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Comparison of the Immunomagnetic Separation/Adenosine Triphosphate Rapid Method and the Modified mTEC Membrane-Filtration Method for Enumeration of *Escherichia coli*

Scientific Investigations Report 2009–5222

U.S. Department of the Interior
U.S. Geological Survey

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U.S. Geological Survey, Reston, Virginia: 2009

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Suggested citation:
Brady, A.M.G., Bushon, R.N., and Bertke, E.E., 2009, Comparison of the immunomagnetic separation/adenosine triphosphate rapid method and the modified mTEC membrane-filtration method for enumeration of *Escherichia coli*: U.S. Geolgogical Survey Scientific Investigations Report 2009–5222, 21 p.

Acknowledgments

The authors thank the following individuals for their technical assistance throughout the study discussed in this report:

Northeast Ohio Regional Sewer District—Mark Citriglia, Eva Hatvani, Lester Stumpe, Ben Tedrick, Cathy Perciado, Eric Soehnlen, and Steve Lizewski.

Cuyahoga County Board of Health—Jill Lis.

Cuyahoga County Sanitary Engineer—Suzanne Britt, Ann McCready-Gliha, and Patricia Boone.

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Conversion Factors

Multiply	By	To obtain
	Length	
inch (in.)	25.4	millimeter (mm)
foot (ft)	.3048	meter (m)
mile (mi)	1.609	kilometer (km)
	Volume	
milliliter (mL)	.03381	fluid ounce (oz)
microliter (µL)	.001	milliliter (mL)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:
$°F=(1.8\times°C)+32$

Bacteria concentrations are given in colony-forming units per 100 milliliters (CFU/100 mL), and bacterial adenosine triphosphate concentrations are given in relative light units per 100 milliliters (RLU/100 mL).

Pore sizes of filters are given in micrometers (µm).

Comparison of the Immunomagnetic Separation/Adenosine Triphosphate Rapid Method and the Modified mTEC Membrane-Filtration Method for Enumeration of *Escherichia coli*

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Abstract

Water quality at beaches is monitored for fecal indicator bacteria by traditional, culture-based methods that can take 18 to 24 hours to obtain results. A rapid detection method that provides estimated concentrations of fecal indicator bacteria within 1 hour from the start of sample processing would allow beach managers to post advisories or close the beach when the conditions are actually considered unsafe instead of a day later, when conditions may have changed. A rapid method that couples immunomagnetic separation with adenosine triphosphate detection (IMS/ATP rapid method) was evaluated through monitoring of *Escherichia coli* (*E. coli*) at three Lake Erie beaches in Ohio (Edgewater and Villa Angela in Cleveland and Huntington in Bay Village).

Beach water samples were collected between 4 and 5 days per week during the recreational seasons (May through September) of 2006 and 2007. Composite samples were created in the lab from two point samples collected at each beach and were shown to be comparable substitutes for analysis of two individual samples. *E. coli* concentrations in composite samples, as determined by the culture-based method, ranged from 4 to 24,000 colony-forming units per 100 milliliters during this study across all beaches. Turbidity also was measured for each sample and ranged from 0.8 to 260 neophelometric turbidity ratio units. Environmental variables were noted at the time of sampling, including number of birds at the beach and wave height. Rainfall amounts were measured at National Weather Service stations at local airports. Turbidity, rainfall, and wave height were significantly related to the culture-based method results each year and for both years combined at each beach. The number of birds at the beach was significantly related to the culture-based method results only at Edgewater during 2006 and during both years combined.

Results of the IMS/ATP method were compared to results of the culture-based method for samples by year for each beach. The IMS/ATP method underwent several changes and refinements during the first year, including changes in reagents and antibodies and alterations to the method protocol. Because of the changes in the method, results from the two years of study could not be combined. Kendall's tau correlation coefficients for relations between the IMS/ATP and culture-based methods were significant except for samples collected during 2006 at Edgewater and for samples collected during 2007 at Villa Angela. Further, relations were stronger for samples collected in 2006 than for those collected in 2007, except at Edgewater where the reverse was observed.

The 2007 dataset was examined to identify possible reasons for the observed difference in significance of relations by year. By dividing the 2007 data set into groups as a function of sampling date, relations (Kendall's tau) between methods were observed to be stronger for samples collected earlier in the season than for those collected later in the season. At Edgewater and Villa Angela, there were more birds at the beach at time of sampling later in the season compared to earlier in the season. (The number of birds was not examined at Huntington.) Also, more wet days (when rainfall during the 24 hours prior to sampling was greater than 0.05 inch) were sampled later in the season compared to earlier in the season. Differences in the dominant fecal source may explain the change in the relations between the culture-based and IMS/ATP methods.

Introduction

The State of Ohio uses *Escherichia coli* (*E. coli*) as an indicator bacterium to assess recreational water quality (Ohio Environmental Protection Agency, 2008). Traditional culture-based methods for determining concentrations of *E. coli* in water require at least 18–24 hours from sample collection to availability of results. Water-quality advisories are issued when concentrations exceed the recreational water-quality standard; however, results are not available until the day after the sample is collected. By that time, bacteria concentrations in the water may have changed significantly (Boehm and others, 2002; Wymer and others, 2005). As a result of the lag between sample collection and analytical results, recreational users may be at risk of contact with water that has exceeded the standard and is not considered safe for recreation. Alternatively, the beach may be declared unsafe when the risk is low, resulting in lost recreational use and revenue. The need for rapid detection methods for monitoring recreational waters is widely recognized (U.S. Congress, 2007; U.S. Environmental Protection Agency, 2007). Many enumeration methods have become available recently that provide results in less than 4 hours, thereby yielding same-day feedback on bacterial concentrations in recreational waters (Griffith and others, 2004; Noble and Weisberg, 2005; Griffith and Weisberg, 2006).

One rapid method for determining concentrations of *E. coli* was developed by researchers at the University of Michigan (Lee and Deininger, 2004) and was further modified and field tested by the U.S. Geological Survey (USGS) in the Cuyahoga River (within the Cuyahoga Valley National Park) during the recreational seasons of 2004–6 (Brady, 2007; Bushon and others, 2007, 2009). The method is based on immunomagnetic separation (IMS), which selectively captures *E. coli* through the use of antibodies attached to magnetic beads. Adenosine triphosphate (ATP) bioluminescence is then measured to quantify the captured *E. coli*. Advantages of the IMS/ATP method include availability of results within 1 hour of the start of sample processing, simplicity and affordability of equipment and supplies, and portability of equipment in the field. Additionally, the IMS/ATP method measures viable cells through the detection of ATP, which is an energy-carrying molecule found in living organisms. In comparison, culture-based methods only measure culturable organisms and would not be able to detect those organisms that have entered a viable but not culturable state.

The USGS, in cooperation with the Northeast Ohio Regional Sewer District (NEORSD), the Cuyahoga County Board of Health, the Office of the Cuyahoga County Sanitary Engineer (CCSE), and the Ohio Water Development Authority, evaluated the use of the IMS/ATP rapid method at three Lake Erie beaches in northeast Ohio.

Purpose and Scope

This report describes the results of a study to test a rapid method at three Lake Erie beaches in an attempt to provide beach managers with a tool that can determine *E. coli* concentrations more rapidly than the culture-based method. Water samples were collected during the recreational seasons (May–September) of 2006 and 2007 at Edgewater, Villa Angela, and Huntington beaches in northeast Ohio. Samples were analyzed for concentrations of *E. coli* using the traditional culture-based method and the IMS/ATP method. Sample results were compared to determine if a relation exists between the results of the two methods. Further, environmental or water-quality factors (for example, turbidity, wave height, and local rainfall) were collected and analyzed to better understand the differences in results between the two methods.

Study Area

The northeastern-Ohio Lake Erie beaches selected for this study (fig. 1) were Edgewater Park and Villa Angela, operated by the Ohio Department of Natural Resources and located within the Cleveland metropolitan area; and Huntington Reservation, operated by Cleveland Metroparks and located in suburban Bay Village to the west of Cleveland. The Edgewater beach bathing area is open to the lake, and a breakwall on the eastern side of beach impedes longshore currents. The Villa Angela beach has four breakwalls built between the beach and the lake to stabilize the beach. At Huntington, the beach is separated into coves by five piers spaced nearly evenly along the beach.

A previous study at Edgewater by Francy and others (2006) investigated potential sources of fecal contamination to the beach. Remote sources, such as the Cuyahoga and Rocky Rivers and Westerly Wastewater Treatment Plant, were shown to have little effect on elevated bacteria concentrations in the bathing waters at the beach. Local sources, such as runoff from parking lots and adjacent areas, reservoirs of *E. coli* in the foreshore sands, and bird feces, were suggested as the dominant sources of fecal contamination to the bathing waters at the beach.

Potential sources of fecal contamination to Villa Angela beach include stormwater and combined-sewer overflows from outfalls from streams to the west and Euclid Creek to the east. At Huntington, potential sources of fecal contamination are largely unknown. At both beaches, birds are also probable sources of *E. coli*.

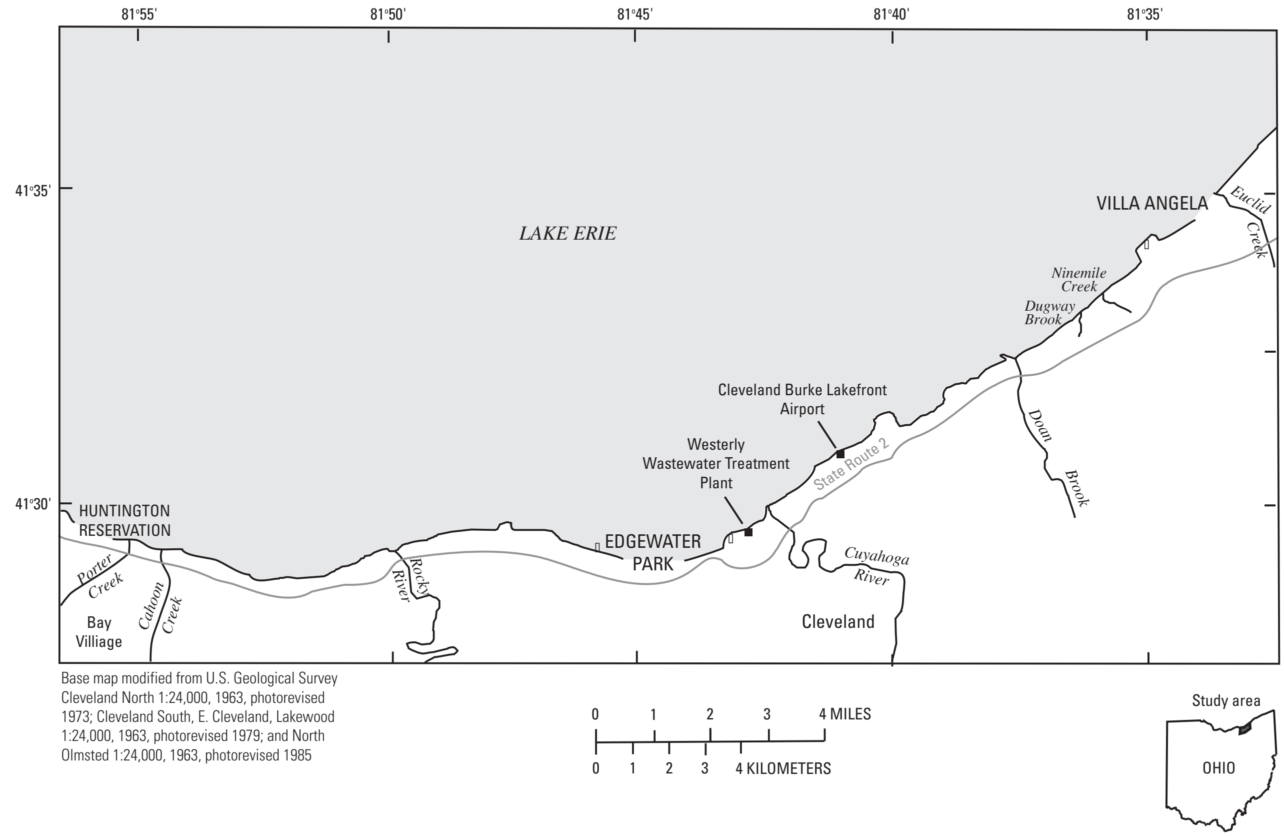

Figure 1. Study area and beach locations, northeastern Ohio.

Previous studies at these beaches have been done by the USGS, in cooperation with local agencies, to evaluate factors related to the concentrations and distribution of *E. coli* and to develop models to predict levels of contamination based on easy-to-measure environmental variables (Francy and Darner, 1998; Francy and others, 2003; Francy and others, 2006; Francy and Darner, 2008). Multiple and diffuse sources, natural variability in bacteria concentrations over space and time, and dynamic currents, weather patterns, and natural processes all contribute to the complexity of estimating bacteria concentrations at these beaches.

Methods of Study

Sampling Frequencies and Locations

Daily water samples for determining *E. coli* concentrations by traditional culture-based methods were collected as part of established monitoring programs. At Edgewater and Villa Angela, water samples were collected by NEORSD five times per week at two locations at each beach. At Huntington, samples were collected by CCSE at two locations four times per week in 2006 and five times per week in 2007. Samples were collected at east and west locations at Edgewater and Villa Angela and at central and west locations at Huntington. Two sampling locations were chosen at each beach so that the heterogeneity of bacterial concentrations across the beach could be evaluated in sample results. Samples were collected during the recreational seasons of 2006 and 2007, starting at the end of May or early June through the end of August or September.

Sample-Collection Methods

Field personnel used USGS sampling methods to collect water samples for analysis by both traditional culture-based and IMS/ATP methods. Water samples were collected between 8 and 9 a.m. in waters ranging from 2.5 to 3 ft in depth by means of a grab-sampling technique that minimized contamination of sterile polypropylene bottles (Myers and others, 2007). Samples were collected approximately 1 ft below the surface of the water. When wave heights were greater than 2 to 3 ft, field personnel waded out to a safe depth and collected the sample or used an extension pole to collect the sample near the routine water depth. Samples were immediately capped and placed in coolers with ice for transport to the lab.

Laboratory Methods

Samples were kept on ice or refrigerated (0–4°C) until analyses could be completed. All samples were analyzed within 6 hours of sample collection. During 2006, samples from Huntington were analyzed by CCSE using the culture-based method and analyzed by NEORSD using the IMS/ATP method. CCSE analyzed Huntington samples using both methods in 2007. NEORSD analyzed samples from Edgewater and Villa Angela using both methods for both years. Composite samples were prepared in the laboratory by adding equal, well-mixed volumes of each of the two samples collected from the sampling locations at each beach into a clean, sterile bottle.

Turbidity was measured by the sample-collecting agency from the prepared composite sample and reported in nephelometric turbidity ratio units (NTRU). At least two aliquots from each sample were measured until two consecutive measurements agreed within 10 percent or within 1 NTRU if readings were less than 10 NTRU.

The Ohio bathing-water single-sample standard for *E. coli* of 235 CFU/100 mL (Ohio Environmental Protection Agency, 2008) was used as a benchmark to evaluate water quality in this study and will henceforth be referred to as “the water-quality standard” (WQS).

Culture-Based Method

Individual and composite samples were analyzed for *E. coli* using the traditional culture-based method of membrane filtration on modified mTEC media (U.S. Environmental Protection Agency, 2006). A series of sample volumes were filtered through 0.45-µm-pore-size filters, placed on agar plates, and incubated for 2 hours at 35°C and then 22 hours at 44.5°C. Any colonies that were red or magenta were counted as positive for *E. coli*. Concentrations were calculated as described by Myers and others (2007) and reported in colony-forming units per 100 milliliters (CFU/100 mL).

Both individual and composite samples were analyzed using the traditional culture-based method to evaluate whether the result of a composite sample yields an *E. coli* concentration that is comparable to the current practice of averaging the results of the individual samples at each beach.

Immunomagnetic Separation/ Adenosine Triphosphate Rapid Method

The IMS/ATP method for *E. coli* was originally described in Lee and Deininger (2004), with further modifications described in Bushon and others (2009a and 2009b). Variations of the method were tested and optimized throughout the project as described below. Because of funding restraints, only the composite sample for each beach was used for IMS/ATP analyses.

For samples collected in 2006, two variations of the IMS/ATP method were tested for each sample. One variation that included preliminary sample processing steps (filtration and elution) was compared to a second variation where samples were analyzed directly with no filtration (direct addition). For the filtration and elution variation, 50 mL of sample were filtered through a 0.45-µm-pore-size filter to capture target bacteria. The bacteria were then eluted from the filter by transferring the filter to a 50-mL centrifuge tube containing phosphate-buffered saline (PBS) with 0.05 percent Tween 20 (Sigma-Aldrich, St. Louis, Mo.) and mixing by hand for 1 minute. The eluate was used for subsequent processing steps. For the direct-addition variation, 5 to 30 mL of sample was added directly to a 50-mL centrifuge tube; for volumes less than 30 mL, the total volume was brought up to 30 mL using PBS.

For samples collected in 2007, only the direct-addition variation was used for sample analyses. The filtration and elution variation of the method was no longer used (see “Sample Processing Versus Direct Addition of Sample” section of this report). The volume of sample analyzed was 25 mL. Further, PBS was no longer used to buffer the sample; instead, ImmTech diluent (ImmTech, Inc., New Windsor, Md.) was used to bring the volume up to 40 mL in a 50-mL centrifuge tube. The ImmTech diluent is designed to reduce nonspecific signal from samples with a complex matrix.

After the initial processing steps (if done), 100 µL of antibody-coated magnetic beads were added to the 50-mL centrifuge tube containing the sample or eluate. In 2006, the processing laboratory coated the beads with antibodies by means of passive adsorption according to directions in TechNote 204 by Bangs Laboratories (1999). The antibodies used in 2006 were goat anti-*E. coli* antibodies (BioDesign International, Saco, Maine). In 2007, the beads were coated with the antibodies by a commercial supplier (ImmTech, Inc.); a rabbit anti-*E. coli* antibody was used because the goat anti-*E. coli* antibodies were no longer available.

Samples containing antibody-coated beads were rotated at 18 rpm for 30 minutes on a sample mixer (Invitrogen, Carlsbad, Calif.). This step permitted the antibodies to adhere to the *E. coli* cells. After mixing, the 50-mL centrifuge tube was placed against a magnet (MPC-50 magnetic particle concentrator; Invitrogen) that pulled the bacteria-antibody-bead complex out of solution. The tube and magnet were rocked gently for 2 minutes before decanting. A washing step was done to decrease contamination from nontarget bacteria. During 2006, the washing step was done with PBS; but in 2007, the PBS was replaced with ImmTech diluent. After the washing step, the beads and associated target bacteria were transferred to a 1.5-mL tube using PBS and were placed against another magnet (MPS-S magnetic particle concentrator; Invitrogen). The tube and magnet were rocked gently for 2 minutes.

The remaining steps were identical for both years and followed the published method (Bushon and others, 2009a). Briefly, the bead complex was washed with a somatic-cell releasing agent (SRA) (New Horizons Diagnostics, Columbia, Md.) to rupture any nonbacterial cells. Another wash step was performed with PBS, and then the bead complex was exposed to a bacterial-cell releasing agent (BRA) (New Horizons Diagnostics) that ruptured bacterial cells, releasing ATP into solution. By using the MPS-S magnet, the solution containing ATP was isolated from the beads and exposed to luciferin-luciferase (LL) (New Horizons Diagnostics). The LL reacted with ATP to produce light. A microluminometer (New Horizons Diagnostics) was used to measure luminescence in relative light units (RLU). Results were reported as RLU/100 mL. Formulations of the reagents involved in the final steps of analysis (SRA, BRA, and LL) were changed by the manufacturer after the sampling season of 2006.

Ancillary Data Collection

Wave height was measured by use of a graduated rod at the time of sample collection. The rod was placed at the sampling location, and the minimum and maximum water heights were noted over the course of 1 minute. The number of birds at the beach were counted before sampling at Edgewater and Villa Angela. Rainfall information was compiled for the National Weather Service stations at local airports (Cleveland Hopkins International[1] and Cleveland Burke Lakefront) (*http://www.erh.noaa.gov*). Hourly rainfall amounts were totaled for the 24 hours prior to 9 a.m. on the reported date. Rainfall data from Cleveland Hopkins International Airport were used for data analysis for Edgewater and Huntington, and rainfall data from Cleveland Burke Lakefront Airport were used for data analysis for Villa Angela.

Quality Assurance/Quality Control

Quality-assurance and quality-control laboratory and field procedures (Myers and others, 2007; Francy and others, 2008) were followed throughout the study. No problems were observed for quality-control samples analyzed during this study. Filter blanks and method blanks were analyzed daily for the culture-based and IMS/ATP methods, respectively. Filter blanks consisted of approximately 50 mL of sterile buffer filtered and plated on modified mTEC agar prior to filtering the sample. Method blanks consisted of 30 mL (2006) or 25 mL (2007) of PBS treated in the same manner as a regular sample and analyzed by means of the IMS/ATP method. Replicate environmental samples were not processed in 2006 because each sample was analyzed by using the filtration and elution variation as well as the direct-addition variation of the IMS/ATP rapid method. In 2007, a total of 27 replicate samples were collected and analyzed by the direct-addition variation of the rapid method (13 replicates processed by NEORSD and 14 replicates processed by CCSE). To examine the variability in the replicates, the absolute value of the relative percent difference (ARPD) was calculated by taking the absolute value of the difference between the two replicates, dividing by the average, and multiplying by 100. For the replicate samples processed by the IMS/ATP method in 2007, the ARPD varied from <1 to 145 percent. The average ARPD was 43 percent, which is high compared to what Bushon and others (2009a) found in replicate samples collected from a river (24 percent).

Data Analysis and Statistics

Scatterplots and correlation analyses were used as exploratory tools to examine the data. Correlation analyses were used to assess the strength of the relation between two variables. Pearson’s r was calculated to measure linear correlation

[1] Not shown in figure 1. Cleveland Hopkins International Airport is about 11 mi southwest of Cleveland Burke Lakefront Airport.

between culture-based method results for the daily average of two individual samples and the associated composite sample. Kendall's tau-b (τ) was calculated to measure the strength of the monotonic relationship between two variables. Kendall's tau-b is a nonparametric procedure that is based on ranks and is resistant to outliers (Helsel and Hirsch, 2002). This procedure was used for comparisons of results of the culture-based method (*E. coli* concentrations, in CFU/100 mL of sample) and results of the IMS/ATP method (luminescence, in RLU/100 mL of sample) and environmental or water-quality factors. In addition, Kendall-Theil robust lines were included on plots to assist with making comparisons among beaches and years of data. The Kendall-Theil robust line regression is a nonparametric method that was chosen for this study because of the number of outliers in the datasets. The slope of the Kendall-Theil robust line is the median of all slopes between any two data points in the dataset; the intercept of this line is calculated by placing the line through the median x and y values (Granato, 2006).

To further examine the relation between environmental or water-quality variables and *E. coli* concentrations, observations were placed into groups of nearly equal size on the basis of magnitude of the explanatory variable. Tukey-Kramer multiple-comparison tests on the data were used to compare all possible pairs of treatment group means of $\log_{10}$-transformed concentrations. This test is powerful even when treatment group sizes are unequal (Helsel and Hirsch, 2002). The alpha (α) level for all tests of significance in this report was 0.05.

During 2006, for individual beaches, 26 to 40 percent of sample results from the IMS/ATP method (direct-addition variation) exceeded the maximum measureable luminescence of the luminometer. These data were described as right-censored because, when plotted in a scatterplot, the censored values fall on the right side of the graph. Because different volumes of sample were analyzed by means of the IMS/ATP method, some sample results for luminescences within the measureable range exceeded those that were censored. For example, if 30 mL of sample A was analyzed and the luminescence was greater than 900,000 RLU, it would be reported as greater than 3,000,000 RLU/100 mL. However, if 5 mL of sample B was analyzed and the luminescence was measureable at 200,000 RLU, it would be reported as 4,000,000 RLU/100 mL. Because only one volume was analyzed per sample, there is no way to know whether the luminescence was higher for sample A or sample B. Therefore, to treat all data equally, any measured values that were greater than 900,000 RLU per 30 mL (highest volume of any sample analyzed by means of the IMS/ATP method) were classified as censored. The additional censoring increased the percentage of censored values to 33 to 55 percent. The censored data were all assigned the same value (3,000,000 RLU/100 mL) for use in nonparametric correlation analysis. Changes in reagents and antibodies and amendments to the procedure for the IMS/ATP method between years eliminated the higher range of luminescence so that no sample results were censored during 2007.

Results

Comparison of Composite Sampling to Average of Multiple-Point Samples

Bacteria concentrations can vary along a beach. Concentrations along the beach may be affected by the location relative to the bacteria source, by breakwaters or groins, or by exposure to sunlight. Because of this spatial variability, agencies who monitor beach water quality may choose to stratify the beach into several areas and collect a sample from each area for analysis. Current practice at Edgewater, Villa Angela, and Huntington is to collect two samples at fixed locations at each beach and analyze them using the culture-based method for determining concentrations of *E. coli*. The arithmetic mean concentration is then computed from the individual concentrations determined for the two samples. The mean concentration is then used to determine whether a beach is posted with an advisory based on the water-quality standard set by Ohio Environmental Protection Agency.

For all samples collected during this study, *E. coli* concentrations in the composited samples were compared to the arithmetic mean of the two individual samples collected for the same beach and date. Prior to statistical analysis, *E. coli* concentrations were $\log_{10}$ transformed. The mean concentration (determined by averaging concentrations from the two point samples) and the composite sample concentrations for Edgewater, Villa Angela, and Huntington beaches combined were highly correlated ($r = 0.976$, $p < 0.0001$) (fig. 2). The mean *E. coli* concentrations in the composite samples were not significantly different from the *E. coli* concentrations determined by averaging the concentrations of two point samples, as determined by paired t-tests at Edgewater ($t = -0.56$, $p = 0.5725$), Villa Angela ($t = -1.86$, $p = 0.0641$), or Huntington ($t = -0.75$, $p = 0.4567$). Combining two point samples into a single composite is more economically feasible than analyzing individual samples by the culture-based method (Bertke, 2007). The difference in cost is even greater for the IMS/ATP method, because the IMS/ATP method is more labor intensive and supplies are more expensive than the culture-based method. Therefore, it is important to determine whether composited-sample results provide similar measures of water quality as results from averaging concentrations from two point samples.

Comparisons were made between the mean and composite concentrations for samples collected at the same beach and on the same date to determine whether one concentration exceeded the WQS whereas the other did not. Discordance occurred in 5.0 percent of the samples collected at all beaches. More discordances were observed at Villa Angela (8.3 percent) than at Edgewater (2.7 percent) and Huntington (3.6 percent). The low percentage of discordances and the strong correlations between the concentrations indicate that composite sampling appears to be a suitable alternative to multiple-point sampling.

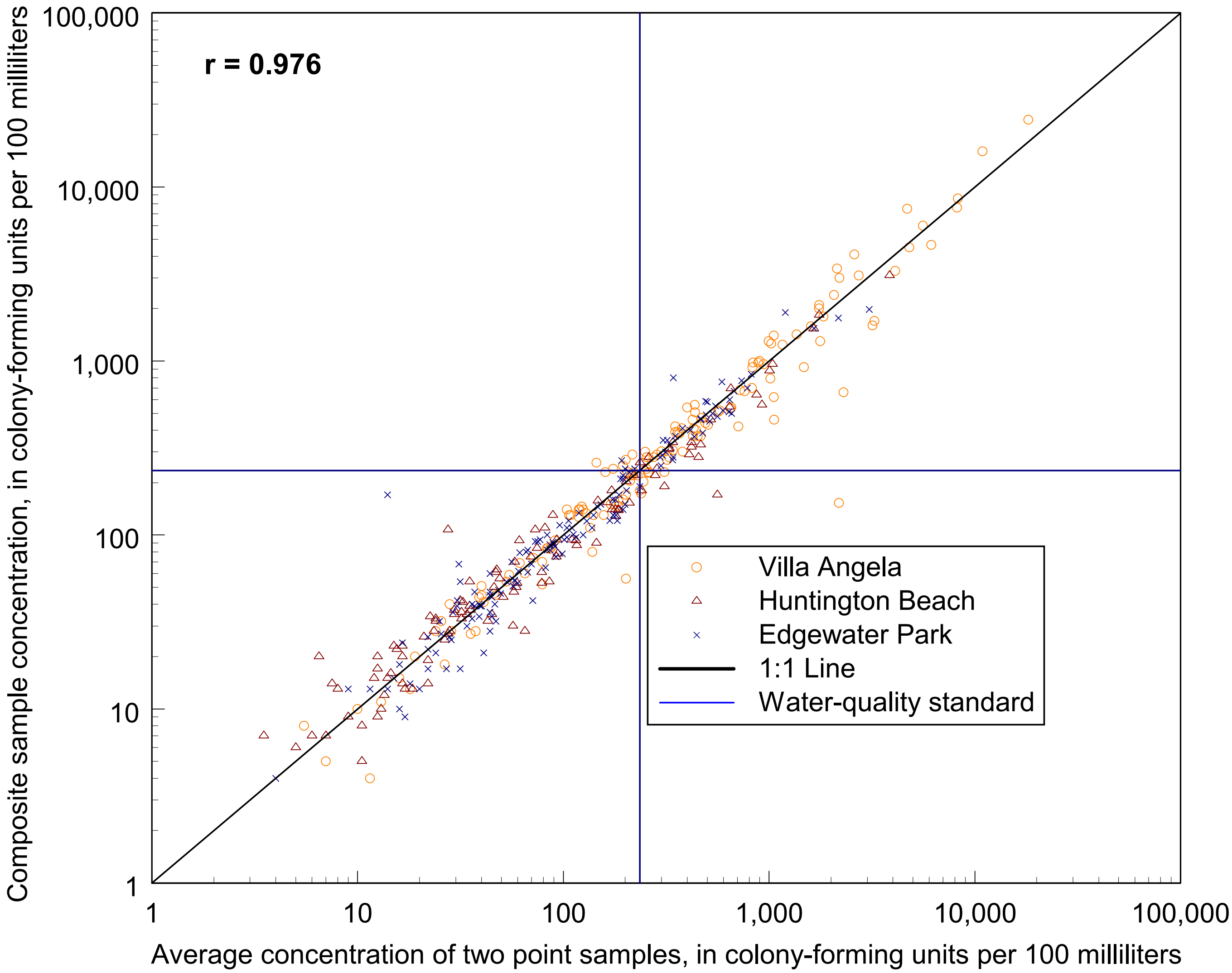

Figure 2. Scatterplot of *Escherichia coli* concentrations from composite samples and the arithmetic average of multiple-point samples at three Lake Erie beaches, May through September 2006 and May through August 2007. The water-quality standard is the Ohio single-sample bathing-water standard for *E. coli* of 235 colony-forming units per 100 milliliters. (r, the Pearson's correlation coefficient for results from all beaches combined.)

General Patterns in *Escherichia coli*, Turbidity, and Rainfall

Including both years of study at all beaches, concentrations of *E. coli* ranged from 4 to 24,000 CFU/100 mL and included several exceedances of the WQS (table 1). At Edgewater, concentration ranges were similar for both years of data collection (4 to 1,900 CFU/100 mL in 2006 and 10 to 2,000 CFU/100 mL in 2007) (table 1). During 2006 at Villa Angela, the median concentration of *E. coli* was near the WQS; but in 2007, the median was above the WQS, and approximately 60 percent of the sample concentrations exceeded the WQS. The largest *E. coli* concentration was observed at Villa Angela on July 17, 2007, a second consecutive day without rainfall on which a large number of birds were observed at the beach. Yearly ranges of concentrations were similar at Huntington: 7 to 3,100 and 5 to 1,800 CFU/100 mL for 2006 and 2007, respectively.

Throughout the duration of the study at all beaches, turbidity ranged from 0.8 NTRU to 260 NTRU (table 2). The largest observed turbidity was at Huntington Beach on August 29, 2006; a large rainfall event occurred the previous day (1.3 in.), and wave heights were recorded at 5 ft—the highest waves observed during this study. Samples were not collected at Edgewater or Villa Angela on this date because of unsafe sampling conditions. Turbidity remained elevated at Huntington over the next 2 days (162 and 83 NTRU, respectively). With the exception of this event, turbidity rarely exceeded 50 NTRU (n=8 for samples with greater than 50 NTRU for all beaches). Median turbidity values for all beaches and years sampled did not exceed 7.9 NTRU.

The largest rainfall amount observed for a 24-hour perior prior to sampling was 3.33 in. at Cleveland Hopkins International Airport on August 8, 2007 (table 3). For both years during reported periods, rainfall in excess of 0.05 in. occurred on approximately 27–29 percent of the days for both airports.

Table 1. Summary statistics for concentrations of *Escherichia coli* as determined by the culture-based method in samples collected from three northeastern-Ohio beaches, May through September 2006 and May through August 2007. The water-quality standard is the Ohio single-sample bathing-water standard of 235 colony-forming units per 100 milliliters.

[WQS, water-quality standard; CFU/100 mL, colony-forming units per 100 milliliters]

Year	Number of samples	Number exceeding WQS (percent)	*Escherichia coli*, in CFU/100 mL			
			Minimum	Median	Maximum	Average
Edgewater						
2006	82	18 (22)	4	90	1,900	180
2007	62	22 (35)	10	110	2,000	280
Villa Angela						
2006	82	39 (48)	4	230	4,100	430
2007	62	37 (60)	10	340	24,000	1,800
Huntington						
2006	53	12 (23)	7	75	3,100	220
2007	60	10 (17)	5	50	1,800	150

Table 2 Summary statistics for turbidity measurements from samples collected at three northeastern-Ohio beaches, May through September 2006 and May through August 2007.

[NTRU, neophelometric turbidity ratio units]

Year	Number of samples	Turbidity, in NTRU			
		Minimum	Median	Maximum	Average
Edgewater					
2006	81	1.7	7.9	46	11
2007	61	1.6	4.0	52	7.4
Villa Angela					
2006	81	1.3	5.1	61	10
2007	61	0.8	3.2	90	7.4
Huntington					
2006	53	1.9	7.6	260	21
2007	60	1.7	7.8	90	14

Table 3. Summary statistics for rainfall at two Cleveland, Ohio, airports on days samples were collected.

[Wet days are days on which rainfall over the previous 24 hours prior to sampling exceeded 0.05 inch.Total rainfall is the sum of all observed rainfall in the previous 24 hours prior to sampling. Source information from the National Weather Service.]

Dates	Number of observations	Number of wet days (percent)	Maximum rainfall, in inches	Total rainfall, in inches
Cleveland Hopkins International Airport				
2006: 5/31–9/28	82	23 (28)	2.26	10.1
2007: 6/5–8/31	62	18 (29)	3.33	12.1
Cleveland Burke Lakefront Airport				
2006: 5/31–9/28	82	24 (29)	1.60	11.3
2007: 6/5–8/31	62	17 (27)	3.18	10.5

Relations Between Environmental or Water-Quality Factors and *E. coli* Concentrations

Correlation analysis and scatterplots were done to evaluate the correlations between *E. coli* concentrations measured by the culture-based method and environmental or water-quality factors. Statistically significant relations were found between log_{10}-transformed *E. coli* concentrations and log_{10}-transformed turbidity, rainfall, and wave height at all three beaches during both years of sampling (table 4). The number of birds at the beach was significantly correlated with *E. coli* concentrations at Edgewater during 2006 and when both years were combined, but the relations were not significant at Villa Angela. At Huntington, the correlations between *E. coli* and turbidity and wave height were strong and persisted over both years of sample collection.

Relations Between Culture-Based and IMS/ATP Methods

Sample Processing Versus Direct Addition of Sample

During sample processing in 2006, the filtration and elution variation was compared to the direct-addition variation for the IMS/ATP method to determine which variation produced results most strongly correlated with those of the culture-based method, as measured by Kendall's tau-b correlation coefficient. The results of the two methods were significantly correlated for each beach. The direct-addition variation at Villa Angela and Huntington produced results that were more strongly correlated with results from the culture-based method (data not shown) than did the filtration and elution variation. At Edgewater, in contrast, the relation between the filtration and elution variation and the culture-based method was slightly stronger than that between the direct addition variation and the culture-based method ($\tau = 0.26$ and $\tau = 0.20$,

Table 4. Summary of Kendall's tau correlations between log_{10}-transformed *Escherichia coli* concentrations in water as measured by the culture-based method and environmental or water-quality variables at three northeastern-Ohio beaches in 2006 and 2007.

[Bold values are significant at $\alpha = 0.05$. na, data not available]

Year	Number of samples	Log_{10}-transformed turbidity	Rainfall	Wave height	Number of birds
		Edgewater			
2006	82	**0.40**	**0.18**	**0.25**	**0.28**
2007	61	**.28**	**.37**	**.37**	–.049
Both years	143	**.30**	**.26**	**.30**	**.15**
		Villa Angela			
2006	81	**.33**	**.35**	**.41**	–.024
2007	61	**.30**	**.11**	**.42**	.15
Both years	142	**.27**	**.24**	**.37**	.011
		Huntington			
2006	53	**.32**	**.31**	**.40**	na
2007	60	**.52**	**.38**	**.55**	na
Both years	113	**.44**	**.35**	**.47**	na

respectively). On the basis of this statistical analysis and increased ease of use of the direct-addition variation compared to the filtration and elution variation, the direct-addition variation was chosen for use during the 2007 field season.

Evaluation of 2006 and 2007 Data

Because of optimization steps and changes in antibodies and reagents between years, the IMS/ATP method results could not be combined across years. However, several observations are worth noting by comparing the datasets between years for the direct-addition variation. The most notable difference between years was the change in magnitude of luminescence observed. During 2006, IMS/ATP results often exceeded the upper limit of the luminometer; however, no samples collected during 2007 required upper-limit censoring. At Edgewater (fig. 3) and Villa Angela (fig. 4), the slopes of the Kendall-Theil robust lines were similar for both years (censored data are not shown on the plots); however, the y-intercepts were different. At Huntington, the slope for 2006 is noticeably steeper and the y-intercept higher than for 2007 (fig. 5).

Correlations between the culture-based and IMS/ATP methods were different between 2006 and 2007 (table 5). At Edgewater, the correlation coefficient was significant in 2007 but was not significant in 2006. At Villa Angela and Huntington, these relations were stronger in 2006 than in 2007. The reason for this difference may be the result of changes in reagents and the antibody used. In addition, differences in correlations between the two methods could be influenced by the censoring of the IMS/ATP method results for some samples collected during 2006.

Although the majority of the correlation coefficients were significant, there was noticeable scatter around the Kendall-Theil roubust line for all plots (figures 3, 4, and 5). This scatter may be the result of differences in the what is being measured by the two different methods, that is, culturable versus viable cells. The culture-based method measures cells that can be cultured on a specific agar plate; whereas, the IMS/ATP method measures culturable cells, as well as viable but not culturable cells that contain ATP.

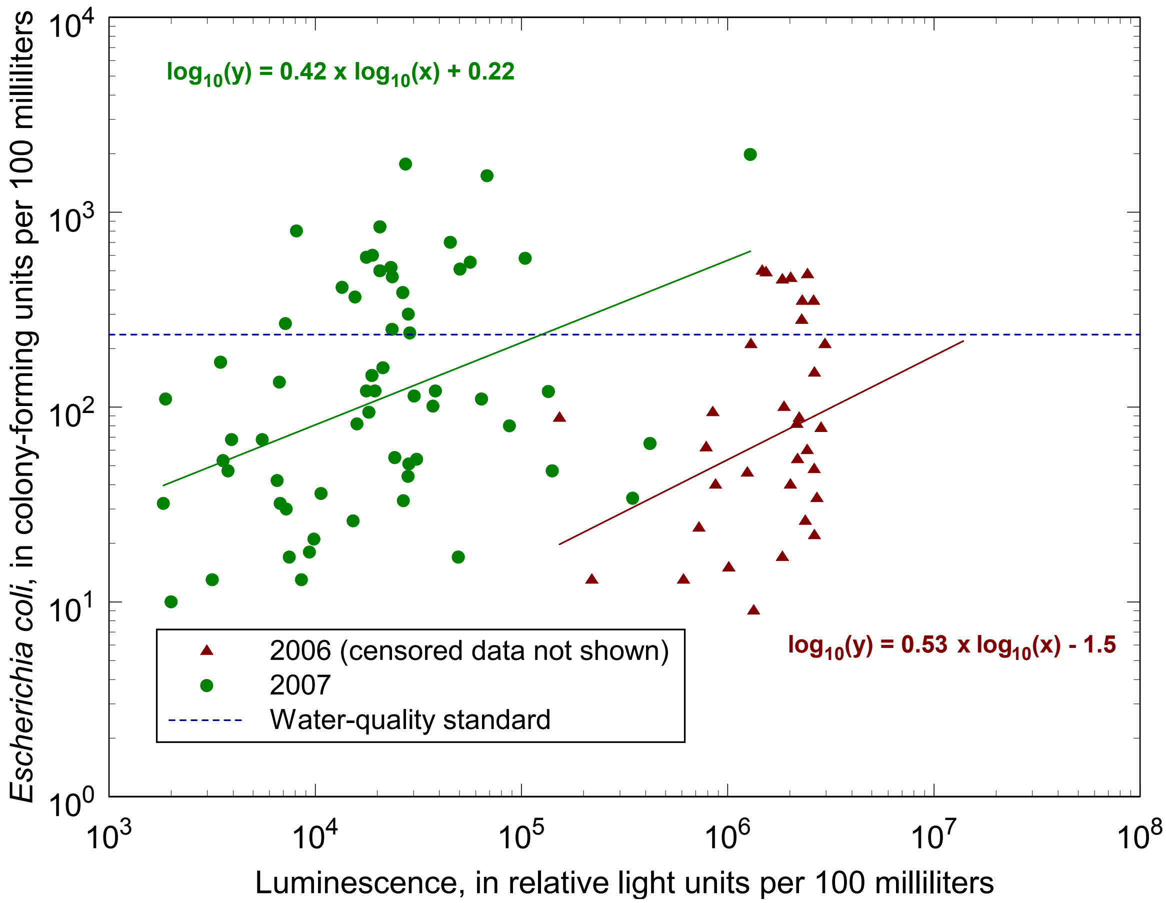

Figure 3. Relation between the culture-based *Escherichia coli* concentrations and IMS/ATP luminescence at Edgewater, Cleveland, Ohio, during May through September 2006 and June through August 2007. The solid line is the Kendall-Theil robust line. The water-quality standard is the Ohio single-sample bathing-water standard for *E. coli* of 235 colony-forming units per 100 milliliters.

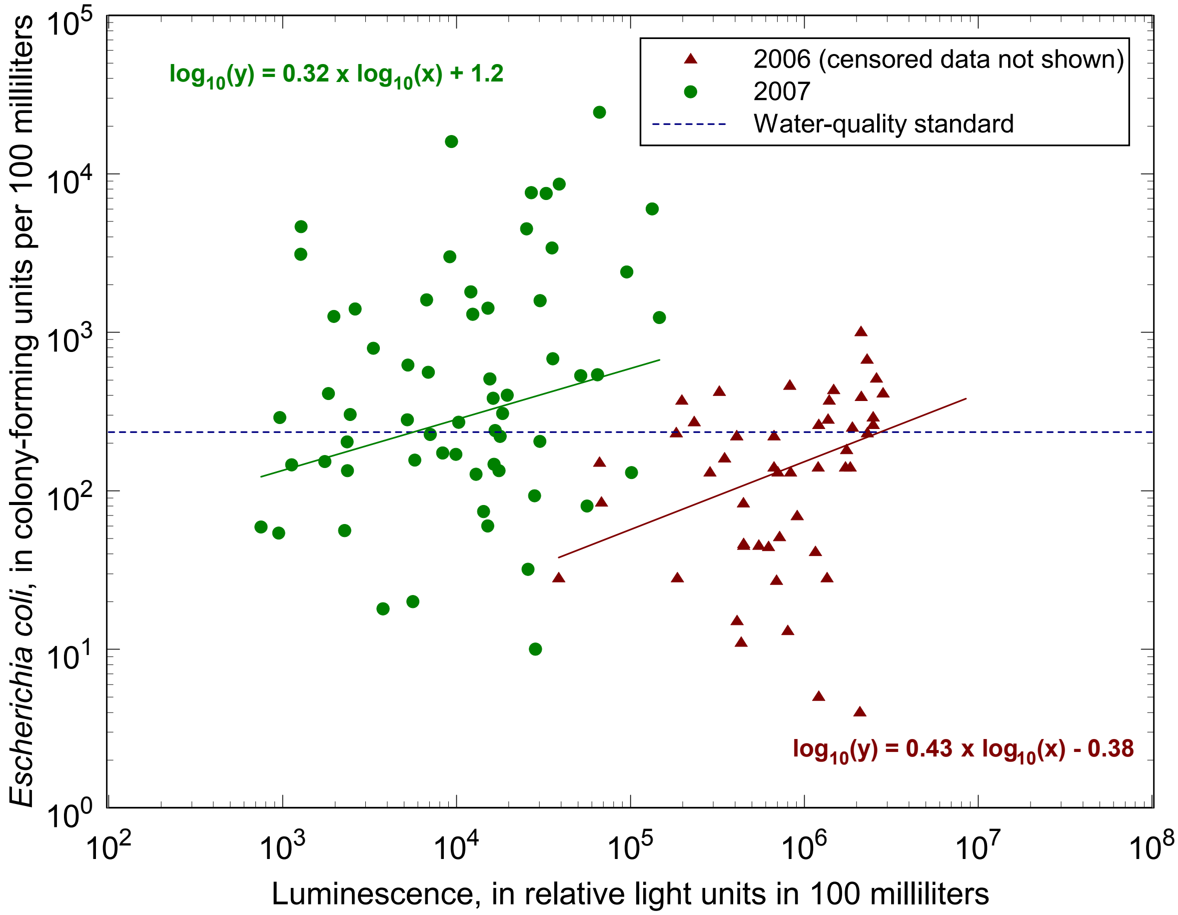

Figure 4. Relation between the culture-based *Escherichia coli* concentrations and IMS/ATP luminescence at Villa Angela, Cleveland, Ohio, during May through September 2006 and June through August 2007. The solid line is the Kendall-Theil robust line. The water-quality standard is the Ohio single-sample bathing-water standard for *E. coli* of 235 colony-forming units per 100 milliliters.

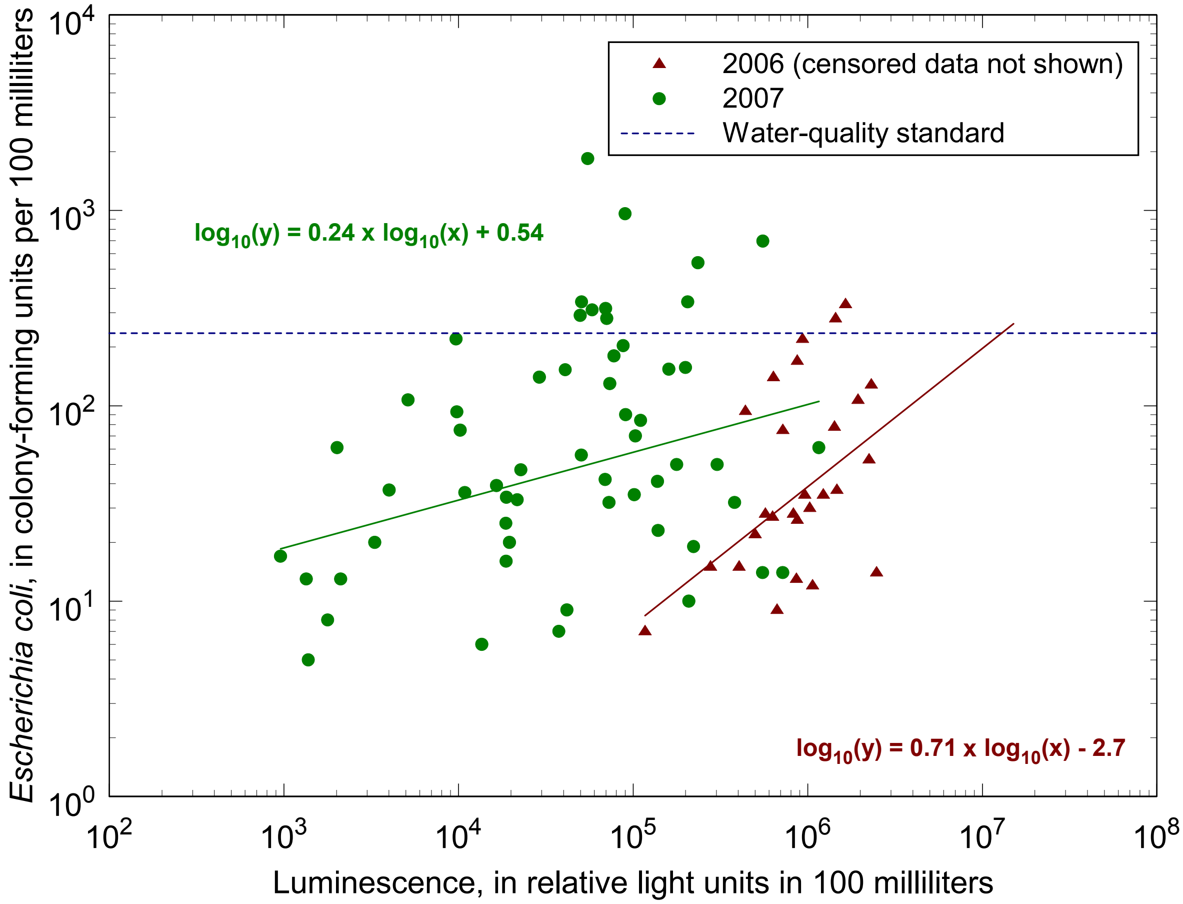

Figure 5. Relation between the culture-based *Escherichia coli* concentrations and IMS/ATP luminescence at Huntington Reservation, Bay Village, Ohio, during May through September 2006 and June through August 2007. The solid line is the Kendall-Theil robust line. The water-quality standard is the Ohio single-sample bathing-water standard for *E. coli* of 235 colony-forming units per 100 milliliters

Table 5. Summary of Kendall's tau correlations between results for the culture-based method and direct-addition variation of the IMS/ATP method at three northeastern-Ohio beaches.

[Bold values are significant at $\alpha = 0.05$.]

Year	Number of samples	Number of censored values (percent)	Kendall's tau correlation coefficient	p value
		Edgewater		
2006	73	40(55)	0.12	0.1699
2007	61	0 (0)	**.24**	.0076
		Villa Angela		
2006	72	24 (33)	**.36**	<.0001
2007	61	0 (0)	.16	.0646
		Huntington		
2006	46	19 (41)	**.40**	.0002
2007	57	0 (0)	**.20**	.0312

Effects of Water-Quality and Environmental Factors

Because of previously described changes in IMS/ATP rapid method, resulting in two distinct datasets, the discussion of data analysis that follows will focus on samples analyzed with the most up-to-date IMS/ATP protocol (2007). The data were divided into groups as a function of environmental factors, water-quality factors, and sampling dates. Groups were formed by dividing the dataset into subsets of nearly equal size on the basis of the magnitude of the factor. Correlations between the IMS/ATP and culture-based methods were determined individually for these groups.

At all beaches, correlations between the two methods were strongest and significant during the month of June (table 6) but were not significant during July or August. Although ranges in the concentration of *E. coli* were similar during these months, other factors may have affected these relations during July and August. For example, there was a significant increase in the number of birds at Edgewater during July compared to June (p = 0.0058, fig. 6) and at Villa Angela during August as compared to June (p = 0.0144, fig. 7). The numbers of birds were not recorded at Huntington. Birds, therefore, may have been a more dominant fecal source late in the summer than early in the summer. Perhaps the relations between culture and IMS/ATP methods were affected by fecal source.

Further, there were more wet days sampled during July and August than in June (table 7). Total rainfall for days sampled in August was larger than in either June or July. The increased number of wet days sampled during July and August and the total rainfall during August may have also resulted in different fecal sources between the early and late summer.

Table 6. Summary of Kendall's tau correlations between results for the culture-based method and direct-addition variation of the IMS/ATP method at three northeastern-Ohio beaches, by month sampled in 2007.

[Bold values are significant at α = 0.05.]

Month	Number of samples	Kendall's tau correlation coefficient	p value
Edgewater			
June	19	**0.38**	0.0228
July	21	.20	.1939
August	21	.22	.1735
Villa Angela			
June	19	**.41**	.0143
July	21	.076	.6290
August	21	<.00001	1.0000
Huntington			
June	18	**.48**	.0050
July	18	.066	.7047
August	21	–.029	.8561

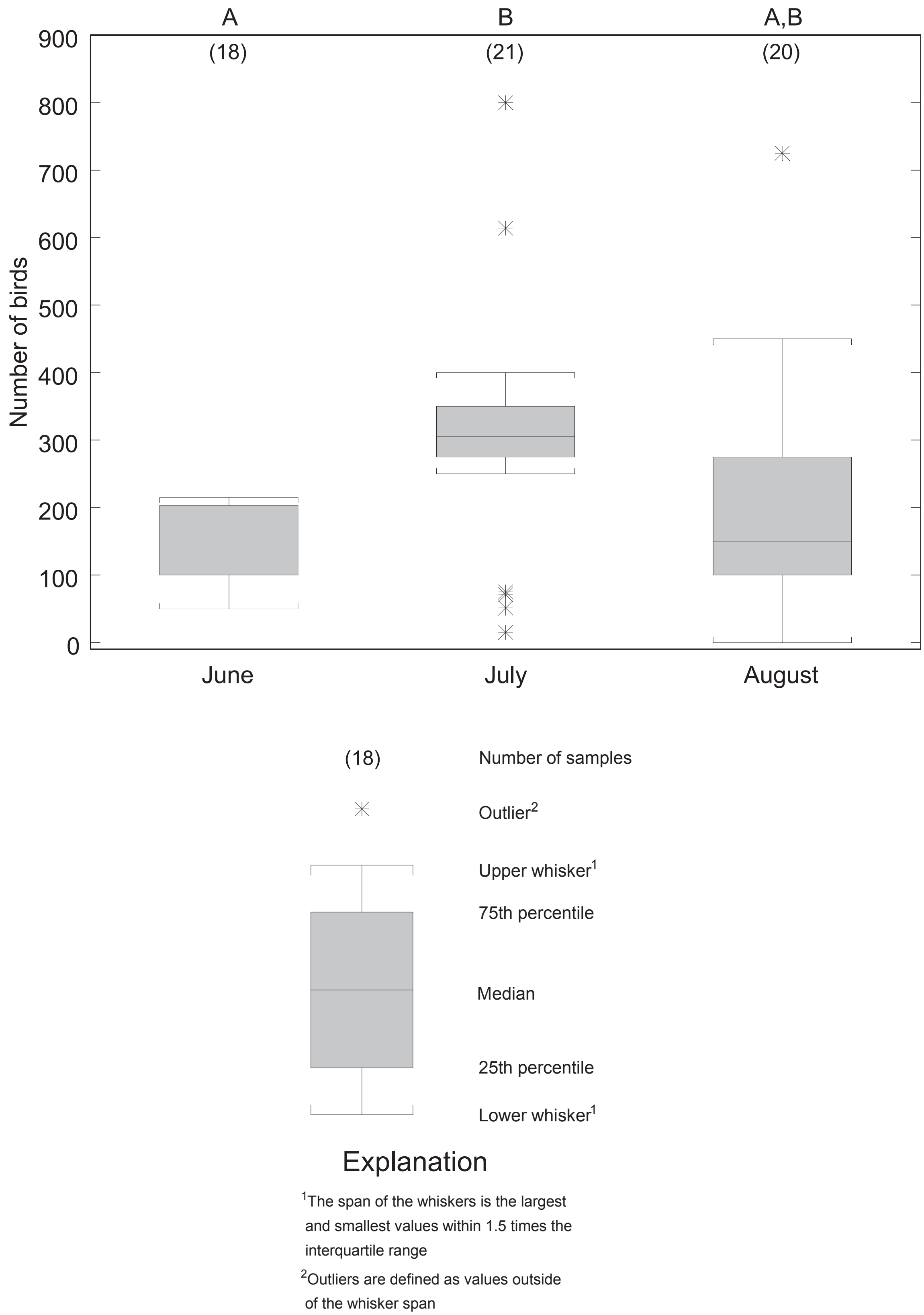

Figure 6. Number of birds at Edgewater Beach at time of sampling as a function of month sampled. (Results of Tukey-Kramer multiple comparison test are presented as letters, and groups with a letter in common do not differ significantly at α = 0.05.)

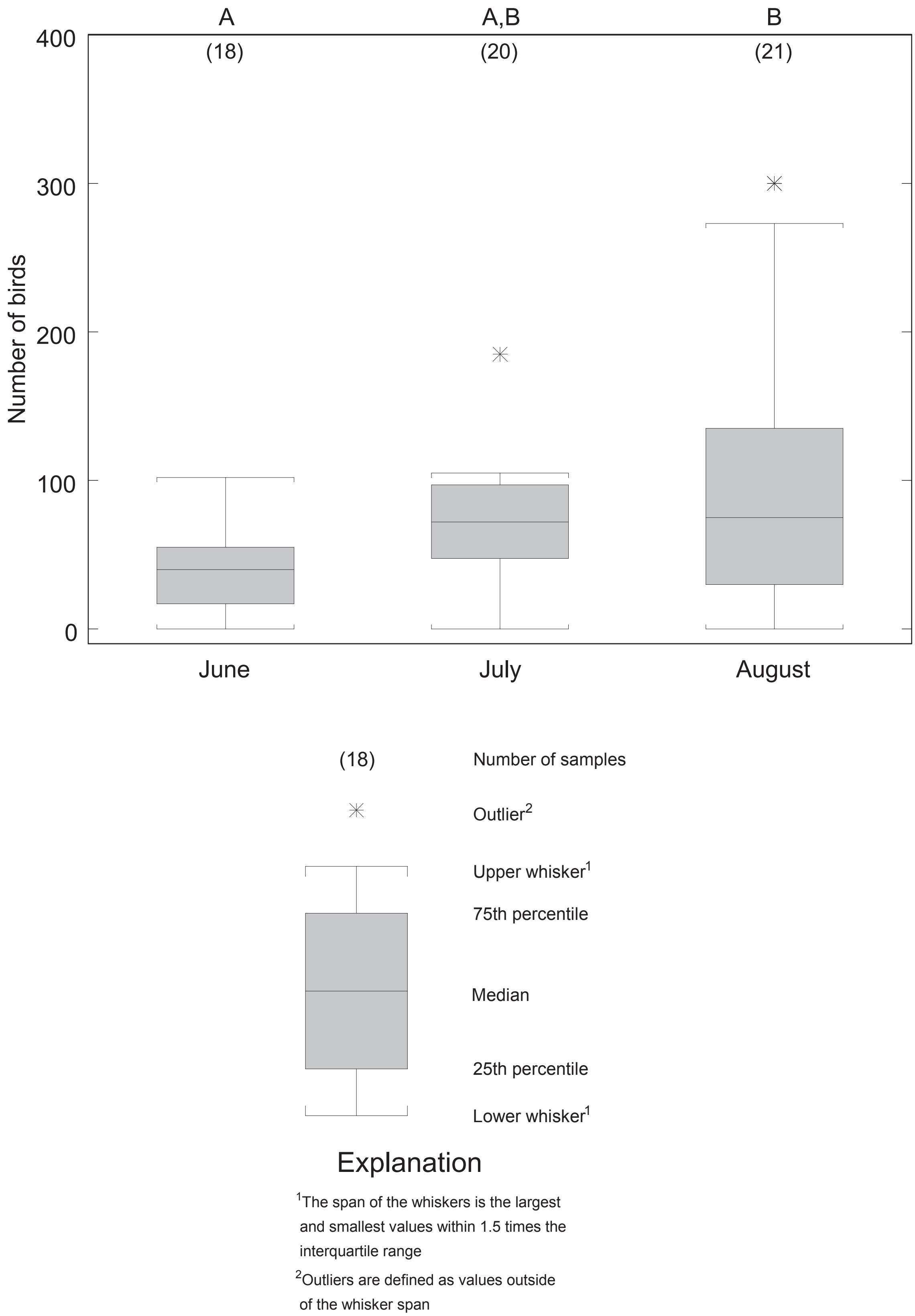

Figure 7. Number of birds at Villa Angela Beach at time of sampling as a function of month sampled. (Results of Tukey-Kramer multiple comparison test are presented as letters, and groups with a letter in common do not differ significantly at $\alpha = 0.05$.

Table 7. Summary statistics for rainfall at two Cleveland, Ohio, airports on days samples were collected during individual months, 2007.

[Wet days are days on which rainfall over the previous 24 hours prior to sampling exceeded 0.05 inch. Total rainfall is the sum of all observed rainfall in the previous 24 hours prior to sampling during individual months. Source information from the National Weather Service.]

Month	Number of observations	Number of wet days (percent)	Maximum rainfall, in inches	Total rainfall, in inches
Cleveland Hopkins International Airport				
June	19	3(16)	0.66	1.2
July	21	8 (38)	.81	2.4
August	22	7 (32)	3.33	8.6
Cleveland Burke Lakefront Airport				
June	19	3 (16)	.50	1.0
July	21	7 (33)	.36	1.6
August	22	7 (32)	3.18	8.0

Future Steps

The IMS/ATP method is a promising tool that has the potential to assist beach managers in determining recreational water quality within an hour of the start of sample processing. However, this method is still experimental, and further optimization is required before this method can be used for regulatory purposes. Ultimately, incorporation of the IMS/ATP method in an epidemiological study is needed so that method results can be directly correlated to human illness rates. Until this is done, further studies are needed to establish the linkage between the IMS/ATP method and culture-based method so that the IMS/ATP results can be used in recreational water settings that use water-quality standards based on culture-based methods.

Summary and Conclusions

Current practices for monitoring concentrations of *E. coli*, a fecal-indicator organism used to assess recreational water quality, require a minimum of 18 hours until availability of results. This time lag may result in recreational users being exposed to unsafe water; or, alternatively, revenue and recreational opportunities may be lost because beaches are posted with an advisory when the water is falsely considered unsafe for recreation. The need is widely recognized for more rapid availability of results so that beach managers can make decisions on closures or advisories based on same-day conditions. The U.S. Geological Survey, in cooperation with the Ohio Water Development Authority, Northeast Ohio Regional Sewer District, Cuyahoga County Board of Health, and Cuyahoga County Sanitary Engineer, evaluated a rapid detection method (IMS/ATP) for *E. coli* that yields results within 1 hour from the start of sample processing. The IMS/ATP method uses magnetic beads coated with antibodies for *E. coli* and an immunomagnetic separation technique to capture the *E. coli* cells in the water sample. Once captured, ATP is released from the bacterial cells and measured with a luminometer. Three Lake Erie beaches in northeastern Ohio were chosen as the sites for this study because of previous studies done at these beaches and the availability of information on bacteria contamination sources and (or) levels.

Beach-water samples were collected during the recreational seasons (May–September) of 2006 and 2007 at Edgewater, Villa Angela, and Huntington beaches. In 2006, 82 samples each were collected at Edgewater and Villa Angela, whereas 53 samples were collected at Huntington. Approximately 60 samples were collected at each of the three beaches in 2007. Each sample was analyzed for *E. coli* by the traditional culture-based method and the IMS/ATP method. Environmental data that may affect bacteria concentrations on the beaches also were collected, including the number of birds at the beach, wave height, sample turbidity, and local rainfall amounts.

Sampling procedures at these beaches involve the collection of two samples from each beach to better represent the heterogeneity of bacteria concentrations. The arithmetic mean concentration is calculated from the results of the two samples and is used to determine whether the water-quality standard has been exceeded. In this study, composite samples were prepared by adding equal volumes of the two samples. The individual samples and the composite sample were analyzed by the culture-based method for *E. coli*. The arithmetic mean concentration of the two samples collected at the beach were highly correlated with the results of the composite sample and had mean concentrations that were not statistically different. A separate study, conducted by the U.S. Geological Survey during 2005 at Edgewater, Villa Angela, and Lakeview (Lorain, Ohio) beaches, also found that compositing the samples yielded similar measures of recreational water quality as the arithmetic average of multiple-point samples at a lower cost (Bertke, 2007). Further support of these results was found in a previous study at a beach in Racine, Wis., in which investigators concluded that analyzing a composited sample was a reasonable and cost-effective alternative to results from computing the mean of the individual samples (Kinzelman and others, 2006). Combining two point samples into a single composite is more economically feasible than analyzing individual samples by the culture-based method. The difference in cost is even greater for the IMS/ATP method, because the IMS/ATP method is more labor intensive and the supplies are more expensive than the culture-based method.

E. coli concentrations determined by the culture-based method in the composite samples ranged from 4 to 24,000 CFU/100 mL for both years at all beaches and included several water-quality exceedances. At Edgewater, the standard was exceeded in 22 and 35 percent of samples in 2006 and 2007, respectively. The standard was exceeded in 48 and 60 percent of samples in 2006 and 2007, respectively, at Villa Angela. At Huntington, the percentage of exceedances was 23 in 2006 and 17 in 2007. The average turbidity measurements at all beaches were higher in 2006 than in 2007. The percentage of wet days sampled (sampling days when rainfall over the previous 24 hours exceeded 0.05 in.) was similar for both years (between 27 and 29 percent of days sampled). *E. coli* concentrations measured by the culture-based method were significantly correlated to turbidity, rainfall, and wave height measurements at all beaches in both years. The number of birds was significantly related to *E. coli* concentrations only at Edgewater in 2006 and when 2006 and 2007 data were combined. The two strongest relations to *E. coli* concentrations were at Huntington in 2007; turbidity ($r = 0.52$) and wave height ($r = 0.55$).

For the IMS/ATP method, two variations of the method were tested during the recreational season of 2006. For Villa Angela and Huntington, results from the direct-addition variation of the method were more strongly correlated than the results of the filtration and elution variation to results from the culture-based method. These findings are consistent with an earlier study in a recreational river, in which the results from the direct-addition variation were found to have a stronger correlation with results from the culture-based method than the two other variations tested (Bushon and others, 2007). In contrast, results for Edgewater from the filtration and elution variation were slightly more strongly correlated than those from the direct-addition variation to results of the culture-based method. On the basis of these statistical analyses and the increased ease of use of the direct-addition variation, only the direct-addition variation was used during the 2007 recreational season.

Differences between years were noticeable in the relations between the culture-based and IMS/ATP methods (direct-addition variation). For Edgewater and Villa Angela, the slopes of the Kendall-Theil robust lines were similar for 2006 and 2007; however, the y-intercepts were quite different. For Huntington, both the slopes and y-intercepts were different. Correlations of the results of both methods were statistically significant for Villa Angela during 2006, for Huntington during 2006 and 2007, and for Edgewater during 2007. Between the 2 years of sampling, the manufacturers made changes to the antibodies and reagents used for the IMS/ATP method; therefore, the data for the 2 years were not combined. Statistically significant relations were also found between the methods at a recreational river (Bushon and others, 2009a) using the original reagents and antibodies. The updated reagents and antibodies used in 2007 were also used in a study by Bushon and others (2009b) in which statistically significant relations between the two methods were found in untreated wastewater samples.

To further understand the relations between the methods in 2007, exploratory data analysis was done by breaking up the data into groups based on explanatory variables, including sampling date. At all beaches, relations between the two methods were stronger in samples collected earlier in the recreational season than in those collected later. At Edgewater and Villa Angela, the number of birds was shown to increase through the season. Also, there were more wet days sampled during July and August compared to June. It is hypothesized that the relations between the two methods could be affected by fecal source, and the differences in the dominant fecal source may explain the change in the relations between the two methods. At Huntington, no observed environmental factors could explain the change in relations between the methods through the season. The number of birds at time of sampling was not recorded at this beach, but counting birds at Huntington is suggested for future studies.

The rapid method shows promise for use in recreational water-quality monitoring although the relations between the culture-based and IMS/ATP rapid methods were not as strong for samples collected in 2007 at Villa Angela and Huntington compared to those collected in 2006. Although promising, the IMS/ATP method is still undergoing optimization and is not ready for use in place of the culture-based method without further refinement. With its lower cost than other currently available rapid methods, IMS/ATP method has an added advantage of being cost-effective for smaller beach-monitoring agencies. In complex systems, such as beaches where numerous contamination sources affect the area, multiple tools may be required to provide beach managers with the best possible information to protect the public. The IMS/ATP rapid method has the potential to become a strong tool to assist in this endeavor.

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Publishing support was provided by the USGS Columbus Publishing
Service Center.

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