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Sharing of Ribotype Patterns of *Escherichia Coli* Isolates During Baseflow and Stormflow Conditions

By Peter G. Hartel, Elizabeth A. Frick, Adrienne L. Funk, Jennifer L. Hill, Jacob D. Summer, and M. Brian Gregory

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Sharing of Ribotype Patterns of *Escherichia Coli* Isolates During Baseflow and Stormflow Conditions

By Peter G. Hartel[1], Elizabeth A. Frick[2], Adrienne L. Funk[2], Jennifer L. Hill[1], Jacob D. Summer[1], and M. Brian Gregory[2]

Abstract

Factors affecting bacterial source tracking are important to understand because they affect the amount of sampling needed to describe fecal sources in a watershed adequately. The study area was a 76-kilometer reach of the Chattahoochee River and its tributaries in Metropolitan Atlanta, Georgia. *Escherichia coli* was isolated from water samples collected during baseflow and stormflow conditions from four mainstem and eight tributary sites; 262 isolates were ribotyped and assessed for their similarity. The vast majority of the *E. coli* ribotype patterns were unshared, whether the comparisons were between baseflow and stormflow conditions at one location, or between one location and another. The data suggest that either baseflow and stormflow conditions affected sharing of ribotype patterns, or that the sample size was too small to characterize the sharing adequately. Regardless, the results suggest that a large sampling of *E. coli* isolates is needed during various flow conditions from watersheds with complex land-use patterns for adequate bacterial source tracking.

Introduction

In recent years, a number of phenotypic and genotypic methods have been developed for bacterial source tracking, which are ways to determine the host origin of fecal bacteria in contaminated waters. Most of the recent research on phenotypic methods has concentrated on multiple antibiotic analysis (Wiggins et al., 1999), whereas most of the research on genotypic methods has concentrated on ribotyping (Parveen et al., 1999), pulsed field gel electrophoresis (Kariuki et al., 1999), and various polymerase chain reaction (PCR)-based (Dombek et al., 2000) methods. All of these methods are based on the assumption that specific markers or strains of bacteria are associated with specific animal species (Amor et al., 2000), and most require a host origin database consisting of one bacterial species isolated from the feces of a number of warm-blooded animals (including humans). By matching environmental isolates to isolates contained in the host origin database, the host source of the fecal contamination can be identified.

In conducting bacterial source tracking, it is important to understand the factors affecting changes in a bacterial subspecies hosted by a population of warm-blooded animals because these factors affect the amount of sampling needed to describe the bacterial population in the watershed adequately. One of these factors is flow conditions. During baseflow conditions, the contributing geographic area and feces from different host animal species are likely relatively constant over time. This contribution contrasts with stormflow conditions, when the contributing geographic area and feces from different host animal species are constantly changing over time, depending on the antecedent rainfall and type of infrastructure present (such as the percent and distribution of impervious areas, stormwater drainage network, settling basins, and the conditions and capacities of sanitary sewer lines).

Whitlock et al. (2002) used antibiotic resistance analysis as the bacterial source tracking method to determine that sources of fecal indicator bacteria varied geographically and temporally in an urban watershed during a 7-month period. In terms of geographic changes, Hartel et al. (2002) noted that the percentage of ribotype patterns matching each other increased with decreased distance between geographic locations for cattle and horses, but not for swine and chickens. The shortest distance between two tested geographic locations was 175 kilometers (km). In a study in which the two geographic locations were closer together, Buchan et al. (2001) used denaturing gradient gel electrophoresis of the 16S-23S intergenic spacer region to obtain 45 unique banding patterns from 51 *E. coli* isolates from two waterways in Georgia, which were 11 km apart. None of the *E. coli* banding patterns from the two sites overlapped. Finally, in studies of temporal changes, little temporal stability was observed in *E. coli* isolates obtained from feral house mice (Gordon, 1997) or cattle (Jenkins et al., 2003).

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To determine the effect of flow conditions on changes of *E. coli* subspecies, isolates of this fecal bacterium were obtained from water samples collected in the Upper Chattahoochee River watershed in Georgia during baseflow and stormflow conditions. Subspecies composition was evaluated by the extent of sharing of ribotype patterns among *E. coli* isolates. Sharing of ribotype patterns was defined as the DNA banding patterns (representing DNA encoding for ribosomal RNA) of two or more environmental isolates matching at or greater than 90 percent similarity.

Materials and Methods

Study area

The study area was a 76-km reach of the Upper Chattahoochee River watershed (fig. 1). The 12 sampling sites comprised 4 mainstem and 8 tributary sites. This reach of the Chattahoochee River was selected because it is one of the most heavily used rivers in Georgia, serving as a resource for drinking water, recreation, and wastewater assimilation for much of Metropolitan Atlanta. During the early 1990s, urban land use in watersheds of tributary streams ranged from approximately 21 percent in James Creek, near the upstream end of the study area, to 80 percent in Rottenwood Creek, near the downstream end of the study area (Evelyn H. Hopkins, Geographer, written commun., U.S. Geological Survey, Atlanta, Georgia, 2000). According to the Georgia Department of Natural Resources (1997), 67 of 77 stream reaches assessed in Metropolitan Atlanta did not meet or only partially met State and Federal water quality standards. Excessive numbers of fecal coliform bacteria were a contributing factor in 63 of these 67 streams. From May to October 1994 and 1995, median numbers of fecal coliforms in the Chattahoochee River increased steadily downstream from less than 20 Most-Probable-Number (MPN) per 100 milliliters (mL) below Buford Dam to 790 MPN per 100 mL downstream from Metropolitan Atlanta (Gregory and Frick, 2000).

Water sampling

Water samples were collected on February 22, 2000, at all sites during winter baseflow conditions during a multiyear drought (fig. 2). For sites where insufficient *E. coli* isolates were obtained, supplemental baseflow samples were collected on March 1, 2000. Water samples were collected on April 2, 2000, at near-peak-flow conditions for one of the larger storms during early 2000. The Chattahoochee River is a regulated river with upstream releases primarily for hydroelectric power generation, water supply for Metropolitan Atlanta, and flood control. Chattahoochee River samples were collected from a bridge or boat. Tributary stream samples were collected by wading during baseflow conditions and from bridges during stormflow conditions. The one exception was the stormflow sample from James Creek, which was a grab sample collected from the side of the creek because flow conditions made it unsafe to collect from a bridge.

Except for the grab sample from James Creek, surface-water samples were collected according to U.S. Geological Survey (USGS) protocols for depth-integrated, equal-width increment samples (Myers and Sylvester, 1999; Wilde et al., 1999). The 1- to 3-L samples collected at each site were composites of isokinetic, depth-integrated samples normally collected from five, approximately equal-width increments. The combination of the constant sampling rate for each vertical transit and the isokinetic property of the samplers results in a discharge-weighted sample that is proportional to total streamflow.

Selection and identification of *E. coli* isolates from water

Samples were transported from the sites to the laboratory on ice in coolers and were processed within 6 hours (h). Each water sample was serially diluted and passed through separate 0.45-micrometer filters. Each filter was placed on a 5-centimeter petri dish containing mTEC medium (Difco Laboratories, Sparks, Md.), and the bacteria were resuscitated at 35.0 ± 0.5 degrees Centigrade (°C) for 2 h before incubating at 44.5 ± 0.2°C for 22 to 24 h according to USGS protocols (Myers and Sylvester, 1999).

The study objective was to obtain 50 *E. coli* isolates from each of the 12 locations during baseflow and stormflow conditions. However, this was not possible because the membrane filters were often contaminated with non-*E. coli* bacteria, particularly during stormflow conditions. If the composite of the membrane filters from a serial dilution had more than 50 yellow to yellowish-brown colonies, then the isolates were arbitrarily selected, streaked onto tryptic soy agar (Difco Laboratories) and incubated at 35°C for 24 h. If the composite of the membrane filters from a serial dilution had 50 or fewer yellow to yellowish-brown colonies, then all of these isolates were selected. All isolates were streaked twice on tryptic soy agar to ensure purity. Each isolate was inoculated into a 24-multiwell tissue culture plate containing separate 1-mL slants of Simmons citrate and urea agar (both from Difco Laboratories). Three bacterial species from the American Type Culture Collection (ATCC; Manassas, Va.) were used as controls: *Escherichia coli* ATCC #11775 (citrate negative, urea hydrolysis negative), *Klebsiella pneumoniae* ATCC #13883 (citrate positive, urea hydrolysis positive), and *Enterobacter aerogenes* ATCC #13048 (citrate positive, urea hydrolysis negative). Isolates that were both citrate negative on Simmons citrate agar and urea hydrolysis negative on urea agar were subjected to an oxidase test. Isolates that were oxidase negative were considered as confirmed *E. coli* isolates and kept for long-term storage.

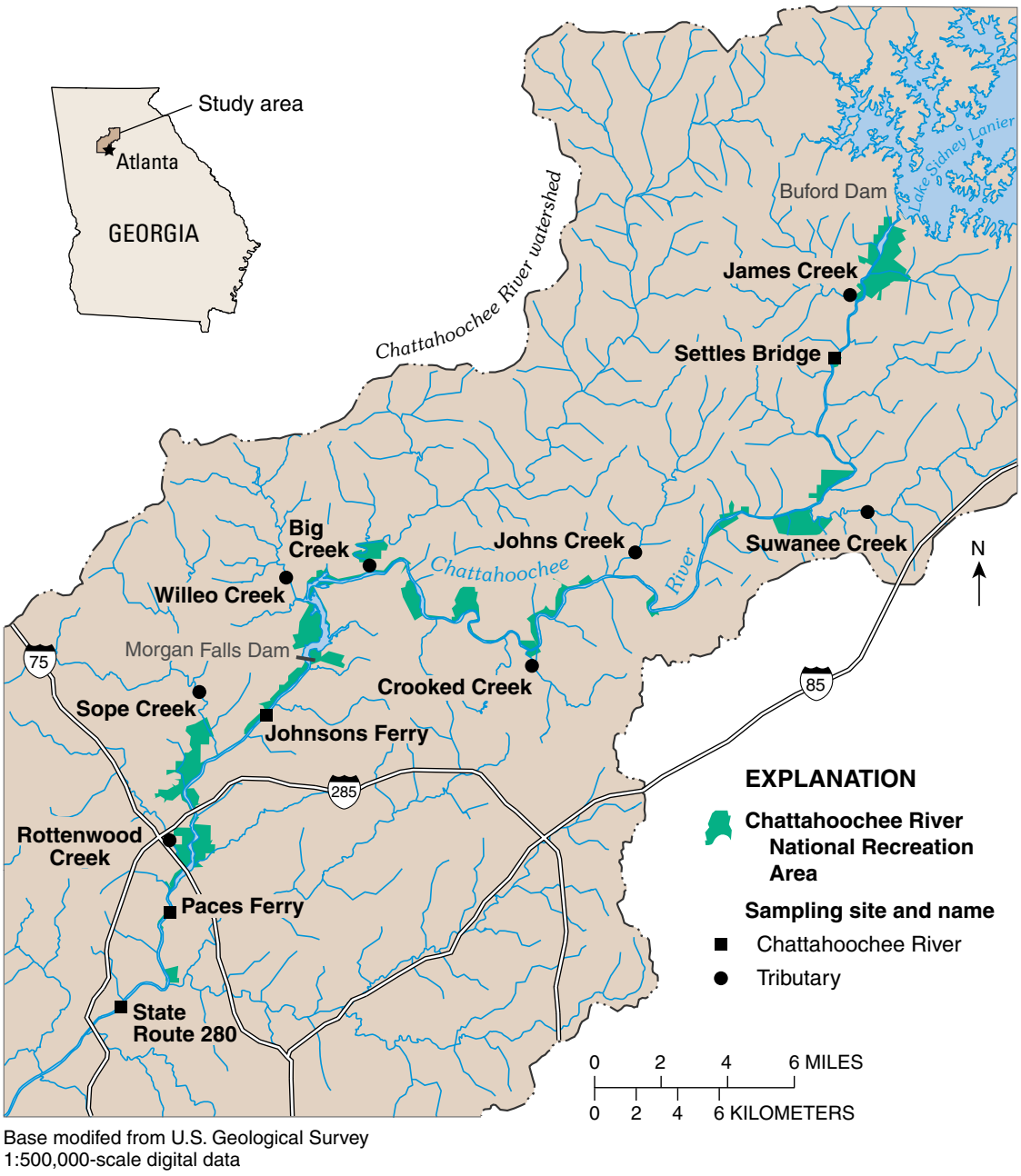

Figure 1. Location of the sampling sites, Upper Chattahoochee River watershed, 2000.

DNA extraction, quantification, and ribotyping of *E. coli* isolates

A standard protocol for extracting, quantifying, and ribotyping the DNA was followed as described by Hartel et al. (2002). Briefly, *E. coli* isolates were streaked on tryptic soy agar and incubated at 35°C for 24 h. A single colony was inoculated into 10 mL of Luria-Bertani broth and was incubated on a rotary shaker at 75 revolutions per minute (rpm) at 35°C. After 18 h, a 2.0-mL sample was removed and the DNA extracted with a commercially available kit. A portion of the DNA was mixed with a fluorescent dye and quantified with a fluorometer using DNA from *E. coli* as a standard.

For ribotyping, two 1-microgram samples of DNA from each isolate were each separately digested overnight with the restriction enzymes *Eco*RI and *Pvu*II. The digested DNA was stained and loaded into a 1-percent agarose gel. The gel was electrophoresed at 58 volts for 3 h using a horizontal gel system. A commercial digoxigenin-labeled (DIG-labeled) molecular weight marker occupied every fifth lane of the gel. Control lanes consisted of a lane with no DNA and a lane

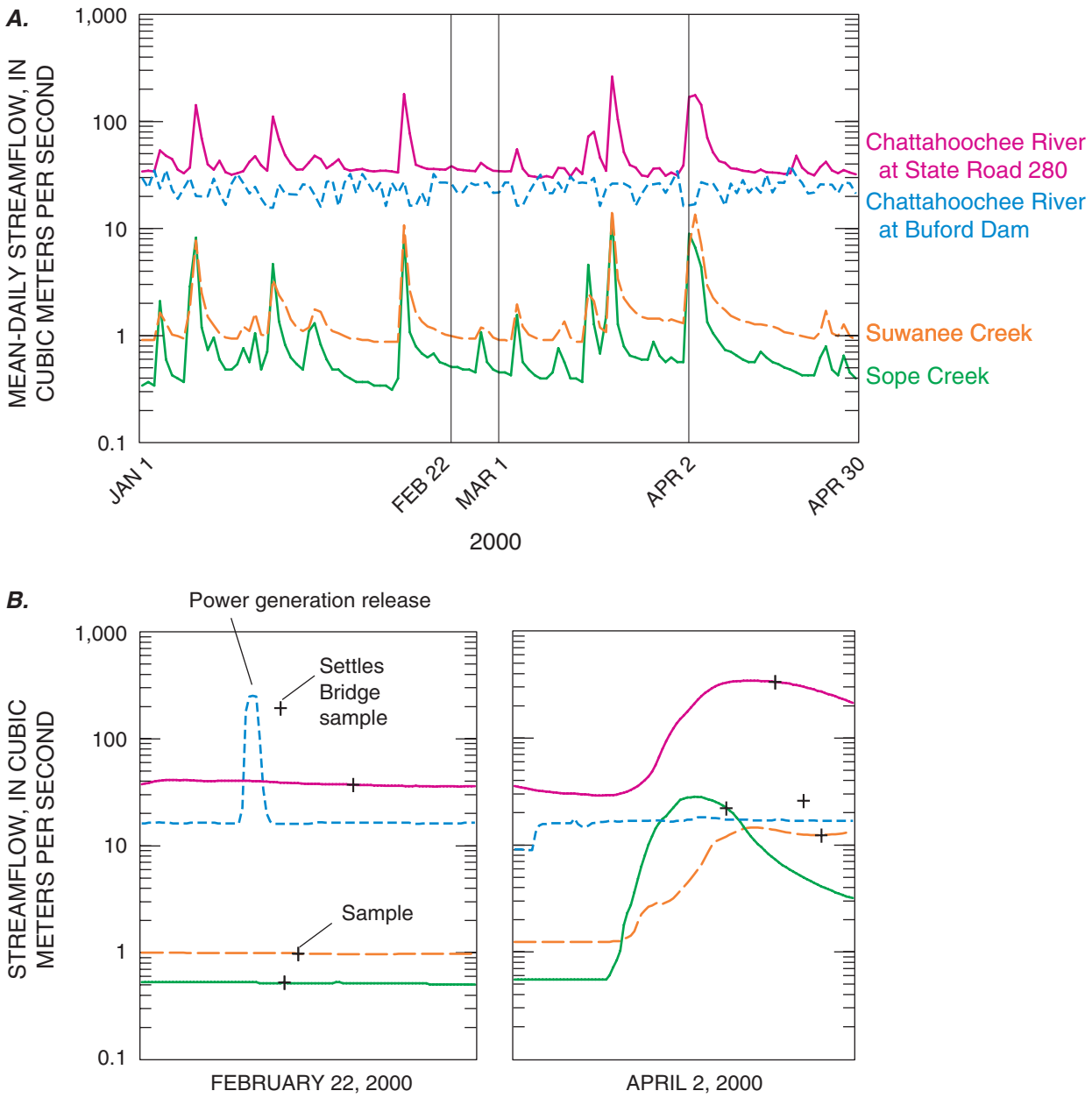

Figure 2. (*A*) Mean-daily streamflow and (*B*) streamflow on primary sampling dates at selected gages on the Chattahoochee River and tributary streams, January–April 2000. The Chattahoochee River at Buford Dam (short dashes) is 7.2 kilometers upstream from Settles Bridge.

with DNA from *E. coli* ATCC #11775. DNA was transferred by Southern blotting to a nylon membrane using a vacuum blotting system and the DNA on the membrane was cross-linked with ultraviolet light. Following a 2-h prehybridization at 42°C, the membrane was hybridized at the same temperature overnight to DIG-labeled cDNA from *E. coli* total ribosomal RNA. Membranes were prepared for chemiluminescence by a series of washing steps before a chemiluminescent substrate for alkaline phosphatase was added. Membranes were placed in an imager and images saved as TIFF files, which were imported into BioNumerics® software (Version 3.0, Applied Maths, Kortrijk, Belgium) for analysis. Typically, gels showed 9 to 11 bands for EcoRI and 11 to 13 bands for *Pvu*II digestion. DNA fragments with less than 1,375 base pairs were ignored because they were often indistinct. Lanes were normalized within the gel with the molecular weight marker and variations among the gels were assessed with the *E. coli* ATCC #11775 strain. Optimization (maximum percentage shift allowed between two different patterns for the patterns to still be considered a match) and tolerance (maximum percentage shift allowed between two bands on different patterns for the bands to still be considered a match) were each

set at 1 percent. The normalized banding patterns for both enzymes were stacked with *Eco*RI on the top and *Pvu*II on the bottom to create one combined ribotype pattern for each isolate, which was used to discriminate among ribotype patterns. Indices were determined using Dice's coincidence index (Dice, 1945) and the distance between clusters calculated using the unweighted pair-group method using arithmetic averages (UPGMA). The banding pattern of the control *E. coli* ATCC #11775 strain varied from gel to gel, and all the banding patterns of the control strain were similar at the 90-percent level. Because the control strain was a single ribotype, the banding patterns of two or more environmental isolates had to have a similarity index greater than or equal to 90 percent to be considered the same ribotype.

Statistical analysis

Significant differences ($p = 0.01$) between the number of presumptive and confirmed *E. coli* isolates obtained during baseflow and stormflow conditions were determined with a binomial proportion comparison test.

To compare isolates during the different flow conditions, isolates obtained at each location were analyzed with the BioNumerics® software, and the sharing of ribotype patterns and isolate sharing were arrayed in a table. To compare isolates obtained from different locations, isolates obtained during baseflow and stormflow conditions at each location were combined and compared to combined isolates at each of the other locations (66 total comparisons). The sharing of ribotype patterns and isolate sharing were converted to percentages (to normalize unequal sample sizes) and were arrayed over distance in a graph. The significance of the correlation coefficient between percent sharing of ribotype patterns and distance, or the percent isolate sharing and distance, was determined using standard statistical tables.

Results

At four mainstem and eight tributary streams sampling sites, numbers of presumptive *E. coli* ranged from 9 to 320 colonies per 100 mL during baseflow conditions, and from 1,300 to 24,000 colonies per 100 mL during stormflow conditions (table 1). The increase in numbers of presumptive *E. coli* during stormflow conditions ranged from about 10-fold higher at Suwanee Creek to more than 2,000-fold higher at the Chattahoochee River at State Route 280 compared numbers of presumptive *E. coli* during baseflow conditions. The numbers of fecal coliforms were 10- to 100-fold higher during stormflow conditions at the same sites in the Chattahoochee River watershed compared to baseflow conditions (Gregory and Frick, 2001). Similar increases have also been observed for *E. coli* on other rivers (Solo-Gabriele et al., 2000).

In general, a higher percentage of presumptive *E. coli* colonies were confirmed to be *E. coli* from water samples collected during baseflow conditions compared with water samples collected during stormflow conditions (table 2). Of 550 presumptive *E. coli* isolates obtained during baseflow conditions, 490 (89%) were subsequently confirmed as *E. coli*. During stormflow conditions, the number of confirmed *E. coli* isolates decreased significantly, and only 174 of 275 presumed *E. coli* isolates (63%) were confirmed as *E. coli*. Therefore, 11 percent and 37 percent of the presumptive *E. coli* isolates on mTEC medium were false positives during baseflow and stormflow conditions, respectively.

Of the 664 confirmed *E. coli* isolates, 262 isolates were ribotyped. When the isolates were compared between baseflow and stormflow conditions, they yielded a total of 163 ribotypes, of which only 6 (3.6%) were shared (table 3). Only 21 of 262 isolates (8.0%) shared ribotype patterns. Therefore, the vast majority of ribotype patterns were unshared, and the vast majority of isolates did not share ribotypes. In fact, 8 of the 12 sampling locations had ribotypes and isolates with no sharing whatsoever.

When the isolates from baseflow and stormflow conditions were combined at each sampling location and compared to combined isolates at each of the other locations, no significant correlation was observed either between the percentage of sharing of ribotype patterns and distance (from 4 to 76 km; $r^2 = 0.03$; fig. 3) or between the percentage of isolates sharing the same ribotype and distance ($r^2 = 0.04$; data not shown). The maximum percentage of sharing of ribotype patterns between any two sites, regardless of distance, was 29 percent. The vast majority of the comparisons (60 of 66; 91%) had less than 15-percent sharing of ribotype patterns, and 10 comparisons (from 18 to 44 km apart) had no sharing of ribotype patterns. Therefore, the percentage of sharing of ribotype patterns did not depend on geographic location.

Discussion

The majority of *E. coli* ribotype patterns were unshared, whether the comparisons were between baseflow and stormflow conditions at one location, or between one location and another. There are two reasonable explanations for the data. First, it is possible that the sharing of *E. coli* ribotype patterns was affected by baseflow and stormflow conditions. The most likely sources to affect this sharing are sewage overflow (Richman, 1996) and overland flow (Entry et al., 2000). Metropolitan Atlanta has well-documented problems with stormwater runoff and sewage overflow (Stevens, 2001). In terms of overland flow, it seems reasonable that fecal material on or near the riverbank would be transported to the river primarily during stormflow conditions. Whitlock et al. (2002) observed that humans and dogs were the dominant sources of fecal contamination in an urban watershed when fecal coliform numbers were low, and wild animal feces the dominant source when fecal coliform numbers were high.

Table 1. Streamflow and colony-forming units of presumptive *E. coli* during baseflow and stormflow conditions at four mainstem and eight tributary stream sampling sites, Upper Chattahoochee River watershed (adapted from Gregory and Frick, 2001).

[USGS, U.S. Geological Survey; ID, identification; CFU 100 mL^{-1}, colony-forming units per 100 milliliters; m^3 sec^{-1}, cubic meters per second; NA, not available]

USGS site ID	Sampling site	Baseflow				Stormflow	
		February 22, 2000		March 1, 2000		April 2, 2000	
		Streamflow (m^3 sec^{-1})	Presumptive *E. coli* (CFU 100 mL^{-1})	Streamflow (m^3 sec^{-1})	Presumptive *E. coli* (CFU 100 mL^{-1})	Streamflow (m^3 sec^{-1})	Presumptive *E. coli* (CFU 100 mL^{-1})
02335420	James Creek	0.3	82	NA	NA	NA	2,300
02334550	Chattahoochee River—Settles Bridge	192[a, b]	32	NA	NA	23[b]	3,300
02334885	Suwanee Creek	0.9	320	0.9[c]	NA	12	3,100
02335078	Johns Creek	0.2	100	NA	NA	20	11,000
02335350	Crooked Creek	0.3	26	0.2[c]	NA	21	10,000[b]
02335741	Big Creek	1.7	44	1.6[b]	44	25	4,800[b]
02335790	Willeo Creek	0.3	93	NA	NA	7	3,800[b]
02335830	Chattahoochee River—Johnsons Ferry	33	60	31	24	128	1,300[b]
02335870	Sope Creek	0.5	190	0.5[c]	NA	23	9,300[b]
02335910	Rottenwood Creek	0.3	25[b]	0.3[b]	46[b]	27	7,400[b]
02336000	Chattahoochee River—Paces Ferry	31	62[b]	30	21	150	3,500[b]
02336490	Chattahoochee River—State Route 280	37	9[b]	33	40	334	24,000[b]

[a] Streamflow was high because of water released upstream for power generation rather than from rainfall (fig. 2b).

[b] Estimated.

[c] Mean daily discharge

Furthermore, little sharing of ribotype patterns was observed between two different locations. A likely explanation for this lack of sharing is that land use varies from approximately 21 percent urban in James Creek, near the upstream end of the study area, to 80 percent urban in Rottenwood Creek, near the downstream end of the study area (Evelyn H. Hopkins, Geographer, written commun., U.S. Geological Survey, Atlanta, Georgia, 2000). Thus, the contribution from wildlife is more likely in the upper portion of the study area compared with the lower portion, which is more likely to have a greater contribution from human sewage. Given that specific markers or strains of bacteria are associated with specific animal species (Amor et al., 2000) and that these animal species likely change with land use, then the lack of ribotype sharing is not surprising. These data also are consistent with Buchan et al. (2001) who observed that none of the 45 banding patterns that they obtained from 51 *E. coli* isolates from two water sources 11 km apart overlapped with each other.

A second explanation is that the two different flow conditions did not affect *E. coli* sharing of ribotype patterns. Samadpour and Chechowitz (1995) suggested that the typical increase in fecal contamination observed after storm events may represent more of the same contributing sources rather than additional sources. Thus, all ribotypes were present in the water at all times, and only small sampling size made it appear as if the *E. coli* ribotype patterns were unshared. Unfortunately, it was not possible to know the diversity of the *E. coli* ribotype population before the experiment was conducted; the study was not designed to test this hypothesis.

Nevertheless, both explanations suggest a large environmental sampling is necessary for a watershed as complex as the Upper Chattahoochee River. Both Buchan et al. (2001), using 16S-23S ribosomal RNA intergenic spacer region analysis, and Hagedorn et al. (1999), using multiple antibiotic resistance analysis, concluded that many bacterial isolates are necessary for bacterial source tracking to describe a watershed. The environmental sampling should include both large numbers of isolates to represent subspecies diversity at a specific point in time, and sample collection during different flow conditions to represent different subspecies composition.

Table 2. Number of presumptive *E. coli* isolates obtained after initial isolation on mTEC agar that were subsequently confirmed as *E. coli* (oxidase negative, citrate negative, and urea hydrolysis negative). The isolates were obtained from water sampled during baseflow (February 22 and March 1, 2000) or stormflow conditions (April 2, 2000) from four mainstem sites and eight tributary streams of the Upper Chattahoochee River watershed.

[USGS, U.S. Geological Survey; ID, identification]

USGS site ID	Location	Baseflow		Stormflow	
		Confirmed *E. coli* / presumptive *E. coli* (number)	False positives (percent)	Confirmed *E. coli* / presumptive *E. coli* (number)	False positives (percent)
02335420	James Creek	50 / 50	0	13 / 14	7
02334550	Chattahoochee River—Settles Bridge	42 / 42	0	24 / 24	0
02334885	Suwanee Creek	48 / 49	2	35 / 40	13
02335078	Johns Creek	49 / 49	0	14 / 16	13
02335350	Crooked Creek	45 / 45	0	17 / 28	39
02335741	Big Creek	23 / 41[a]	44	6 / 16	63
02335790	Willeo Creek	48 / 48	0	7 / 15	53
02335830	Chattahoochee River—Johnsons Ferry	34 / 50[a]	32	10 / 16	38
02335870	Sope Creek	48 / 48	0	14 / 37	62
02335910	Rottenwood Creek	44 / 44[a]	0	3 / 16	81
02336000	Chattahoochee River—Paces Ferry	36 / 45[a]	20	16 / 16	0
02336490	Chattahoochee River—State Route 280	23 / 39[a]	41	15 / 37	59
Totals		**490 / 550**	**11**	**174 / 275**	**37****

[a] Site was resampled on March 1, 2000, to obtain more isolates.

**Significant at the 0.01 probability level.

The percentage of *E. coli* isolates that were false positive on mTEC agar increased significantly during stormflow conditions (37%) compared with baseflow conditions (11%). Typically, freshwater samples plated on the mTEC agar have a 15-percent false positive rate (Dufour et al., 1981). The relatively high percentage of false positive *E. coli* isolates observed when stormflow conditions were sampled suggests that some caution is needed in counting yellow and yellow-brown isolates as presumptive *E. coli* after isolation on mTEC agar from stormflow samples. The manufacturer of the medium notes that high fecal counts may not provide accurate urease results (Difco Laboratories, 1998).

Conclusions

- In bacterial source tracking, it is important to understand factors affecting changes in a bacterial subspecies that enter streams because these factors determine the amount of sampling needed to describe the watershed adequately.
- The majority of *E. coli* ribotype patterns identified in this study were unshared, whether the comparisons were between baseflow and stormflow at one location, or between one location and another. One explanation is that land-use patterns and baseflow and stormflow conditions affected this sharing.
- An alternative explanation is that baseflow and stormflow conditions had no effect on *E. coli* sharing of ribotype patterns and only the relatively small sampling size made it appear as if the ribotype patterns were unshared.
- The results underscore the need for extensive environmental sampling in a watershed as complex as the Upper Chattahoochee River, and the need for an extensive host origin database for genotypic methods using such databases.

Table 3. Shared and unshared ribotype patterns and isolates obtained from four mainstem and eight tributary stream sampling sites on the Chattahoochee River during baseflow and stormflow conditions.

[USGS, U.S. Geological Survey; ID, identification]

USGS site ID	Location	Number of isolates		Number shared		Number unshared	
		Baseflow	Stormflow	Ribotype patterns	Isolates	Ribotype patterns	Isolates
02335420	James Creek	12	10	0	0	9	22
02334550	Chattahoochee River—Settles Bridge	29	23	3	12	32	40
02334885	Suwanee Creek	11	15	1	2	17	24
02335078	Johns Creek	12	10	0	0	9	22
02335350	Crooked Creek	11	11	0	0	14	22
02335741	Big Creek	14	1	0	0	9	15
02335790	Willeo Creek	10	9	0	0	13	19
02335830	Chattahoochee River—Johnsons Ferry	5	8	1	5	7	8
02335870	Sope Creek	6	4	1	2	8	8
02335910	Rottenwood Creek	27	2	0	0	14	29
02336000	Chattahoochee River—Paces Ferry	10	13	0	0	18	23
02336490	Chattahoochee River—State Route 280	4	5	0	0	7	9
Totals		**151**	**111**	**6**	**21**	**157**	**241**

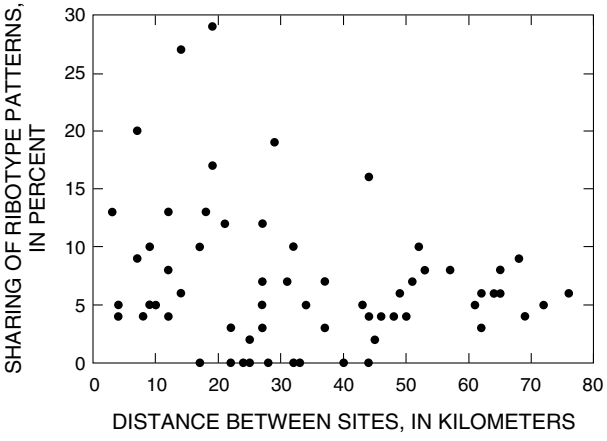

Figure 3. Percentage of ribotype patterns shared plotted against distance between sites for each of 66 possible site pairs in the Chattahoochee River watershed, February–April 2000. Distances between sites were determined from Carter et al. (1989) and U.S. Army Corps of Engineers (1985).

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References

Amor, K., Heinrichs, D.E., Frirdich, E., Ziebell, K., Johnson, R.P., and Whitfield, C., 2000, Distribution of core oligosaccharide types in lipopolysaccharides from *Escherichia coli*: Infection and Immunity 68(3), p. 1116–1124.

Buchan, A., Alber, M., and Hodson, R.E., 2001, Strain–specific differentiation of environmental *Escherichia coli* isolates via denaturing gradient gel electrophoresis (DGGE) analysis of the 16S-23S intergenic spacer region: FEMS Microbiology Ecology 35, p. 313–321.

Carter, R.F., Hopkins, E.H., and Perlman, H.A., 1989, Low-flow profiles of the upper Chattahoochee River and tributaries in Georgia: U.S. Geological Survey Water–Resources Investigations Report 89–4056, 194 p.

Dice, L.R., 1945, Measures of the amount of ecologic association between species: Ecology 26, p. 297–302.

Difco Laboratories, 1998, Difco manual, 11th ed.: Becton Dickinson, Sparks, Md.

Dombek, P.E., Johnson, L.K., Zimmerley, S.T., and Sadowsky, M.J., 2000, Use of repetitive DNA sequences and the PCR to differentiate *Escherichia coli* isolates from human and animal sources: Applied and Environmental Microbiology 66(6), p. 2572–2577.

Dufour, A.P., Strickland, E.R., and Cabelli, V.J., 1981, Membrane filter method for enumerating *Escherichia coli*: Applied and Environmental Microbiology 41(5), p. 1152–1158.

Entry, J.A., Hubbard, R.K., Thies, J.E., and Fuhrmann, J.J., 2000, The influence of vegetation in riparian filterstrips on coliform bacteria—I. Movement and survival in water: Journal of Environmental Quality 29(4), p. 1206–1214.

Georgia Department of Natural Resources, 1997, Water quality in Georgia, 1994–95: Environmental Protection Division, Atlanta, 119 p.

Gordon, D.M., 1997, The genetic structure of *Escherichia coli* populations in feral house mice: Microbiology 144, p. 2233–2240.

Gregory, M.B., and Frick, E.A., 2000, Fecal-coliform bacteria concentrations in streams of the Chattahoochee River National Recreation Area, Metropolitan Atlanta, Georgia, May–October 1994 and 1995: U.S. Geological Survey Water-Resources Investigations Report 00-4139, 8 p. Accessed online January 14, 2004, at *http://ga.water.usgs.gov/publications/wrir00-4139.pdf*.

Gregory, M.B., and Frick, E.A., 2001, Indicator-bacteria concentrations in streams of the Chattahoochee River National Recreation Area, March 1999–April 2000: *in* K. J. Hatcher (ed.), Proceedings of the 2001 Georgia Water Resources Conference, March 26–27, 2001, Univ. of Georgia, Athens, p. 510–513. Accessed online January 14, 2004, at *http://ga.water.usgs.gov/nawqa/IndicatorBacteria_99_00_final.pdf*.

Hagedorn, Charles, Robinson, S.L., Filtz, J.R., Grubbs, S.M., Angier, T.A., and Reneau, Jr., R.B., 1999, Determining sources of fecal pollution in a rural Virginia watershed with antibiotic resistance patterns in fecal streptococci: Applied and Environmental Microbiology 65(12), p. 5522–5531.

Hartel, P.G., Entry, J., Summer, J., Hill, J.L., Collins, J.V., and Segars, W.I., 2002, Geographic variability of *Escherichia coli* ribotypes from animals in Idaho and Georgia: Journal of Environmental Quality 31(4), p. 1273–1278.

Jenkins, M.B., Hartel, P.G., Olexa, T.J., and Stuedemann, J.A., 2003, Putative temporal variability of *Escherichia coli* ribotypes from yearling steers: Journal of Environmental Quality 32(1), p. 305–309.

Kariuki, S., Gilks, C., Kimari, J., Obanda, A., Muyodi, J., Waiyaki, P., and Hart, C.A., 1999, Genotype analysis of *Escherichia coli* strains isolated from children and chickens living in close contact: Applied and Environmental Microbiology 65(2), p. 472–476.

Myers, D.N., and Sylvester, M.A., 1999, National field manual for the collection of water quality data, Biological indicators, Fecal indicator bacteria: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 9, Chap. A7, Sec. 7.1, variously paginated. Accessed online January 14, 2004, at *http://water.usgs.gov/owq/FieldManual/*.

Parveen, Salina, Portier, K.M., Robinson, Kevin, Edmiston, Lee, and Tamplin, M.L., 1999, Discriminant analysis of ribotype profiles of *Escherichia coli* for differentiating human and nonhuman sources of fecal pollution: Applied and Environmental Microbiology 65(7), p. 3142–3147.

Richman, Michael, 1996, Sewer separation lowers fecal coliform levels: Water Environment and Technology 8(11), p. 20–22.

Samadpour, Mansour, and Chechowitz, Naomi, 1995, Little Soos Creek microbial source tracking: Report to Surface Water Management Division, King County Department of Public Works, Seattle, Wash., 36 p.

Solo-Gabriele, H.M., Wolfert, M.A., Desmarais, T.R., and Palmer, C.J., 2000, Sources of *Escherichia coli* in a coastal subtropical environment: Applied and Environmental Microbiology 66(1), p. 230–237.

Stevens, Pat, 2001, Metro Atlanta water resources overview: *in* K.J. Hatcher (ed.), Proceedings of the 2001 Georgia Water Resources Conference, March 26–27, 2001, Univ. of Georgia, Athens, p. 9–14.

U.S. Army Corps of Engineers, 1985, Florida–Georgia stream mileage tables with drainage areas: Mobile, Ala., 233 p.

Whitlock, J.E., Jones, D.T., and Harwood, V.J., 2002, Identification of the sources of fecal coliforms in an urban watershed using antibiotic resistance analysis: Water Research 36(17), p. 4273–4282.

Wiggins, B.A., Andrews, R.W., Conway, R.A., Corr, C.L., Dobratz, E.J., Dougherty, D.P., Eppard, J.R., Knupp, S.R., Limjoco, M.C., Mettenburg, J.M., Rinehardt, J.M., Sonsino, J., Torrijos, R.L., and Zimmerman, M.E., 1999, Use of antibiotic resistance analysis to identify nonpoint sources of fecal pollution: Applied and Environmental Microbiology 65(8), p. 3483–3486.

Wilde, F.D., Radtke, D.B., Gibs, J., and Iwatsubo, R.T., 1999, National field manual for the collection of water-quality data. Collection of water samples: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 9, Chap. A4, variously paginated. Accessed online January 14, 2004, at *http://water.usgs.gov/owq/FieldManual/.*