (Eppendorf, New York, N.Y.). The forward primer used in this study was 5'-TAT GAA AGC AAC AGC ACA AGT T-3'; the reverse primer was 5'-ACG TCG AAA GTT CGA TTT CC-3' (Scott and others, 2005). Cycle conditions for the PCR were as follows: initial denaturation at 95ºC for 3 minutes, followed by 35 cycles of denaturation at 94ºC for 1 minute, annealing at 58ºC for 1 minute, and extension at 72ºC for 1 minute. PCR products were separated on a 1.0 percent, high-resolution agarose gel, stained using SybrGreen I nucleic acid stain, and photodocumented under UV light. Each gel included several 1 Kbp ladder wells that helped confirm the presence of the 680 bp *esp* target. Gel images were exported into BioNumerics software (Applied Maths, Austin, Tex.) for final analysis.

Quality Assurance and Quality Control

Quality-assurance and quality-control (QA/QC) practices are considered an integral part of all data-collection activities. Field and laboratory protocols were distributed to all personnel to ensure that procedures were followed correctly and consistently. The USGS made several on site QA/QC checks of procedures performed by field and laboratory personnel throughout each recreational season, and corrective actions were taken as needed. Procedures for QA/QC laboratory practices are described in Francy, Bushon, and others (2005).

Field quality-control samples were collected to measure sampling and analytical variability or contamination potential. At least 10 percent of *E. coli* samples were field QC samples, including split replicates, interagency split replicates, and field blanks. Split replicates consisted of two samples collected by the same agency, each bottle analyzed twice. Interagency split replicates consisted of one bottle collected by NEORSD and one collected by the USGS concurrently at the same sampling point. Each bottle was analyzed twice, once by NEORSD and once by the USGS. Field blanks were used to measure contamination potential during sample collection and handling. To collect a field blank, 200–500 mL of sterile buffer was poured into the bottle under actual field conditions. The field blank was processed the same as a regular sample for *E. coli* or for *Bacteroides* or enterococci markers.

In the laboratory, filter blanks were included for at least every three *E. coli* samples (and every sample for *Bacteroides* or enterococci markers) to document that filtration equipment and buffered water were not contaminated. Positive-control reference cultures for *E. coli* were analyzed periodically. Positive-control reference cultures were pure cultures of *E. coli* ATCC 10798 (American Type Culture Collection, Rockville, Md.) prepared by the USGS and distributed to laboratory personnel by overnight mail. At the same time, personnel in the USGS laboratory plated the pure culture, and results were compared. Results from QC samples were carefully monitored, and retests and (or) corrective measures were taken when needed. The results from QC samples did not require removal of any data from the datasets.

Positive-control DNA (Delaware, Ohio, wastewater influent sample) and negative-control DNA (Delaware, Ohio, cattle feces) were similarly processed for the presence of the *Bacteroides* and enterococci markers. The presence of the general *Bacteroides* marker (Bac32) was used as evidence that the PCR reaction was successful; in the absence of Bac32, a matrix spike was included. For the enterococci marker, matrix spikes were included for each environmental sample to identify any possible matrix inhibition. PCR reagent blanks were included to test for contamination of PCR reagents with amplifiable target DNA.

Data Analysis and Statistics

Analysis of variance (ANOVA) was used to compare more than two groups of data. For data that were not normally distributed, the nonparametric rank transform test was done. Contingency-table analysis was used to assess the relations between MAR indices and fecal source; chi-square distributions were used to evaluate results from contingency tests (Helsel and Hirsch, 2002, p. 378–382).

Lake-level data were obtained from National Oceanic and Atmospheric Administration (NOAA) station in Cleveland for Edgewater (NOAA ID 9063053) (National Oceanic and Atmospheric Administration, 2005a).

Rainfall data were compiled from National Weather Service stations at Hopkins International Airport for Edgewater and at Ashtabula County Airport for Lakeshore (National Oceanic and Atmospheric Administration, 2005b). These sites are 5.5 and 11 mi inland from the Lake Erie shoreline, respectively. For 2005, local rainfall data for Lakeshore were available from a private source, located approximately 0.5 mi east of Lakeshore along the shoreline. The relation between local rainfall and *E. coli* concentrations at Lakeshore was stronger than between *E. coli* concentrations and airport rainfall (data not shown), so local data were used when available. For 2005 at Edgewater, data were also obtained from a NEORSD-operated gage at John Marshall High School, 3 mi inland from the Lake Erie shoreline; data from this site were used to augment data collected at the airport gage.

Two rainfall variables were used to aid in data interpretation. “R_{d-1}” was the amount of rain, in inches, that fell in the 24-hour period (9 a.m. to 9 a.m.) preceding the day of sampling. Rainfall weighted 72 hours (“Rw72”) included 3 days of rainfall with the most recent rainfall receiving the most weight, as follows:

$$Rw72 = (3* R_{d-1} + 2* R_{d-2} + R_{d-3})$$

where R_{d-1}, R_{d-2}, and R_{d-3} were amounts of rain that fell in 24-hour periods 1 day, 2 days, and 3 days preceding sample collection, respectively.

Spatial Distributions of *Escherichia coli*

Edgewater

At Edgewater, data from nearshore lake sites were used to identify potential regional origins of fecal contamination (such as rivers or outfalls) and determine *E. coli* concentrations along transport pathways to the beach. Measurements of *E. coli* concentrations in the bathing area were used to assess spatial patterns and identify local contamination. The piezometer studies were done to determine *E. coli* concentrations in shallow ground water and determine if the interactions between ground water, precipitation, lake levels, and wave height could explain the source or mechanisms of transport of *E. coli* to the beach.

Nearshore Lake

Data from 2003 and 2004 at nearshore lake sites (fig. 1*A*) were grouped into two datasets (fig. 5): (*A*) 10 “dry” days when $R_{d-1} = 0$ in., and (*B*) 9 “wet” days when $R_{d-1} > 0.1$ in. On the dry days, average and median *E. coli* concentrations were highest at the mouth of the Rocky River (site J), low at other nearshore lake sites, and slightly higher in the Edgewater bathing area. The bathing-area samples were the average concentrations at sites C and F on the 19 days that samples were collected at nearshore lake sites. On wet days, average *E. coli* concentrations were very high at the mouths of the Rocky River (site J) and Cuyahoga River (site Q), decreased along transport pathways to Edgewater from site Q, were low at the Edgewater nearshore lake site (site M), but were elevated in the Edgewater bathing area. On wet days, median *E. coli* concentrations were above 235 CFU/100 mL in the bathing area and at sites J, P, and Q. Differences in *E. coli* concentrations between the Edgewater bathing area (sites C and F) and the nearshore lake sites not near river mouths or treatment facilities (sites K, L, M, N, and O) may have been due to water depth. The samples at C and F were collected at 3-ft water depths within the bathing area; the other samples were collected at average depths ranging from 5 ft (site L) to 19.6 ft (site O). The effect of water depth on *E. coli* concentrations was not investigated during this study.

Bathing Area

Spatial studies were done at Main and Middle Beach bathing-area sites in 2005 (fig. 1*B*). In order to simplify data presentation, samples collected from sites that were spatially similar, located at similar water depths, and generally had the same range of *E. coli* concentrations were pooled into three groups.

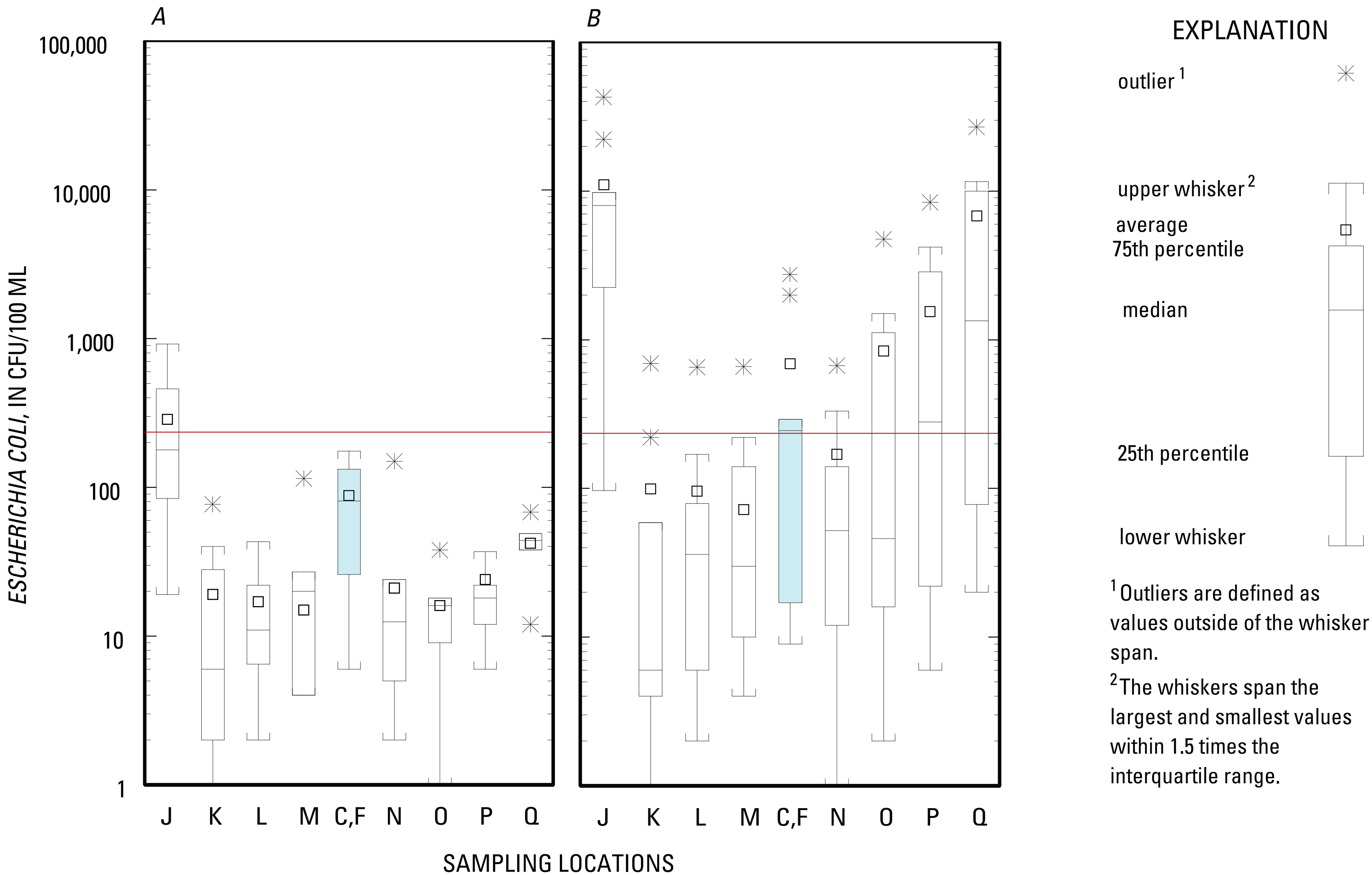

Figure 5. Distribution of *Escherichia coli* concentrations at nearshore lake sampling sites in and around Edgewater, Cleveland, Ohio, 2003 and 2004, on *A*, 10 dry and *B*, 9 wet days. (All samples were collected about 150 feet offshore except for bathing-area sites C and F. Sampling locations are as follows: J - Rocky River; K - W 117th; L - Edgewater outfall; M - Edgewater nearshore lake; C,F - Edgewater bathing area; N - Piers; O - Breakwalls; P - CSTOF; Q - Cuyahoga River. The Ohio single-sample standard of 235 colony-forming units per 100 mL (CFU/100 mL) is indicated by a red line and is used as a point of reference. Bathing-area samples are designated by a solid blue box and are averages of sites C and F.)

An average concentration was calculated for each day for the three groups (fig. 6): (1) Main Beach samples collected at 3-ft water depths (sites C and F, circles and solid connecting lines), (2) Main Beach samples collected at 1- and 2-ft water depths (sites A, B, E, and D, triangles), and (3) Middle Beach samples collected at 3-ft depths (sites G, H, and I, squares). Elevated average *E. coli* concentrations (greater than 235 CFU/100 mL) were usually associated with increased wave heights and (or) rainfall, but not always (fig. 6). The patterns of *E. coli* concentrations were similar at the Main and Middle beaches; but on most days when concentrations were elevated, concentrations were higher at the Middle Beach than the Main Beach. Similarly, concentrations of *E. coli* collected at 1- and 2-ft water depths were often higher than those collected at 3-ft depths at the Main beach.

The Edgewater outfall, which discharges into the western end of Middle Beach, overflowed twice during the summer of 2005—June 10 at 5:15 p.m. and July 21 at 4:15 a.m. *E. coli* concentrations were not elevated at the Main Beach until two days after the discharge (June 12) that occurred on June 10 (R_{d-1} was 0 in. at the airport gage and 0.83 in. at the John Marshall gage for June 11). In contrast, on July 21, Main Beach and Middle Beach *E. coli* concentrations were quickly elevated in response to the overflow (R_{d-1} was 0.77 in. at the airport gage and 1.15 in. at the John Marshall gage for July 21). On June 10 and 11, there were no waves or noticeable currents, whereas on June 12 and July 21, wave heights were 1 ft with northwesterly and southerly currents, respectively. This pattern indicates that currents and waves may play a significant role in delivering nearshore contaminants to the Main Beach.

Shallow Ground Water

Shallow ground water was sampled at Edgewater to understand the relations of *E. coli* concentrations in shallow ground water in foreshore sands to those in lake water: Is shallow ground water a source of *E. coli* to the lake, is the lake providing a source of *E. coli* to shallow ground water, or is it a combination of both mechanisms? Additionally, are the backshore sands (sands not infiltrated by lake water during the season) and areas upgradient from the beach a source of *E. coli* to the beach? What is the role of local rainfall, lake level, and wave action in affecting *E. coli* concentrations in shallow ground water? These questions were addressed by determining the direction of ground-water flow (as measured by hydraulic gradients), by determining concentrations of *E. coli* in ground water and the lake simultaneously, and by examining environmental and meteorological data.

The hydraulic gradient is the difference in hydraulic head between two points divided by the distance between the points. If these differences are measured in a nearly horizontal plane (on a well at distance from another at a similar elevation), this is generally considered the horizontal hydraulic gradient. If the differences are measured vertically (between a shallow and deep piezometer at nearly the same location), it is the vertical hydraulic gradient. The hydraulic gradient is expressed as a dimensionless quantity and is used to determine flow direction.

Ground water was sampled on June 30 and July 28–29, 2004, at the east location of Edgewater (fig. 1*B*) to determine shallow horizontal hydraulic gradients and spatial patterns of *E. coli* concentrations in shallow ground water in foreshore and backshore sands. Measured water levels, in all instances, showed that shallow ground-water flow was toward the lake (fig. 7), although slight variations in the July water levels indicated that wave action may reverse the gradients less than 10 ft inland from the edge of water (“near foreshore area”) (fig. 7*B*). Concentrations of *E. coli* in piezometers ranged from <1 to 400 MPN/100 mL. Concentrations of *E. coli* in lake-water samples in the two studies ranged from 17 to 190 CFU/100 mL. The highest concentration of *E. coli* was found 50 ft inland from the edge of water in June. In July, the highest concentrations were found in the near foreshore area. The higher random concentration in backshore sands in June indicates a possible source of *E. coli* inland from the beach; the high concentrations in the near foreshore in July indicate that interactions between the lake and shallow ground water may contribute to high concentrations of *E. coli.*

Additional short-term ground-water studies were done June 23 and July 20–21, 2005, at the west location of Edgewater (fig. 1*B*) to confirm the horizontal hydraulic gradients measured in 2004, determine vertical hydraulic gradients, and identify spatial patterns of *E. coli* in the near foreshore area. Weather conditions were different during the two studies done in 2005. On June 23, wave heights were measured as 1.5 ft, and the area received no rain (R_{d-1}= 0 in.) at the airport and John Marshall gages. On July 20, wave heights were measured at 0.42 ft with no rain at both gages; on July 21, wave heights were measured at 1.0 ft, R_{d-1} was 0.76 and 1.15 in. at the airport and John Marshall gages, respectively, and the Edgewater outfall discharged.

During both 2005 studies, temporal water-level measurements confirmed that the general horizontal hydraulic gradient was towards the lake; however, as in 2004, near-foreshore gradients were reversed at least once during each day (fig. 8). Vertical hydraulic gradients were assessed with shallow (≈0.5 ft) and deep (≈3 ft) piezometer pairs installed 6 ft inland from the edge of water and from lakebed piezometers (gradient differences are not always visible on fig. 8, because the differences are very small). Vertical hydraulic gradients were shifting and variable in 2005. On June 23, the morning water-level measurements in inland piezometers indicated a slight upward gradient (0.03 ft/ft); noon and afternoon measurements showed a downward gradient (0.07 and 0.01 ft/ft, respectively). Also in June, data from the lakebed piezometer showed that vertical hydraulic gradients between the lake and shallow lake bottom were always upward (0.14-0.3 ft/ft). In contrast, during July, inland vertical hydraulic gradients were always downward, and the vertical hydraulic gradients between the lake and lakebed piezometer were variable and ranged from downward (0.07 ft/ft) to upward (0.13 ft/ft).

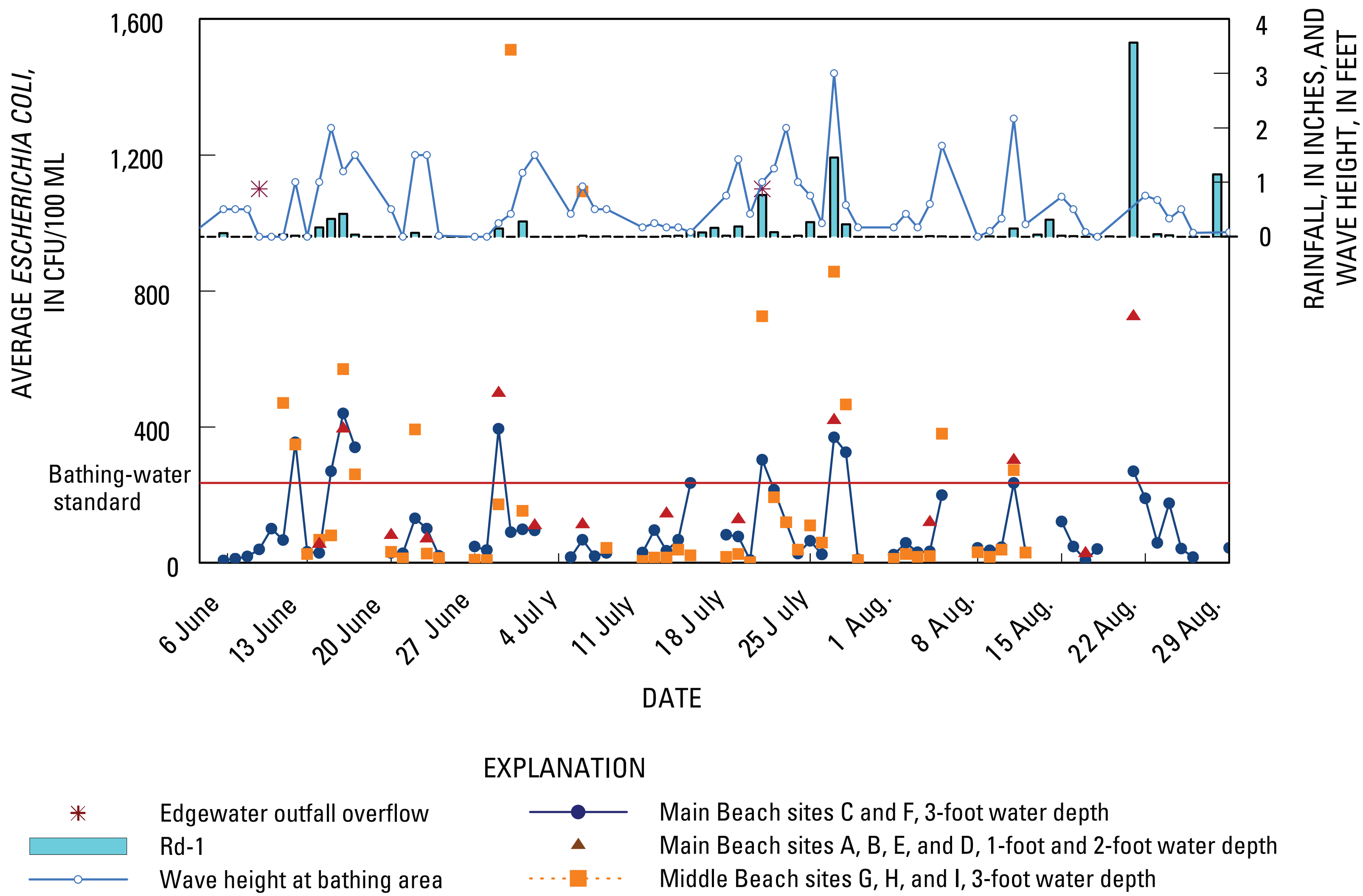

Figure 6. Average concentrations of *Escherichia coli* at Main and Middle Beach bathing-area sites, Edgewater, Cleveland, Ohio, 2005. (R_{d-1} is the rainfall amount at Hopkins International Airport in the 24-hour period preceding sampling. Main Beach consecutive daily samples are connected by a solid line to provide a frame of reference. CFU/100 mL is colony-forming units per 100 milliliters.)

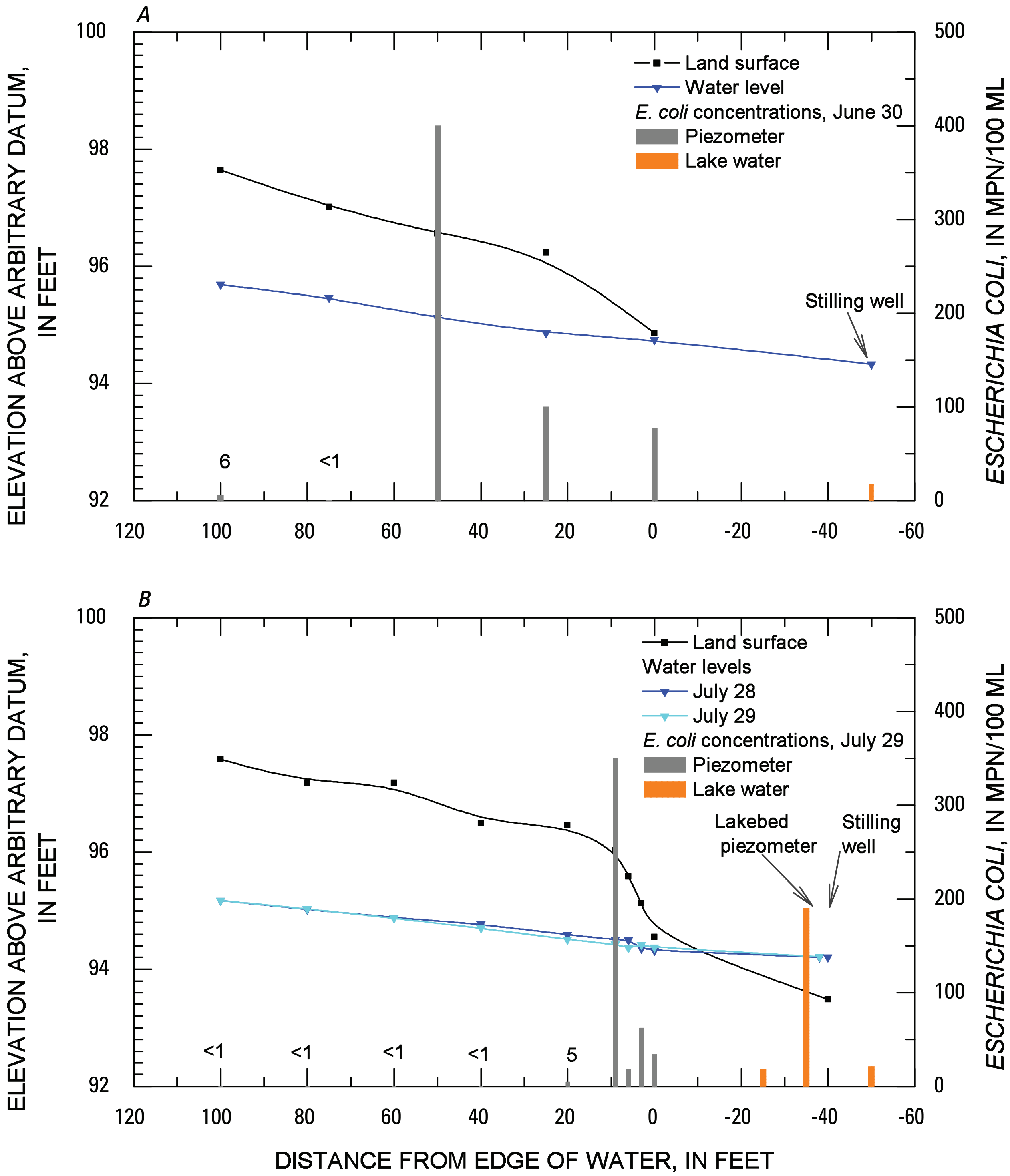

Figure 7. Land surface, water levels, and *Escherichia coli* (*E. coli*) concentrations in shallow ground water and lake water at the east sampling location at Edgewater, Cleveland, Ohio, 2004, in *A*, June, and *B*, July. (MPN/100 mL is the most probable number per 100 milliliters; *E. coli* concentrations not visually apparent are written numerically.)

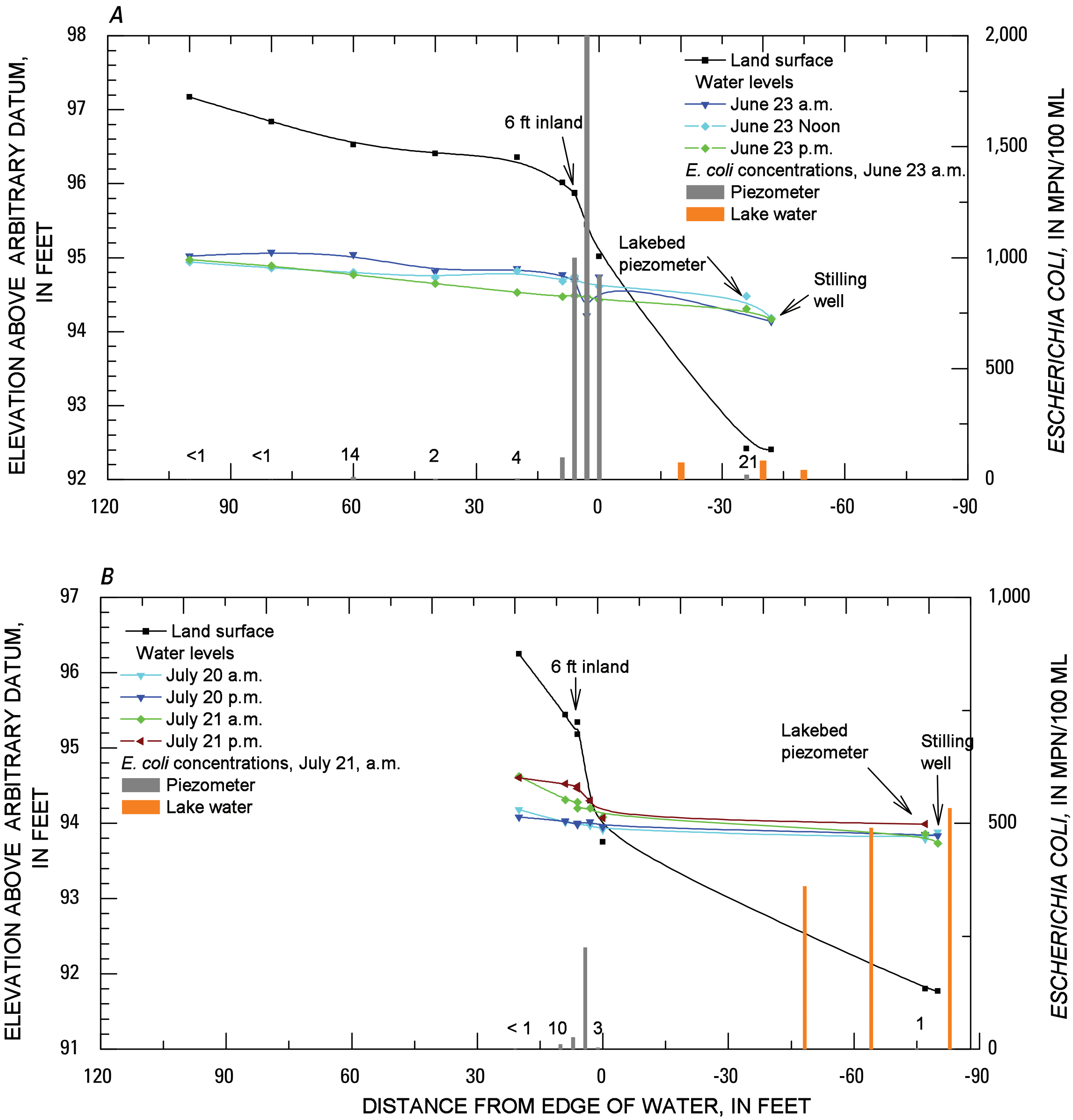

Figure 8. Land surface, water levels, and *Escherichia coli* (*E. coli*) concentrations in shallow ground water and lake water at the west sampling location at Edgewater, Cleveland, Ohio, 2005, in *A*, June, and *B*, July. (Piezometer 6 feet inland is an average of shallow and deep *E. coli* concentrations. Although *E. coli* concentrations were measured three times in July, one sampling is shown as an example for simplicity. MPN/100 mL is the most probable number per 100 milliliters.)

Concentrations of *E. coli* in piezometers ranged from <1 to 2,000 and from <1 to 225 MPN/100 mL in June and July, 2005, respectively. The highest concentrations of *E. coli* in both June and July in piezometers were found in the near foreshore area; the highest *E. coli* concentration further inland than 10 ft was found in June 2005 at 60 ft inland (14 MPN/100 mL; fig. 8*A*). In June 2005, *E. coli* concentrations were higher in piezometer samples than in lake-water samples (fig. 8*A*). In contrast, in July 2005, *E. coli* concentrations from lake-water samples were higher than concentrations found in piezometer samples (fig. 8*B*). *E. coli* concentrations from the lake were always higher than concentrations found in lakebed piezometers.

A monthlong ground-water study was done late summer 2005 at the west location at Edgewater to better define temporal relations between *E. coli* concentrations in the near foreshore area and the lake (fig. 9). The piezometers were placed at 6 ft and 9 ft from the edge of water on August 16. The piezometer originally placed 6 ft from the edge of water was more than 25 ft from the edge of water after August 30, owing to seasonal lake-level decline; the piezometer originally placed 9 ft from the edge of water was dry after August 30 (fig. 9*A*).

During this study, data were collected after two heavy rain events—August 21 and 31. After receiving 3.56 in. of R_{d-1} on August 21, concentrations of *E. coli* in both piezometers increased to >2,400 MPN/100 mL on August 22, whereas the lake *E. coli* concentration increased to only 220 MPN/100 mL. The highest *E. coli* concentration (6,900 MPN/100 mL) was found in the 6-ft-inland piezometer on August 23. This delayed increase of *E. coli* concentrations may have been in response to the high waves that occurred on August 22 but did not occur on August 21. The high waves may have accelerated infiltration of surface *E. coli* into beach sands; the distance to the edge of water from the 6-ft inland piezometer was only 7–8 ft. The long antecedent dry period before August 21 also may have contributed to elevated *E. coli* concentrations. In contrast, on September 1 after 2.39 in. of R_{d-1} on August 31, the piezometer *E. coli* concentration remained low (8 MPN/100 mL) while the lake *E. coli* concentration increased to 1,400 MPN/100 mL. From August 31 to September 2, wave heights were elevated; however, the distance to the edge of water (> 25 ft) may have diminished any influence from the waves; a short antecedent dry period (<3 days) may have also contributed to low *E. coli* concentrations in shallow ground water.

During the monthlong ground-water study, *E. coli* concentrations from shallow ground water generally decreased as the distance from the piezometers to the lake (edge of water) increased (fig. 9*A*). Overall, lake *E. coli* concentrations remained about the same or increased slightly during the sampling period. Lake level decreased about 0.5 ft during this period, whereas water levels in the piezometers fluctuated but were at the same level at the end as the beginning of the study (fig. 9*B*). The decrease in lake levels with steady water levels in piezometers suggests that local precipitation and infiltration maintain the hydraulic head in shallow ground water in beach sands and that regionwide seasonal precipitation affects lake levels.

Lakeshore

At Lakeshore, sediment samples were collected to determine the potential for storage of *E. coli* in lakebed sediments from nearshore lake and bathing-area sites and in sediments from the gravel parking lot. Nearshore lake and bathing-area water samples were collected to identify potential origins of fecal contamination along the shoreline and west of Pinney Dock (fig. 2).

Sediments

Concentrations of *E. coli* in 29 bed sediment samples collected at bathing-area sites (sites A–C) ranged from 8 to >500 CFU/g_{DW} sediment (table 1). Among bathing-area sites, the median concentration was highest at site B, although statistically significant differences were not found between sites (ANOVA, data not shown). At nearshore lake sites (sites E–L), median concentrations of *E. coli* in bed sediments were highest at sites I and J. *E. coli* concentrations in 12 parking-lot sediment samples at four sites were high and variable, ranging from 440 to 20,000 CFU/g_{DW} sediment (table 1).

Nearshore Lake and Bathing Areas

Patterns of *E. coli* concentrations in water at nearshore lake and bathing-water sites are shown for three days in 2004 with different antecedent rainfall amounts (fig. 10). During dry conditions (fig. 10*A*, June 24), samples were collected at four nearshore lake and two bathing-area sites; the *E. coli* concentration was above the standard at site F and below at all other sites. After the first sampling, additional nearshore lake sites and a central bathing-area site were added to the sampling scheme to better characterize spatial patterns. On a day with a moderate amount of R_{d-1} (fig. 10*B*, August 18), *E. coli* concentrations were near or above the bathing-water standard at site J and at bathing-water sites (A–C). On a day with heavy Rw72 (fig. 10*C*, July 14), *E. coli* concentrations were very high at bathing-water sites and considerably lower at other nearshore lake sites. *E. coli* concentrations in water were low during the three days sampled at sites I, K, and L, which are further from the shoreline than the other nearshore lake sites (fig. 2).

Physical evidence obtained on July 12, 2004, showed that runoff from the gravel parking lot caused degradation of water quality at the beach. On that morning, it rained 0.68 in. from 6 to 8 a.m. Flowing discharge was observed from a pipe draining the parking lot to the beach at site B (fig. 11). The *E. coli* concentration from the parking-lot drain was 26,000 CFU/100 mL.

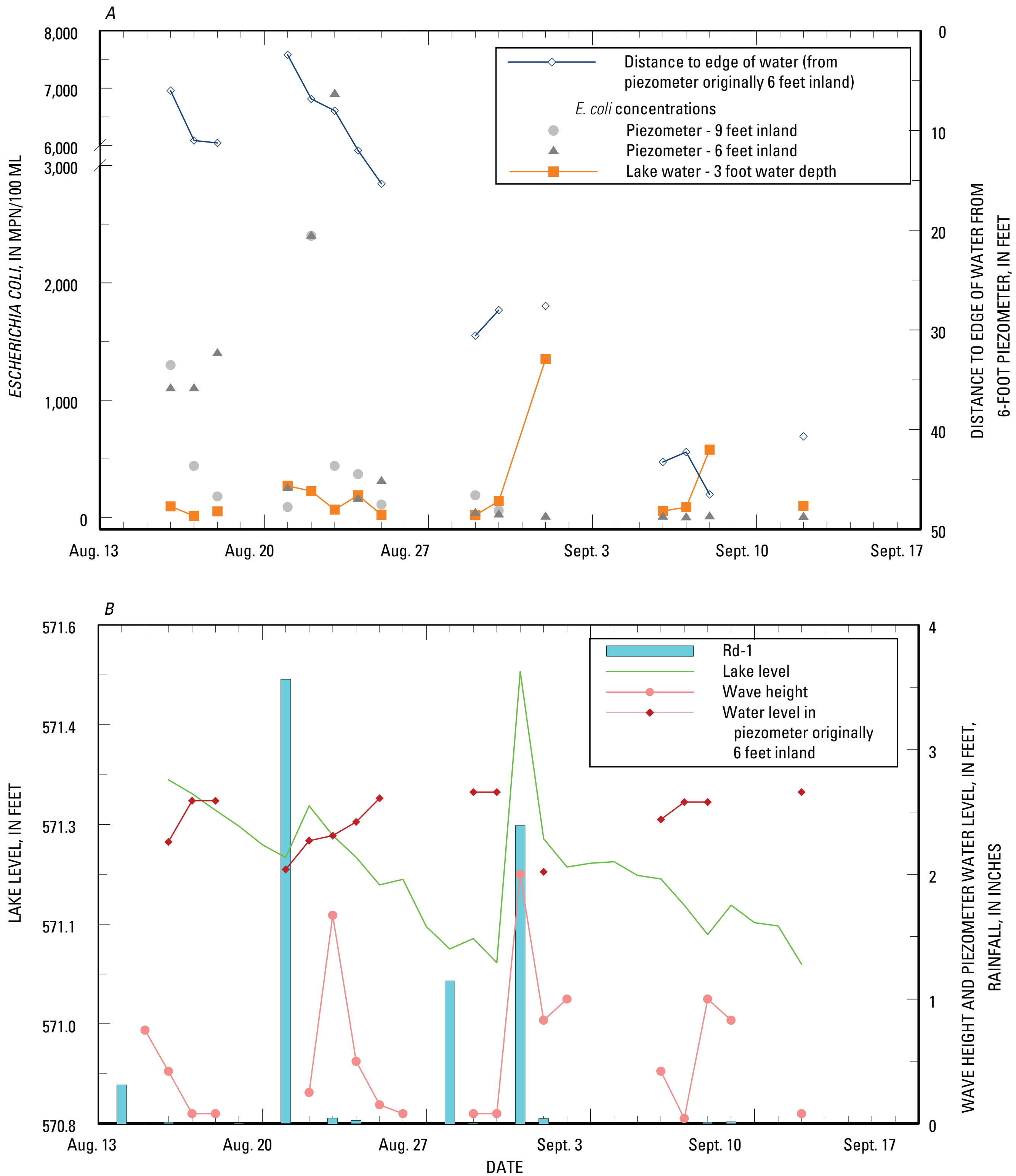

Figure 9. *Escherichia coli* (*E. coli*) concentrations in shallow ground water and lake water at the west sampling location, Edgewater, Cleveland, Ohio, 2005; associated with *A*, distance to edge of water; and *B*, R_{d-1}, lake level, wave height, and the water level in the piezometer 6 feet inland. (MPN/100 mL is the most probable number per 100 milliliters; R_{d-1} is the rainfall amount at Hopkins International Airport in the 24-hour period preceding sampling; lake-level data were obtained from the National Oceanic and Atmospheric Administration station in Cleveland, Ohio; connecting lines are included to provide a point of reference.)

Table 1. *Escherichia coli* concentrations in bed and parking-lot sediments in bathing-area, nearshore, and parking-lot sites at Lakeshore, Ashtabula, Ohio, 2004 and 2005.

[MPN/g_{dw}, most probable number per gram dry weight]

Site ID	Site description	Number of samples	*Escherichia coli*, in MPN/g_{dw} sediment		
			Minimum	Maximum	Median
		Bathing area			
A	East	11	14	320	63
B	Central	7	8	>500	120
C	West	11	18	350	47
		Nearshore			
E	West of beach groin	3	20	200	160
F	In front of pavilion, 3 foot depth	1	8	8	8
G	In front of pavilion, 4 foot depth	2	18	66	42
H	Near pond drainage	3	14	>290	14
I	West side of west groin	3	200	>510	490
J	Boat ramp	3	470	1,200	470
K	Center point	2	16	25	20
L	Pinney Dock	3	59	170	59
		Parking lot			
AA	Northwest corner	3	1,900	2,700	2,500
BB	West side	3	920	8,200	3,200
CC	East side	3	1,400	11,000	3,700
DD	Southwest corner	3	440	20,000	2,000

In 2005, spatial sampling was done on 12 days at sites that had high *E. coli* concentrations in 2004 and that were accessible from the shoreline; these included bathing-water sites and sites F, H, and J (fig. 2). *E. coli* concentrations were above the bathing-water standard at sites F, H, and J on 3, 6, and 10 days, respectively (fig. 12). The three days when *E. coli* exceeded the standard at site F were associated with rainfall. In contrast, the exceedance at site F in 2004 was on a dry day (fig. 10). Runoff from the pavilion roof may have contributed to high *E. coli* concentrations in 2005 at site F. At sites H and J, elevated *E. coli* concentrations were often associated with local rainfall, but not always.

Multiple Antibiotic Resistance Indices of *Escherichia coli* Isolates and Presence of Human Markers

Microbial source tracking methods were used to help better interpret spatial findings and determine whether sources of fecal contamination at Edgewater and Lakeshore were of human or nonhuman origin. MAR results were used as the first level of testing to distinguish between wastewater (representing human) and waterfowl sources of *E. coli*. A positive result for the *Bacteroides* and (or) the enterococcus human marker provided corroborating evidence of human fecal contamination; a negative result of both markers indicated that the markers were not found but did not preclude the possibility of human fecal contamination.

Because spreading of colonies on MAR plates from well to well was a common problem, procedures were developed to consistently identify antibiotic resistance and obtain the most useable data possible. A plate was discarded if more than one-half the plate was affected by spreading colonies. If a colony spread out on a small area of a plate (less than five wells) and technicians were able to discern the original well of the spreader, it was coded as one resistant colony for that area. If a colony spread out on a large area of the plate or technicians could not discern the original well of the spreader, all affected isolates were removed from the dataset. For antibiotics tested with two concentrations, the plate with the higher number of countable wells was used for MAR index calculations.

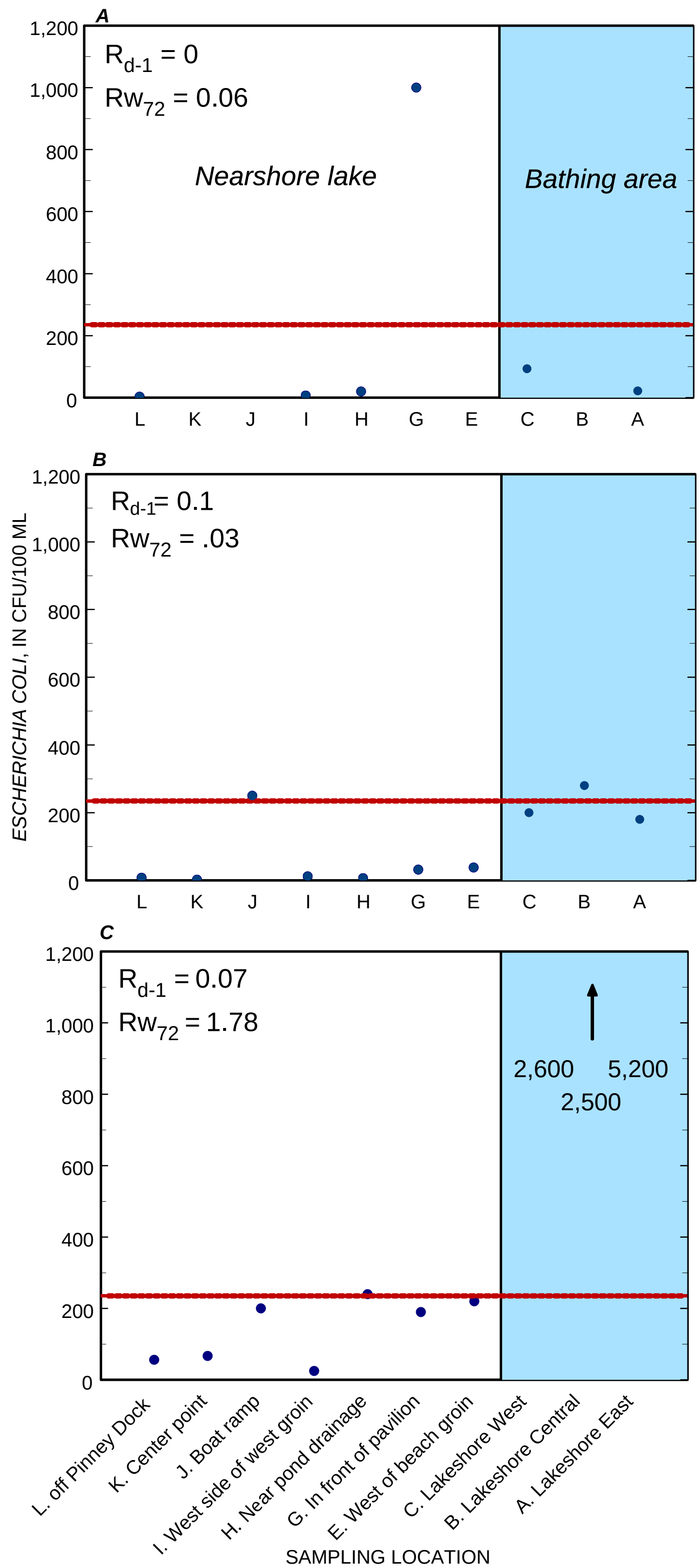

Figure 10. Concentrations of *Escherichia coli* in water collected at nearshore lake and bathing-water sites at Lakeshore, Ashtabula, Ohio, on three days in 2004. *A*, Dry conditions – June 24. *B*, Moderate rain – August 18. *C*, Heavy rain – July 14. (R_{d-1} is the rainfall amount from Ashtabula County Airport, in inches, in the 24-hour period preceding sampling. Rw_{72} is the weighted sum of rainfall amount in the 72-hour period preceding sampling, with the most recent rainfall receiving the most weight. The Ohio single-sample maximum bathing-water standard of 235 colony-forming units per 100 milliliters (CFU/100 mL) is indicated by dotted lines and is used as a point of reference.)

Figure 11. Stormwater runoff from a pipe draining the parking lot to the beach at Lakeshore, Ashtabula, Ohio, July 12, 2004. (Photograph by Timothy Roberts, U.S. Geological Survey)

MAR patterns and indices were different between the two source samples; bird feces and wastewater. MAR data from source samples on multiple sampling days were combined for each beach for data analysis. Out of 1,055 tests run on *E. coli* isolates from bird feces at Lakeshore, only 11 tests showed resistance: 7 to streptomycin, 1 to tetracycline, and 3 to ampicillin (data not shown). Similarly at Edgewater, out of 1,479 tests, 10 tests from bird feces showed resistance: 6 to streptomycin, 1 to tetracycline, and 3 to sulfathiazole. In contrast, in wastewater samples, 151 out of 1,004 tests at Lakeshore and 59 out of 655 tests at Edgewater showed resistance; resistant *E. coli* isolates were found for every antibiotic. (The dataset was smaller at Edgewater because plates were overgrown with spreading colonies on two dates.) For wastewater samples, individual antibiotic resistance indices ranged from 0.02 for nalidixic acid at Edgewater to 0.21 for streptomycin at Lakeshore (data not shown). At both beaches, overall indices for wastewater were about 10 times higher and significantly different from those found for bird feces (table 2).

Site-specific MAR indices for source samples were statistically compared to indices for environmental samples using Chi-square analysis (table 2). At Edgewater, a definitive result was found for the bathing-water sample collected on September 1. In this sample, the index was significantly different from bird feces (chi-square = 67.0, p = <0.0001) but not from wastewater (chi-square = 0.91, p = 0.3411), indicating a predominant wastewater source. In the other four samples from Edgewater, the index was significantly different from both bird feces and wastewater. This result suggests mixed sources, although to varying degrees. For example, the bathing-water sample collected on July 13 may be more affected by wastewater than by bird feces, as shown by a lower chi-square and higher *p* value for wastewater. At Lakeshore, definitive results indicating a bird source were found for the bathing-water sample collected on July 14 and for all samples collected on August 15. In these samples, the indices for environmental samples, ranging from 0.005 to 0.014, were significantly different from wastewater, but not from bird feces. In the other four samples at Lakeshore, MAR indices suggested mixed sources.

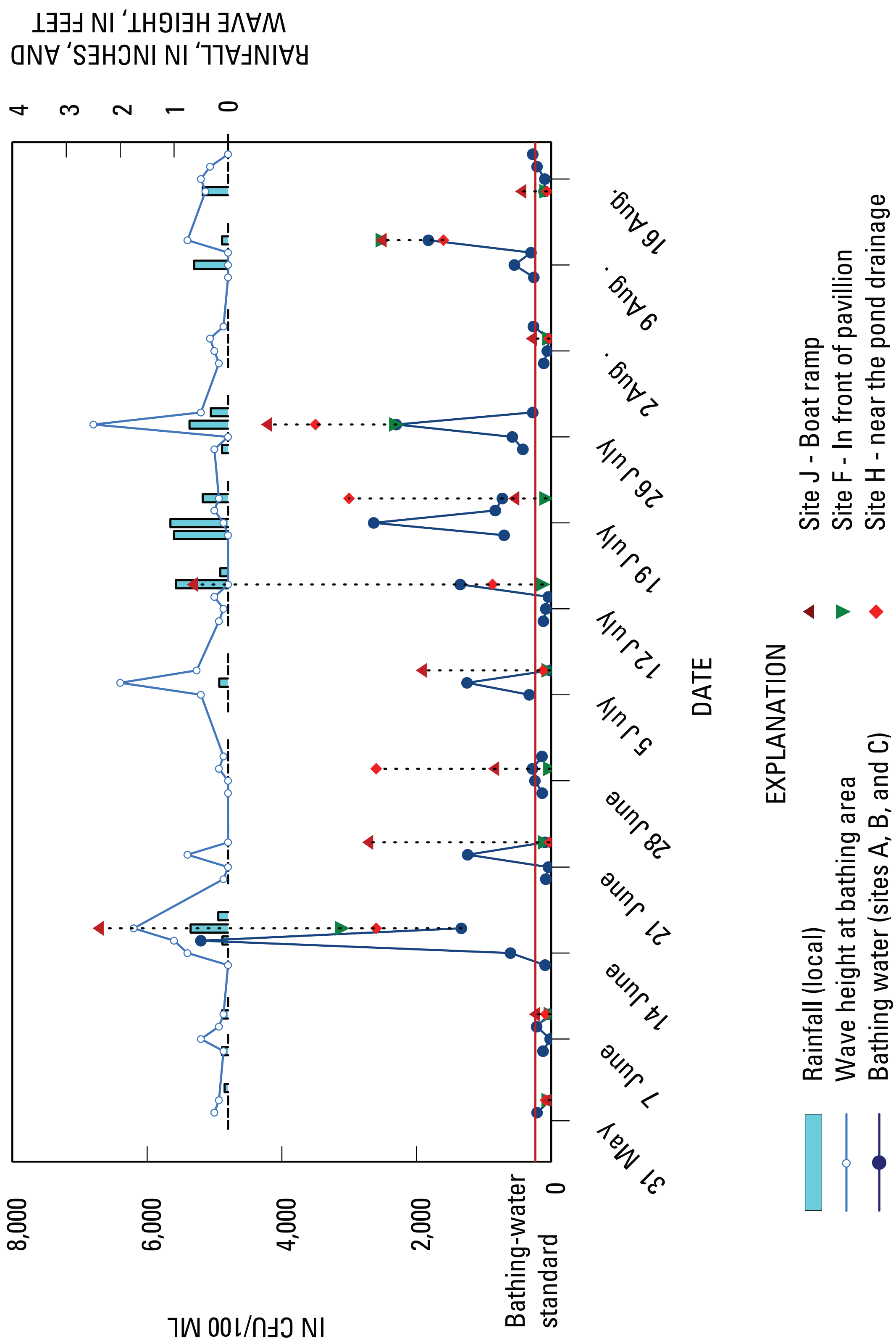

Figure 12. Average concentrations of *Escherichia coli* at nearshore lake sites, Lakeshore, Ashtabula, Ohio, 2005. (Rainfall amounts are from a local, privately operated gage. CFU/100 mL is colony-forming units per 100 milliliters. Gaps in rainfall data are because no data were collected on weekends.)

Table 2. Multiple antibiotic resistance (MAR) indices for samples collected at Edgewater and Lakeshore, 2005, and their relations to MAR indices of source samples.

[Chi-square is determined by use of contingency-table analysis; *p* is the significance of the relation; MAR indices for source samples and results from contingency-table analysis between the two source samples are shown in bold text]

			Relation to source samples			
			Wastewater		Bird feces	
Date	Source or environmental sample	MAR index[a]	Chi-square	*p*	Chi-square	*p*
EDGEWATER						
	Wastewater source	**0.090**			**139.0**	**<0.0001**
	Bird feces source	**.007**				
June 29	Bathing water	.026	13.4	0.0003	9.4	0.0022
July 13	Bathing water	.040	9.2	.0024	25.8	<.0001
Aug. 21	Bathing water	.033	11.1	.0009	16.2	<.0001
Sept. 1	Bathing water	.073	.91	.3411	67.0	<.0001
Sept. 1	Ground water	.032	5.8	.0164	10.0	.0015
LAKESHORE						
	Wastewater source	**.150**			**100.7**	**<.0001**
	Bird feces source	**.010**				
June 28	Bathing water	.032	30.4	<.0001	7.6	<.0001
July 14	Bathing water	.010	60.6	<.0001	.02	.8761
Aug. 15	Bathing water	.014	47.8	<.0001	.33	.5699
Aug. 15	Parking Lot	.005	59.2	<.0001	.84	.3605
Aug. 15	Site N	.005	63.9	<.0001	1.04	.3093
Sept. 1	Bathing water	.056	23.5	<.0001	26.5	<.0001
Sept. 1	Parking Lot	.045	28.9	<.0001	17.1	<.0001
Sept. 1	Site I	.041	31.7	<.0001	14.6	<.0001

[a]The MAR index is calculated as the number of resistant isolates/number of antibiotics tested X total isolates tested.

Human markers for *Bacteroides* and enterococci were used in conjunction with MAR indices as multiple lines of evidence (table 3). At Edgewater, both human markers were found on September 1 in bathing-water samples, further confirming the MAR results of predominantly wastewater sources. On September 1, the area received the remnants of Hurricane Katrina, including 3- to 5-ft wave heights and heavy Rw72; *E. coli* and enterococci concentrations were elevated. In all other samples at Edgewater, the MAR index indicated a mixed source, yet neither human marker was found. *E. coli* and enterococci concentrations were lower in these samples than those collected on September 1.

At Lakeshore, both human markers were found on July 14 in bathing water, yet the results from the MAR index indicated bird feces as a predominant source; *E. coli* and enterococci concentrations were elevated in this sample. In contrast, on August 15, results from MAR indexes and human markers were in agreement: human markers were not found in any samples, and the MAR indices were consistent with a bird fecal source. At site I on September 1, the *Bacteroides* marker was found, the enterococci marker was not found, and MAR indicated mixed sources. Parking-lot samples were determined to be from bird sources on August 15 and mixed sources on September 1 by use of the MAR index; the enterococci marker was not found.

Table 3. Weather conditions, indicator concentrations, and results of microbial source tracking at bathing-water and other sites at Edgewater and Lakeshore, 2005.

[*E. coli, Escherichia coli;* Ent, enterococci; CFU/100 mL, colony-forming units per 100 milliliters; MPN/g_{dw}, most probable number per gram dry weight; MAR is multiple antibiotic resistance and is based on comparisons of indices in environmental samples to those of sources samples; Bact is the *Bacteroides* human marker and *Esp* is the enterococci *esp* human marker, reported as negative (neg) or positive (pos); M, mixed source; W, wastewater; B, bird feces; --, no data]

Date	Rainfall (inches)	Indicator concentration (CFU/100 mL)		Microbial source tracking			Indicator concentration (CFU/100 mL)		Microbial source tracking			Indicator concentration (MPN/g_{dw})		Microbial source tracking		
		E. coli	Ent	MAR	Bact	*Esp*	*E. coli*	Ent	MAR	Bact	*Esp*	*E. coli*	Ent	MAR	Bact	*Esp*
EDGEWATER																
	Airport, Rw_{72}[a]	**Bathing water**					**Ground water**									
June 29	0.15	51	63	M	neg	neg	--	--	--	--	--					
July 13	.01	21	2	M	neg	neg	--	--	--	--	--					
August 21	3.6	270	100	M	neg	neg	92	160	--	neg	neg					
Sept. 1	2.4	1,400	380	W	pos	pos	7	21	M	neg	neg					
LAKESHORE																
	Local, R_{d-1}[b]	**Bathing water**					**Site I or N**					**Parking Lot**				
June 28	0	500	84	M	neg	neg	--	--	--	--	--	--	--	--	--	--
July 14	.97	1,100	430	B	pos	pos	--	--	--	--	--	--	--	--	--	--
August 15	.5	82	42	B	neg	neg	980	380	B	neg	neg	1,400	70,000	B	--	neg
Sept. 1	3.7[c]	220	190	M	neg	neg	410	370	M	pos	neg	10,000	83,000	M	--	neg

[a]Rw_{72} was the weighted sum of rainfall, in inches, at Hopkins International Airport, Cleveland, Ohio, in the 72-hour period before sampling, with the most recent rainfall receiving the most weight.

[b]R_{d-1} was the rainfall amount, in inches, at a local rainfall gage, in the 24-hour period preceding sampling.

[c]Local rainfall was not available; rainfall is from Ashtabula County Airport.

Discussion and Conclusions

A multiple-method approach was used to help identify sources of fecal contamination at two Lake Erie beaches: an urban beach (Edgewater) and a beach in a small city (Lakeshore). Sampling identified spatial patterns in *E. coli* concentrations and yielded information on the physical pathways of contamination. MST testing provided information on whether the source was from wastewater or bird feces and if a human-specific marker was present; however, MST did not provide information on the pathways of contamination.

Edgewater

The first step at Edgewater was to identify the importance of remote sources of fecal contamination in this highly urbanized area. During 2003 and 2004, *E. coli* concentrations near the mouths of rivers and outfalls remote to the beach were elevated during wet conditions but generally decreased along transport pathways to the beach. Further, concentrations were lower in nearshore lake samples typically collected 150 ft from the shoreline than in those collected within the bathing area at Edgewater, indicating a potential local source to the beach. Nearshore lake samples, however, were not collected at 3-ft depths and similar proximities to the shoreline as bathing-water samples; nearshore lake samples collected closer to the shoreline are needed to strengthen the local-source hypothesis. Nevertheless, a transport pathway to the beach from nearshore lake sites with high *E. coli* concentrations was not evident, and it seems unlikely that fecal contamination would hug the shoreline and not be transported at least 150 ft offshore. This line of thinking downgraded the importance of remote sources to the beach, and subsequent source-tracking efforts concentrated on the beach itself.

In 2005, *E. coli* concentrations were compared in Main and Middle Beach bathing-water samples at Edgewater. Elevated *E. coli* concentrations were generally associated with rainfall and (or) high waves; however, there were days when *E. coli* was elevated in the absence of rainfall or high waves. Samples collected at the Middle Beach had generally higher *E. coli* concentrations than those collected at the Main Beach. The different responses of *E. coli* concentrations on two days the outfall flowed in 2005 suggested that waves and currents may be important factors in transporting *E. coli* from the Middle to the Main Beach. Further, frequent elevated *E. coli* concentrations in the absence of a discharge at the Edgewater outfall indicated that outfall discharges were not a major source of fecal contamination at Edgewater. Because the Middle beach is not guarded, borders a wooded area, and tends to be visited by boaters and dog owners and not by swimmers, potential sources include the illegal discharge of wastes from boaters; wastes from dogs, stray cats, and wildlife; and resuspension of *E. coli* from lakebed sediments through the actions of jet skis, boats, and waves. The Middle beach also has the potential to receive stormwater from an abandoned storm sewer system.

Sources at the Middle Beach may not be the only ones contributing to elevated *E. coli* in the Main beach. In earlier studies (Francy and others, 2003), turbidity and wave heights were statistically related to *E. coli* concentrations, providing circumstantial evidence that resuspension of *E. coli* from lakebed sediments may be important at Edgewater. In the present study, samples collected at 1- and 2-ft water depths at the Main Beach had higher *E. coli* concentrations than those collected at 3-ft water depths. This may be from resuspension of *E. coli* from lakebed sediments or from storage and accumulation of *E. coli* from shallow ground water in the foreshore area.

Short-term shallow ground-water studies at Edgewater in 2004 and 2005 showed that ground water generally flows toward the lake, with slight temporal reversals in flow in the near foreshore area, probably a result of ground-water response to wave action. An *E. coli* concentration peak at a seemingly random location in June 2004 showed the heterogeneity of *E. coli* concentrations in beach sands. This suggests a local surface source, such as feces from the large numbers of gulls that congregate on the beach. Infiltration of bird feces through sand with surface water from rainfall or high waves may be a mechanism of concentrating *E. coli* in shallow ground water. Because concentrations of *E. coli* generally declined with increasing distance inland from the lake, another inland source may be the interaction of the lake with shallow ground water in the near foreshore area; this interaction may sustain higher levels of *E. coli* in the shallow ground water close to the lake, even after lake *E. coli* concentrations decline. A monthlong study (August 16 through September 12, 2005) supported the hypothesis that the interaction of shallow ground water and waves is a mechanism of *E. coli* storage and accumulation; as the distance to the edge of water increased, *E. coli* concentrations in shallow ground water generally decreased. The monthlong study also supported the hypothesis that infiltration of precipitation and waves through beach sands is a likely mechanism of *E. coli* transport to ground water.

In other studies, investigators found that fecal-indicator bacteria were abundant in beach sands, may be a reservoir for enteric organisms, and may affect recreational water quality (Alm and others, 2003; Kinzelman and others, 2004a). It is well known that bacteria survive longer in sediments than in water because sediments provide a nutrient-rich environment and protection from sunlight inactivation and protozoan grazing (Alm and others, 2003; LaLiberte and Grimes, 1982). At a Lake Michigan beach frequented by large populations of seagulls, investigators found that deeper beach grooming practices without leveling resulted in lower *E. coli* concentrations in foreshore sands than shallow practices with leveling (Kinzelman and others, 2004b). The former practice helped to aerate the sand and allowed for exposure of bacteria to sunlight and predators. The investigators suggested that significant populations of seagulls contribute to the accumulation of *E. coli* in beach sands and that waves washing over beach sands could transport bacteria from sediments to lake waters as they recede. Investigators in the United Kingdom found that pathogenic species of *Campylobacter* were more prevalent in sand at beaches that did not meet the European Commission (EC) Bathing Water Directive than at beaches that met the Directive (Bolton and others, 1999). The EC Bathing Water Directive specifies the microbial standards for recreational waters in Europe. Their results indicated that sand may be acting as a natural filter leading to concentration of pathogens on areas of the beach used by the public and may be an important mechanism for reseeding of bathing water with pathogens.

A complicated scenario emerges at Edgewater—spatial patterns of *E. coli* indicated that local sources of fecal contamination were dominant; the Edgewater outfall was not a major source of fecal contamination; elevated *E. coli* concentrations were often associated with rainfall and (or) high waves, but not always; and elevated *E. coli* concentrations were found in shallow ground water, especially in the active near foreshore area. MST methods indicated that mixed sources were present (bird feces and wastewater) on all days sampled except for September 1, which was affected by atypical weather patterns associated with Hurricane Katrina. Human markers were found only on September 1, when the MAR index indicated a dominant wastewater source and bacterial indicator concentrations were high. Concentrations of bacterial indicators may have been too low to detect human markers on the other days when MAR indices indicated mixed sources. Scott and others (2005) proposed that the absence of the human enterococci marker could indicate a nonhuman source of contamination, but only in association with high counts of enterococci.

Lakeshore

At Lakeshore, low concentrations of *E. coli* at nearshore lake sites furthest from the shoreline indicated that fecal contamination was most likely of local origin. High concentrations of *E. coli* in water and sediments at several nearshore lake sites close to the shoreline showed that contamination was emanating from several points. Bathing-area bed sediments and the gravel parking-lot sediments appeared to be important reservoirs of *E. coli*; physical evidence confirmed that runoff from the gravel parking lot was an important contributor. A dry-weather source at site F (in front of the pavilion) caused high *E. coli* concentrations on one day in 2004 but not on the days sampled in 2005. Elevated concentrations of *E. coli* at sites H (near pond drainage) and J (boat ramp) indicated that sources of fecal contamination were concentrated in these areas in both dry and wet weather.

At Lakeshore bathing-water sites, MST results indicated mixed sources on three out of four days tested. On a day with moderate rainfall and elevated *E. coli* and enterococci concentrations (July 14), both human markers were found in bathing waters, yet the MAR index indicated bird feces as the dominant source. On another day (August 15) that received 0.5 in. of local rainfall in past 24 hours and indicator concentrations were not elevated, results from MAR indexes and human markers were in agreement that bird feces was the dominant source. MAR indices indicated mixed or bird sources at sites I (west side of west groin) or N (east side of west groin) and at parking-lot sites. Both human markers are almost always found in sewage from a wastewater-treatment plant that serves large populations, but only the *Bacteroides* marker was consistently found in individual fecal samples (Troy Scott, Biological Consulting Services of N. Florida, Inc., oral commun., 2006). On September 1, the *Bacteroides* marker was found at site I, but not the enterococci marker. This suggests that the source of human fecal contamination may be from a small contributing population, such as one or more septic systems, and not from wastewater with a large contributing population.

Using a Multiple-Method Approach to Source Tracking

The studies at Edgewater and Lakeshore indicate that a multiple-method approach is needed to identify sources of fecal contamination at coastal beaches. These include (1) documenting the spatial variability of fecal contamination in the area and at the beach, (2) identifying the environmental and meteorological factors that affect indicator concentrations, and (3) applying multiple MST techniques to help understand earlier findings. This type of approach was used successfully to track sources of fecal contamination to a recreational area in southern California (Noble and others, 2006). Investigators found that levels of fecal-indicator bacteria were not discerning because they were consistent throughout the watershed. By adding other tools to the investigation, such as presence of human enterovirus and human-specific *Bacteroides* marker, researchers were able to identify the most important sources.

Birds are an important source of fecal contamination at Lake Erie beaches and elsewhere. Canadian researchers found a significant correlation ($r = 0.88$) between fecal-coliform concentrations and number of birds at bathing-beach locations on a spring-fed lake (Lévesque and others, 1993). When food was spread on the sand, the number of gulls on the beach and fecal-coliform concentrations in the water increased rapidly. In a Great Lakes study, high concentrations of *E. coli* (10^5–10^9 CFU/g) associated with gull feces suggested that gulls may be a major contributor to fecal contamination to beaches (Fogarty and others, 2003). Unfortunately, markers that indicate bird contamination are not available; for example, in one recent study, Fogarty and Voytek (2005) were unable to detect fecal contamination by avian species using *Bacteroides* markers.

Several MST methods are needed in order to help distinguish human from wildlife sources of fecal contamination at beaches. Studies should include both *Bacteroides* and enterococcus human markers to provide definitive evidence of a human source. However, the absence of either marker does not necessarily imply the absence of a human source of fecal contamination, especially when indicator concentrations are low. MAR indices can help to identify sources, even when indicator concentrations are low and human markers are not found. In a study of *E. coli* source isolates in southern Ontario, average MAR indices for human, livestock, and wildlife isolates were 0.1339, 0.0966, and 0.027, respectively (Guan and others, 2002); these are similar to those found in sources at Lakeshore and Edgewater (table 2). This consistency between sites further illustrates the usefulness of MAR indices.

More work needs to be done during a variety of weather and wave conditions that combines spatial sampling with MST tools to better understand the sources of fecal contamination at Edgewater and Lakeshore. At Edgewater, additional days would need to be sampled, including days with high indicator concentrations and no rainfall or waves. A season-long shallow ground-water study that incorporates MST tools would help to further reveal the interactions between shallow ground water and lake water at Edgewater. At Lakeshore, more work needs to be done at nearshore lake sites that combines spatial sampling with MST and includes several consecutive days when *E. coli* concentrations are elevated during dry and wet weather.

Summary

Because of the complexity of coastal environments and the prevalence of nonpoint sources of fecal contamination, a multiple-method approach is practical for identifying sources of fecal contamination at coastal beaches. This approach was applied to two Lake Erie bathing beaches: Edgewater in Cleveland, Ohio, and Lakeshore in Ashtabula, Ohio. Steps included identifying spatial patterns of *E. coli* concentrations in each area, determining weather patterns that caused elevated *E. coli*, and applying microbial source tracking (MST) techniques to specific sites.

Edgewater and Lakeshore are popular recreational areas with beaches in a highly urbanized area and a small municipality, respectively. The beach at Edgewater is potentially affected by effluents from wastewater-treatment plants and septic systems, stormwater runoff, CSO's, and wildlife. Lakeshore is potentially affected by treated wastewater, septic systems, drainage from inland ponds, stormwater runoff from the park and parking lots, wildlife, and illegal discharges from boats. At Edgewater, spatial sampling for *E. coli* was done at bathing-area, nearshore lake, and shallow-ground-water sites during 2003–05. At Lakeshore, spatial sampling for *E. coli* was done at bathing, nearshore lake, and parking-lot sites in 2004 and 2005. Weather data were compiled from the nearest National Weather Service station at both beaches or from a local source, when available. Samples were collected for MST testing on four days during 2005. These included bird fecal samples and wastewater from local treatment plants as source samples from both beaches. Environmental samples for MST testing included bathing-water samples at both beaches; shallow ground-water samples were collected at Edgewater and nearshore lake and parking-lot samples were also collected at Lakeshore. Three MST methods were used during this study: multiple antibiotic resistance (MAR) indexing of *E. coli* isolates and the presence of human-specific genetic markers within two types of bacteria, *Bacteroides* and enterococcus.

At Edgewater, spatial patterns of *E. coli* indicated that local sources of fecal contamination were dominant; the Edgewater outfall was not a major source of fecal contamination; elevated *E. coli* concentrations were often associated with rainfall and (or) high waves, but not always; and elevated *E. coli* concentrations were found in shallow ground water. *E. coli* concentrations near the mouths of rivers and outfalls remote to the beach were elevated during wet conditions, but concentrations decreased along transport pathways to the beach. In addition, concentrations of *E. coli* were lower in nearshore lake samples collected 150 ft from the shoreline than in those collected within the bathing area. Further supporting the theory of local sources, samples collected within the bathing area at 1- and 2-ft water depths had higher *E. coli* concentrations than those collected at 3-ft water depths. Measured hydraulic gradients showed that shallow ground-water flow was generally towards the lake. *E. coli* concentrations were often elevated in shallow ground water in the near foreshore area and were occasionally elevated in random backshore locations. The interaction of the lake with shallow ground water in the near foreshore area may sustain higher levels of *E. coli* in the shallow ground water close to the lake, even after lake *E. coli* concentrations decline. The infiltration of bird feces through beach sands by precipitation and waves is also a likely mechanism of *E. coli* transport to ground water.

At Lakeshore, low concentrations of *E. coli* in the nearshore lake sites furthest from the shoreline indicated that fecal contamination was most likely of local origin. Spatial sampling at Lakeshore indicated elevated concentrations during wet and dry weather at bathing-area sites, in parking-lot sediments, and at nearshore lake sites at the boat ramp (site J) and near the pond drainage (site H); fecal contamination may therefore be emanating from several points along the shoreline. Actual physical evidence confirmed that runoff from the parking lot leads to the degradation of water quality at the beach.

MST samples were collected in 2005 to help interpret spatial findings and determine whether sources of fecal contamination were of human or nonhuman origin. MAR indices (number of resistant isolates / (number of antibiotics tested × total isolates tested)) were useful in distinguishing between bird feces and wastewater sources. MAR indices for bird feces were 0.007 and 0.010 at Edgewater and Ashtabula, respectively. At the same two beaches, MAR indices for wastewater (0.090 and 0.150) were about 10 times higher than those for bird feces. The results from MAR indices agreed with results from the two human-specific markers in some but not all of the samples tested. On one day at Edgewater, the MAR index of bathing-water samples indicated a wastewater source, and both *Bacteroides* and enterococci markers were found to corroborate this finding. Similarly, on one day at Lakeshore, the MAR index indicated a bird feces source, and no human markers were found in bathing-water samples. On three days at Edgewater and two days at Lakeshore, the MAR index indicated a mixed source, but neither marker was found; this may be because bacterial indicator concentrations were too low to detect a marker. Contradictory results were found at Lakeshore on one day when the MAR index in bathing-water samples indicated a predominant bird source, yet both human markers were found; *E. coli* and enterococci concentrations were elevated in bathing-water samples collected that day.

Multiple tools are needed to identify sources of fecal contamination at coastal beaches. Spatial sampling identified patterns in *E. coli* concentrations and yielded information on the physical pathways of contamination. MST methods provided information on whether the source was likely of human or nonhuman origin only; however, MST did not provide information on the pathways of contamination. As was found in other studies, waterfowl are an important source of fecal contamination at Lake Erie beaches. In order to distinguish between human and bird sources, several MST methods should be applied. *Bacteroides* and enterococci human markers provide evidence of a human source; however, the absence of both markers does not imply the absence of a human source, especially when indicator concentrations are low. MAR indices can help distinguish between human and waterfowl sources, even when indicator concentrations are low.

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