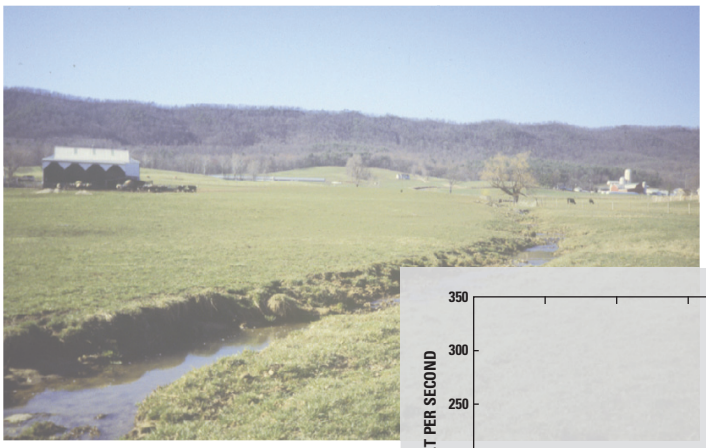
U.S. Department of the Interior
U.S. Geological Survey

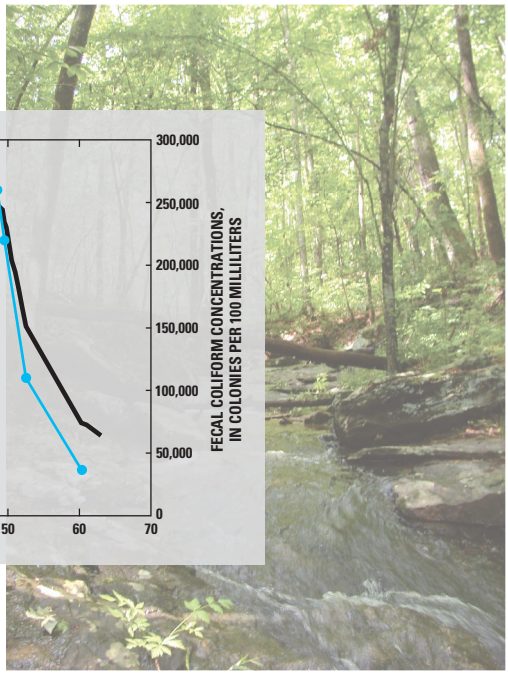
Prepared in cooperation with:

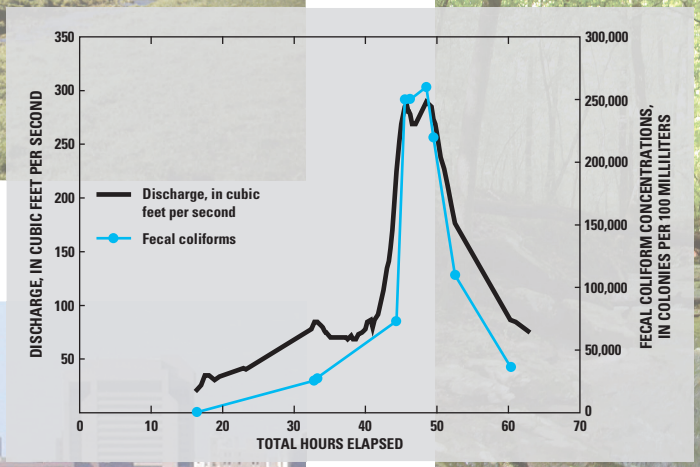
Virginia Department of Conservation and Recreation

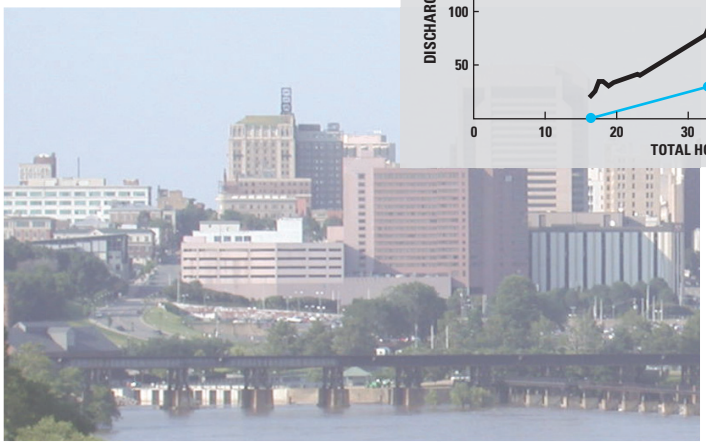
Patterns and Sources of Fecal Coliform Bacteria in Three Streams in Virginia, 1999-2000

Water-Resources Investigations Report 03-4115









USGS
science for a changing world

Representative agricultural, urban, and forested streams (Photographs by K.E. Hyer and D.L. Moyer, U.S. Geological Survey)

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

Patterns and Sources of Fecal Coliform Bacteria in Three Streams in Virginia, 1999-2000

By Kenneth E. Hyer and Douglas L. Moyer

Water-Resources Investigations Report 03-4115

Prepared in cooperation with:

Virginia Department of Conservation and Recreation
Virginia Department of Environmental Quality
Fairfax County, Virginia

Richmond, Virginia
2003

U.S. DEPARTMENT OF THE INTERIOR
GALE A. NORTON, *Secretary*

U.S. GEOLOGICAL SURVEY
Charles G. Groat, *Director*

For additional information write to:

District Chief
U.S. Geological Survey
1730 East Parham Road
Richmond, VA 23228
dc_va@usgs.gov

Copies of this report can be purchased from:

U.S. Geological Survey
Branch of Information Services
Box 25286, Federal Center
Denver, CO 80225-0286

Information about water resources in Virginia is available on the World Wide Web at http://va.water.usgs.gov

CONTENTS

Figures

Tables

Appendix

CONVERSION FACTORS, DATUM, AND ABBREVIATED WATER-QUALITY UNITS

Multiply	By	To obtain
	Length	
inch (in.)	25.4	millimeter
foot (ft)	0.3048	meter
mile (mi)	1.609	kilometer
	Area	
square mile (mi^2)	259.0	hectare
square mile (mi^2)	2.590	square kilometer
	Volume	
gallon (gal)	3.785	liter
gallon (gal)	0.003785	cubic meter
	Flow	
cubic foot per second (ft^3/s)	0.02832	cubic meter per second
gallons per day	0.003785	cubic meter per day
million gallons per day (Mgal/d)	0.04381	cubic meter per second

Horizontal coordinate information is referenced to the North American Datum of 1927 (NAD27).

Temperature: Temperature is reported in degrees Celsius (°C), which can be converted to degrees Fahrenheit (°F) as follows: °F = 1.8 (°C) + 32°

Abbreviated water-quality units: Chemical concentration is reported in milligrams per liter (mg/L) or micrograms per liter (µg/L). Milligrams per liter is a unit expressing the concentration of chemical constituents in solution as mass (milligrams) of solute per unit volume (liter) of water. One-thousand micrograms per liter is equivalent to 1 milligram per liter. For concentrations less than 7,000 mg/L, the numerical value is the same as for concentrations in parts per million. Bacterial concentrations are reported in units of colonies per 100 milliliters (col/100mL). Specific electrical conductance of water is reported in microsiemens per centimeter at 25 degrees Celsius (µS/cm). Turbidity is reported in nephelometric turbidity units (NTU).

Patterns and Sources of Fecal Coliform Bacteria in Three Streams in Virginia, 1999-2000

By Kenneth E. Hyer and Douglas L. Moyer

ABSTRACT

Surface-water impairment by fecal coliform bacteria is a water-quality issue of national scope and importance. In Virginia, more than 175 stream segments are on the Commonwealth's 1998 303(d) list of impaired waters because of elevated concentrations of fecal coliform bacteria. These fecal coliform-impaired stream segments require the development of total maximum daily load (TMDL) and associated implementation plans, but accurate information on the sources contributing these bacteria usually is lacking. The development of defendable fecal coliform TMDLs and management plans can benefit from reliable information on the bacteria sources that are responsible for the impairment. Bacterial source tracking (BST) recently has emerged as a powerful tool for identifying the sources of fecal coliform bacteria that impair surface waters. In a demonstration of BST technology, three watersheds on Virginia's 1998 303(d) list with diverse land-use practices (and potentially diverse bacteria sources) were studied. Accotink Creek is dominated by urban land uses, Christians Creek by agricultural land uses, and Blacks Run is affected by both urban and agricultural land uses. During the 20-month field study (March 1999–October 2000), water samples were collected from each stream during a range of flow conditions and seasons. For each sample, specific conductance, dissolved oxygen concentration, pH, turbidity, flow, and water temperature were measured. Fecal coliform concentrations of each water sample were determined using the membrane filtration technique. Next, *Escherichia coli* (*E. coli*) were isolated from the fecal coliform bacteria and their sources were identified using ribotyping (a method of "genetic fingerprinting").

Study results provide enhanced understanding of the concentrations and sources of fecal coliform bacteria in these three watersheds. Continuum sampling (sampling along the length of the streams) indicated that elevated concentrations of fecal coliform bacteria (maximum observed concentration of 290,000 colonies/100 milliliters (col/100mL) could occur along the entire length of each stream, and that the samples collected at the downstream monitoring station of each stream were generally representative of the entire upstream reach. Seasonal patterns were observed in the base-flow fecal coliform concentrations of all streams; concentrations were typically highest in the summer and lowest in the winter. Fecal coliform concentrations were lowest during periods of base flow (typically 200–2,000 col/100mL) and increased by 3–4 orders of magnitude during storm events (as high as 700,000 col/100mL). Multiple linear regression models were developed to predict fecal coliform concentrations as a function of streamflow and other water-quality parameters. The source tracking technique provided identification of bacteria contributions from diverse sources that included (but were not limited to) humans, cattle, poultry, horses, dogs, cats, geese, ducks, raccoons, and deer. Seasonal patterns were observed in the contributions of cattle and poultry sources. There were relations between the identified sources of fecal coliform bacteria and the land-use practices within each watershed. There were only minor differences in the distribution of bacteria sources between low-flow periods and high-flow periods. A coupled approach that utilized both a large available source library and a smaller, location-specific source library provided the most success in identifying the unknown *E. coli* isolates. BST data should provide valuable support and guidance for producing more defend-

able and scientifically rigorous watershed models. Incorporation of these bacteria-source data into watershed management strategies also should result in the selection of more efficient source-reduction scenarios for improving water quality.

INTRODUCTION

Surface-water impairment by fecal coliform bacteria is a water-quality issue of national scope and importance. Section 303(d) of the Clean Water Act requires that each State identify surface waters that do not meet applicable water-quality standards. In Virginia, more than 175 stream segments are on the Commonwealth's 1998 303(d) list of impaired waters because of violations of the fecal coliform bacteria standard (an instantaneous water-quality standard of 1,000 col/100 mL, or a geometric mean water-quality standard of 200 col/100 mL). Fecal coliform concentrations that violate either standard indicate an increased risk to human health when these waters are contacted through swimming or other recreational activities.

In Virginia, total maximum daily load (TMDL) plans will need to be developed over the next 10 years for all impaired water bodies identified on the State's 1998 303(d) list. TMDL plans provide a quantitative representation of all the contaminant contributions to a stream:

$$TMDL = \sum WLAi + \sum LAi + MOS \quad (1)$$

where $\sum WLAi$ represents the sum of all the point-source loadings, $\sum LAi$ represents the sum of all the nonpoint-source loadings, and MOS represents a margin of safety. The sum of these loading terms and assigned margin of safety constitutes the TMDL and represents the fecal coliform loading that the surface-water body can assimilate without violating the state's water-quality standards. For a TMDL plan to be approved by the U.S. Environmental Protection Agency (USEPA), all major fecal coliform contributions to the stream must be identified and quantified. Once a TMDL plan is established, fecal coliform source-load contributions are then reduced (through implementation of source-control management practices) until the target TMDL is achieved.

Establishing TMDLs in waters contaminated by fecal coliform bacteria is difficult because the specific sources of the bacteria are numerous and the magnitude of their contributions is commonly unknown. Potential sources of fecal coliform bacteria include all warm-blooded animals (humans, pets, domesticated livestock, birds, and wildlife). The lack of information on bacteria sources makes it difficult to develop accurate load allocations, technically defensible TMDLs, and appropriate source-load reduction measures. Information about the major fecal coliform sources that impair surface-water quality would represent a major improvement in the development of technically defensible TMDLs.

Bacterial source tracking (BST) recently has emerged as a tool for identifying the sources of fecal coliform bacteria that impair surface waters. In application, this technology identifies specific differences among the fecal coliform bacteria that are present in the feces of different animal species. Time, diet, environment, and many other factors may have contributed to produce these evolutionary distinctions; these distinctions are used in BST to identify the animal source of fecal coliform bacteria that have been isolated from a waterbody.

BST is a rapidly growing technology with various analytical techniques available, depending on the goals of the study. In general, these techniques rely on molecular, genetics-based approaches (also known as "genetic fingerprinting"), or phenotypic (relating to the physical characteristics of an organism) distinctions between the bacteria of different sources. Three primary genetic techniques are available for BST. Ribotyping characterizes a small, specific portion of the bacteria's DNA sequence (Samadpour and Chechowitz, 1995). Pulsed-field gel electrophoresis (PFGE) is similar to ribotyping but typically is performed on the entire genome of the bacteria (Simmons and others, 1995). Polymerase chain reaction (PCR) amplifies selected DNA sequences in the bacteria's genome (Makino and others, 1999). Phenotypic techniques generally involve an antibiotic resistance analysis, where resistance patterns for a suite of different concentrations and types of antibiotics are developed (Wiggins, 1996; Hagedorn and others, 1999).

Although all these techniques show promise for bacteria source identification, the ribotyping technique was chosen for this study. Ribotyping involves an analysis of the specific DNA sequence that codes for the production of ribosomal RNA (ribonucleic acid). Ribotyping has been demonstrated to be an effective technique for distinguishing bacteria from the feces of

multiple animal sources (Carson and others, 2001); it has been performed successfully and used to identify fecal coliform bacteria sources in both freshwater (Samadpour and Chechowitz, 1995) and estuarine systems (Ongerth and Samadpour, 1994). Furthermore, the technique has been used to identify the sources of bacteria contributing to impairments in both urban (Herrera Environmental Consultants, Inc., 1993) and wilderness systems (Farag and others, 2001). The broad applicability of ribotyping makes it well suited for use in this study.

This study was performed to demonstrate the field application of BST technology and to identify the sources of fecal coliform bacteria in three streams on Virginia's 1998 303(d) list of impaired waters. The three streams sampled during this study were selected because they represent a range of land uses (urban, agricultural, and mixed urban/agricultural) and most of the potential fecal coliform sources that are likely to be encountered throughout the Commonwealth. The three streams were sampled over a period of 20 months (March 1999–October 2000) and over a wide range of hydrological conditions. For all samples, the fecal coliform concentration, specific conductance, turbidity, pH, water temperature, and dissolved oxygen concentration were determined. Ribotyping was used to identify the sources of the fecal coliform bacteria. The results of this study have broad implications for the development of fecal coliform watershed models, selection of TMDL allocation scenarios, and the identification of effective strategies for reducing fecal coliform contributions to streams. The U. S. Geological Survey (USGS) conducted this study in cooperation with the Virginia Department of Environmental Quality (DEQ), Virginia Department of Conservation and Recreation (DCR), and Fairfax County, Virginia.

Purpose and Scope

This report demonstrates the field application of bacterial source tracking technology, which was used to identify the sources of fecal coliform bacteria in three streams that are on Virginia's 1998 303(d) list of impaired waters. Streamwater data were collected from March 1999 through October 2000, under both base-flow and storm-flow conditions. Concentrations of fecal coliform bacteria were determined at the stream gage and 4–5 other locations in each watershed; bacterial source tracking was performed only on the samples that were collected at the stream gage in each watershed. In addition to identifying the sources of fecal coliform bacteria in the three streams, the report describes (1) seasonal and discharge-related patterns in the concentrations of fecal coliform bacteria, (2) multiple linear regression models for predicting fecal coliform concentrations as a function of supporting water-quality field parameters, (3) seasonal and discharge-related patterns in the identified bacteria sources of each stream, and (4) the effect of source-library size on the identification of bacteria. Study results have broad implications for the interpretation of source-tracking data and the development of TMDL plans in impaired streams.

Acknowledgments

The authors acknowledge USGS employees Michael Gearheart, Trisha Baldwin, and Russ Lotspeich for providing outstanding technical support on this project. Don Stoeckel and Michael Focazio of USGS are thanked for providing technical reviews of this report. The following organization and persons assisted in collecting the scat samples that were used for developing a portion of the known-source database: The Wildlife Center of Virginia, Michael Noto (James Madison University), Kay Rutledge (Fairfax County, Wastewater Treatment Division), Robert Heavener (Rockingham County Sewer Authority), Melissa Harris (Augusta County Service Authority), and Greg Zell (Arlington County, Department of Parks, Recreation, and Community Resources). Michael Noto also assisted in collecting additional continuum samples in Blacks Run. Lastly, we are indebted to the residents, farmers, and stakeholders in all three watersheds for their enthusiastic participation in this study.

DESCRIPTION OF THE STUDY AREAS

Three stream segments on Virginia's 1998 303(d) list were selected for this study. The streams in Virginia that are impaired by fecal coliform bacteria drain watersheds that generally can be categorized into one of three land-use practices: agricultural, urban, and mixed urban/agricultural. To represent a range of land uses and potential sources of fecal contamination, a representative study site was selected from each of these land-use types. The criteria evaluated for site

selection included (1) presence of a stream gage, (2) size of watershed (about 100 mi^2 or smaller), (3) well-defined and stable land-use patterns, (4) availability of historical water-quality data, (5) availability of up-to-date geographic information system (GIS) coverages, and (6) support from the local community. The three sites selected for this study (fig. 1) were Accotink Creek (representing urban land use), Christians Creek (agricultural land use), and Blacks Run (mixed urban and agricultural land use). The data collected during this study are being used in a separate watershed modeling and TMDL development study by the USGS (Moyer and Hyer, in press).

Accotink Creek

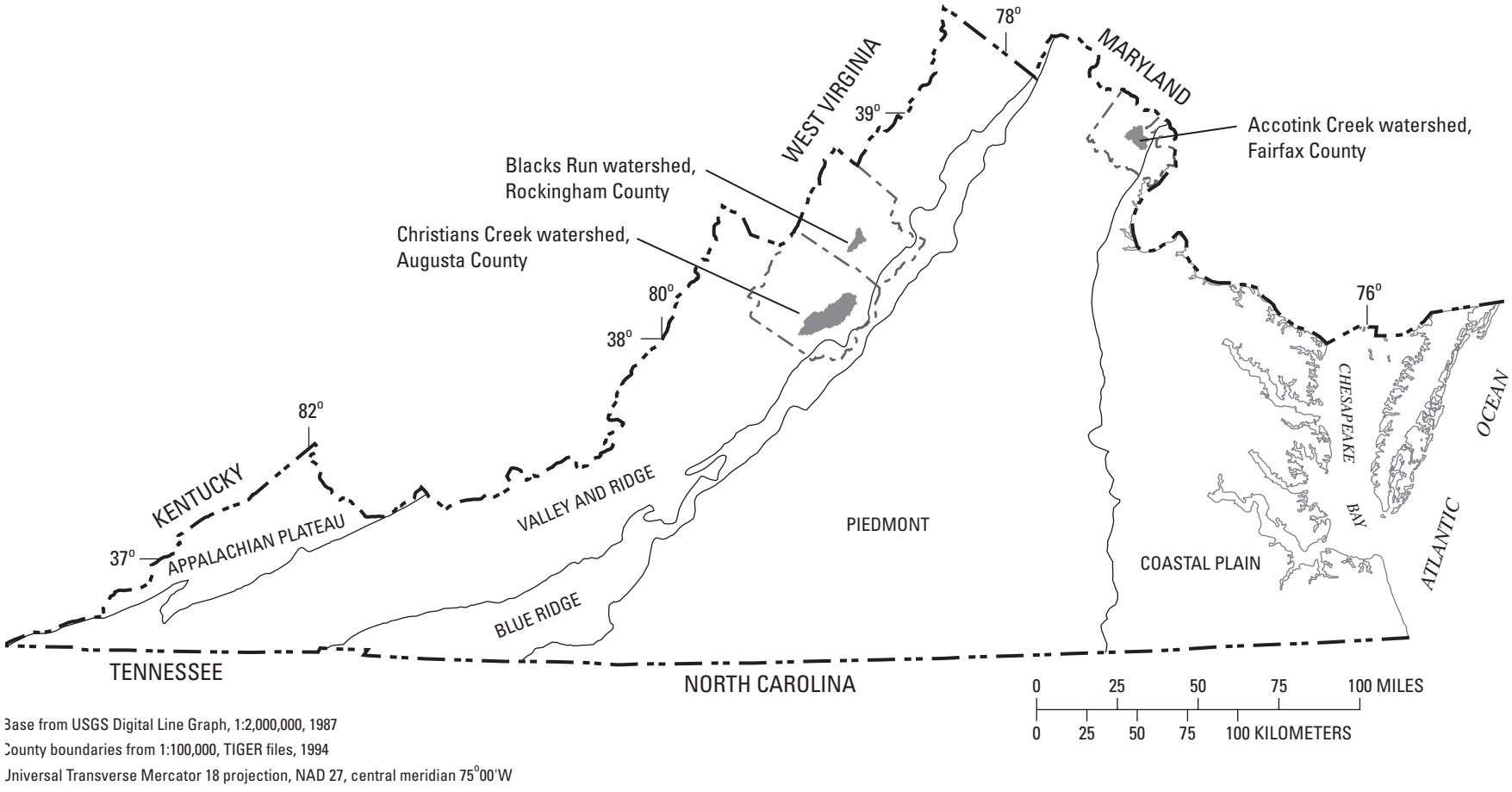
Accotink Creek near Annandale, Va., is the urban watershed selected for this study (fig. 2). The headwaters of Accotink Creek are in the city of Fairfax, Va., and the creek flows for approximately 10.9 mi before it drains into Lake Accotink, located in Fairfax County. The impaired stream reach is a 4.5-mi-long section just upstream of Lake Accotink. The portion of the Accotink Creek watershed studied has a drainage basin area of 25 mi^2 and a population of more than 110,000 (2000 U.S. Census Bureau data). Approximately 600 ft upstream from the bridge at Route 620 (Braddock Road) is a stream gage that has been active since 1949 and is managed by DEQ (USGS station number 01654000). DEQ has performed quarterly sampling for fecal coliform bacteria at the bridge at Route 620 since 1990. Currently, there are no permitted fecal coliform point source dischargers within the watershed (J. Crowther, Virginia Department of Environmental Quality, written commun., 1999).

Although portions of the watershed are forested (especially adjacent to the stream), urban and residential land uses dominate the majority of the watershed. Potential sources of fecal contamination in this urban watershed include domestic pets (such as dogs and cats), wildlife (such as raccoons, opossum, rats, squirrels, and deer), waterfowl (such as geese, ducks, and sea gulls), and humans (as contributed by cross-pipes, leaking or overflowing sewer lines, and failing septic systems).

The Accotink Creek watershed lies in the Piedmont physiographic province, and is underlain by crystalline igneous and metamorphic rocks (Froelich and Zenone, 1985). The surficial geology of the watershed is composed of five formations. The Wissahickon Formation dominates the watershed and is composed of quartz-mica schist, phyllite, and quartzite (Johnston, 1964). The Greenstone Contact Complex is present in some headwater areas of the catchment and is composed of chlorite schist, sericite-chlorite schist, chlorite-quartz schist, talc schist and small amounts of quartzite (Johnston, 1962). Granitic rocks are distributed throughout the watershed; these rocks are of variable composition and include biotite granite, muscovite granite, biotite-muscovite granite, granodiorite, quartz monzonite, and quartz diorite (Johnston, 1964). A small portion of the watershed is underlain by the Sykesville Formation, which includes muscovite or sericite-biotite-quartz schist and gneiss, quartzite, epidote quartzite, and muscovite-biotite quartzite (Johnston, 1964). Alluvial material (composed of clay and sand, as well as quartz cobbles and pebbles) also is present along the channel and in the floodplain of Accotink Creek (Johnston, 1962).

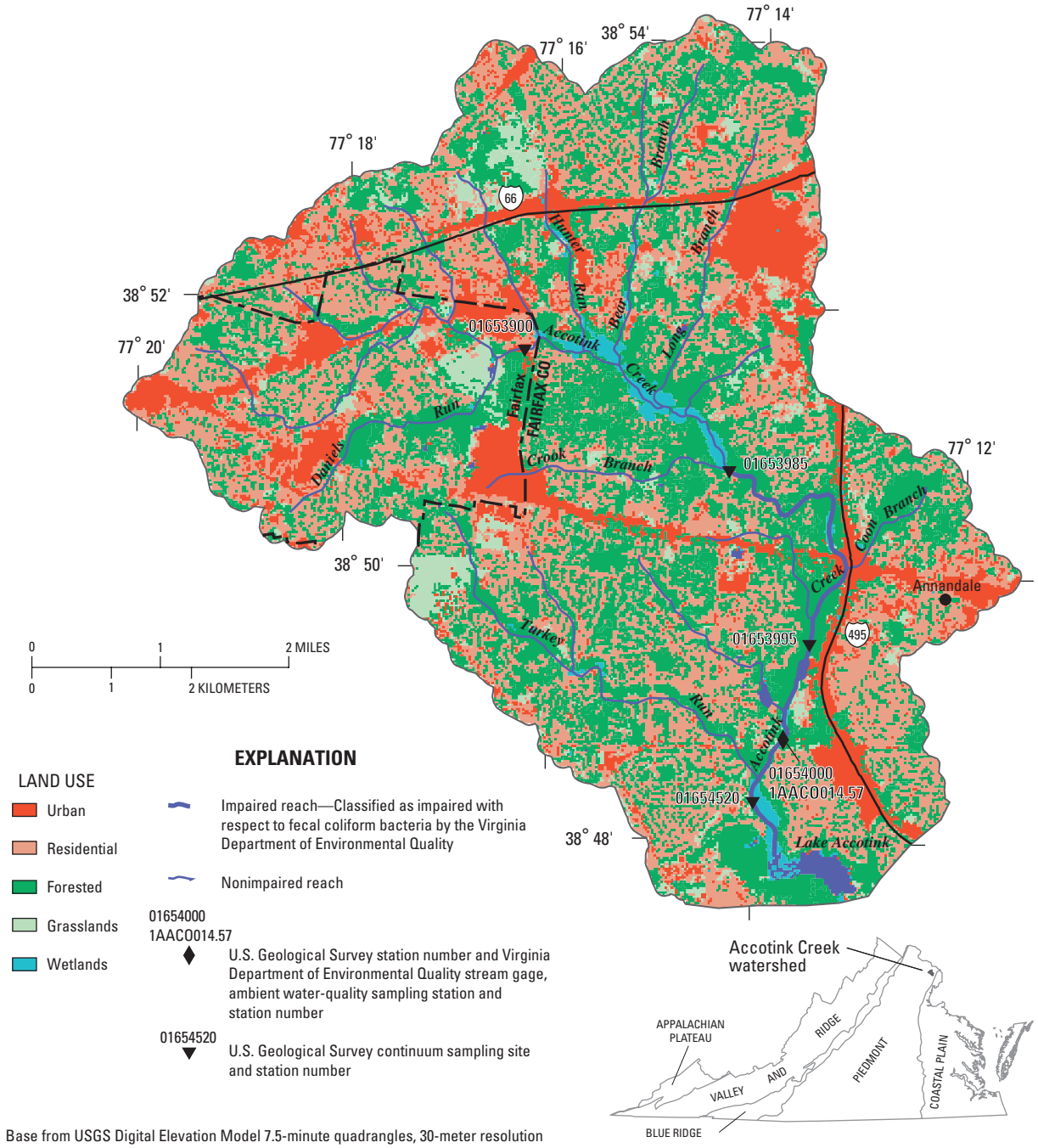
The soils of the Accotink Creek watershed are present as three distinct soil associations, described by Porter and others (1963). The Glenelg-Elioak-Manor association has developed from the weathering of the crystalline bedrock of the Piedmont. These well-drained (and, in some places, excessively drained) silt-loam soils dominate the watershed. The Fairfax-Beltsville-Glenelg association comprises a relatively small portion of the watershed (limited to the headwater areas) and formed from the residuum of Piedmont bedrock and fluvial Coastal Plain sediments. These soils are present as silt or sand loams, and range from somewhat poorly drained to well drained. The Chewacla-Wehadkee association occurs only on a limited basis within the watershed, generally in the bottomland and in floodplains along streams. These silt-loam soils range from moderately well drained to poorly drained and have developed from alluvial material that was washed from the Piedmont uplands.

Most water-quality data for this study were collected from the Accotink Creek stream gage (station number 01654000); this site also is a DEQ ambient water-quality sampling station. Four additional stations where data were collected (continuum sampling sites) along Accotink Creek are at Route 237 (Pickett Road, station number 01653900), Route 846 (Woodburn Road, station number 01653985), Woodlark Drive (station number 01653995), and Lonsdale Drive (station number 01654520).



Base from USGS Digital Line Graph, 1:2,000,000, 1987
County boundaries from 1:100,000, TIGER files, 1994
Universal Transverse Mercator 18 projection, NAD 27, central meridian 75°00'W

Figure 1. Location of Accotink Creek, Blacks Run, and Christians Creek watersheds, and physiographic provinces in Virginia.



Base from USGS Digital Elevation Model 7.5-minute quadrangles, 30-meter resolution

Land use from U.S. Environmental Protection Agency Multi-Resolution Land Characteristics Region 3, 1993, 30-meter resolution

Hydrography digitized from 1:24,000, 7.5-minute quadrangles

County boundaries from 1:100,000, TIGER files, 1994

Universal Transverse Mercator 18 projection, NAD 27, central meridian 75°00'W

Figure 2. Land use, streams, and sampling stations in the Accotink Creek watershed, Fairfax County, Virginia.

Christians Creek

Christians Creek, located in Augusta County, is the agricultural watershed selected for this study (fig. 3). Christians Creek originates northwest of Greenville, Va., and extends to the confluence with the Middle River. The entire 31.5-mi-long reach is classified as impaired with respect to fecal coliform bacteria. The watershed has a drainage area of 107 mi^2. The population of the watershed is estimated to be 12,000 (1990 U.S. Census Bureau data). There is a recently (1997) deactivated stream gage (still operational for instantaneous stage determinations) at Route 794 (Sangers Lane, station number 01624800), with a period of record from 1967 to 1997. DEQ has sampled for fecal coliform bacteria at Route 794 and Route 831 (Old White Hill Road, station number 1BCST021.76) on a monthly basis since 1991. The ambient water-quality sampling station at Route 794 was the primary Christians Creek sampling location for this study.

There are 18 permitted point source dischargers in the watershed (B.K. Fowler, Virginia Department of Environmental Quality, written commun., 2000; table 1). The Fishersville Sewage Treatment Plant discharges into Christians Creek about 1,500 ft upstream from one of the USGS and DEQ water-quality sampling locations (Route 794). On various occasions, the outfall from this sewage-treatment plant was sampled to check that it was not an important contributor of fecal coliform bacteria to the stream. As permitted, none of these point sources contributes greater than 200 col/100 mL to Christians Creek. None of these point sources represents a large flow contribution to Christians Creek; cumulatively, these sources account for less than 5 percent of the daily flow in the creek. The 12 private permitted dischargers in the watershed are 9 family residences and 3 small businesses.

Land use within the watershed is dominated by agricultural practices that are potential sources of fecal coliform bacteria within the watershed. Major components of animal husbandry in this watershed include the production of beef cattle, dairy cattle, heifers, broilers, and turkeys. Other potential fecal coliform bacteria sources within the watershed include humans (as contributed by failing septic systems, leaking or overflowing sewer lines, cross-pipes, and straight pipes), domestic animals (such as dogs and cats), waterfowl (such as geese, ducks, and sea gulls), and wildlife (such as deer, raccoons, opossum, rabbits, muskrats, ground hogs, foxes, and beaver).

The Christians Creek watershed lies within the Valley and Ridge physiographic province. The surficial geology that underlies the drainage basin is composed of 10 formations and is dominated by limestone and dolomite; information about each formation is summarized from Rader (1967). The Martinsburg Formation (calcareous shale and sandstone) is the dominant formation within the basin. Other formations in the watershed include the Edinburg Formation (argillaceous limestone and shale), Lincolnshire Formation (cherty limestone), New Market Limestone (limestone with dolomite beds near the base), Beekmantown Formation (dolomite and limestone), Chepultepec Formation (limestone and dolomite), Conococheague Formation

Table 1. Permitted point-source dischargers of fecal coliform bacteria in Christians Creek watershed during 2000, Augusta County, Virginia (B.K. Fowler, Virginia Department of Environmental Quality, written commun., 2000)

[Mgal/d, million gallons per day]

Discharger	Discharge (Mgal/d)	Latitude	Longitude
Fishersville Sewage Treatment Plant	0.7	38º07'41"	78º59'46"
Staunton Plaza Sewage Treatment Plant	.09	38º06'45"	79º03'18"
Brookwood Interchange Sewage Treatment Plant	.03	38º04'26"	79º04'56"
Riverheads High School Sewage Treatment Plant	.014	38º01'47"	79º08'27"
Southern States Cooperative	0	38º06'09"	79º04'24"
Woodlawn Village Mobile Home Park	.007	38º08'53"	78º55'06"
12 private permitted dischargers	.001	Various	Various

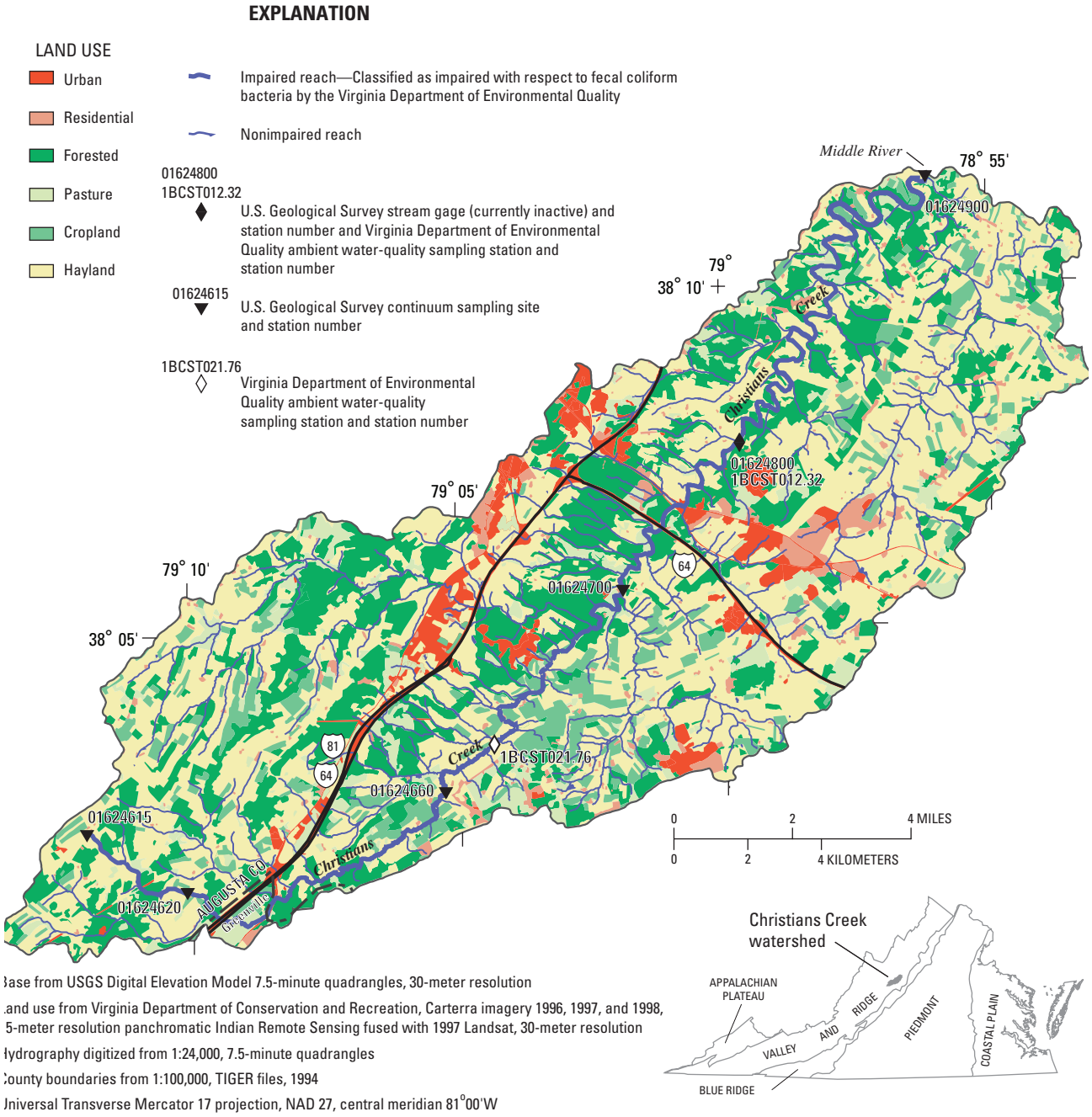


Figure 3. Land use, streams, and sampling stations in the Christians Creek watershed, Augusta County, Virginia.

(limestone, dolomite, and sandstone), and Elbrook Formation (limestone and dolomite). Alluvial material (composed of sand and clay) is present in portions of the floodplain adjacent to Christians Creek. Small amounts of fault breccia (large blocks of dolomite and limestone with crush conglomerate) also are present in the basin.

The soils of the Christians Creek watershed have been described thoroughly (Hockman and others, 1979) and are best classified as derived from the parent material from which they were formed. Much of the soil in the watershed has formed from the residuum of interbedded limestone, dolomite, and calcareous shale. Three soil assemblages have been identified in this category. The Frederick-Christian-Rock outcrop assemblage consists of deep, well-drained, silt loam or fine sandy loam soils with limestone outcrop areas. The Frederick-Bookwood-Christian assemblage consists of deep to moderately deep, well-drained, silt loam or fine sandy loam soils; scattered sinkholes or rock outcrops also may be present. The Chilhowie-Edom assemblage consists of deep to moderately deep, well-drained, silt loam or silty clay loam soils with occasional bedrock outcrops. Soil also has formed from the residuum of shale and thin interbedded sandstone and limestone. These soils are a part of the Berks-Weikert-Sequoia assemblage, which consists of shallow to deep, well-drained, silt loam or shaly silt loam soils. On floodplains and terraces, soils have formed in the alluvial or colluvial material. Although not extensive within the watershed, these soils are part of the Buchanan-Wheeling-Buckton assemblage, which consists of deep, somewhat poorly drained to well-drained soils. Generally these soils consist of silt loam, loam, or fine sandy loam, although some soils are gravelly or cobbly.

Most water-quality data were collected from Christians Creek below the bridge at Route 794 (Sangers Lane, station number 01624800); this site also is a DEQ ambient water-quality sampling station. Five additional sampling stations (continuum sampling sites) along Christians Creek were at the spring near Route 693 (Berry Moore Road, station number 01624615), Route 604 (McClures Mill Road, station number 01624620), Route 340 (Stuarts Draft Highway, station number 01624660), Route 635 (Barterbrook Road, station number 01624700), and Route 612 (Laurel Hill Road, station number 01624900).

Blacks Run

Blacks Run, located in Rockingham County, is the mixed urban and agricultural watershed selected for this study (fig. 4). Blacks Run originates on the north side of the city of Harrisonburg and extends to the confluence of Cooks Creek. The entire 10.7-mi-long reach is classified as impaired with respect to fecal coliform bacteria. The watershed has a drainage area of 20 mi^2 and an estimated population of 34,700 (1990 U.S. Census Bureau data). The city of Harrisonburg is the primary urban area within the watershed. This stream, like many in Virginia, did not have a stream gage, so one was installed (station number 01621470) at Route 704 (Cecil Wampler Road) in 1999. DEQ has sampled for fecal coliform bacteria at this station on a monthly basis since 1991.

There are no sewage-treatment plants in the Blacks Run watershed, but there are two private permitted dischargers, one family residence and one small business (B.K. Fowler, Virginia Department of Environmental Quality, written commun., 2000). Under the discharge permits, the treated wastewater discharge may not exceed 1,000 gallons per day and may not contain fecal coliform bacteria concentrations that exceed 200 col/100 mL.

Approximately two-thirds of the watershed (generally the portion closer to the headwaters) is dominated by urban land uses. In this urban area, potentially major contributors of fecal coliform bacteria include humans (as contributed by cross-pipes, failing septic systems, and leaking or overflowing sewer lines), domestic animals (such as dogs and cats), waterfowl (such as geese, ducks, and sea gulls), and wildlife (such as raccoons, opossum, rats, squirrels, and deer). The remaining one-third of the watershed (the lower portion of the watershed, closer to the stream gage) is dominated by agricultural land uses. Major components of the animal husbandry in this watershed include the production of beef cattle, dairy cattle, heifers, chickens, broilers, and turkeys. Other potential contributors in this agricultural area include humans (as contributed by failing septic systems, leaking or overflowing sewer lines, cross-pipes, and straight pipes), domestic animals (such as dogs and cats), waterfowl (such as geese, ducks, and sea gulls), and wildlife (such as deer, raccoons, opossum, rabbits, muskrats, ground hogs, foxes, and beaver).

The Blacks Run watershed lies within the Valley and Ridge physiographic province. The surficial geol

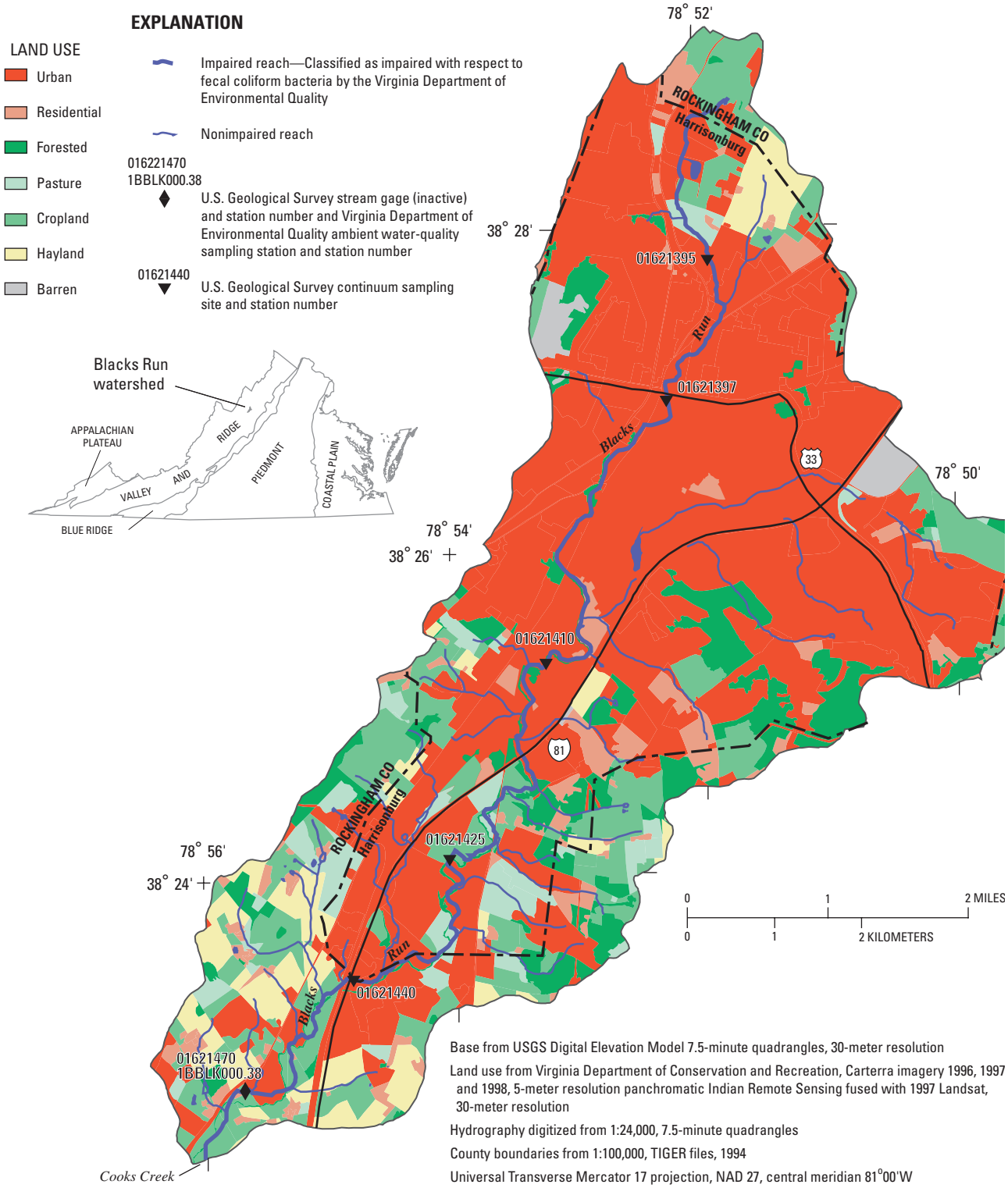


Figure 4. Land use, streams, and sampling stations in the Blacks Run watershed, Rockingham County, Virginia. Streams that appear disconnected are continuous; however, development activities within the watershed have captured these streams and routed the streamflow under portions of the city of Harrisonburg. Barren areas are primarily quarries.

ogy of the watershed is composed of seven formations and is dominated by limestone and dolomite; information about each formation is summarized in Gathright and Frischmann (1986). The primary formations within the watershed include the Martinsburg Formation (calcareous slate, argillite, and sandstone), Beekmantown Group (limestone and dolomite), New Market Limestone (limestone with dolomite beds near the base), Lincolnshire Formation (cherty limestone), Oranda Formation (limestone and calcareous shale), and Edinburg Formation (limestone and calcareous shale). Karst features are evident in portions of the watershed. Alluvial material (composed of unconsolidated fine sand, silt, and minor clay) is present in portions of the floodplain adjacent to Blacks Run.

The soils of the Blacks Run watershed have been described thoroughly (Hockman and others, 1982) and are best classified as derived from the parent material from which they were formed. Most of the soil in the watershed has formed from the residuum of limestone, dolomite, and calcareous shale. Three soil assemblages have been identified in this category. The Frederick-Lodi-Rock outcrop assemblage consists of deep, well-drained, silt loam soils with limestone or dolomite outcrop areas. The Endcav-Carbo-Rock outcrop assemblage consists of deep and moderately deep, well-drained, silt loam soils; sinkholes and limestone outcrops are common in this assemblage. The Chilhowie-Edom assemblage consists of deep to moderately deep, well-drained, silt loam or silty clay loam soils with occasional bedrock outcrops. On floodplains and terraces, soils have formed in the alluvial or colluvial material. Although not extensive within the watershed, these soils are part of the Monongahela-Unison-Cotaco assemblage, which consists of deep, well-drained or moderately well drained soils. Generally these soils consist of fine sandy loam soils, although some soils are cobbly.

Most water-quality data for this study were collected from Blacks Run below the bridge at Route 704 (Cecil Wampler Road, station number 01621470); this site also is a DEQ ambient water-quality sampling station. Five additional sampling stations (continuum sampling sites) along Blacks Run were at Route 753 (Liberty Street, station number 01621395), Water Street (station number 01621397), Route 726 (Stone Spring Road, station number 01621410), Route 679 (Pleasant Valley Road, station number 01621425), and Route 988 (station number 01621440).

METHODS

Water-sample collection for bacteria

Intensive streamwater sampling at the ambient water-quality sampling station of each watershed was done to provide an understanding of the temporal patterns in fecal coliform concentrations and the specific sources of these bacteria at each sampling site. Streamwater samples were collected over a wide range of hydrological conditions. Low-flow samples were collected from each stream approximately every 6 weeks, and approximately 4 of these low-flow samplings in each watershed were performed on the recession limbs of storm events. Typically, between four and eight depth-integrated samples were collected at each sampling site during each low-flow sampling. Width integration was accomplished by sampling at three locations across the width of the stream (the center of the channel and approximately halfway to each stream bank). The depth-integrated samples were collected at 5-minute intervals, providing time integration during each sampling. Five storm events were sampled on each stream. During each storm event, at least 10 water samples were collected from approximately the center of the streamflow. When possible, the storm samples were collected such that the first three samples were collected on the rising limb of the hydrograph, the next four samples were collected around the peak in the hydrograph, and the last three samples were collected on the falling limb of the hydrograph (fig. 5).
All samples were collected using sterile, 160-ml, narrow-mouth, borosilicate glass bottles. The samples were collected from the stream using the hand-dip method or a weighted-bottle sampler, depending on the site and flow conditions. Samples were immediately chilled on ice and processed in the field within 6 hours of collection.

Continuum sampling sites were established at 2- to 4-mi intervals along each of the three stream reaches, resulting in a total of four or five continuum sites on each reach. These continuum sites were sampled at various times during this study to evaluate whether the intensive sampling at the ambient water-quality sampling station represented the entire watershed. Each continuum sample was collected as a single, depth-integrated sample from the approximate center of the streamflow.

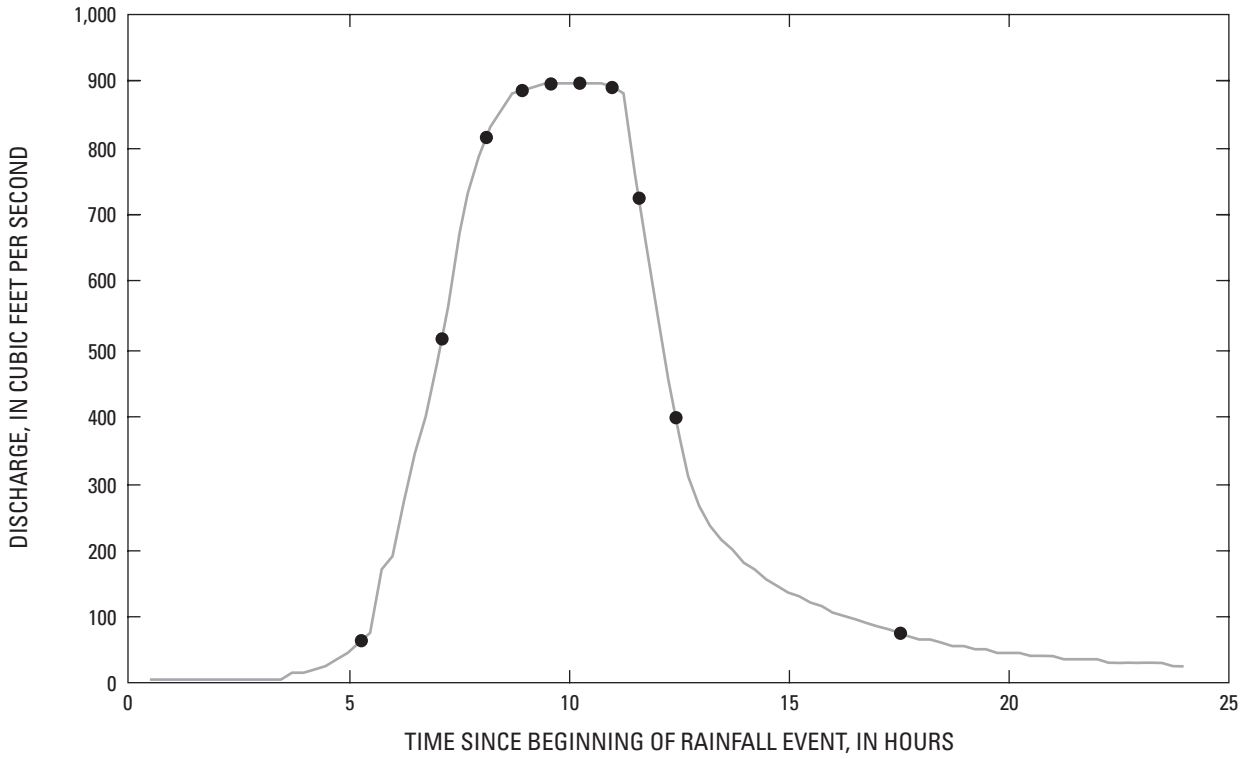


Figure 5. Storm-flow sampling design for bacterial source tracking study in Accotink Creek, Blacks Run, and Christians Creek watersheds, Virginia.

Synoptic samples of Accotink Creek were collected on June 5, 2000, following a major storm event. Various storm drains, major stream tributaries, and main channel sites were sampled to determine whether the entire watershed was contributing fecal coliform bacteria to the stream. Rhodamine WT dye was injected into the stream headwaters, and synoptic samples were collected while moving downstream at a rate that was consistent with the stream velocity and the injected dye. A single water sample (a grab sample) from the approximate center of the streamflow was collected from each sampling site. During this synoptic survey, a consistent water parcel was sampled as it traveled from the headwaters to the stream gage.

Supporting field measurements

Streamwater discharge and field water-quality parameters (pH, turbidity, dissolved oxygen concentration, water temperature, and specific conductance) were measured during the collection of each of the water samples for bacteria enumeration. Discharge measurements were made following standard USGS methods (Rantz and others, 1982). All field parameters were determined in accordance with the standard methods of the USGS (Wilde and Radke, 1998). The pH, water temperature, and specific conductance were measured using a YSI Model 63 handheld field meter. The dissolved oxygen concentration was measured using a YSI Model 95 handheld field meter. Turbidity was determined using a HACH 2100P handheld portable turbidimeter. All meters were calibrated (or quality assured, as appropriate) at the start of each field day, in accordance with the manufacturers' instructions. Specific conductance, dissolved oxygen concentration, pH, and water temperature were measured in situ by positioning the probes in the center (or as close as possible to the center) of the streamflow. Turbidity was measured on aliquots obtained from the water samples that were processed for bacteria.

Fecal coliform enumeration

All samples for the enumeration of fecal coliform bacteria were collected and processed according to USGS standard methods (Myers and Sylvester, 1997).

Water samples were processed in the field by membrane filtration (using gridded, 0.7-µm pore size membrane filters), and filters were incubated on a media of m-FC broth. Through this technique, fecal coliform bacteria are defined operationally as organisms that produce blue colonies in whole or in part after incubation for 18 to 22 hours at 44.5 ± 0.2ºC. A range of sample dilutions was always prepared in an effort to have at least one filter with colonies in the ideal counting range (20-60 colonies). The filter apparatus, bench tops, and necessary equipment were sterilized between the processing of each water sample. Start and end sample blanks were processed to ensure that the equipment initially was sterile, and that between-dilution rinsing procedures were adequate. Replicates were processed on 6 percent of the samples. After incubation, fecal coliform colonies were counted and the concentration of bacteria in the streamwater sample was calculated (as col/100 mL) based on the volume of filtered sample.

E. coli enumeration

About 150 fecal coliform samples (approximately 50 from each watershed) also were enumerated for *Escherichia coli* (*E. coli*) concentrations. *E. coli* were enumerated following standard USGS methods (Myers and Sylvester, 1997). Water samples were processed in the field by membrane filtration (using gridded, 0.45-µm pore size membrane filters), and filters were incubated on m-TEC agar. Through this technique, *E. coli* bacteria are defined operationally as organisms that produce yellow or yellow-brown colonies after resuscitation at 35.0 ± 0.5ºC for 2 hours and incubation for 22 to 24 hours at 44.5 ± 0.2ºC. A range of sample dilutions was always prepared in an effort to have at least one filter with colonies in the ideal counting range (20-80 colonies). The filter apparatus, bench tops, and necessary equipment were sterilized between the processing of each water sample. Start and end sample blanks were processed to ensure that the equipment initially was sterile, and that between-dilution rinsing procedures were adequate. After incubation, *E. coli* colonies were counted and the concentration of the streamwater sample was calculated (as col/100 mL) based on the volume of filtered sample.

A paired comparison of fecal coliform and *E. coli* concentrations was performed to verify that *E. coli* were the primary component of the fecal coliform bacteria that were observed in the streams. This verification was important because the ribotyping was performed on *E. coli*, and the Commonwealth of Virginia determined the water quality of streams and rivers on the basis of a fecal coliform standard. A strong correlation is present between fecal coliform and *E. coli* concentrations (fig. 6); most of the fecal coliforms collected in these three streams (67 percent) were *E. coli*. These results justify the use of *E. coli* bacteria for ribotyping even though the water-quality standard is based on fecal coliform bacteria.

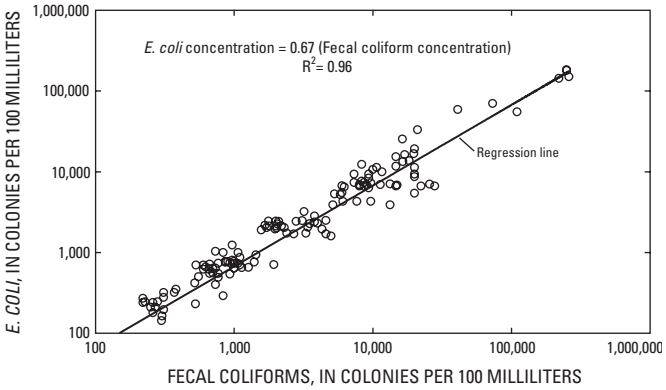


Figure 6. Relation of fecal coliform and *E. coli* concentrations in water samples collected March 1999 through October 2000 in the Accotink Creek, Blacks Run, and Christians Creek watersheds, Virginia.

Bacterial source tracking

Ribotyping was selected as the BST technique for this study because it offered definitive source identification and produced results that should be applicable to detailed TMDL development. Dr. Mansour Samadpour's Microbial Source Tracking Laboratory at the University of Washington (UWMSTL) performed the bacterial source tracking for all samples in this study. Although the specific application to field-based source identification is relatively new, ribotyping is a well-established tool in molecular biology (Tarkka and others, 1994; Schalch and others, 1997; Dalla-Costa and others, 1998; Samadpour, 2001). Conceptually, ribotyping is successful for this application because individual *E. coli* strains generally are host-species specific—only infrequently does an *E. coli* strain colonize a foreign host species. Subtle genetic differences are present among *E. coli* strains, and ribotyping is able to characterize these differences. After isolating and characterizing an *E. coli* strain from an unknown source,

the strain is compared with a known source database (developed from the feces of potential source animals) to identify the source of the *E. coli*. The ribotyping technique makes use of the portion of the *E. coli* genome that codes for the production of ribosomal RNA (ribonucleic acid). This portion of the *E. coli* genome is believed to be stable and intolerant of genetic mutations. Consequently, individual *E. coli* strains should maintain the same genes for ribosomal RNA production over many generations, and the occurrence of each *E. coli* strain can be tracked over extended time periods.

Standard microbiological and molecular biology techniques were used in the ribotyping analysis. The following is a brief description of the steps used in the ribotyping procedure:

1. *Isolation of E. coli bacteria*: For each water sample, a single fecal coliform plate was sent to the UWMSTL, where it was logged into the tracking system. Between 3 and 5 *E. coli* colonies were isolated from each fecal coliform plate. Colonies were cultured on MacConkey Agar following standard techniques and confirmed using biochemical tests (indole production from tryptophane, and lack of growth on a citrate media).
2. *Preservation of pure cultures*: Isolated *E. coli* colonies were stored by freezing at –80°C in a nutrient broth that contained 15-percent glycerol.
3. *Isolation of genetic material*: Isolated *E. coli* colonies also were cultured on a nutrient medium for isolation of their genetic material. Cells were collected from the nutrient medium and lysed (broken open). After various cleanup and extraction steps, the free DNA material was isolated from the remainder of the cellular material.
4. *Digestion of the DNA material using restriction enzymes*: The isolated DNA material was digested (cut into fragments of variable length that depended on the specific base sequence that the enzyme recognized) in separate reactions using a pair of restriction enzymes (*Eco*RI and *Pvu*II). Each enzyme produces a different, but highly specific digestion of the DNA.
5. *Gel electrophoresis to separate the digested DNA material*: The DNA fragments were loaded into an agarose gel and an electrical field was applied to the gel. Because the DNA fragments are negatively charged, the induced current causes them to migrate away from the negative electrode; the agarose gel is sufficiently permeable that the small DNA fragments migrate faster than the larger fragments. After 17 hours, the DNA fragments become separated according to the size of the fragment and the current is discontinued. One specific *E. coli* isolate (labeled isolate #3915) was included with every gel to allow size comparisons among individual gels. Following electrophoresis, the DNA fragments in the gel were stained with ethidium bromide (which fluoresces under an ultraviolet light source), and the gel was placed under an ultraviolet light to ensure that complete digestion occurred and that the electrophoresis was successful. If digestion and electrophoresis are successful, a fluorescent band of DNA will generally extend from the lower edge of the gel (where the DNA fragments were initially loaded) to the upper edge of the gel (near the positive electrode).
6. *Transfer of the DNA fragments from the agarose gel onto a nylon membrane*: Once electrophoresis was completed, the DNA material was manipulated further before being transferred onto a nylon membrane. First, the DNA fragments were cut up further (using hydrochloric acid) to allow an easier transfer from the gel onto the paper. Second, the DNA was denatured (using sodium hydroxide) into single strands to allow recombination with the gene probe. Neither of these treatments affected the positioning of the DNA within the gel. After these two manipulations, the single-stranded DNA fragments were transferred from the agarose gel onto the nylon membrane. This procedure is known as the Southern blot procedure. After the transfer was complete, the nylon membrane was air dried and baked to fix the single-stranded DNA fragments to the membrane.
7. *Hybridization with the radiolabeled cDNA probe:* The radiolabeled cDNA probe was prepared by extension of random hexanucleotide primers. The probe and the nylon membrane then were combined in a hybridization solution, and the probe was given time to hybridize, or bind with any complementary, single-stranded DNA fragments on the nylon membrane. Following hybridization, the nylon membrane was washed to remove any non-specific binding of probe material and then allowed to dry. Only regions containing single-stranded DNA complementary to the cDNA probe retained the radioactive label.

8. *Generation of the autoradiograph:* The dry membranes were exposed to X-ray films; the hybridized regions appear as dark bands on the radiograph (fig. 7). This specific banding pattern is the "ribotype" for a particular *E. coli* isolate.
9. *Comparison of the unknown E. coli banding pattern to the known-source library of patterns*: The unknown ribotype was compared to the known-source library (described below) to see if the unknown pattern matched a source that was already sampled. Analysis and identification of the unknown isolate banding pattern was performed by assigning a numerical value to each ribotype based on the distance between bands. Bands that were more than 3 mm apart were counted as single bands, whereas bands that were within 3 mm of each other were counted as double or triple bands (for example, two bands that were closer than 3 mm to each other were designated a "2" and three bands with 3 mm or less between each band were designated a "3." In this manner, each banding pattern was assigned a specific numeric value. Two isolates with the same numeric value but different banding patterns (because the actual bands may be shifted and not identical) were assigned letters to differentiate the two ribotypes; for example, 2122111A and 2122111B would identify two isolates with similar but slightly offset banding patterns. Isolates with the same numeric values for their ribotypes were deemed to be members of the same ribogroup. The known-source library of ribotype patterns was stored in an electronic database that also included information on the animal source from which each known isolate was obtained. Unknown isolates were queried against this database, based on the numeric value. If an unknown isolate had the same numeric value as any in the known-source library, the unknown ribotype was compared directly to all the known-source isolates that were members of this particular ribogroup. The unknown isolate was identified only if the banding pattern of the unknown isolate visually matched an isolate in the library for both the restriction enzymes. Any unknown isolate that did not match a sample in the known source library was labeled "unknown."

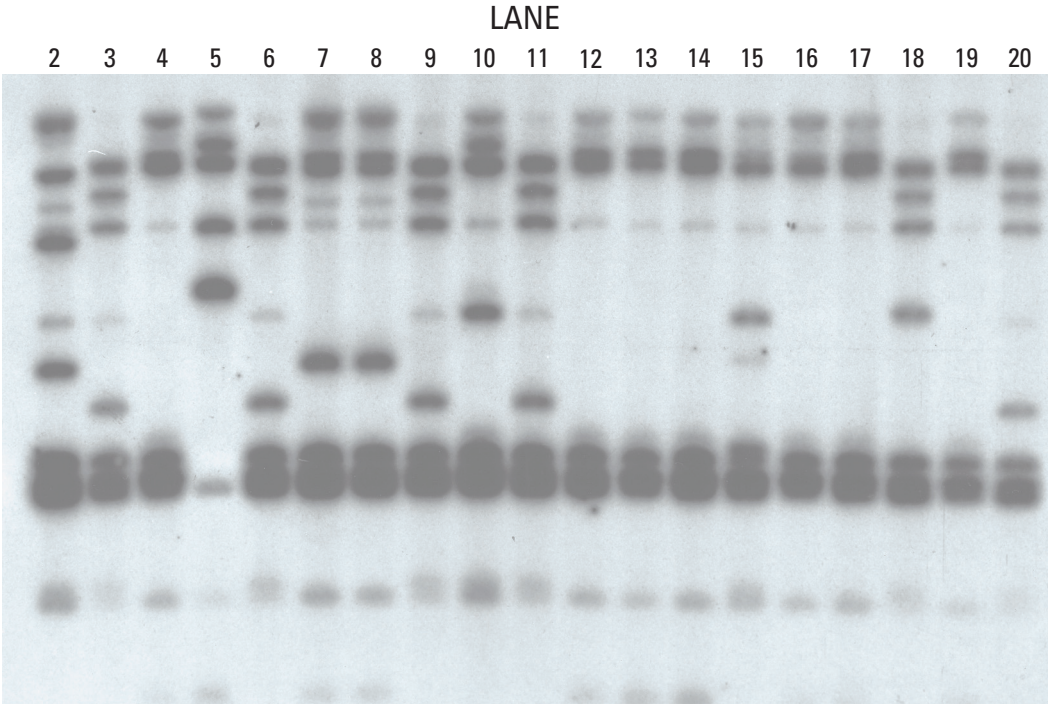


Figure 7. Example of the banding patterns produced by the ribotyping procedure. Each lane represents the pattern generated by a single *E. coli* isolate.

Source-sample collection and source library development

A source-sample library is necessary for a successful source tracking study. The source-sample library provides a set of known ribotype patterns with which the unknown isolates can be compared and identified. The extensive source library at the UWMSTL contained approximately 50,000 isolates. In addition, a site-specific source library was developed for this study by collecting known-source fecal samples from most of the potentially contributing animal sources in each of the three watersheds studied. Fresh fecal samples (of known origin) were collected from farms, animal shelters, veterinary clinics, animal rehabilitation centers, sewage-treatment plants, forested areas, and public parks. These fecal samples were collected aseptically, placed in sterile specimen containers, labeled by source, and sent by overnight delivery to the UWMSTL. At the laboratory, a single *E. coli* isolate was cultured from each fecal sample, ribotyped, and added to the source-library database.

PATTERNS AND SOURCES OF FECAL COLIFORM BACTERIA

Overview of the water samples collected

A total of 605 water samples was collected from the three study streams during this investigation. The distribution of the total number and the type of water samples collected are presented in table 2. Approximately two-thirds of the samples from the ambient water-quality sampling stations in each watershed were collected during low-flow conditions; the remaining one-third of all samples were collected during storm-flow periods. The collection of water samples during both low-flow and storm-flow periods is critical for accurately describing both bacteria concentrations and bacteria sources in a surface-water system. Continuum samples were also collected to investigate the spatial patterns of the fecal coliform concentrations along the length of each study stream.

Fecal coliform analyses

Spatial patterns in the fecal coliform concentrations

The continuum streamwater samples provided evidence that the fecal coliform concentrations observed at the ambient water-quality sampling station of each stream were reflective of the water-quality conditions for the entire stream (table 3). Although concentrations of fecal coliforms were variable among continuum sites on a given day, the streamwater quality (relative to the water-quality standard) generally was consistent among sites; if the water-quality standard was violated at the ambient water-quality sampling station, then the standard typically was violated at the other continuum sampling sites on that day. Similarly, if the water-quality standard was met at the ambient water-quality sampling station, then the other continuum sampling sites also were generally in compliance with the standard. Several of the continuum samples had extremely elevated fecal coliform concentrations (Accotink Creek on June 6 and August 8, 2000; Blacks Run on March 22, July 22, September 5, and October 4, 1999). All six of these sampling events were performed under storm-affected flow conditions (there had been appreciable rainfall within the past 48 hours and the flow was still receding). These storm-affected samples provided evidence that the fecal coliform concentrations increase during storm-flow periods. Another Accotink Creek sampling event on August 11, 1999, is of interest because it occurred during extended drought conditions and the stream had been reduced to a series of disconnected pools; the samples from these disconnected

Table 2. Number and type of streamwater samples collected from March 1999 through October 2000 in three watersheds in Virginia

Watershed	Number of samples collected			
	Low flow	Storm flow	Continuum	Total
Accotink Creek	104	53	36	193
Christians Creek	104	66	18	188
Blacks Run	99	56	69	224

Table 3. Fecal coliform concentrations of the continuum samples in three watersheds in Virginia, 1999-2000

[Location of stations on figures 2-4; col/100 mL, colonies per 100 milliliters; –, no sample collected]

Accotink Creek watershed

Continuum sample number	Station number	Fecal coliform concentration (col/100mL)			
		Sampling date			
		3/18/99	8/11/99	6/6/00[a]	8/8/00[a]
1	01653900	320	190	38,000	13,000
2	01653985	200	25	18,000	15,000
3	01653995	50	54	23,000	17,000
4	01654000[b]	73	37	13,000	13,000
5	01654520	64	42	–	9,300

Christians Creek watershed

Continuum sample number	Station number	Fecal coliform concentration (col/100mL)		
		Sampling date		
		3/25/99	7/27/99	8/1/00
1	01624615	5	71	7
2	01624620	87	1,500	300
3	01624660	230	2,000	3,800
4	01624700	23	6,400	1,900
5	01624800[b]	15	790	1,800
6	01624900	9	140	830

Blacks Run watershed

Continuum sample number	Station number	Fecal coliform concentration (col/100mL)												
		Sampling date												
		3/22/99[a]	7/22/99[a]	8/19/99	9/5/99[a]	10/4/99[a]	11/17/99	12/17/99	1/22/00	2/25/00	3/28/00	4/27/00	5/13/00	8/15/00
1	01621395	290,000	–	–	86,000	2,300	–	–	–	16	200	450	–	–
2	01621397	–	23,000	760	62,000	4,600	300	1,100	20,000	120	770	340	2,000	6,000
3	01621410	100	54,000	610	41,000	4,300	69	160	–	26	42	170	770	520
4	01621425	400	81,000	820	22,000	2,300	33	110	13	80	130	610	1,700	2,000
5	01621440	2,000	39,000	94	21,000	1,100	10	18	10	13	160	73	200	380
6	01621470[b]	2,200	7,200	22,000	65,000	6,300	410	6,000	20	390	180	61	580	700

[a] Storm-affected sample (rain had occurred in the last 48 hours and flow was receding)
[b] Sampling station is co-located with a stream gage

pools had some of the lowest fecal coliform concentrations that were observed during the study.

The synoptic sampling of Accotink Creek on June 6, 2000, provided further evidence that the entire watershed was contributing fecal coliforms (table 4). This synoptic sampling was performed immediately following a storm event and included samples from various storm drains, major stream tributaries, and main channel sites (all samples were collected while moving in the downstream direction). All sampled storm drains and stream tributaries had elevated concentrations of fecal coliform bacteria, and all samples collected during this synoptic survey exceeded the Commonwealth's instantaneous water-quality standard for fecal coliform bacteria (1,000 col/100 mL). However, because these samples were collected at different times and on different portions of the storm recession (while the water quality of the entire stream system was changing rapidly), direct comparisons of fecal coliform concentrations among sites would not be meaningful. For example, these data do not support the conclusion that the fecal coliform contributions from Daniels Run were greater than the contributions from Coon Branch because these two samples were collected approximately 9 hours apart and on different portions of the hydrograph. Rather, one could conclude that elevated concentrations of fecal coliform bacteria occurred throughout the watershed and that all areas sampled contributed to these elevated concentrations.

Temporal patterns in the fecal coliform concentrations

Seasonal patterns were evaluated in the fecal coliform concentrations at each ambient water-quality sampling station. Water samples were collected over a 20-month period, with 15 sampling events at each site during low-flow periods. Between four and eight water samples were collected during each low-flow sampling event, and these low-flow fecal coliform concentrations are summarized (fig. 8). For some of the low-flow sampling events, stream discharge records and meteorological data indicated that the streamflow was receding and that rain had fallen within the last 48 hours. These recession-flow samples (identified in figure 8) represent a subset of the low-flow samples. Most of the low-flow samples, however, were not collected under periods of recession flow, and this other subset of low-flow samples is referred to as base-flow samples.

In Accotink Creek, base-flow fecal coliform concentrations were generally below the instantaneous water-quality standard of 1,000 col/100 mL (fig. 8); however, the recession-flow samples occasionally exceeded the water-quality standard. The recession-flow fecal coliform concentrations were significantly elevated relative to the base-flow fecal

Table 4. Fecal coliform concentrations of water samples collected in the Accotink Creek watershed, Virginia, during synoptic sampling, June 6, 2000

[col/100 mL, colonies per 100 milliliters; samples collected and stations listed in downstream order; storm-drain station numbers increase in the downstream direction]

Main channel station	Fecal coliform (col/100mL)	Stream tributary sampling station	Fecal coliform[a] (col/100mL)	Storm-drain sampling station number	Fecal coliform (col/100mL)
Above Daniels Run	33,000	Daniels Run	100,000	1	21,000
01653900	38,000	Hunters Run	22,000	2	31,000
01653985	18,000	Bear Branch	22,000	3	10,000
01653995	23,000	Long Branch	30,000	4	10,000
01654000	13,000	Crook Branch	18,000	5	2,000
		Coon Branch	13,000		
		Turkey Run	1,100		

[a] Upstream sites were sampled immediately following a storm; more downstream sites were sampled later on the recession curve of the storm hydrograph

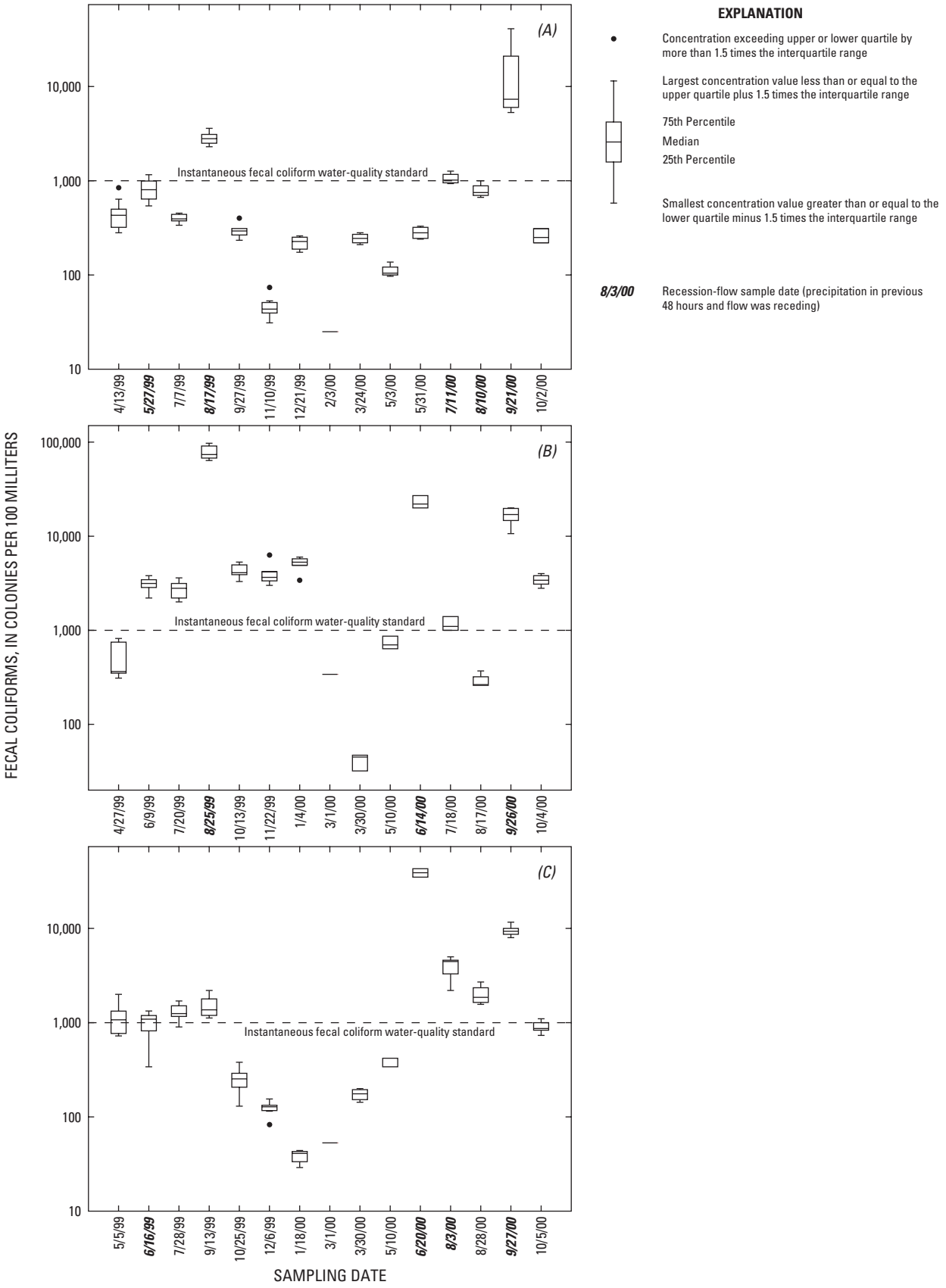


Figure 8. Fecal coliform concentrations during low-flow sampling of Accotink Creek *(A)*, Blacks Run *(B)*, and Christians Creek *(C)* watersheds, Virginia, 1999-2000.

coliform concentrations ($p < 0.05$ using a Wilcoxon rank-sum test). The timing of the collection of some of the recession-flow samples makes a seasonal evaluation difficult; however, there appeared to be a slight seasonality with slightly lower fecal coliform concentrations during the winter and slightly higher concentrations during the warmer months. Pronounced seasonality in the fecal coliform concentrations was not expected in Accotink Creek because the land-use practices and potential fecal coliform sources in the watershed can be considered constant throughout the year.

Low-flow fecal coliform concentrations in Blacks Run were elevated relative to the Commonwealth's water-quality standard (fig. 8). Similar to Accotink Creek, the recession-flow water samples had fecal coliform concentrations that were significantly higher than those observed during base-flow conditions ($p < 0.05$ using a Wilcoxon rank-sum test). More than half of the base-flow water samples had fecal coliform concentrations that exceeded the 1,000 col/100 mL fecal coliform standard. A seasonal pattern was present that was similar to, but more pronounced than the one observed in Accotink Creek. The highest base-flow fecal coliform concentrations occurred during the summer and into the fall. During the winter, fecal coliform concentrations decreased to a minimum and then increased during the spring. This seasonal pattern is consistent with the animal management practices in the watershed. Livestock numbers typically are greatest during the summer and fall, and during these warm months the animals (particularly cattle) spend more time closer to and sometimes wading into the stream. This increased association of animals with the stream likely results in both direct deposition of feces into the stream and deposition of feces closer to the stream than during other times of the year. In addition to animal management practices, it also is possible that seasonally different fecal coliform survival rates (greater bacteria survival during the warm summer, relative to the cold winter, for example) may have affected these observed fecal coliform concentrations and contributed to the observed seasonal patterns.

Low-flow fecal coliform concentrations in Christians Creek also demonstrated a seasonal pattern (fig. 8). Approximately half of the base-flow water samples had fecal coliform concentrations that exceeded the Commonwealth's water-quality standard. Recession-flow samples had fecal coliform concentrations that were significantly elevated relative to the base-flow samples ($p < 0.05$ using a Wilcoxon rank-sum test). The seasonal variation in the Christians Creek fecal coliform concentrations was more pronounced and followed the same pattern as at Blacks Run and Accotink Creek. The highest base-flow fecal coliform concentrations occurred during the warm summer months, concentrations decreased through the fall, reached a minimum during the winter, and then concentrations increased through the spring. This pattern again is consistent with the animal practices in the watershed (increased animal density and activity around the streams during the hot summer months) and possible seasonal differences in survival rates of fecal coliform bacteria. Similar seasonal patterns have been described in other studies of fecal coliform concentrations and loads (Christensen and others, 2001; Baxter-Potter and Gilliland, 1988).

Fecal coliform concentrations were also monitored during five storm events on each study stream. At least 10 water samples were collected during each storm event, and as possible, the entire storm hydrograph (rising limb, plateau, and falling limb) was sampled. The fecal coliform concentrations observed during these storm events (fig. 9) were significantly elevated ($p < 0.05$ using Wilcoxon rank-sum test) relative to the observed base-flow fecal coliform concentrations (the recession-flow samples were not included for this analysis), and the water-quality standard was usually exceeded during these storm events. A large range of concentrations was observed during the individual storms because of the comprehensive sampling over the entire hydrograph. Peak fecal coliform concentrations observed during storm events on each study stream were 340,000 col/100 mL in Accotink Creek; 260,000 col/100 mL in Blacks Run; and 730,000 col/100 mL in Christians Creek. These elevated fecal coliform concentrations during storm events were anticipated on the basis of the results of previous studies (Christensen and others, 2001; Bolstad and Swank, 1997). In other studies, these elevated storm-flow concentrations have been interpreted as a combination of a flushing response (whereby fecal coliform bacteria that were deposited near the stream are washed off the land surface and into the stream) and a re-suspension of streambed sediments containing fecal coliform bacteria (Hunter and others, 1992; McDonald and Kay, 1981). Similar mechanisms likely were responsible for the storm-flow fecal coliform concentrations observed in these study streams, although other sources (including cross-pipes, failing septic sys

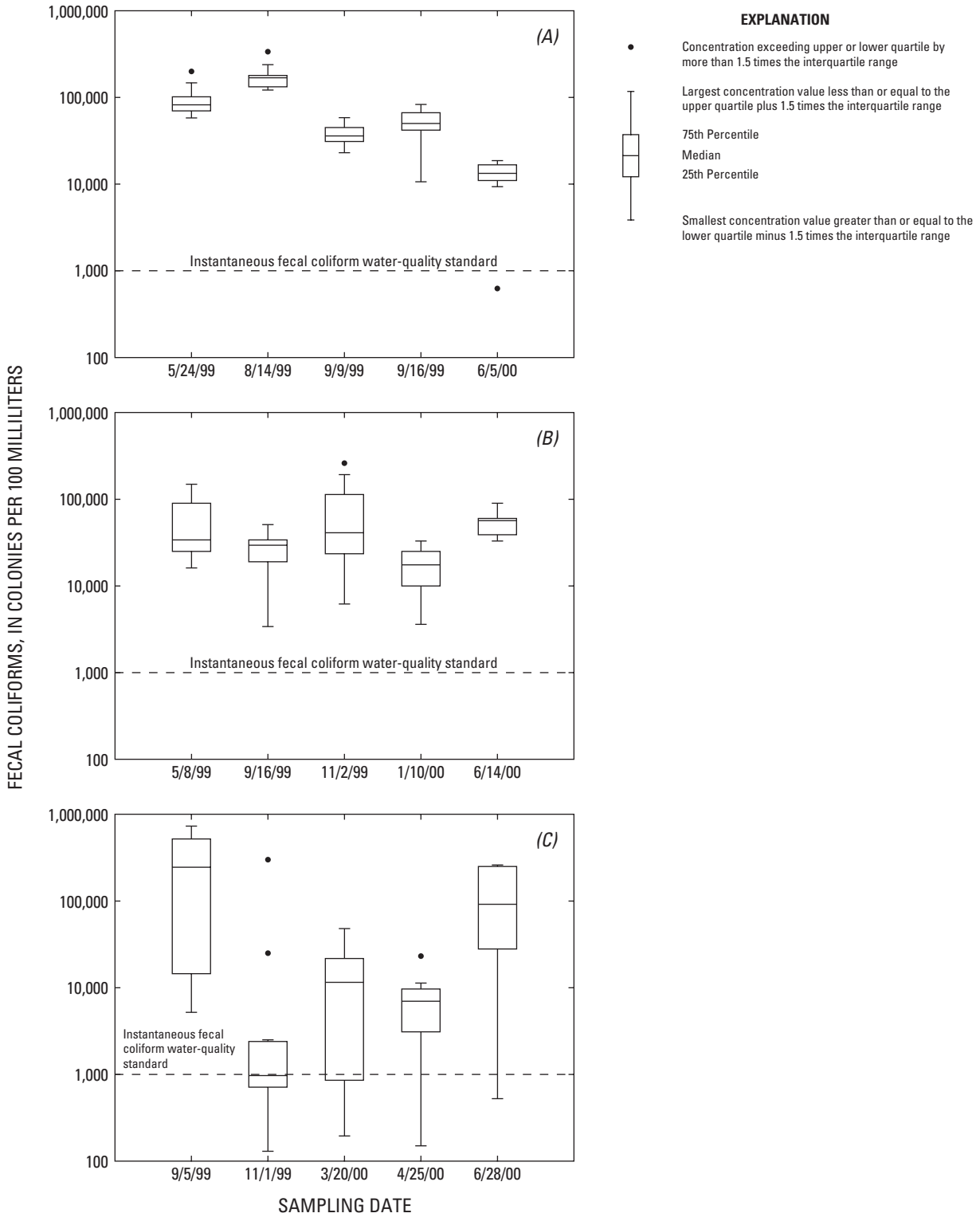


Figure 9. Fecal colifrom concentrations during storm-flow sampling of Accotink Creek *(A)*, Blacks Run *(B)*, and Christians Creek *(C)* watersheds, Virginia, 1999-2000.

tems, and leaking or overflowing sewer lines) also may have contributed during storms.

Overall, the storm-flow responses of the fecal coliform bacteria and the supporting water-quality parameters were consistent among the three study streams and with the responses observed in previous studies. The data from the intensive sampling of the storm events are presented as a series of chemographs (figures demonstrating the time-course evolution of the stream-water composition during a storm event); a single, representative storm event from each study stream is presented (fig. 10). In general, the data demonstrated an increased fecal coliform concentration on the rising limb of the storm hydrograph, peak fecal coliform concentrations around the hydrograph peak, and decreased fecal coliform concentrations on the falling limb of the hydrograph. In a minor variation of this pattern, fecal coliform concentrations in Accotink Creek usually were slightly decreased during the peak in the hydrograph. Data on the supporting water-quality parameters (turbidity, specific conductance, pH, and dissolved oxygen concentration) were typically collected during storm events at a frequency slightly greater than that used for fecal coliforms. Turbidity levels always increased during storm events, generally reaching a maximum concentration about the time of the peak discharge. Increased turbidity levels are reflective of the suspended sediments that enter the water column because of surface runoff, re-suspension of the streambed sediments, or stream bank erosion (Kronvang and others, 1997; Jeje and others, 1991). The pH generally decreased slightly during storm events in all streams. Declines in pH are commonly observed during storms, as relatively more acidic rainfall, runoff, and interflow contributes to the streamflow. These acidic contributions consume buffering capacity and reduce the overall pH (Whitfield and others, 1993; Gburek and Pionke, 1993). As observed in earlier studies (Laudon and Slaymaker, 1997; Caissie and others, 1996), specific conductance generally decreased during storm events–an indication that the new water that was added to the stream during the storm had a relatively lower specific conductance than what was already resident in the stream. Although the initial runoff (also referred to as the "first flush") from a watershed may contain relatively high concentrations of dissolved material (and an elevated specific conductance), subsequent runoff and incident rainfall generally are much more dilute and result in an overall reduction in the streamwater specific conductance during storm events (De Boer and Campbell, 1990). Dissolved oxygen concentrations typically decreased during storm events, a response that has been observed in other studies (Bolstad and Swank, 1997). This decrease in dissolved oxygen concentrations generally is attributed to rapid inputs of readily degraded organic material in the surface runoff, and potentially an increased oxygen demand by the re-suspended streambed sediments.

Correlations between fecal coliform concentrations and stream-water parameters

Correlations were examined between the observed fecal coliform concentrations and the supporting streamwater parameters to develop multiple linear regression models for predicting fecal coliform concentrations at each of the ambient water-quality sampling stations. Parameters considered for these empirical models included discharge, specific conductance, turbidity, pH, water temperature, and dissolved oxygen concentration. The multiple linear regression models were developed using the approach described by Helsel and Hirsch (1992). On the basis of their sample distributions, the fecal coliform concentration, discharge, and turbidity variables were transformed logarithmically (log base 10) to reduce skew and produce more normally distributed residual and partial plots. Best subsets regression was used to identify the most promising multiple linear regression models. These candidate models were subsequently screened for significance of all variables, and the best models were selected based on a minimized Mallows Cp and maximized adjusted R^2. Plots of the model residuals also were evaluated to ensure that the residuals were normally distributed and had a constant variance.

Although best subsets regression is the optimal method for developing multiple linear regression models, stepwise multiple linear regression also may be used (Helsel and Hirsch, 1992). As confirmation, stepwise multiple linear regressions also were performed on the fecal coliform concentration data, and the same supporting streamwater parameters were used as independent variables. The stepwise multiple linear regressions identified the same models as those selected using the best subsets regression.

Multiple linear regression models were developed for the ambient water-quality sampling station of each individual study stream, as well as a combined overall model of all three monitoring stations. These models predicted fecal coliform concentrations as a function of

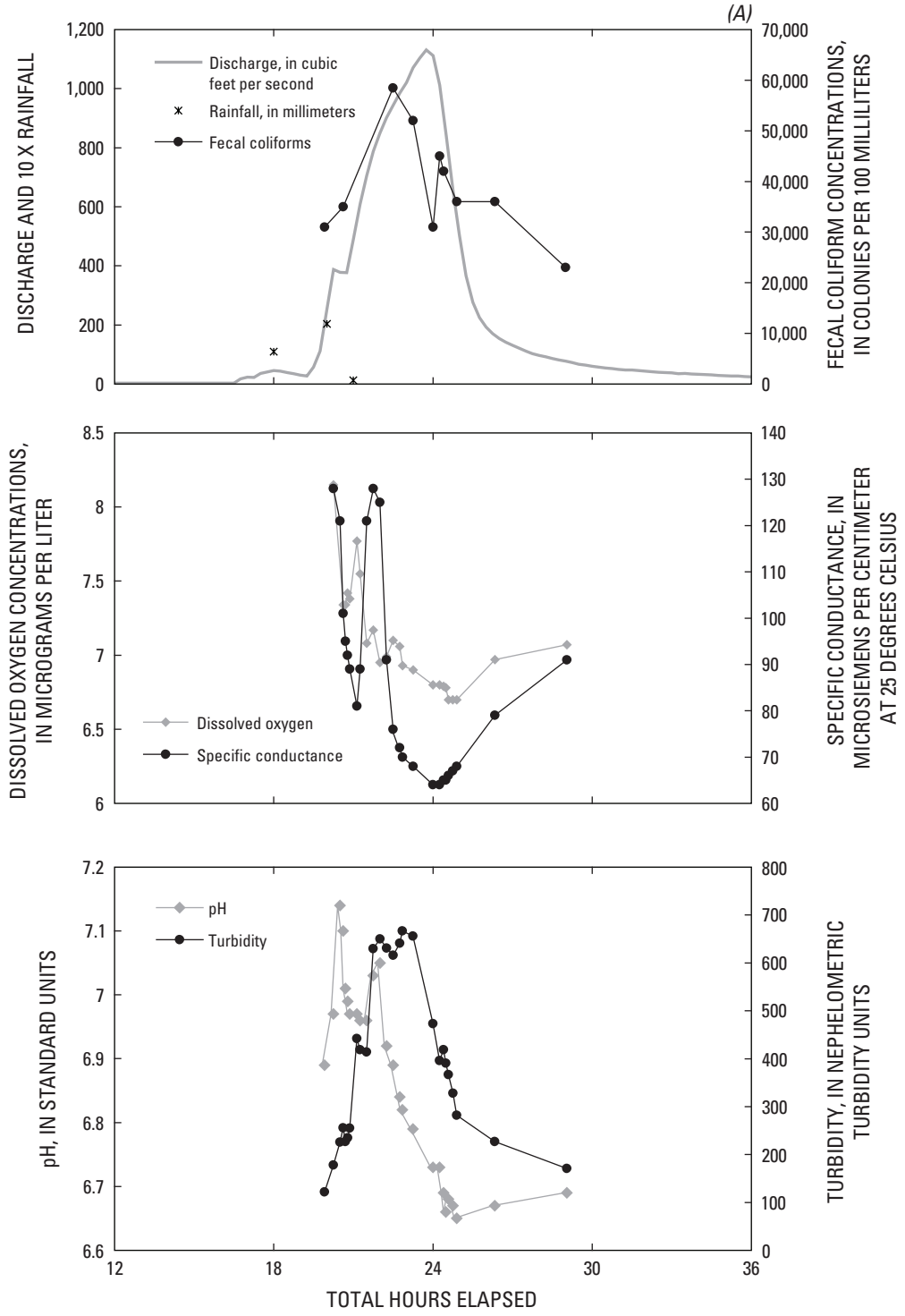


Figure 10. Changes in discharge, fecal coliform concentrations, and supporting water-quality parameters during storm events September 9-10, 1999, Accotink Creek *(A)*, September 15-16, 1999, Blacks Run *(B)*, and June 27-29, 2000, Christians Creek *(C)* watersheds, Virginia.

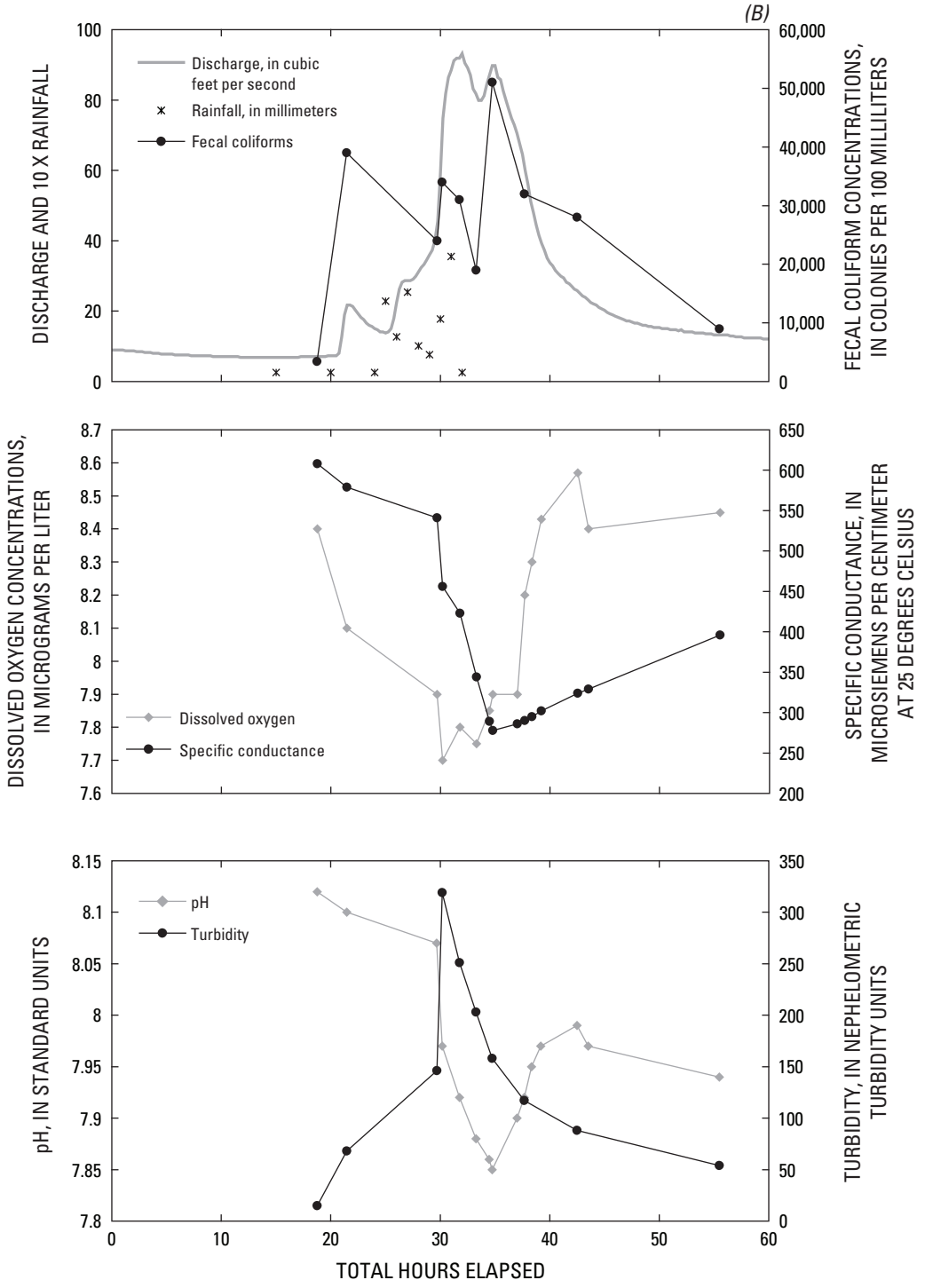


Figure 10. Changes in discharge, fecal coliform concentrations, and supporting water-quality parameters during storm events September 9-10, 1999, Accotink Creek *(A)*, September 15-16, 1999, Blacks Run *(B)*, and June 27-29, 2000, Christians Creek *(C)* watersheds, Virginia—Continued.

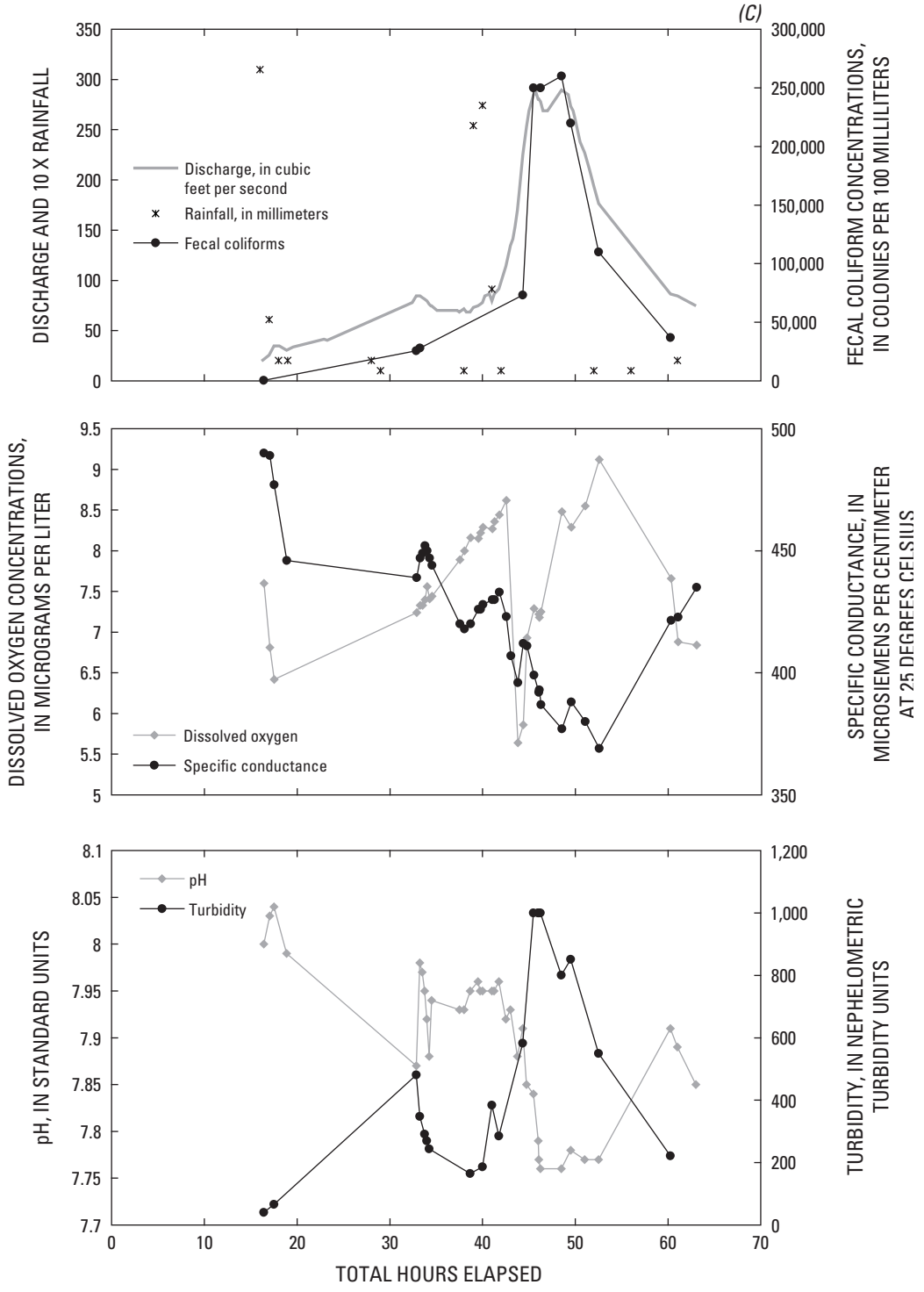


Figure 10. Changes in discharge, fecal coliform concentrations, and supporting water-quality parameters during storm events September 9-10, 1999, Accotink Creek *(A)*, September 15-16, 1999, Blacks Run *(B)*, and June 27-29, 2000, Christians Creek *(C)* watersheds, Virginia—Continued.

some of the supporting streamwater parameters. In all the models, turbidity was identified as the parameter that explained the greatest variance and was most significant in the model. In addition to turbidity, parameters such as water temperature, pH, and dissolved oxygen also were useful in explaining some of the variability in the fecal coliform concentrations. The regression equations and correlation coefficients for these models are:

Accotink Creek:

$$\log[FC] = 1.130\ (\log[Turb]) + 0.044\ (WT) + 1.068 \quad (2)$$
$$R^2 = 0.88$$

Blacks Run:

$$\log[FC] = 0.768\ (\log[Turb]) - 0.086\ (DO) - 0.025\ (WT) + 3.825 \quad (3)$$
$$R^2 = 0.68$$

Christians Creek:

$$\log[FC] = 1.314\ (\log[Turb]) + 0.668\ (pH) - 3.908 \quad (4)$$
$$R^2 = 0.64$$

All three streams combined:

$$\log[FC] = 1.222\ (\log[Turb]) + 1.688 \quad (5)$$
$$R^2 = 0.71,$$

where *FC* represents the fecal coliform concentration,
Turb is the turbidity,
WT is the water temperature, and
DO is the dissolved oxygen concentration.

A comparison between the model predictions and the observed data for all sites is presented (fig. 11). In general, these models explained between 64 percent and 88 percent of the observed variability in fecal coliform concentrations, depending on the study stream. The ability to predict fecal coliform concentrations from these easily measured water-quality parameters is useful, particularly when estimates of fecal coliform concentrations are needed quickly (18-22 hours of incubation are required before fecal coliform concentrations can be determined). Additionally, the parameters used in these predictive models are easier and less expensive to analyze for than fecal coliform; this regression approach may be especially useful in cases where monitoring cost is a special concern.

Although it appears that these empirical models can be used to predict fecal coliform concentrations, independent verification is needed before these models should be applied. After verification, these models would be relevant to the conditions and streams in which they were developed. Given the variability in the observed fecal coliform concentrations (relative to the predicted concentrations), these empirical models may be best suited for situations that require only an approximate fecal coliform concentration, or that call for evaluating the likelihood of a water sample exceeding a specific water-quality standard or criterion.

Correlations between turbidity and fecal coliform concentrations have been observed previously (Christensen and others, 2001; Francy and Darner, 1998). Conceptually, the strong relation between fecal coliform concentrations and turbidity may result because both constituents are "flushed" into the stream during storm events (fecal coliforms are transported in runoff from parking lots, pastures, fields, and other surfaces; sediments are generally eroded off the land surface and carried into the stream). A distinction must be made, however, between this correlation and any inference of causality. Although turbidity is an effective predictor of fecal coliform concentrations, it cannot be inferred that the sediments (measured as turbidity) are the primary source of the fecal coliforms; rather, it can be concluded that conditions that favor elevated fecal coliform concentrations also favor elevated turbidity levels.

Analysis of replicate fecal coliform enumerations

Knowledge of the variability inherent in the fecal coliform enumeration process is important for comparing fecal coliform concentrations of different water samples. Replicate fecal coliform enumerations were performed on 6 percent of the samples collected (7 duplicate fecal coliform enumerations and 24 triplicate fecal coliform enumerations). The replicate fecal coliform enumerations were generally performed as multiple analyses of a single water sample (in a few cases, paired water samples were collected simultaneously and analyzed as duplicate samples). These replicate enumerations were analyzed using a percent difference calculation, given as:

$$\text{Sample \% difference} = \frac{\text{(Sample concentration) - (Mean of replicate)}}{\text{(Mean of replicate)}} \times 100\% \quad (6)$$

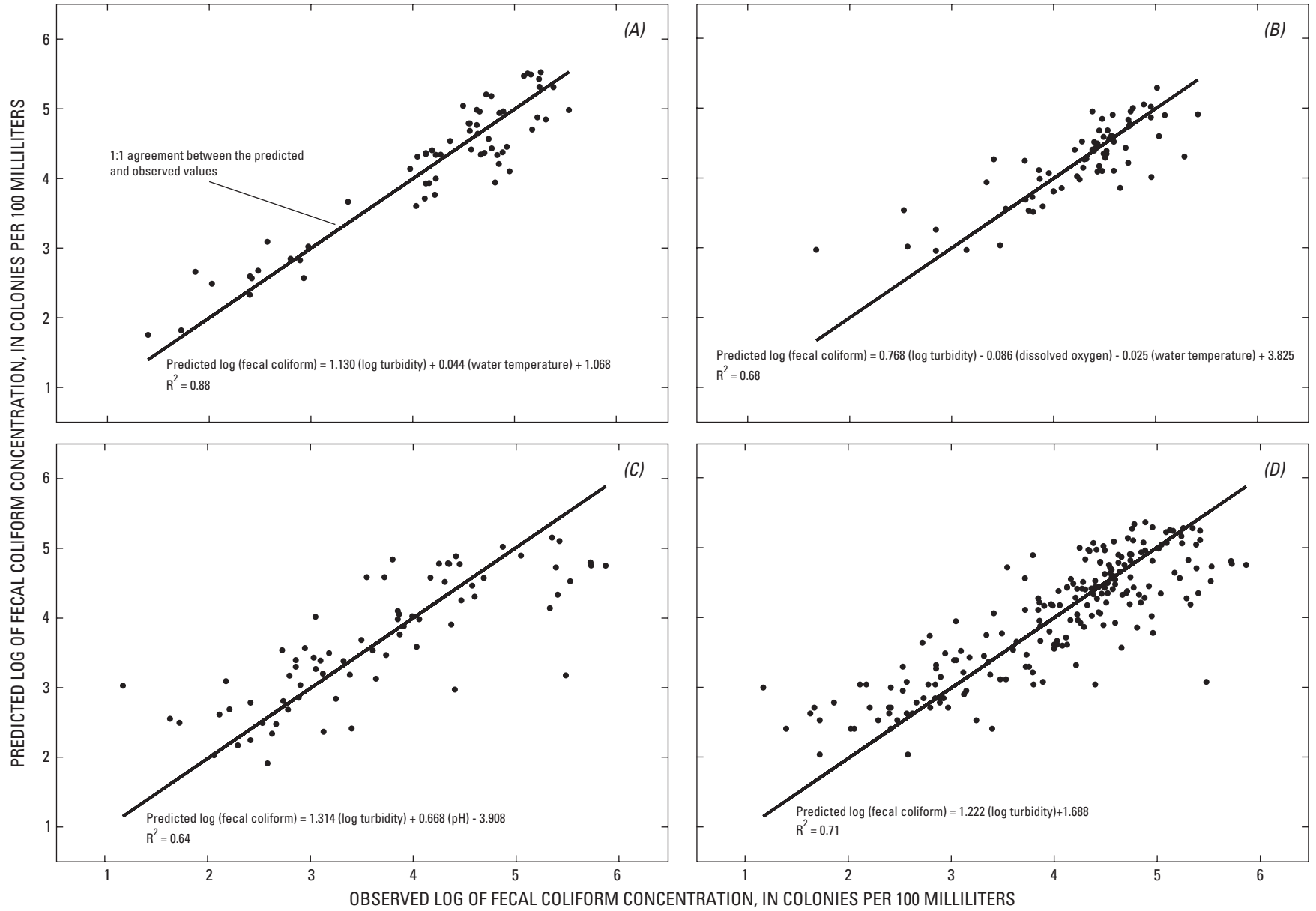


Figure 11. Relations of observed and predicted fecal coliform concentrations as a function of water-quality parameters, from streamwater samples collected March 1999 through October 2000 for Accotink Creek *(A)*, Blacks Run *(B)*, and Christians Creek *(C)*, and all three streams combined *(D)*.

The percent difference values for each individual enumeration (n=86) were summarized to evaluate the variability in the fecal coliform enumeration technique. The percent difference term was normally distributed, with a mean percent difference value of 0.00 percent and a standard deviation of 12.2 percent. This normal distribution of the percent difference term can be used to calculate the probability of observing the specific percent difference value that is present between two fecal coliform concentrations (Johnson and Bhattacharyya, 1985).

Bacteria sources in the three streams

Samples submitted for source tracking

In performing this BST study, a large number of samples were collected over 20 months. Only the water samples collected from the ambient water-quality sampling station of each study stream were submitted for the source-tracking analysis; none of the continuum samples were submitted for ribotyping. This source tracking design was selected because it allowed the development of an understanding of the spatial and temporal patterns in fecal coliform concentrations throughout each study stream and it provided knowledge of the bacteria sources affecting water quality at the ambient water-quality sampling station for each stream.

Results of the bacterial source tracking

A total of 1,285 unknown *E. coli* isolates was ribotyped from the three watersheds during this investigation (table 5). Overall, 65 percent of those isolates were matched to a known-source isolate in the source library. Identification of 65 percent of the unknown isolates is considered successful and is consistent with previous ribotyping studies (Farag and others, 2001; Samadpour and Chechowitz, 1995). The distribution of the number and the type of isolates that were ribotyped is presented in table 5. About 61 percent of the source-tracked isolates were selected from low-flow samples, and about 39 percent of all isolates were from storm-flow samples. Similarly, about 59 percent of the identified *E. coli* were from low-flow samples, and 41 percent were from storm-flow samples. The collection and identification of *E. coli* isolates from both low-flow and storm-flow periods were important for identifying the dominant sources of bacteria in the watersheds.

Procedures for quantifying and interpreting BST data are still being developed; few standard protocols exist to handle the complexities of these data and the methods used to generate them (Simpson and others, 2002). As this technology is applied under different field settings and as the science of BST matures, more uniform approaches may be developed. One unresolved issue involves the number of known-source isolates that are needed to accurately quantify the distribution of bacteria sources. A sample size of about 1,000 *E. coli* isolates represents only a small fraction of the total number of fecal coliform bacteria that are transported by the three streams. The frequency with which samples should be collected during any BST study is also unresolved. More frequent sampling is expensive but may be necessary for evaluating seasonal patterns that may be present in the bacteria sources that are contributing to a stream. The value of storm-flow sampling is unresolved. Point sources are likely to be the primary contributors of fecal coliform bacteria to a stream during base-flow conditions, and nonpoint-source contributions likely dominate during storm-flow periods, but these patterns have yet to be investigated. There remain questions regarding the number of bacteria isolates to source-track from each individual water sample. Evaluating many isolates from a single water sample

Table 5. Number of *E. coli* isolates ribotyped, and percentage of those isolates from low-flow samples collected from three watersheds in Virginia, March 1999 through October 2000. Number (and percentage) of isolates that were identified, and the number (and percentage) of identified isolates from low-flow and high-flow samples

Watershed	Total isolates (percent low flow)	Identified isolates (percent)	Identified low-flow isolates (percent)	Identified storm-flow isolates (percent)
Accotink Creek	404 (64.6)	279 (69.1)	174 (62.4)	105 (37.6)
Blacks Run	451 (60.1)	285 (63.2)	173 (60.7)	112 (39.3)
Christians Creek	430 (59.5)	274 (63.7)	146 (53.3)	128 (46.7)
Total	**1,285(61.3)**	**838 (65.2)**	**493 (58.8)**	**345 (41.2)**

may provide a more detailed understanding of that particular sample, but restrictions in the scope of a study may result in fewer water samples collected and source-tracked. Although these questions remain unresolved, our intensive sampling over a 20-month period, incorporation of low-flow and storm-flow sampling, and identification of more than 270 isolates in each watershed should allow these data to be treated in a semi-quantitative manner and for inferences to be drawn regarding the bacteria sources that are impairing these three streams.

Before presenting the bacteria sources that were identified in the three watersheds, the unidentified *E. coli* isolates must be considered. Approximately 35 percent of the isolates were unidentified. These unidentified isolates represent *E. coli* that were not yet present in the known-source library. Based on knowledge of the potential fecal coliform contributors in these watersheds and the sources represented in the known-source library, the presence of a significant yet unrepresented fecal coliform contributor in these watersheds (lions, for example) is unlikely. It is likely that the unidentified isolates are from sources that are common in these watersheds (humans, dogs, and raccoons, for example) but that the particular ribotype was not yet included in the known-source library. Collection of additional known-source isolates likely would reduce the number of unidentified isolates. On the basis of the diversity of the 50,000-isolate known-source library that was used in this study, it is reasonable to assume that the sources of the unidentified isolates had a distribution that was identical to the source distribution observed in each watershed. The implication of this assumption is that the identified isolates could be used to describe the overall distribution of *E. coli* sources (and, therefore, fecal coliform sources) that impaired each watershed.

The identified bacteria sources in the three watersheds demonstrate that a diverse collection of fecal sources contributed to the impairment of each stream (fig. 12). Two source categories are discussed in more detail. The first source category that was treated differently is poultry, which represents a combination of chicken and turkey sources. The ribotyping technique sometimes was able to distinguish chickens from turkeys (and the two are labeled separately in figure 12b and c); in other cases, an isolate was identified as either a chicken or a turkey isolate (in this case, the isolate is labeled as poultry). This lack of specificity may have occurred for three reasons: (1) identical *E. coli* were found in both birds; (2) different *E. coli* were found in chickens and turkeys, but the ribotyping analysis produced banding patterns that were identical; or (3) the ribotype from the source library that matched the unknown isolate was identified during the source collection process as poultry litter and did not indicate whether the sample was from chickens or turkeys. For data-interpretation and watershed-modeling purposes, the chicken, turkey, and poultry categories were combined into a total poultry category. The second category that was treated differently is avian, a source which was identified in all three watersheds. The avian category represents *E. coli* isolates that occurred in multiple bird species. Whereas the poultry category is specific to chickens and turkeys, the avian category encompasses all birds. For data-interpretation and watershed-modeling purposes, this avian category was distributed among all the observed bird sources, which included geese, ducks, sea gulls, crows, poultry, and swans. Quantitatively, it was assumed that the avian component was distributed proportionally, according to the occurrence of each individual bird source shown in figure 12. For example, if the goose contribution for an individual stream was 25 percent of all the bird sources that were identified, then 25 percent of the avian contribution was attributed to geese. In this way, the avian contribution was distributed among all the identified bird sources.

After combining the poultry sources and distributing the avian component, the *E. coli* sources of each stream were re-plotted (fig. 13). The plot for each stream was arranged from the greatest contributor to the least contributor. No single source accounted for more than 30 percent of the identified *E. coli*; a range of sources contributed fecal coliforms to all three stream systems. In Accotink Creek, the greatest contributors were geese and human sources, followed by dogs, ducks, cats, sea gulls, and raccoons (fig. 13a). Cattle, poultry, human sources, and dogs were the top four sources in both Blacks Run and Christians Creek (fig. 13b and c). Cats also were an important source in Blacks Run, whereas horses and deer were additional sources to Christians Creek. All other observed sources were minor, providing less than 5 percent of the total source observed in these streams. Although they were independently considered minor, these minor sources may be cumulatively important to the overall water quality in these streams.

The bacteria-source data can also be grouped by their general animal categories (humans, pets, waterfowl, wildlife, and agricultural; fig. 14). Accotink

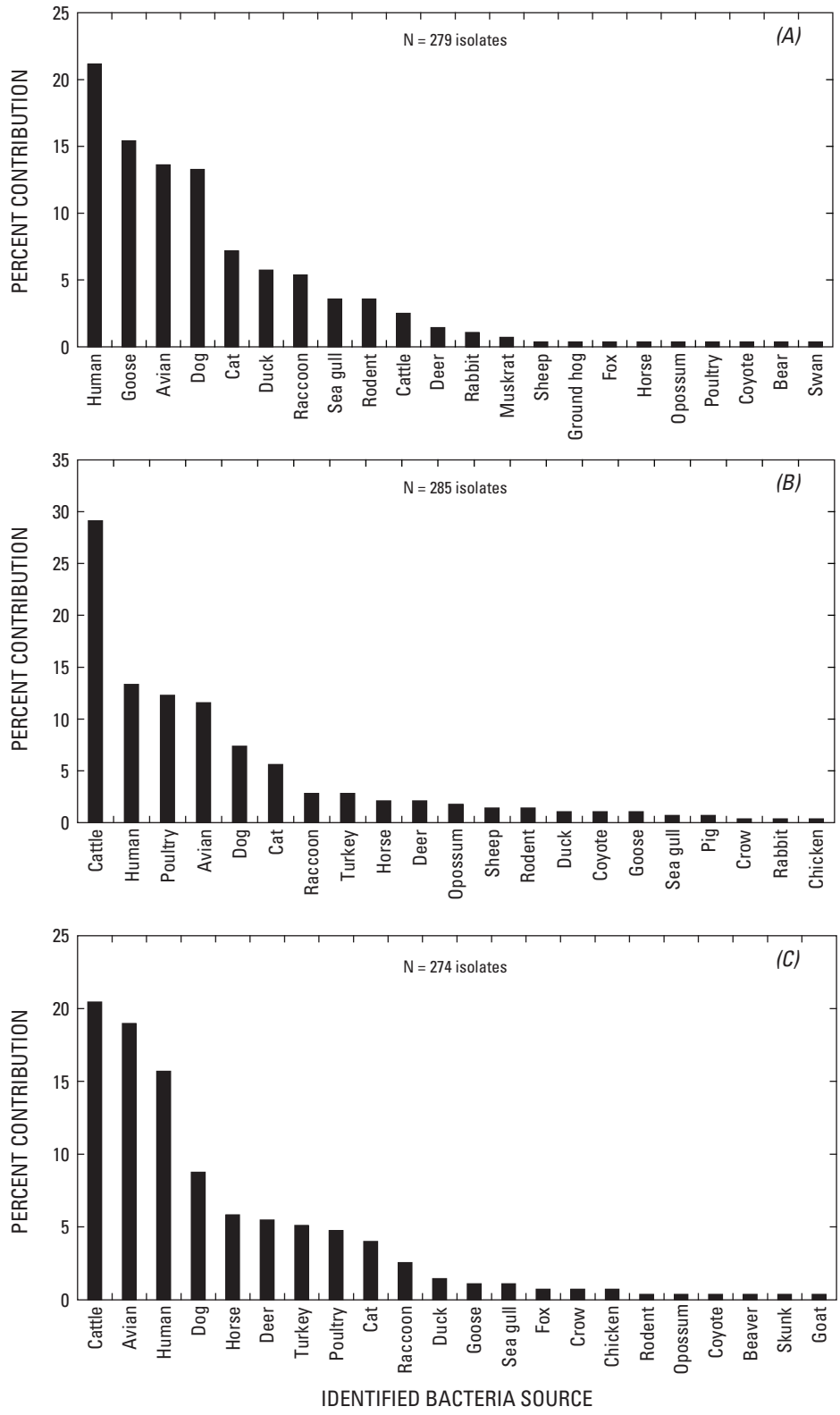


Figure 12. Distribution of the bacteria isolates that were identified in streamwater samples collected from March 1999 through October 2000 in Accotink Creek *(A)*, Blacks Run *(B)*, and Christians Creek *(C)*, Virginia.

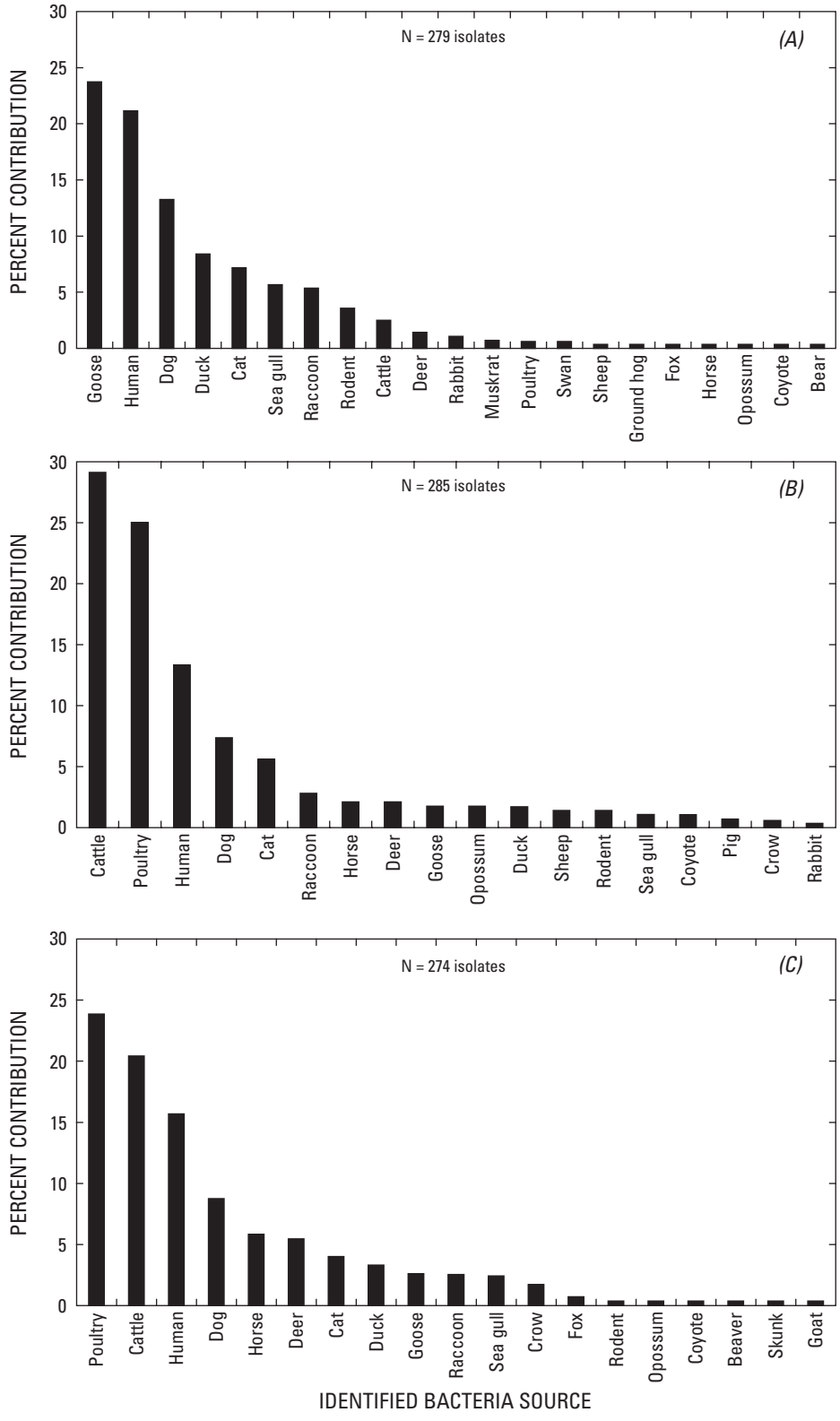


Figure 13. Distribution of the bacteria isolates that were identified in streamwater samples collected from March 1999 through October 2000 in Accotink Creek *(A)*, Blacks Run *(B)*, and Christians Creek *(C)*, Virginia, after combining the poultry sources and distributing the avian source.

Creek was dominated by waterfowl sources (geese, ducks, sea gulls, and swans), followed by almost equal contributions from human sources and pets (dogs and cats). Wildlife also made an important contribution to Accotink Creek, whereas agricultural sources were relatively minor. Both Blacks Run and Christians Creek were dominated by agricultural sources, followed by contributions from human sources, pets, and wildlife. Both Blacks Run and Christians Creek also had relatively minor contributions from waterfowl. In addition to the differences in the general categories that contributed to the impairment of each stream, the data indicate that a range of sources contributed fecal coliforms to each stream; no one group of sources accounted for more than 60 percent of the identified *E. coli* in these stream systems.

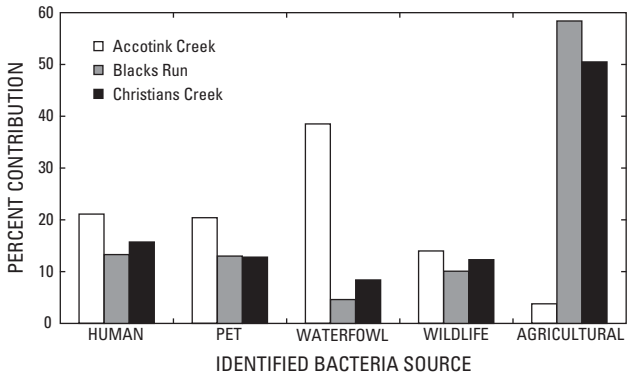


Figure 14. Bacteria sources identified in streamwater samples collected March 1999 through October 2000 from three watersheds in Virginia, grouped by animal category.

Comparison of the BST results (figs. 13 and 14) with the land use of each watershed (fig. 15) demonstrates relations between the dominant activities within each watershed and the observed bacteria sources. The land use of each watershed can be used to infer the source category that would be expected to contribute bacteria to these three streams. Although information about the land use can aid in verifying the presence of an observed source, the BST data from this study do not provide information on the specific mechanisms by which the bacteria are entering these streams.

The Accotink Creek watershed is primarily urban, but still contains large amounts of forested areas and smaller amounts of open, grassland areas; its bacteria sources reflect this land-use pattern. The human population in the watershed is estimated to be about 110,000; therefore, the presence of human-source bac-

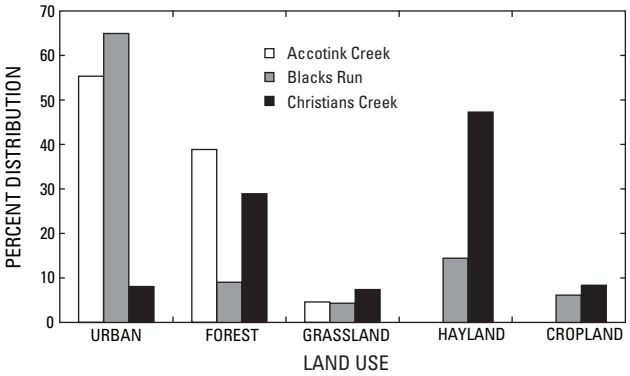


Figure 15. Land use in the Accotink Creek, Blacks Run, and Christians Creek watersheds, Virginia. Urban represents residential and commercial uses; grassland in the Accotink Creek watershed is primarily parks, golf courses, and residential lawns; grassland in the Blacks Run and Christians Creek watersheds is primarily pastureland. See figures 2-4 for sources of land-use data.

teria is not surprising. It is unknown, however, whether this human waste source is contributed by failing septic systems, leaking sewer lines, cross-connected sewer and storm drains, or straight pipes. Similarly, the dominant contributions from waterfowl are not surprising, given the large resident goose and waterfowl populations in the watershed. Waterfowl populations in the area are large because of an abundance of golf course ponds, development lakes, public parks, and other standing water bodies throughout the watershed. The proximity of waterfowl to the stream (and its tributaries) is also likely an important component of the large waterfowl contribution. The significant contributions from dogs and cats are indicative of a large pet population. Wildlife was also an important contributor, and wildlife populations have adapted to both the urban and forested areas of this watershed.

Land use in the Blacks Run watershed reflects the urban activities of the city of Harrisonburg and the agricultural activities that dominate the downstream portions of the watershed. The human population of the watershed is approximately 34,700, providing a source of human waste that could enter the stream through multiple pathways. Agricultural activities in the watershed are demonstrated by the areas of cropland, hayland, and pastureland; however, the agricultural activities also include intensive cattle and poultry farming (County of Rockingham, Department of Planning and Zoning, 1997). The intensive cattle and poultry farming are likely the source of the cattle and poultry contributions in Blacks Run; however, the mechanisms by which these bacteria are transported into the stream are uncertain. Erosion of field-applied manure is one

potential mechanism. Direct deposition of waste into the stream by cattle (in areas where cattle have direct access to the stream) also may be important. Human activities in both the urban and agricultural areas are likely responsible for the pet contributions of bacteria to the stream.

The Christians Creek watershed is dominated by agricultural activities and forested areas; urban areas are minimal. The human population of the watershed is approximately 12,000—considerably smaller than either of the other two watersheds. Although fewer people live in the Christians Creek watershed, *E. coli* of human origin were detected and were an important contributor to the stream. Christians Creek may have a higher occurrence of near-stream contributors than the other study streams. Three straight pipes have been identified in the watershed; these pipes may route untreated wastewater from three houses directly into the stream. These three straight pipes may or may not be contributing an appreciable quantity of the human *E. coli* that are observed at the ambient water-quality sampling station; however, they demonstrate the potential for other straight pipes and a condition in which a single, near-stream source (a straight pipe, for example) may contribute more bacteria than another mechanism (numerous failing septic systems that are located a considerable distance away from the stream, for example). Human activities in the watershed are likely responsible for the pet contributions of *E. coli* to the stream. Agricultural practices are dominant in this watershed; however, the density of these agricultural activities is lower than in the Blacks Run watershed. Similar to Blacks Run, cattle and poultry production accounts for the primary livestock populations in the watershed; these livestock generate large amounts of feces that may be routed into the stream. Numerous horse farms are also located in this watershed, providing a source for the horse waste in the stream. The mixture of forested and agricultural land produces a habitat that is conducive to populations of white-tailed deer and other wildlife.

As an emerging technology, published BST studies are limited; however, other studies have presented field results that can be compared to the results from this study. Four Mile Run (a nearby watershed, approximately 5 miles east of Accotink Creek) was studied by Simmons and others (2000). The Four Mile Run watershed has similar land-use practices and watershed characteristics as Accotink Creek. Simmons and others (2000) used a different method of BST (pulsed-field gel electrophoresis) and a different sampling protocol than used here; however, their identified bacterial sources were similar to those observed in Accotink Creek (fig. 16). Waterfowl, human sources, and dogs all were identified as major contributors of bacteria to both systems. Even though less similar contributions were observed for raccoons, deer, and cats, both studies identified these animals as contributors. Studies in analogous watersheds are not available for direct comparison with the study results in Christians Creek (the agricultural watershed) and Blacks Run (the mixed urban and agricultural watershed); however, others have performed source-tracking (using antibiotic resistance analysis) studies in agricultural watersheds. Cattle have been identified as the primary contributor of fecal coliform bacteria in some agricultural watersheds in Virginia (Hagedorn and others, 1999; Wiggins, 1996). Although the contributions were less than those observed in Christians Creek and Blacks Run, Wiggins (1996) also documented bacteria contributions from both poultry and human sources in some agricultural watersheds.

Despite the wide-spread occurrence of elevated fecal coliform concentrations in surface waters, this water-quality condition appears to be reversible. In two watersheds (one dominated by wildlife sources, the other dominated by agricultural sources), previous studies demonstrated that reducing the dominant sources of fecal pollution identified by BST methods may result in significantly improved water quality (Hagedorn and others, 1999; Simmons and others, 1995). Hagedorn and others (1999) observed an average fecal coliform concentration reduction of 94 percent following the implementation of source-control measures.

Temporal variability in the bacteria sources

The effects of flow on the distribution of bacteria sources were evaluated by comparing the distribution of bacteria sources during low-flow periods and storm-flow periods (fig. 17). It was expected that the bacteria sources would differ between these low-flow and high-flow periods as runoff processes occurred and waste from different sources was flushed into the streams. Although there were small variations in the source contributions, the data indicated that distributions of bacteria sources were relatively uniform during both sampling periods; major contributors during low-flow periods were major contributors during

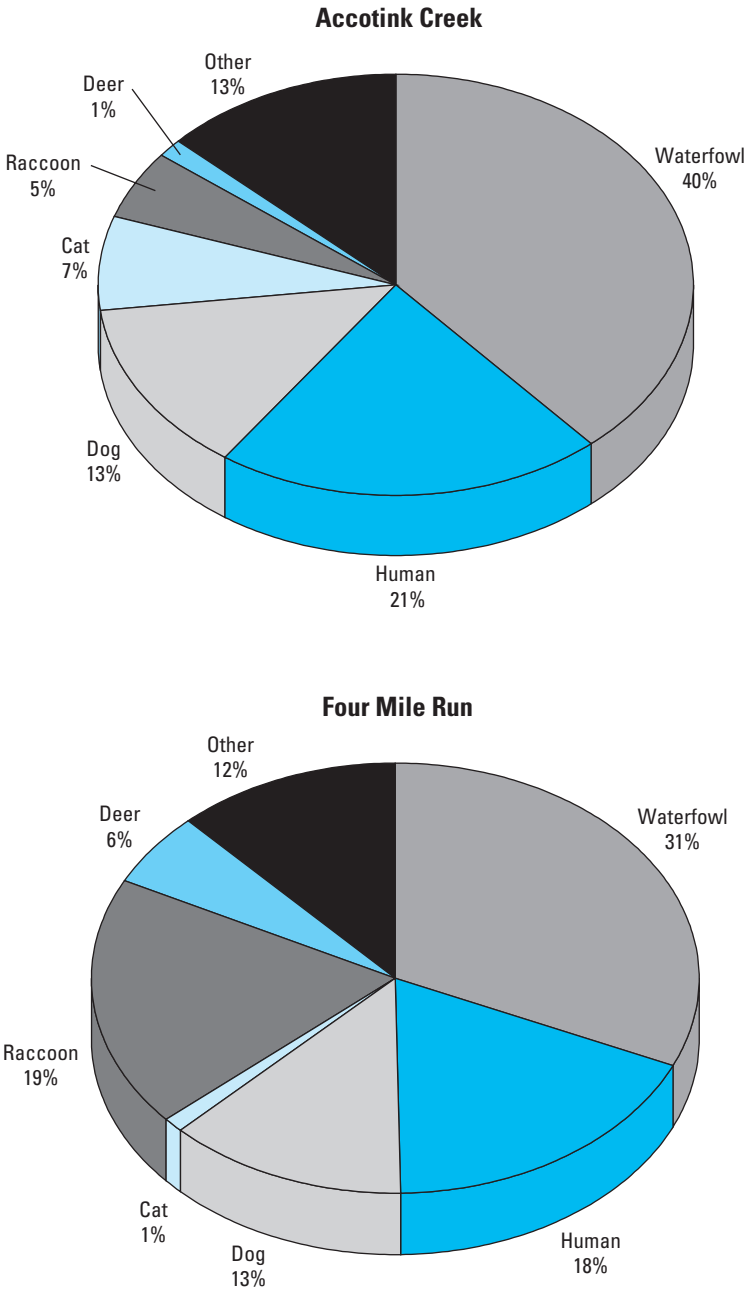


Figure 16. Distribution of identified bacteria sources in two neighboring watersheds, Accotink Creek and Four Mile Run, Virginia. (Four Mile Run data from Don Waye, Northern Virginia Regional Commission, written commun, 2001.)

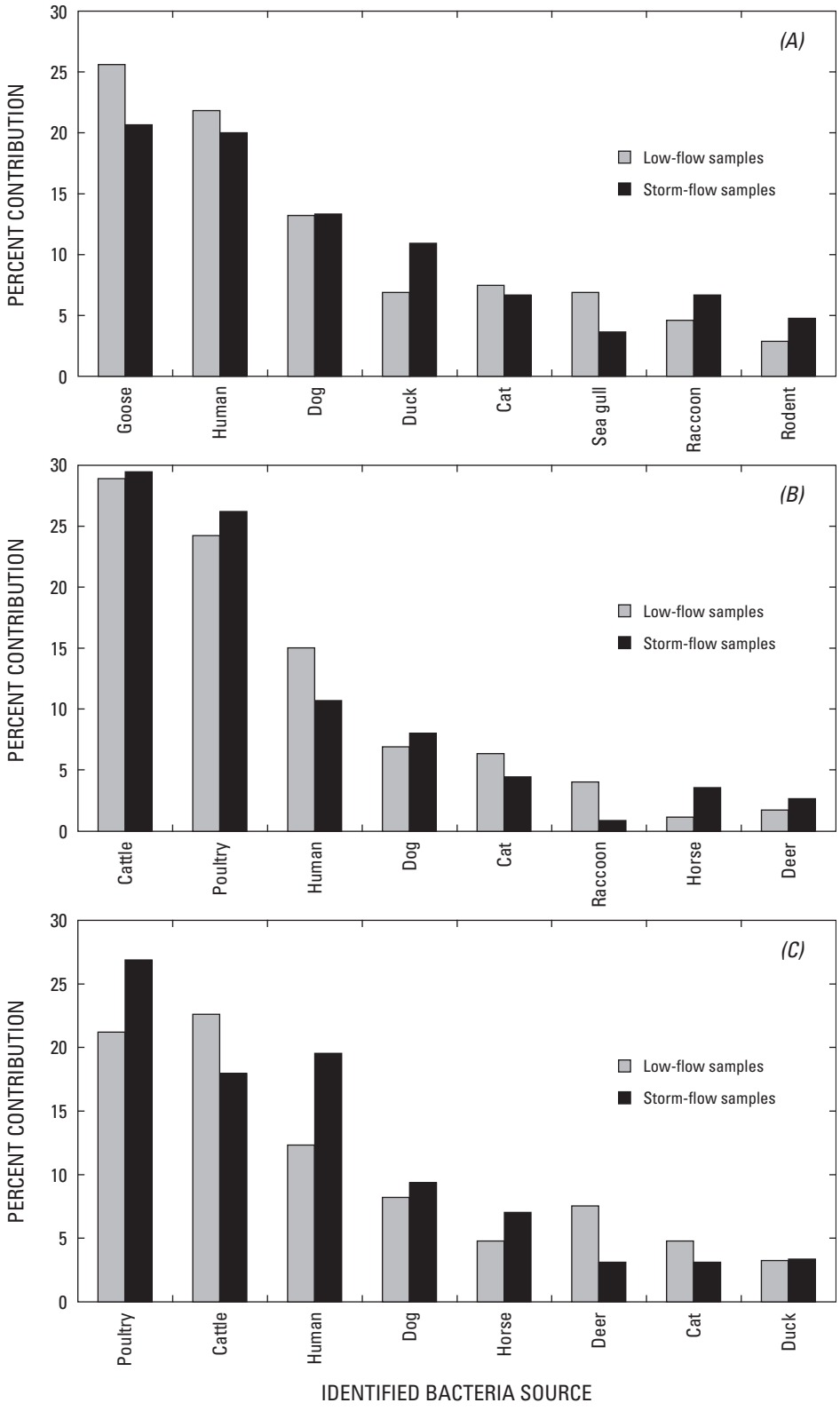


Figure 17. Top eight bacteria sources from low-flow and storm-flow streamwater samples collected March 1999 through October 2000 in Accotink Creek *(A)*, Blacks Run *(B)*, and Christians Creek *(C)*, Virginia.

storm-flow periods, and minor contributors during low-flow periods were minor contributors during storm-flow periods. Although no statistical analysis was performed to establish error bars on these plots (fig. 17), the relatively small number of isolates in each flow category and data analysis indicate that differences of 5 percent or less would be inconclusive. Using this criterion, the Christians Creek data indicated that there might be a slight increase in poultry and human sources during storm events; however, these increases were not indicated in either the Accotink Creek or Blacks Run data.

The observation of relatively uniform distributions of bacteria sources during both low-flow and high-flow periods remains largely unexplained. This pattern may indicate that the bed-sediment reservoir of these streams is a significant source of fecal coliform contamination in the water column. In this scenario, a "sloughing off" of bacteria from the bed-sediment surface may produce the low-flow distributions of fecal coliforms. During storm-flow periods, these same bed sediments are re-suspended into the water column. If no other factors were affecting the streamwater fecal coliform bacteria composition, this situation would result in similar distributions of low-flow and storm-flow bacteria sources. Because streamflow generation, suspended-sediment transport, and fecal coliform transport are complex processes, however, this scenario is probably oversimplified. Alternatively, the complex runoff processes that are initiated during storm events may combine to produce a similar bacteria source distribution to that observed in these three streams during low-flow periods. To our knowledge, no other studies have reported the effects of flow on bacteria-source distributions. The potential for a variation in the distributions of fecal coliform bacteria sources between low-flow and storm-flow periods requires further investigation.

Seasonal patterns in the bacteria-source distributions also were investigated (fig. 18). To have enough isolates in each seasonal category for a meaningful analysis, the seasonal evaluation only involved a comparison of the relatively warm months (April-September) with the relatively cool months (October-March). Only the low-flow samples were used for this analysis to ensure that slight differences between low-flow and storm-flow distributions were not misinterpreted as seasonal patterns. Although some variability was evident in the data, the Accotink Creek results failed to demonstrate seasonality. Seasonal patterns were not necessarily expected in Accotink Creek because the populations of fecal coliform sources in the watershed remain stable over the entire year. The Blacks Run data indicated seasonality in the poultry contributions, with higher percent contributions during the cool months and lower percent contributions during the warm months. This seasonal pattern is logical because the early spring and the late fall (the cool months) are generally when poultry litter is applied to the agricultural fields for fertilizer and as a method of waste disposal. If this field-applied manure were being washed off the fields and into Blacks Run, a larger poultry contribution would be expected during and immediately after application to fields. A similar seasonal pattern was also observed in Christians Creek; in addition to the increased importance of poultry contributions during cool months, however, there also appeared to be an increase in the percentage of cattle contributions during warm months. This seasonal pattern is consistent with the animal-management practices in the Christians Creek watershed. Similar to the Blacks Run watershed, poultry litter applications generally occur in the late fall and early spring. Many cattle herds had direct access to Christians Creek, and during the warmer months, cattle were observed wading into streams and spending many hours wallowing (and sometimes defecating) in the stream. During the cooler months, cattle still visited the streams as a water source, but their time spent in direct contact with the water was reduced greatly compared to the warmer months. This pattern of animal behavior would produce the observed relative dominance by cattle sources during the warm months and a shift to dominance by poultry sources during the cool months. A review of the Blacks Run data indicated a 7-percent increase in the cattle contributions during the warm months. Although this increase in the Blacks Run cattle contribution may not be significant, it lends additional support to the observed seasonal pattern. A similar increase in the contributions of cattle sources during the hot summer months was also observed by Bower (2001). Although the observed seasonal patterns in this study are consistent with the land-use and agricultural practices in each watershed, additional sampling and more detailed discretization (consideration of four seasons) would be needed to confirm these seasonal patterns and further explore the more subtle changes that might be occurring in the contributions from the less dominant fecal coliform sources.

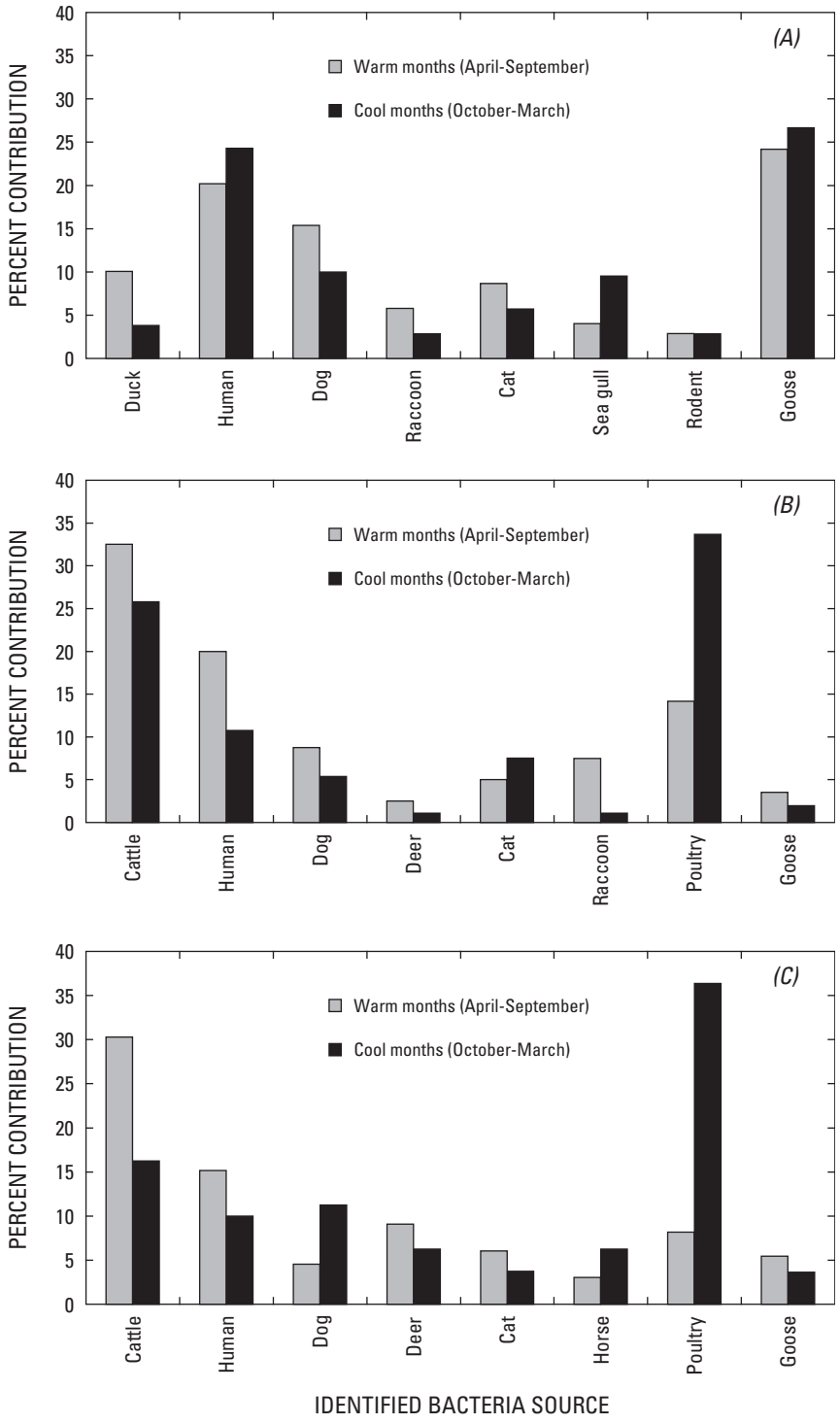


Figure 18. Top eight bacteria sources from low-flow streamwater samples collected April through September 1999 and October 1999 through March 2000 from Accotink Creek *(A)*, Blacks Run *(B)*, and Christians Creek *(C)*, Virginia.

Quality control for the ribotyping results

Quality control for the ribotyping method was done through a blind isolate experiment. In this experiment, 23 known *E. coli* source isolates were randomly selected from the source library at the UWMSTL and sent to the USGS Virginia District for preparation and blinding. The 23 original source isolates were prepared as single, duplicate, or triplicate blind isolates and re-labeled with a key that was known only to USGS personnel (the number of blind isolates prepared from each original source isolate also was not revealed to the UWMSTL). A total of 66 blind isolates was then returned to the UWMSTL for ribotyping analysis. The UWMSTL used the ribotype patterns to identify which blind isolates were replicates (*E. coli* from the same original source isolate) and to match the blind isolate with the original source isolate from the known-source library (table 6). The UWMSTL successfully identified all replicate isolates and associated the blind isolates with the original 23 isolates from the known-source library. This quality-control experiment supports the capacity of the ribotyping method to generate reproducible, isolate-specific banding patterns, and supports the utility of ribotyping for fingerprinting *E. coli*.

The observations of poultry waste in Christians Creek and Blacks Run were supported by Hancock and others (2000), who examined arsenic concentrations in Christians Creek streamwater during both low-flow and storm-flow periods. The bedrock and soils of the Christians Creek watershed are not considered an arsenic source; however, feed amendments containing arsenic (such as Roxarsone, 3-nitro-4-hydroxyphenylarsonic acid) are commonly used in the poultry industry. The arsenic generally passes through the birds (Aschbacher and Feil, 1991) and is excreted with their feces (Morrison, 1969; Kunkle and others, 1981). Field application of poultry litter (which may contain this excreted arsenic) and transport during subsequent storm events may flush poultry-derived arsenic into the streams. Hancock and others (2000) found that detectable concentrations of total arsenic were present during low-flow conditions and that the total arsenic concentration increased during a storm event, supporting the hypothesis that field-applied poultry waste was flushed into streams. The poultry litter that was flushed into streams was also a likely source of the poultry contributions observed here.

In these streams, the presence of fecal coliform bacteria from humans was not unexpected; however, the identification of humans as one of the top three contributors in each stream was unexpected. The presence of human waste in these streams also was indicated by the presence of caffeine and cotinine, both of which can be used as chemical tracers of human wastewater (S.D. Zaugg, U.S. Geological Survey, written commun., 2002). Caffeine is a stimulant that is commonly found in many beverages (like coffee and soda) whereas cotinine is a metabolite of nicotine (the primary source being cigarettes). Some caffeine passes unchanged through the human body, whereas cotinine is produced as a metabolite; both compounds can then be excreted in human waste. Identification of these two compounds in streamwater is an indication of the presence of human waste, but does not indicate the mechanism by which the waste is entering the stream. During a single sampling of all three streams, detectable concentrations of both caffeine and cotinine were measured at the ambient water-quality sampling station of each watershed (fig. 19). Cotinine concentrations are estimated because of the method reporting limit. These data cannot be used to quantify the amount of human waste in

Table 6. Design of the quality-control experiment for the ribotyping analysis used in this study

[UWMSTL, University of Washington Microbial Source Tracking Laboratory; USGS, U.S. Geological Survey; –, no replicate]

UWMSTL library identification number	USGS replicate identification number		
24221	72	–	–
24269	70	2	–
25145	67	5	69
26102	64	8	66
26623	61	11	63
26830	58	14	60
13043	52	20	54
13083	49	23	51
13949	46	26	48
14229	43	29	45
14653	40	32	42
15894	37	35	39
16113	34	38	36
18762	28	44	30
18964	25	47	27
19446	22	50	24
19585	19	53	21
19966	16	56	18
22178	7	65	9
24183	1	71	3
17042	73	80	79
21075	84	81	86
24049	87	85	75

the streams, but they do provide additional, independent evidence of the presence of human waste in all three of these streams.

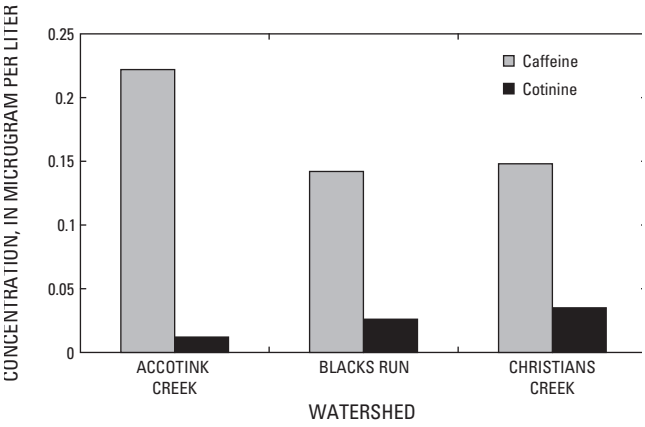


Figure 19. Caffeine and estimated cotinine concentrations measured in Accotink Creek on August 8, 2000, Blacks Run on August 15, 2000, and Christians Creek on August 1, 2000.

Source-library development and application

Successful application of *E. coli*-based BST methods requires the development of an extensive known-source library that represents all major contributors of feces to a particular watershed. The UWMSTL's ribotyping method involved direct comparison of known-source with unknown-source isolate banding patterns, with an exact match in the banding patterns required for positive source identification. Isolates that differed by even a single band were not considered matches. Because of these stringent matching requirements, this method cannot identify any isolates/ribotypes that are not already a part of the known-source database. Two known-source libraries were used in the study. These two libraries consisted of the UWMSTL's large database (containing approximately 50,000 isolates) and the UWMSTL's Virginia-specific database (containing approximately 450 isolates). The Virginia-specific library consisted of source isolates that were collected during previous investigations unrelated to this study.

To enhance the rate of positive source identification, 723 known-source samples were also collected from the three watersheds investigated in this study (table 7). Of these 723 samples, only 559 unique banding patterns were obtained (some of the isolates exhibited the same ribotype). These 559 unique isolates were then compared to the UWMSTL's large database and the Virginia-specific database. More than half (62.8 percent) of the site-specific source isolates that were collected during this study were already present in the UWMSTL's large database. Although the Virginia-specific database was relatively small (compared to the UWMSTL database), nearly 13 percent of the site-specific source isolates that were collected were already present in this database. Of the new known-source isolates collected, 4.3 percent were classified as transient strains of *E. coli* (strains that have been observed in more than one animal classification). Source samples from this study were compared with those already in the UWMSTL's large database and the Virginia-specific database; 27.5 percent of the isolates were identified as new ribotypes, added to the Virginia-specific source library, and used to identify the unknown isolates from this study. The large percentage of source isolates already present in the UWMSTL's large source database (62.8 percent) supports the conclusion that this database had national relevance and, therefore, a national database approach was reasonable for this ribotyping method. In addition, although many of the known-source isolates in this study were already included in the existing source libraries, the contribution of 154 new known-source isolates to the Virginia-specific source library was important and supports the need to collect site-specific fecal samples.

An examination of the databases used to identify the unknown isolates provided further support for using both a database of national scope and a site-specific database. For most cases, a record is available of which database was used to identify each unknown isolate (table 8). Most of the unknown isolates (60.5 percent) were identified using the UWMSTL's large database; however, an appreciable percentage of the unknowns (12.9 percent) were identified using only the Virginia-specific database (this database did include the 154 new known-source isolates that were collected as part of this study). A portion of the unknown isolates (16.1 percent) could be identified using either database, and in some cases (10.5 percent), the database used for the identification was inadvertently not recorded. These results highlight the utility of a large database for the ribotyping method; however, the results also demonstrate the need to supplement a large existing database with locally collected known-source isolates. If only one of these known-source databases had been used for identifying the unknown isolates, the number of identified isolates would have decreased considerably (from 65 percent to 29 percent if only the Virginia-specific

Table 7. Summary of source samples collected in the Accotink Creek, Blacks Run, and Christians Creek watersheds, Virginia, from March 1999 through October 2000, and comparison of the isolates from these source samples with the available source-library databases

[Unique source isolates identified represents the number of genetically distinct source isolates that were observed; this value is generally smaller than the number of source samples collected because clones were occasionally observed between source samples. The sum of the 5 columns to the right of the unique source isolates identified column is equal to this unique source isolates identified column; UWMSTL, University of Washington Microbial Source Tracking Laboratory]

Source	Number of source samples collected	Unique source isolates identified	Isolates already in the Virginia-specific database	Isolates already in the UWMSTL large available database	Isolates already in both databases	Isolates identified as transient	New source isolates added to the Virginia database
Human	220	168	4	103	15	7	39
Pets							
Dog	66	51	3	31	2	4	11
Cat	30	22	1	12	2	1	6
Livestock							
Cow	132	83	7	51	4	4	17
Turkey	39	39	3	22	2	0	12
Chicken	28	23	1	15	1	1	5
Horse	16	12	0	5	2	0	5
Sheep	5	5	0	2	0	2	1
Goat	3	3	0	0	0	1	2
Donkey	1	1	0	0	0	0	1
Mule	1	1	0	1	0	0	0
Pig	1	1	0	0	0	0	1
Poultry	1	1	0	0	0	0	1
Wildlife							
Goose	47	32	2	17	2	1	10
Duck	28	17	1	9	3	1	3
Deer	21	18	1	8	2	0	7
Muskrat	10	10	2	4	0	1	3
Groundhog	9	9	2	2	1	0	4
Rabbit	9	9	0	3	0	1	5
Squirrel	9	8	0	3	2	0	3
Fox	8	8	1	3	1	0	3
Opossum	7	6	0	3	1	0	2
Raccoon	5	5	2	1	1	0	1
Skunk	5	5	0	2	1	0	2
Hawk	4	4	0	3	0	0	1
Bird	3	3	0	2	0	0	1
Crow	3	3	0	2	0	0	1
Rat	3	3	0	1	0	0	2
Beaver	2	2	0	0	0	0	2
Pigeon	2	2	0	1	0	0	1
Osprey	1	1	0	1	0	0	0
Quail	1	1	0	0	0	0	1
Robin	1	1	0	0	0	0	1
Starling	1	1	0	1	0	0	0
Waterfowl	1	1	0	1	0	0	0
Totals	**723**	**559**	**30**	**309**	**42**	**24**	**154**
Percentages[a]		**100**	**5.4**	**55.3**	**7.5**	**4.3**	**27.5**

[a] Percentages are based on the number of unique source isolates identified.

Table 8. Summary of databases used to identify the source of each isolate for this study

[UWMSTL, University of Washington Microbial Source Tracking Laboratory]

Watershed	Total number of isolates	UWMSTL large database (percent)	Virginia-specific database (percent)	Both databases (percent)	Unspecified database (percent)
Accotink Creek	279	177 (63.4)	30 (10.8)	43 (15.4)	29 (10.4)
Blacks Run	285	176 (61.8)	43 (15.1)	43 (15.1)	23 (8.1)
Christians Creek	274	154 (56.2)	35 (12.8)	49 (17.9)	36 (13.1)
Total	**838**	**507 (60.5)**	**108 (12.9)**	**135 (16.1)**	**88 (10.5)**

database had been used for the source identification). The large size of the UWMSTL database is likely the reason it was able to identify the majority of the unknown isolates; the percentage of isolates identified likely would have increased if an even larger known-source database had been used. Although the size of the UWMSTL large database is important, the local nature of the Virginia-specific database is also important. In general terms, the fecal sources that have been sampled for the Virginia-specific source library should be more similar to the actual fecal sources that are found in Virginia waterways. Based on this work, the best source tracking results are likely produced from a coupled approach that utilizes a large available source database combined with a location-specific (or site-specific) source database.

FUTURE DIRECTIONS

In general, future studies (not just at these three impaired watersheds) would be useful in the following areas:

- BST studies would benefit from the development of standard protocols for sampling and data interpretation, including the total number of isolates to source-track in a stream system, the number of isolates to source-track from each water sample, and the design and frequency of sampling. In developing these protocols, the different objectives of the BST studies must be considered.
- The transport mechanisms by which bacteria can be routed into a stream should be identified.
- After the transport mechanisms have been identified and source-management practices have been implemented, the capacity of these practices to reduce source inputs to streams should be evaluated.
- BST data should provide support and guidance for the production of more defendable and scientifically rigorous watershed models. Incorporation of these source-tracking data into watershed-management strategies should result in the selection of more efficient source-reduction scenarios for improving water quality.
- Presently, BST studies are probably too expensive to be performed in all impaired stream systems. Cost-effective strategies are needed for generating bacteria-source information that can be applied to the large number of watersheds for which fecal coliform watershed models still must be developed.

SUMMARY AND CONCLUSIONS

The U.S. Geological Survey, in cooperation with the Virginia Department of Environmental Quality, Virginia Department of Conservation and Recreation, and Fairfax County, began a 3-year study in 1999 to perform bacterial source tracking (BST) on three streams in Virginia. The three streams selected for this study were Accotink Creek, Christians Creek, and Blacks Run, because they represented a range of different land-use practices (urban, agricultural, and mixed urban/agricultural, respectively) and potential fecal coliform sources. The Virginia Department of Environmental Quality classified these three streams as impaired by fecal coliform bacteria because of violations of the of the State's water-quality standard (1,000 col/100mL). This study was performed to demonstrate the field application of BST technology and to identify the sources of fecal coliform bacteria in these three impaired streams. The three streams were sampled over a period of 20 months (March 1999–October 2000) and over a wide range of hydrological conditions. The ribotyping technique was used to identify the sources of the fecal coliform bacteria.

This study demonstrated the utility of BST technology and provided an enhanced understanding of the fecal coliform concentrations and sources that impaired the Accotink Creek, Blacks Run, and Christians Creek watersheds in Virginia. The major findings and conclusions of this study are:

- Fecal coliform concentrations were lowest during periods of base flow (typically 200–2,000 col/100mL) and increased by 3–4 orders of magnitude during storm events (as high as 700,000 col/100mL).
- Multiple linear regression models can be developed to predict fecal coliform bacteria concentrations in these streams as a function of water-quality parameters (turbidity, pH, water temperature, and dissolved oxygen concentration).
- The major contributors of fecal coliform bacteria in each watershed, in order of importance, were:
  - **Accotink Creek:** geese, humans, dogs, ducks, cats, seagulls, and raccoons.
  - **Blacks Run:** cattle, poultry, humans, dogs, and cats.
  - **Christians Creek:** poultry, cattle, humans, dogs, horses, and deer.
- Identified bacteria sources were related to the land-use practices within each watershed.
- For Christians Creek and Blacks Run, seasonal patterns were present in the contributions of *E. coli* from cattle and poultry sources. Cattle sources were more prevalent during the warm months (April–September), whereas poultry sources were more prevalent during the cool months (October–March).
- There were only minor differences in the distribution of bacteria sources between low-flow periods and storm-flow periods.
- A coupled approach that utilized both a large available source library and a smaller, location-specific source library provided the most success in identifying unknown *E. coli* isolates.
- Future studies would benefit from the development of more cost-effective, standardized protocols for BST techniques, sampling strategies, and data analyses.

REFERENCES CITED

Aschbacher, P.W., and Feil, V.J., 1991, Fate of [C-14] arsanilic acid in pigs and chickens: Journal of Agricultural and Food Chemistry, v. 39, no. 1, p. 146-149.

Baxter-Potter, W.R., and Gilliland, M.W., 1988, Bacterial pollution in runoff from agricultural lands: Journal of Environmental Quality, v. 17, no. 1, p. 27-34.

Bolstad, P.V., and Swank, W.T., 1997, Cumulative impacts of landuse on water quality in a Southern Appalachian watershed: Journal of the American Water Resources Association, v. 33, no. 3, p. 519-533.

Bower, R.J., 2001, A case study in Tillamook Bay, Oregon - Fecal source identification using antibiotic resistance analysis: Puget Sound Notes, no. 45, p. 3-8.

Caissie, Daniel, Pollock, T.L., and Cunjak, R.A., 1996, Variation in stream water chemistry and hydrograph separation in a small drainage basin: Journal of Hydrology, v. 178, p. 137-157.

Carson, C.A., Shear, B.L., Ellersieck, M.R., and Asfaw, Amha, 2001, Identification of fecal *Escherichia coli* from humans and animals by ribotyping: Applied and Environmental Microbiology, v. 67, no. 4, p. 1503-1507.

Christensen, V.G., Rasmussen, P.P., Ziegler, A.C., and Jian, Xiaodong, 2001, Continuous monitoring and regression analysis to estimate bacteria loads: Water Environment Federation, TMDL Science Issues Conference, St. Louis, March 4-7, 2001, Onsite Program, p. 405-411.

County of Rockingham, Department of Planning and Zoning, 1997, The sweet smell of agriculture in Rockingham County, Virginia: accessed June 4, 2003, at URL http://www.co.rockingham.va.us/rockag.htm

Dalla-Costa, L.M., Irino, K., Rodrigues, J., Rivera, I.N.G., and Trabulsi, L.R., 1998, Characterisation of diarrhoeagenic *Escherichia coli* clones by ribotyping and ERIC-PCR: Journal of Medicinal Microbiology, v. 47, p. 227-234.

De Boer, D.H, and Campbell, I.A., 1990, Runoff chemistry as an indicator of runoff sources and routing in semi-arid, badland drainage basins: Journal of Hydrology, v. 121, p. 379-394.

Farag, A.M., Goldstein, J.N., Woodward, D.F., and Samadpour, Mansour, 2001, Water quality in three creeks in the backcountry of Grand Teton National Park, U.S.A: Journal of Freshwater Ecology, v. 16, no. 1, p. 135-143.

Francy, D.A., and Darner, R.A., 1998, Factors affecting *Escherichia coli* concentrations at Lake Erie public bathing beaches: U.S. Geological Survey Water-Resources Investigations Report 98-4241, 41 p.

Froelich, A.J., and Zenone, Chester, 1985, The relation of water quality to geology and land use changes in Fairfax County and vicinity, Virginia: U.S. Geological Survey Miscellaneous Investigations Series Map I-1561, scale 1:48,000.

Gathright, T.M., and Frischmann, P.S., 1986, Geology of the Harrisonburg and Bridgewater quadrangles, Virginia: Charlottesville, Virginia Division of Mineral Resources, Publication 60, scale 1:24,000.

Gburek, W.J., and Pionke, H.B., 1993, Analysing the short-term reduction in streamflow pH resulting from acidic precipitation: Hydrological Sciences, v. 38, no. 6, p. 497-518.

Hagedorn, Charles, Robinson, S.L., Filtz, J.R., Grubbs, S.M., Angier, T.A., and Reneau, R.B., 1999, Determining sources of fecal pollution in a rural Virginia watershed with antibiotic resistance patterns in fecal streptococci: Applied and Environmental Microbiology, v. 65, no. 12, p. 5522-5531.

Hancock, T.C., Miller, C.V., Denver, J.M., and Riedel, G.F., 2000, Fate and transport of arsenical feed amendments in Chesapeake Bay watersheds [abs]: Society of Environmental Toxicology and Chemistry Annual Meeting, 21st, Nashville, November 12-16, 2000.

Helsel, D.R., and Hirsch, R.M., 1992, Statistical methods in water resources: New York, Elsevier Science, 529 p.

Herrera Environmental Consultants, Inc., 1993, Pipers Creek - Bacteriological source tracking investigation: Seattle, Consultants report prepared for Seattle Engineering Department, Drainage and Wastewater Utility, 40 p.

Hockman, J.R., McKinney, J.C., Burruss, T.R., Jones, David, Modesitt, R.E., Manhart, L.G., and Waite, W.R., 1979, Soil survey of Augusta County, Virginia: U.S. Department of Agriculture, 249 p.

Hockman, J.R., Neal, C.F., Racey, D.L., and Wagner, D.F., 1982, Soil survey of Rockingham County, Virginia: U.S. Department of Agriculture, 233 p.

Hunter, Colin, McDonald, Adrian, and Beven, Keith, 1992, Input of fecal coliform bacteria to an upland stream channel in the Yorkshire Dales: Water Resources Research, v. 28, no. 7, p. 1869-1876.

Jeje, L.K., Ogunkoya, O.O., and Oluwatimilehin, J.M., 1991, Variation in suspended sediment concentration during storm discharges in three small streams in Upper Osun Basin, Central Western Nigeria: Hydrological Processes, v. 5, p. 361-369.

Johnston, P.M., 1962, Geology and ground-water resources of the Fairfax quadrangle, Virginia: U.S. Geological Survey Water-Supply Paper 1539-L, 61 p.

Johnston, P.M., 1964, Geology and ground-water resources of Washington D.C., and vicinity: U.S. Geological Survey Water-Supply Paper 1776, 97 p.

Johnson, R.A., and Bhattacharyya, G.K., 1985, Statistics, principles and methods: New York, John Wiley and Sons, 524 p.

Kronvang, Brian, Laubel, Anker, and Grant, Ruth, 1997, Suspended sediment and particulate phosphorus transport and delivery pathways in an arable catchment, Gelbaek Stream, Denmark: Hydrological Processes, v. 11, p. 627-642.

Kunkle, W.E., Carr, L.E., Carter, T.A., and Bossard, E.H., 1981, Effect of floor type on the levels of nutrients and heavy metals in poultry litter: Poultry Science, v. 60, no. 6, p. 1160-1164.

Laudon, Hjalmar, and Slaymaker, Olav, 1997, Hydrograph separation using stable isotopes, silica and electrical conductivity - an alpine example: Journal of Hydrology, v. 201, p. 82-101.

Makino, Sou-ichi, Asakura, Hiroshi, Shirahata, Toshikazu, Ikeda, Tetsuya, Takeshi, Koichi, Arai, Keisuke, Nagasawa, Motohiro, Abe, Tamotsu, and Sadamoto, Terukazu, 1999, Molecular epidemiological study of a mass outbreak caused by enteropathogenic *Escherichia coli* O157-H45: Microbiology and Immunology, v. 43, no. 4, p. 381-384.

McDonald, Adrian, and Kay, David, 1981, Enteric bacterial concentrations in reservoir feeder streams - Baseflow characteristics and response to hydrograph events: Water Research, v. 15, p. 961-968.

Morrison, J.L., 1969, Distribution of arsenic from poultry litter in broiler chicken, soils, and crops: Journal of Agricultural and Food Chemistry, v. 17, no. 6, p. 1288-1290.

Moyer, D.L., and Hyer, K.E., in press, Use of the Hydrologic Simulation Program—FORTRAN and bacterial source tracking for development of the fecal coliform Total Maximum Daily Load (TMDL) for Accotink Creek, Fairfax County, Virginia: U.S. Geological Survey Water-Resources Investigations Report 03-4160.

Moyer, D.L., and Hyer, K.E., in press, Use of the Hydrologic Simulation Program—FORTRAN and bacterial source tracking for development of the fecal coliform Total Maximum Daily Load (TMDL) for Blacks Run, Rockingham County, Virginia: U.S. Geological Survey Water-Resources Investigations Report 03-4161.

Moyer, D.L., and Hyer, K.E., in press, Use of the Hydrologic Simulation Program—FORTRAN and bacterial source tracking for development of the fecal coliform Total Maximum Daily Load (TMDL) for Christians Creek, Augusta County, Virginia: U.S. Geological Survey Water-Resources Investigations Report 03-4162.

Myers, D.N., and Sylvester, M.A., 1997, Fecal Indicator Bacteria, Chapter 7.1 in National field manual for the collection of water-quality data: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, variously paged.

Ongerth, J.E., and Samadpour, Mansour, 1994, A practical method for differentiating between fecal coliforms of human and of animal origin in shellfish and shellfish waters: Water Microbiology for the 21st Century, Seattle, March 23-24, 1994 [Proceedings].

Porter, H.C., Derting, J.F., Elder, J.H., Henry, E.F., and Pendleton, R.F., 1963, Soil survey of Fairfax County, Virginia: U.S. Department of Agriculture, 103 p.

Rader, E.K., 1967, Geology of the Staunton, Churchville, Greenville, and Stuarts Draft quadrangles, Virginia: Charlottesville, Virginia Division of Mineral Resources, Report of Investigations 12, scale 1:24,000.

Rantz, S.E., and others, 1982, Measurement and computation of streamflow - Volume 1. Measurement of stage and discharge: U.S. Geological Survey Water-Supply Paper 2175, 284 p.

Samadpour, Mansour, 2001, Molecular typing of *Pseudomonas aeruginosa* in distribution systems: Denver, AWWA Research Foundation and American Water Works Association, 114 p.

Samadpour, Mansour, and Chechowitz, Naomi, 1995, Little Soos Creek Microbial Source Tracking: Seattle, University of Washington Department of Environmental Health, 36 p.

Schalch, B., Bjorkroth, J., Eisgruber, H., Korkeala, H., and Stolle, A., 1997, Ribotyping for strain characterization of *Clostridium perfringes* isolates from food poisoning cases and outbreaks: Applied and Environmental Microbiology, v. 63, no. 10, p. 3992-3994.

Simmons, G.M., Herbein, S.A., and James, C.M., 1995, Managing nonpoint fecal coliform sources to tidal inlets: Water Resources Update, Universities Council on Water Resources, no. 100, p. 64-74.

Simmons, G.M., Waye, D.F., Herbein, S.A., Myers, Sharon, and Walker, Ellen, 2001, Estimating nonpoint fecal coliform sources in Northern Virginia's Four Mile Run watershed: Fairfax, Virginia, Northern Virginia Regional Commission: accessed June 4, 2003, at URL http://www.novaregion.org/4MileRun/bacteria.html, 20 p.

Simpson, J.M., Santo Domingo, J.W., and Reasoner, D.J., 2002, Microbial source tracking: State of the science: Environmental Science and Technology, v. 36, no. 24, p. 5279-5288.

Tarkka, E., Ahman, H., and Siitonen, A., 1994, Ribotyping as an epidemiologic tool for *Escherichia coli*: Epidemiology and Infection, v. 112, p. 263-274.

Whitfield, P.H., Rousseau, Normand, and Michnowsky, Eric, 1993, Rainfall induced changes in chemistry of a British Columbia coastal stream: Northwest Science, v. 67, no. 1, p. 1-6.

Wiggins, B.A., 1996, Discriminant analysis of antibiotic resistance patterns in fecal streptococci, a method to differentiate human and animal sources of fecal pollution in natural waters: Applied and Environmental Microbiology, v. 62, no. 11, p. 3997-4002.

Wilde, F.D., and Radtke, D.B., eds., 1998, Field measurements, Chapter A6 in National field manual for the collection of water-quality data: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, variously paged.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
			Accotink Creek		
04/13/99	1000	WAC06	23470	Feline	E, V
04/13/99	1000	WAC06	23471	Goose	V
04/13/99	1005	WAC07	23473	Opossum	n.r.
04/13/99	1005	WAC07	23472	–	n.a.
04/13/99	1010	WAC08	23474	Avian	E
04/13/99	1010	WAC08	23475	Avian	E
04/13/99	1015	WAC09	23477	Dog	E
04/13/99	1015	WAC09	23476	–	n.a.
04/13/99	1020	WAC10	23478	Dog	E
04/13/99	1020	WAC10	23479	Dog	E
04/13/99	1025	WAC11	23480	–	n.a.
04/13/99	1025	WAC11	23481	–	n.a.
04/13/99	1030	WAC12	23482	–	n.a.
04/13/99	1030	WAC12	23483	–	n.a.
04/13/99	1035	WAC13	23485	Dog	E
04/13/99	1035	WAC13	23484	–	n.a.
05/24/99	1115	WAC15	23787	Raccoon	E
05/24/99	1115	WAC15	23788	Raccoon	E
05/24/99	1130	WAC16	23790	Human	E
05/24/99	1130	WAC16	23789	–	n.a.
05/24/99	1145	WAC17	23792	Human	E
05/24/99	1145	WAC17	23791	Human	E
05/24/99	1200	WAC18	23794	Human	E
05/24/99	1200	WAC18	23793	–	n.a.
05/24/99	1230	WAC19	23796	Feline	E
05/24/99	1230	WAC19	23795	Raccoon	E
05/24/99	1300	WAC20	23798	Duck	E
05/24/99	1300	WAC20	23797	–	n.a.
05/24/99	1330	WAC21	23799	Dog	E
05/24/99	1530	WAC22	23800	Dog	E
05/24/99	1530	WAC22	23801	–	n.a.
05/24/99	1730	WAC23	23802	Goose	V
05/24/99	1730	WAC23	23803	Goose	V
05/24/99	1900	WAC24	23805	Duck	E
05/24/99	1900	WAC24	23804	Transient	n.r.
05/27/99	1115	WAC25	23869	Dog	E
05/27/99	1120	WAC26	23870	Dog	E
05/27/99	1125	WAC27	23872	Canine	n.r.
05/27/99	1125	WAC27	23871	Fox	E

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
05/27/99	1130	WAC28	23874	Goose	E
05/27/99	1130	WAC28	23873	–	n.a.
05/27/99	1135	WAC29	23876	Dog	E
05/27/99	1135	WAC29	23875	–	n.a.
05/27/99	1140	WAC30	23878	Human	E, V
05/27/99	1140	WAC30	23877	Sea Gull	E
05/27/99	1145	WAC31	23879	Human	E, V
05/27/99	1150	WAC32	23880	–	n.a.
05/27/99	1150	WAC32	23881	–	n.a.
07/07/99	1100	WAC33	24353	Dog	E
07/07/99	1100	WAC33	24354	Human	E
07/07/99	1105	WAC34	24356	Duck	E
07/07/99	1105	WAC34	24355	–	n.a.
07/07/99	1110	WAC35	24358	Feline	E
07/07/99	1110	WAC35	24357	–	n.a.
07/07/99	1115	WAC36	24359	Avian	E
07/07/99	1115	WAC36	24360	Human	E
07/07/99	1120	WAC37	24361	Avian	E
07/07/99	1120	WAC37	24362	Dog	E
07/07/99	1125	WAC38	24363	Avian	E
07/07/99	1125	WAC38	24364	Avian	E
07/07/99	1125	WAC38	24365	Feline	E
07/07/99	1130	WAC39	24368	Goose	V
07/07/99	1130	WAC39	24367	Human	E, V
07/07/99	1135	WAC40	24366	Human	E, V
07/07/99	1135	WAC40	24369	–	n.a.
07/07/99	1135	WAC40	24370	–	n.a.
08/14/99	1640	WAC47	24830	Goose	E
08/14/99	1640	WAC47	24828	Rodent	E
08/14/99	1640	WAC47	24829	–	n.a.
08/14/99	1745	WAC48	24831	Avian	E
08/14/99	1745	WAC48	24832	Avian	E
08/14/99	1745	WAC48	24833	Goose	E, V
08/14/99	1915	WAC49	24834	Duck	E
08/14/99	1915	WAC49	24835	Human	E
08/14/99	1945	WAC50	24836	Duck	E
08/14/99	2000	WAC51	24838	Dog	E
08/14/99	2000	WAC51	24837	Human	E
08/14/99	2000	WAC51	24839	Raccoon	E
08/14/99	2010	WAC52	24842	Dog	E
08/14/99	2010	WAC52	24840	Dog	E, V

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
08/14/99	2010	WAC52	24841	Goose	E, V
08/14/99	2025	WAC53	24845	Bovine	E
08/14/99	2025	WAC53	24844	Duck	E
08/14/99	2025	WAC53	24843	Feline	E, V
08/14/99	2100	WAC54	24847	Feline	E, V
08/14/99	2100	WAC54	24848	Goose	E
08/14/99	2100	WAC54	24846	–	n.a.
08/14/99	2145	WAC55	24849	Avian	n.r.
08/14/99	2145	WAC55	24850	Rodent	E
08/14/99	2145	WAC55	24851	Rodent	E
08/15/99	15	WAC56	24852	Goose	V
08/15/99	15	WAC56	24853	–	n.a.
08/15/99	15	WAC56	24854	–	n.a.
08/17/99	1005	WAC59	24855	Goose	E, V
08/17/99	1010	WAC60	24856	–	n.a.
08/17/99	1010	WAC60	24857	–	n.a.
08/17/99	1010	WAC60	24858	–	n.a.
08/17/99	1020	WAC62	24859	Duck	E, V
08/17/99	1020	WAC62	24860	–	n.a.
08/17/99	1030	WAC64	24861	Goose	E, V
08/17/99	1030	WAC64	24862	Human	E
09/10/99	2055	WAC65	25306	Avian	E
09/10/99	2055	WAC65	25305	Dog	E
09/10/99	2137	WAC66	25308	Dog	n.r.
09/10/99	2137	WAC66	25307	Human	V
09/10/99	2330	WAC67	25310	Rodent	E
09/10/99	2330	WAC67	25311	Rodent	E
09/10/99	2330	WAC67	25309	–	n.a.
09/10/99	2330	WAC67	25312	–	n.a.
09/10/99	15	WAC68	25315	Digested Sludge	E
09/10/99	15	WAC68	25314	Feline	E, V
09/10/99	15	WAC68	25313	Human	E
09/10/99	15	WAC68	25316	–	n.a.
09/10/99	100	WAC69	25319	Goose	E
09/10/99	100	WAC69	25320	Goose	E
09/10/99	100	WAC69	25317	Sludge	n.r.
09/10/99	100	WAC69	25318	–	n.a.
09/10/99	124	WAC71	25321	Dog	E
09/10/99	124	WAC71	25323	Dog	E
09/10/99	124	WAC71	25324	Duck	E
09/10/99	124	WAC71	25322	Human	E, V

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
09/10/99	124	WAC71	25325	Human	E
09/10/99	154	WAC72	25326	Goose	E, V
09/10/99	154	WAC72	25327	Human	E
09/10/99	154	WAC72	25328	Human	E
09/10/99	320	WAC73	25330	Feline	V
09/10/99	320	WAC73	25331	Feline	V
09/10/99	320	WAC73	25329	–	n.a.
09/10/99	320	WAC73	25332	–	n.a.
09/10/99	603	WAC74	25333	Goose	E
09/15/99	640	WAC75	25707	Human	E
09/15/99	640	WAC75	25704	Goose	E
09/15/99	640	WAC75	25706	Human	E
09/15/99	640	WAC75	25705	–	n.a.
09/15/99	900	WAC76	25710	Dog	E
09/15/99	900	WAC76	25708	Duck	E, V
09/15/99	900	WAC76	25709	–	n.a.
09/15/99	945	WAC77	25713	Sea Gull	E
09/15/99	945	WAC77	25711	–	n.a.
09/15/99	945	WAC77	25712	–	n.a.
09/15/99	945	WAC77	25714	–	n.a.
09/15/99	1115	WAC78	25715	Bovine	E
09/15/99	1115	WAC78	25717	Goose	E, V
09/15/99	1115	WAC78	25718	Goose	E, V
09/15/99	1115	WAC78	25716	–	n.a.
09/15/99	1230	WAC79	25722	Dog	E
09/15/99	1230	WAC79	25721	Human	E, V
09/15/99	1230	WAC79	25719	Muskrat	V
09/15/99	1230	WAC79	25720	Muskrat	V
09/15/99	1315	WAC80	25725	Dog	E
09/15/99	1315	WAC80	25723	–	n.a.
09/15/99	1315	WAC80	25724	–	n.a.
09/15/99	1315	WAC80	25726	–	n.a.
09/15/99	1430	WAC81	25729	Septage	n.r.
09/15/99	1430	WAC81	25727	–	n.a.
09/15/99	1430	WAC81	25728	–	n.a.
09/15/99	1545	WAC82	25733	Goose	E
09/15/99	1545	WAC82	25732	Human	E
09/15/99	1545	WAC82	25731	Human	E, V
09/15/99	1545	WAC82	25730	Sea Gull	E
09/15/99	1730	WAC83	25734	–	n.a.
09/15/99	2100	WAC84	25735	Coyote	E

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
09/15/99	2100	WAC84	25736	–	n.a.
09/16/99	900	WAC85	25738	combined sewer overflow	E
09/16/99	900	WAC85	25737	Raccoon	E
09/16/99	900	WAC85	25739	–	n.a.
09/27/99	1105	WAC86	25847	Rodent	E
09/27/99	1105	WAC86	25849	Sea Gull	E
09/27/99	1105	WAC86	25848	–	n.a.
09/27/99	1110	WAC87	25851	Raccoon	E
09/27/99	1110	WAC87	25850	–	n.a.
09/27/99	1110	WAC87	25852	–	n.a.
09/27/99	1110	WAC87	25853	–	n.a.
09/27/99	1115	WAC88	25855	Avian	E
09/27/99	1115	WAC88	25854	Dog	E, V
09/27/99	1115	WAC88	25856	–	n.a.
09/27/99	1115	WAC88	25857	–	n.a.
09/27/99	1120	WAC89	25858	Bovine	E, V
09/27/99	1120	WAC89	25861	Goose	E
09/27/99	1120	WAC89	25859	Goose	E, V
09/27/99	1120	WAC89	25860	–	n.a.
09/27/99	1125	WAC90	25863	Dog	E
09/27/99	1125	WAC90	25865	Dog	E
09/27/99	1125	WAC90	25862	–	n.a.
09/27/99	1125	WAC90	25864	–	n.a.
09/27/99	1130	WAC91	25868	Avian	E
09/27/99	1130	WAC91	25869	Avian	E
09/27/99	1130	WAC91	25867	Duck	E
09/27/99	1130	WAC91	25866	Septage	n.r.
09/27/99	1135	WAC92	25870	Avian	E
09/27/99	1135	WAC92	25871	Avian	E
09/27/99	1135	WAC92	25873	Dog	n.r.
09/27/99	1135	WAC92	25872	–	n.a.
09/27/99	1140	WAC93	25876	Avian	E
09/27/99	1140	WAC93	25877	Raccoon	E, V
09/27/99	1140	WAC93	25874	–	n.a.
09/27/99	1140	WAC93	25875	–	n.a.
09/27/99	1140	WAC93B	25880	Avian	E
09/27/99	1140	WAC93B	25878	–	n.a.
09/27/99	1140	WAC93B	25879	–	n.a.
09/27/99	1140	WAC93B	25881	–	n.a.
09/27/99	1140	WAC93C	25882	Dog	E
09/27/99	1140	WAC93C	25883	Raccoon	E, V

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
09/27/99	1140	WAC93C	25884	–	n.a.
09/27/99	1140	WAC93C	25885	–	n.a.
11/10/99	940	WAC94	26511	–	n.a.
11/10/99	940	WAC94	26512	Human	E
11/10/99	940	WAC94	26513	Septage	n.r.
11/10/99	940	WAC94	26514	Bovine	E, V
11/10/99	945	WAC95	26515	Human	E
11/10/99	945	WAC95	26516	Goose	E, V
11/10/99	945	WAC95	26517	Feline	E
11/10/99	945	WAC95	26518	–	n.a.
11/10/99	950	WAC96	26519	Goose	E, V
11/10/99	950	WAC96	26520	Goose	E
11/10/99	950	WAC96	26521	Human	E
11/10/99	950	WAC96	26522	–	n.a.
11/10/99	955	WAC97	26523	Human	E
11/10/99	955	WAC97	26524	Duck	E
11/10/99	955	WAC97	26525	–	n.a.
11/10/99	955	WAC97	26526	Bovine	E, V
11/10/99	1000	WAC98	26527	Goose	E, V
11/10/99	1000	WAC98	26528	Raccoon	E
11/10/99	1000	WAC98	26529	–	n.a.
11/10/99	1000	WAC98	26530	Raccoon	E
11/10/99	1005	WAC99	26531	Sea Gull	n.r.
11/10/99	1005	WAC99	26532	Rabbit	E
11/10/99	1005	WAC99	26533	Rabbit	E
11/10/99	1005	WAC99	26534	Deer	E
11/10/99	1010	WAC100	26535	Deer	E
11/10/99	1010	WAC100	26536	Dog	V
11/10/99	1010	WAC100	26537	Human	E
11/10/99	1010	WAC100	26538	–	n.a.
11/10/99	1015	WAC101	26539	–	n.a.
11/10/99	1015	WAC101	26540	Human	E, V
11/10/99	1015	WAC101	26541	Human	E, V
11/10/99	1015	WAC101	26542	Feline	E
11/10/99	1015	WAC101B	26543	Goose	E
11/10/99	1015	WAC101B	26544	Dog	E
11/10/99	1015	WAC101B	26545	–	n.a.
11/10/99	1015	WAC101B	26546	–	n.a.
11/10/99	1015	WAC101B	26547	Avian	E
11/10/99	1015	WAC101C	26548	Avian	n.r.
11/10/99	1015	WAC101C	26549	Avian	n.r.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
11/10/99	1015	WAC101C	26550	Feline	E
11/10/99	1015	WAC101C	26551	Dog	E
12/21/99	1215	WAC102	26864	Avian	E
12/21/99	1215	WAC102	26865	Goose	n.r.
12/21/99	1215	WAC102	26866	Goose	n.r.
12/21/99	1220	WAC103	26868	Sea Gull	E
12/21/99	1220	WAC103	26870	Sea Gull	E
12/21/99	1220	WAC103	26867	–	n.a.
12/21/99	1220	WAC103	26869	–	n.a.
12/21/99	1225	WAC104	26874	Digested Sludge	E
12/21/99	1225	WAC104	26873	Goose	E
12/21/99	1225	WAC104	26871	–	n.a.
12/21/99	1225	WAC104	26872	–	n.a.
12/21/99	1230	WAC105	26877	Goose	E
12/21/99	1230	WAC105	26878	Goose	V
12/21/99	1230	WAC105	26875	Human	E
12/21/99	1230	WAC105	26876	Sheep	V
12/21/99	1235	WAC106	26881	Goose	V
12/21/99	1235	WAC106	26879	–	n.a.
12/21/99	1235	WAC106	26880	–	n.a.
12/21/99	1235	WAC106	26882	–	n.a.
12/21/99	1240	WAC107	26883	Human	E
12/21/99	1240	WAC107	26884	Sea Gull	E
12/21/99	1240	WAC107	26886	Sea Gull	E
12/21/99	1240	WAC107	26885	–	n.a.
12/21/99	1245	WAC108	26890	Avian	n.r.
12/21/99	1245	WAC108	26887	Canine	E
12/21/99	1245	WAC108	26888	Goose	V
12/21/99	1245	WAC108	26889	–	n.a.
12/21/99	1250	WAC109	26892	Dog	E
12/21/99	1250	WAC109	26893	Duck	E
12/21/99	1250	WAC109	26891	Human	V
12/21/99	1250	WAC109	26894	Human	E, V
12/21/99	1250	WAC109B	26896	Feline	n.r.
12/21/99	1250	WAC109B	26897	Goose	E
12/21/99	1250	WAC109B	26895	–	n.a.
12/21/99	1250	WAC109B	26898	–	n.a.
12/21/99	1250	WAC109C	26899	Dog	n.r.
12/21/99	1250	WAC109C	26900	Goose	E, V
03/24/00	1315	WAC111	27877	Human	n.r.
03/24/00	1315	WAC111	27875	–	n.a.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
03/24/00	1315	WAC111	27876	–	n.a.
03/24/00	1320	WAC112	27878	Avian	E
03/24/00	1320	WAC112	27879	Human	n.r.
03/24/00	1320	WAC112	27880	–	n.a.
03/24/00	1325	WAC113	27882	Dog	E
03/24/00	1325	WAC113	27883	Human	V
03/24/00	1325	WAC113	27881	Human	n.r.
03/24/00	1330	WAC114	27884	Human	V
03/24/00	1330	WAC114	27886	Transient	n.r.
03/24/00	1330	WAC114	27885	–	n.a.
03/24/00	1330	WAC114B	27887	Avian	E
03/24/00	1330	WAC114B	27888	Bear	E
03/24/00	1330	WAC114B	27889	–	n.a.
03/24/00	1330	WAC114C	27891	Rodent	E
03/24/00	1330	WAC114C	27892	Rodent	E
03/24/00	1330	WAC114C	27890	–	n.a.
05/03/00	1315	WAC115	29752	Avian	E
05/03/00	1315	WAC115	29753	Avian	E
05/03/00	1315	WAC115	29751	–	n.a.
05/03/00	1320	WAC116	29754	Human	E
05/03/00	1320	WAC116	29756	Human	V
05/03/00	1320	WAC116	29755	Swan	V
05/03/00	1325	WAC117	29757	Human	V
05/03/00	1325	WAC117	29758	Human	E
05/03/00	1325	WAC117	29759	Rodent	E
05/03/00	1330	WAC118	29762	Avian	E, V
05/03/00	1330	WAC118	29760	Human	V
05/03/00	1330	WAC118	29761	–	n.a.
05/03/00	1330	WAC118B	29764	Raccoon	V
05/03/00	1330	WAC118B	29763	–	n.a.
05/03/00	1330	WAC118B	29765	–	n.a.
05/03/00	1330	WAC118C	29767	Avian	E
05/03/00	1330	WAC118C	29766	–	n.a.
05/03/00	1330	WAC118C	29768	–	n.a.
05/31/00	1100	WAC119	31168	Avian	n.r.
05/31/00	1100	WAC119	31169	Duck	E
05/31/00	1100	WAC119	31170	–	n.a.
05/31/00	1105	WAC120	31172	Avian	E
05/31/00	1105	WAC120	31173	Human	V
05/31/00	1105	WAC120	31171	Raccoon	E
05/31/00	1110	WAC121	31175	Avian	E

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
05/31/00	1110	WAC121	31174	Human	E
05/31/00	1110	WAC121	31176	Raccoon	E
05/31/00	1115	WAC122	31179	Goose	V
05/31/00	1115	WAC122	31177	–	n.a.
05/31/00	1115	WAC122	31178	–	n.a.
05/31/00	1115	WAC122B	31181	Bovine	E, V
05/31/00	1115	WAC122B	31182	Feline	E
05/31/00	1115	WAC122B	31180	–	n.a.
05/31/00	1115	WAC122C	31183	Feline	E
05/31/00	1115	WAC122C	31184	–	n.a.
05/31/00	1115	WAC122C	31185	–	n.a.
06/05/00	2000	WAC123	31502	Dog	E
06/05/00	2000	WAC123	31501	Sea Gull	E
06/06/00	815	WAC124	31504	Duck	E
06/06/00	815	WAC124	31505	Duck	E
06/06/00	815	WAC124	31503	–	n.a.
06/06/00	935	WAC125	31507	Deer	E
06/06/00	935	WAC125	31508	Deer	E
06/06/00	935	WAC125	31506	–	n.a.
06/06/00	1000	WAC126	31509	Goose	n.r.
06/06/00	1000	WAC126	31510	Goose	n.r.
06/06/00	1000	WAC126	31511	–	n.a.
06/06/00	1015	WAC127	31513	Dog	E
06/06/00	1015	WAC127	31514	Human	E, V
06/06/00	1015	WAC127	31512	–	n.a.
06/06/00	1030	WAC128	31516	Ground Hog	V
06/06/00	1030	WAC128	31515	Rabbit	E
06/06/00	1030	WAC128	31517	–	n.a.
06/06/00	1045	WAC129	31519	Bovine	E
06/06/00	1045	WAC129	31518	Feline	E
06/06/00	1045	WAC129	31520	Human	E
06/06/00	1050	WAC130	31521	–	n.a.
06/06/00	1050	WAC130	31522	–	n.a.
06/06/00	1050	WAC130	31523	–	n.a.
06/06/00	1130	WAC131	31526	Human	E, V
06/06/00	1130	WAC131	31524	Raccoon	E
06/06/00	1130	WAC131	31525	Raccoon	E
06/06/00	1310	WAC132	31528	Avian	n.r.
06/06/00	1310	WAC132	31529	Avian	n.r.
06/06/00	1310	WAC132	31527	–	n.a.
06/06/00	2037	WAC133	31531	Avian	E

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
06/06/00	2037	WAC133	31532	Avian	E
06/06/00	2037	WAC133	31530	–	n.a.
07/11/00	1100	WAC134	33258	Duck	E, V
07/11/00	1100	WAC134	33257	Feline	E
07/11/00	1100	WAC134	33259	Feline	E
07/11/00	1105	WAC135	33260	Feline	E
07/11/00	1105	WAC135	33261	Human	E
07/11/00	1105	WAC135	33262	Human	E
07/11/00	1110	WAC136	33263	Poultry	n.r.
07/11/00	1110	WAC136	33264	–	n.a.
07/11/00	1110	WAC136	33265	–	n.a.
07/11/00	1115	WAC137	33266	Avian	n.r.
07/11/00	1115	WAC137	33267	Goose	V
07/11/00	1115	WAC137	33268	Goose	V
07/11/00	1115	WAC137B	33270	Horse	E
07/11/00	1115	WAC137B	33269	Rodent	E
07/11/00	1115	WAC137B	33271	–	n.a.
07/11/00	1115	WAC137C	33273	Feline	E
07/11/00	1115	WAC137C	33272	Human	E
07/11/00	1115	WAC137C	33274	Human	V
08/10/00	745	WAC143	34687	Goose	E, V
08/10/00	745	WAC143	34688	Goose	E, V
08/10/00	745	WAC143	34686	–	n.a.
08/10/00	750	WAC144	34691	Human	V
08/10/00	750	WAC144	34689	–	n.a.
08/10/00	750	WAC144	34690	–	n.a.
08/10/00	750	WAC145	34692	–	n.a.
08/10/00	750	WAC145	34693	–	n.a.
08/10/00	750	WAC145	34694	–	n.a.
08/10/00	755	WAC146	34695	Human	E, V
08/10/00	755	WAC146	34696	–	n.a.
08/10/00	755	WAC146	34697	–	n.a.
08/10/00	800	WAC147	34699	Avian	E
08/10/00	800	WAC147	34700	Avian	E
08/10/00	800	WAC147	34698	–	n.a.
08/10/00	800	WAC148	34703	Dog	E
08/10/00	800	WAC148	34702	–	n.a.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
			Blacks Run		
04/27/99	1430	WBR06	23551	Human	E
04/27/99	1430	WBR06	23552	Raccoon	V
04/27/99	1435	WBR07	23554	Bovine	E, V
04/27/99	1435	WBR07	23553	Dog	E
04/27/99	1440	WBR08	23556	Human	E
04/27/99	1440	WBR08	23555	–	n.a.
04/27/99	1445	WBR09	23557	Bovine	E
04/27/99	1445	WBR09	23558	–	n.a.
04/27/99	1450	WBR10	23559	Bovine	E
04/27/99	1450	WBR10	23560	Human	E
04/27/99	1455	WBR11	23561	Bovine	E
04/27/99	1455	WBR11	23562	–	n.a.
04/27/99	1500	WBR12	23564	Dog	E, V
04/27/99	1500	WBR12	23563	–	n.a.
04/27/99	1505	WBR13	23565	Avian	E
04/27/99	1505	WBR13	23566	–	n.a.
05/08/99	300	WBR14	23674	Bovine	E
05/08/99	300	WBR14	23675	Bovine	E
05/08/99	345	WBR15	23676	Bovine	E
05/08/99	345	WBR15	23677	Sea Gull	E
05/08/99	445	WBR16	23678	Feline	E, V
05/08/99	445	WBR16	23679	Sea Gull	E
05/08/99	545	WBR17	23681	Bovine	V
05/08/99	545	WBR17	23680	Poultry	E
05/08/99	745	WBR18	23683	Horse	E
05/08/99	745	WBR18	23682	Human	E
05/08/99	1015	WBR19	23684	–	n.a.
05/08/99	1015	WBR19	23685	–	n.a.
05/08/99	1115	WBR20	23686	–	n.a.
05/08/99	1115	WBR20	23687	–	n.a.
05/08/99	1345	WBR21	23688	Dog	E
05/08/99	1345	WBR21	23689	Horse	E
05/08/99	1445	WBR22	23690	Bovine	E, V
05/08/99	1445	WBR22	23691	–	n.a.
05/08/99	1645	WBR23	23692	Bovine	E
05/08/99	1645	WBR23	23693	–	n.a.
06/09/99	1425	WBR24	24128	Dog	E
06/09/99	1425	WBR24	24129	–	n.a.
06/09/99	1430	WBR25	24131	Bovine	E, V

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
06/09/99	1430	WBR25	24130	Poultry	E
06/09/99	1435	WBR26	24133	Human	E
06/09/99	1435	WBR26	24132	–	n.a.
06/09/99	1440	WBR27	24135	Bovine	E, V
06/09/99	1440	WBR27	24134	Dog	E
06/09/99	1445	WBR28	24136	Bovine	V
06/09/99	1445	WBR28	24137	Duck	V
06/09/99	1450	WBR29	24139	Dog	E
06/09/99	1450	WBR29	24138	–	n.a.
06/09/99	1455	WBR30	24140	Feline	E
06/09/99	1455	WBR30	24141	Poultry	E, V
06/09/99	1500	WBR31	24143	Human	E
06/09/99	1500	WBR31	24142	Poultry	E, V
07/20/99	925	WBR32	24615	Human	V
07/20/99	925	WBR32	24616	Human	V
07/20/99	930	WBR33	24617	–	n.a.
07/20/99	930	WBR33	24618	Bovine	n.r.
07/20/99	935	WBR34	24619	Bovine	n.r.
07/20/99	935	WBR34	24620	–	n.a.
07/20/99	940	WBR35	24621	Bovine	E
07/20/99	940	WBR35	24622	Bovine	E
07/20/99	945	WBR36	24623	Bovine	E
07/20/99	945	WBR36	24624	–	n.a.
07/20/99	950	WBR37	24625	Human	E
07/20/99	950	WBR37	24626	Bovine	E, V
07/20/99	955	WBR38	24627	Avian	E
07/20/99	955	WBR38	24628	–	n.a.
07/20/99	920	WBR39	24629	Bovine	E
07/20/99	920	WBR39	24630	–	n.a.
08/25/99	1250	WBR51	24892	–	n.a.
08/25/99	1250	WBR51	24893	–	n.a.
08/25/99	1250	WBR51	24894	–	n.a.
08/25/99	1255	WBR52	24896	Bovine	E
08/25/99	1255	WBR52	24898	Bovine	E, V
08/25/99	1255	WBR52	24895	Poultry	V
08/25/99	1255	WBR52	24897	–	n.a.
08/25/99	1300	WBR53	24900	Bovine	E
08/25/99	1300	WBR53	24899	–	n.a.
08/25/99	1300	WBR53	24901	–	n.a.
08/25/99	1305	WBR54	24903	Feline	E
08/25/99	1305	WBR54	24902	–	n.a.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
08/25/99	1305	WBR54	24904	–	n.a.
08/25/99	1310	WBR55	24906	Human	E
08/25/99	1310	WBR55	24907	Human	E
08/25/99	1310	WBR55	24905	–	n.a.
08/25/99	1315	WBR56	24909	Human	E
08/25/99	1315	WBR56	24908	–	n.a.
08/25/99	1315	WBR56	24910	–	n.a.
08/25/99	1320	WBR57	24911	Poultry	V
08/25/99	1320	WBR57	24912	–	n.a.
08/25/99	1320	WBR57	24913	–	n.a.
08/25/99	1325	WBR58	24915	Poultry	V
08/25/99	1325	WBR58	24914	–	n.a.
08/25/99	1325	WBR58	24916	–	n.a.
09/15/99	1845	WBR59	25741	Bovine	E
09/15/99	1845	WBR59	25740	Poultry	E
09/15/99	1845	WBR59	25742	–	n.a.
09/15/99	1845	WBR59	25743	–	n.a.
09/15/99	2127	WBR60	25744	–	n.a.
09/15/99	2127	WBR60	25745	–	n.a.
09/15/99	2127	WBR60	25746	–	n.a.
09/15/99	2127	WBR60	25747	–	n.a.
09/15/99	542	WBR61	25749	Bovine	E
09/15/99	542	WBR61	25748	–	n.a.
09/15/99	542	WBR61	25750	–	n.a.
09/15/99	542	WBR61	25751	–	n.a.
09/15/99	610	WBR62	25752	Avian	E
09/15/99	610	WBR62	25753	–	n.a.
09/15/99	610	WBR62	25754	–	n.a.
09/15/99	610	WBR62	25755	–	n.a.
09/15/99	745	WBR63	25758	Bovine	E
09/15/99	745	WBR63	25756	Poultry	n.r.
09/15/99	745	WBR63	25757	–	n.a.
09/15/99	745	WBR63	25759	–	n.a.
09/15/99	916	WBR64	25762	Bovine	E, V
09/15/99	916	WBR64	25760	Horse	E
09/15/99	916	WBR64	25761	Human	V
09/15/99	916	WBR64	25763	Poultry	E, V
09/15/99	1045	WBR65	25767	Human	n.r.
09/15/99	1045	WBR65	25765	Opossum	n.r.
09/15/99	1045	WBR65	25766	Poultry	V
09/15/99	1045	WBR65	25764	–	n.a.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
09/15/99	1340	WBR66	25770	Bovine	E
09/15/99	1340	WBR66	25768	Poultry	n.r.
09/15/99	1340	WBR66	25769	Raccoon	E
09/15/99	1340	WBR66	25771	–	n.a.
09/15/99	1830	WBR67	25773	Avian	E
09/15/99	1830	WBR67	25775	Deer	E
09/15/99	1830	WBR67	25774	Poultry	V
09/15/99	1830	WBR67	25772	Sheep	E
09/15/99	730	WBR68	25776	Avian	E
09/15/99	730	WBR68	25777	Avian	E
09/15/99	730	WBR68	25778	Bovine	E
09/15/99	730	WBR68	25779	Poultry	V
010/13/99	930	WBR69	26130	Avian	n.r.
010/13/99	930	WBR69	26131	Poultry	V
010/13/99	930	WBR69	26129	–	n.a.
010/13/99	930	WBR69	26132	–	n.a.
010/13/99	935	WBR70	26134	Avian	E
010/13/99	935	WBR70	26133	–	n.a.
010/13/99	935	WBR70	26135	–	n.a.
010/13/99	935	WBR70	26136	–	n.a.
010/13/99	940	WBR71	26139	Bovine	E, V
010/13/99	940	WBR71	26140	Bovine	E, V
010/13/99	940	WBR71	26138	Human	V
010/13/99	940	WBR71	26137	–	n.a.
010/13/99	945	WBR72	26143	Dog	E
010/13/99	945	WBR72	26144	Sanitary Sewer	E
010/13/99	945	WBR72	26141	–	n.a.
010/13/99	945	WBR72	26142	–	n.a.
010/13/99	950	WBR73	26147	Avian	n.r.
010/13/99	950	WBR73	26145	Cat	E
010/13/99	950	WBR73	26148	Raccoon	E
010/13/99	950	WBR73	26146	–	n.a.
010/13/99	955	WBR74	26151	Bovine	E
010/13/99	955	WBR74	26152	Bovine	E
010/13/99	955	WBR74	26149	–	n.a.
010/13/99	955	WBR74	26150	–	n.a.
010/13/99	1000	WBR75	26156	Avian	n.r.
010/13/99	1000	WBR75	26155	Turkey	E
010/13/99	1000	WBR75	26153	Turkey	V
010/13/99	1000	WBR75	26154	–	n.a.
010/13/99	1005	WBR76	26160	Bovine	E

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
010/13/99	1005	WBR76	26158	Horse	E
010/13/99	1005	WBR76	26157	–	n.a.
010/13/99	1005	WBR76	26159	–	n.a.
010/13/99	1005	WBR76B	26164	Bovine	V
010/13/99	1005	WBR76B	26161	Bovine	E
010/13/99	1005	WBR76B	26162	Bovine	E
010/13/99	1005	WBR76B	26163	Bovine	E
010/13/99	1005	WBR76C	26166	Avian	n.r.
010/13/99	1005	WBR76C	26165	Bovine	E
010/13/99	1005	WBR76C	26168	Feline	E
010/13/99	1005	WBR76C	26167	–	n.a.
11/03/99	15	WBR77	26444	Horse	E
11/03/99	15	WBR77	26441	Human	E
11/03/99	15	WBR77	26442	–	n.a.
11/03/99	15	WBR77	26443	–	n.a.
11/03/99	630	WBR78	26445	Bovine	E
11/03/99	630	WBR78	26447	Bovine	V
11/03/99	630	WBR78	26448	Dog	E
11/03/99	630	WBR78	26446	–	n.a.
11/03/99	1300	WBR79	26451	Coyote	E
11/03/99	1300	WBR79	26452	Poultry	V
11/03/99	1300	WBR79	26449	–	n.a.
11/03/99	1300	WBR79	26450	–	n.a.
11/03/99	1515	WBR80	26454	Bovine	V
11/03/99	1515	WBR80	26453	–	n.a.
11/03/99	1515	WBR80	26455	–	n.a.
11/03/99	1515	WBR80	26456	–	n.a.
11/03/99	1630	WBR81	26459	Bovine	V
11/03/99	1630	WBR81	26458	Bovine	E, V
11/03/99	1630	WBR81	26460	Bovine	E, V
11/03/99	1630	WBR81	26457	Dog	E
11/03/99	1645	WBR82	26463	Human	E
11/03/99	1645	WBR82	26464	Human	E
11/03/99	1645	WBR82	26461	–	n.a.
11/03/99	1645	WBR82	26462	–	n.a.
11/03/99	1700	WBR83	26465	Avian	E
11/03/99	1700	WBR83	26468	Bovine	E, V
11/03/99	1700	WBR83	26466	Poultry	V
11/03/99	1700	WBR83	26467	–	n.a.
11/03/99	1715	WBR84	26471	Bovine	E, V
11/03/99	1715	WBR84	26470	Human	E

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
11/03/99	1715	WBR84	26469	Human	V
11/03/99	1715	WBR84	26472	Poultry	V
11/03/99	1730	WBR85	26476	Bovine	E
11/03/99	1730	WBR85	26474	Dog	E, V
11/03/99	1730	WBR85	26475	Turkey	E, V
11/03/99	1730	WBR85	26473	–	n.a.
11/03/99	1845	WBR86	26479	Human	E
11/03/99	1845	WBR86	26480	Turkey	E, V
11/03/99	1845	WBR86	26477	–	n.a.
11/03/99	1845	WBR86	26478	–	n.a.
11/03/99	2230	WBR87	26483	Dog	E
11/03/99	2230	WBR87	26481	Poultry	E
11/03/99	2230	WBR87	26484	Turkey	E, V
11/03/99	2230	WBR87	26482	–	n.a.
11/03/99	1100	WBR88	26488	Human	V
11/03/99	1100	WBR88	26486	Poultry	E
11/03/99	1100	WBR88	26485	Sheep	V
11/03/99	1100	WBR88	26487	–	n.a.
11/22/99	1050	WBR89	26713	Bovine	V
11/22/99	1050	WBR89	26712	–	n.a.
11/22/99	1050	WBR89	26714	–	n.a.
11/22/99	1050	WBR89	26715	–	n.a.
11/22/99	1055	WBR90	26717	Bovine	V
11/22/99	1055	WBR90	26718	Bovine	E, V
11/22/99	1055	WBR90	26716	Feline	E
11/22/99	1055	WBR90	26719	Poultry	V
11/22/99	1100	WBR91	26720	Bovine	V
11/22/99	1100	WBR91	26722	Poultry	V
11/22/99	1100	WBR91	26723	Poultry	V
11/22/99	1100	WBR91	26721	–	n.a.
11/22/99	1105	WBR92	26724	–	n.a.
11/22/99	1105	WBR92	26725	–	n.a.
11/22/99	1105	WBR92	26726	–	n.a.
11/22/99	1105	WBR92	26727	–	n.a.
11/22/99	1110	WBR93	26730	Avian	n.r.
11/22/99	1110	WBR93	26728	Avian	E
11/22/99	1110	WBR93	26729	Rodent	E
11/22/99	1110	WBR93	26731	Rodent	E
11/22/99	1115	WBR94	26732	Avian	E
11/22/99	1115	WBR94	26735	Bovine	E, V
11/22/99	1115	WBR94	26734	Bovine	E

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
11/22/99	1115	WBR94	26733	–	n.a.
11/22/99	1120	WBR95	26736	Bovine	E, V
11/22/99	1120	WBR95	26739	Bovine	E, V
11/22/99	1120	WBR95	26737	–	n.a.
11/22/99	1120	WBR95	26738	–	n.a.
11/22/99	1125	WBR96	26743	Avian	n.r.
11/22/99	1125	WBR96	26741	Bovine	E
11/22/99	1125	WBR96	26740	Dog	V
11/22/99	1125	WBR96	26742	–	n.a.
11/22/99	1125	WBR96B	26746	Avian	E
11/22/99	1125	WBR96B	26747	Avian	E
11/22/99	1125	WBR96B	26744	Human	E
11/22/99	1125	WBR96B	26745	–	n.a.
11/22/99	1125	WBR96C	26748	Avian	E
11/22/99	1125	WBR96C	26750	Avian	E
11/22/99	1125	WBR96C	26751	Horse	E
11/22/99	1125	WBR96C	26749	–	n.a.
01/04/00	1100	WBR97	26908	Poultry	V
01/04/00	1100	WBR97	26909	Avian	n.r.
01/04/00	1100	WBR97	26910	–	n.a.
01/04/00	1100	WBR97	26911	Dog	E
01/04/00	1105	WBR98	26912	Human	E, V
01/04/00	1105	WBR98	26913	Human	E
01/04/00	1105	WBR98	26914	Duck	E
01/04/00	1105	WBR98	26915	Feline	E
01/04/00	1110	WBR99	26916	–	n.a.
01/04/00	1110	WBR99	26917	Poultry	E
01/04/00	1110	WBR99	26918	–	n.a.
01/04/00	1110	WBR99	26919	Poultry	E
01/04/00	1115	WBR100	26920	–	n.a.
01/04/00	1115	WBR100	26921	Opossum	E
01/04/00	1115	WBR100	26922	Feline	E
01/04/00	1115	WBR100	26923	–	n.a.
01/04/00	1120	WBR101	26924	Deer	E
01/04/00	1120	WBR101	26925	Sheep	E
01/04/00	1120	WBR101	26926	Poultry	E
01/04/00	1120	WBR101	26927	Pig	E
01/04/00	1125	WBR102	26928	Poultry	E
01/04/00	1125	WBR102	26929	–	n.a.
01/04/00	1125	WBR102	26930	Turkey	E, V
01/04/00	1125	WBR102	26931	–	n.a.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
01/04/00	1130	WBR103	26932	Turkey	E, V
01/04/00	1130	WBR103	26933	Feline	E
01/04/00	1130	WBR103	26934	–	n.a.
01/04/00	1130	WBR103	26935	Feline	E
01/04/00	1135	WBR104	26936	Bovine	E
01/04/00	1135	WBR104	26937	Bovine	E
01/04/00	1135	WBR104	26938	Opossum	E
01/04/00	1135	WBR104	26939	Human	E
01/04/00	1135	WBR104B	26940	Bovine	E
01/04/00	1135	WBR104B	26941	Dog	E
01/04/00	1135	WBR104B	26942	Bovine	E
01/04/00	1135	WBR104B	26943	Opossum	n.r.
01/04/00	1135	WBR104C	26944	Human	E, V
01/04/00	1135	WBR104C	26945	Bovine	E
01/04/00	1135	WBR104C	26946	Human	E
01/04/00	1135	WBR104C	26947	–	n.a.
01/10/00	1003	WBR105	26972	Dog	E
01/10/00	1003	WBR105	26973	Avian	E
01/10/00	1003	WBR105	26974	Feline	E
01/10/00	1003	WBR105	26975	Bovine	E
01/10/00	1300	WBR108	26976	–	n.a.
01/10/00	1300	WBR108	26977	Avian	E
01/10/00	1300	WBR108	26978	–	n.a.
01/10/00	1300	WBR108	26979	Feline	E
01/10/00	1400	WBR111	26980	Bovine	E
01/10/00	1400	WBR111	26982	Bovine	E
01/10/00	1400	WBR111	26981	Coyote	E
01/10/00	1400	WBR111	26983	Pig	n.r.
01/10/00	1618	WBR112	26985	Avian	n.r.
01/10/00	1618	WBR112	26987	Bovine	E
01/10/00	1618	WBR112	26984	–	n.a.
01/10/00	1618	WBR112	26986	–	n.a.
01/10/00	1641	WBR113	26988	Bovine	E
01/10/00	1641	WBR113	26990	Bovine	E
01/10/00	1641	WBR113	26989	Coyote	E
01/10/00	1641	WBR113	26991	Duck	V
01/10/00	1700	WBR114	26992	Bovine	E, V
01/10/00	1700	WBR114	26995	Opossum	E
01/10/00	1700	WBR114	26993	Sheep	E
01/10/00	1700	WBR114	26994	–	n.a.
01/10/00	1715	WBR115	26998	Bovine	E, V

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
01/10/00	1715	WBR115	26997	Deer	E
01/10/00	1715	WBR115	26996	Feline	E
01/10/00	1715	WBR115	26999	Rodent	E
01/10/00	1900	WBR116	27002	Bovine	E
01/10/00	1900	WBR116	27003	Bovine	E
01/10/00	1900	WBR116	27000	–	n.a.
01/10/00	1900	WBR116	27001	–	n.a.
01/10/00	2205	WBR117	27007	Dog	E
01/10/00	2205	WBR117	27004	Human	n.r.
01/10/00	2205	WBR117	27006	Poultry	V
01/10/00	2205	WBR117	27005	–	n.a.
01/10/00	925	WBR118	27009	Bovine	V
01/10/00	925	WBR118	27008	Human	n.r.
01/10/00	925	WBR118	27010	–	n.a.
01/10/00	925	WBR118	27011	–	n.a.
01/10/00	1215	WBR119	27012	–	n.a.
01/10/00	1215	WBR119	27013	–	n.a.
01/10/00	1215	WBR119	27014	–	n.a.
01/10/00	1215	WBR119	27015	–	n.a.
03/30/00	1240	WBR121	27992	Human	E
03/30/00	1240	WBR121	27990	–	n.a.
03/30/00	1240	WBR121	27991	–	n.a.
03/30/00	1245	WBR122	27995	Avian	E
03/30/00	1245	WBR122	27993	Goose	E
03/30/00	1245	WBR122	27994	Human	E, V
03/30/00	1250	WBR123	27998	Poultry	n.r.
03/30/00	1250	WBR123	27997	Poultry	V
03/30/00	1250	WBR123	27996	–	n.a.
03/30/00	1250	WBR123B	28001	Avian	E
03/30/00	1250	WBR123B	27999	Human	E
03/30/00	1250	WBR123B	28000	Poultry	n.r.
03/30/00	1250	WBR123C	28003	Dog	E
03/30/00	1250	WBR123C	28002	Poultry	n.r.
03/30/00	1250	WBR123C	28004	–	n.a.
05/10/00	1205	WBR124	30228	Bovine	E
05/10/00	1205	WBR124	30226	Deer	E
05/10/00	1205	WBR124	30227	Deer	E
05/10/00	1210	WBR125	30229	–	n.a.
05/10/00	1210	WBR125	30230	–	n.a.
05/10/00	1210	WBR125	30231	–	n.a.
05/10/00	1215	WBR126	30233	Crow	n.r.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
05/10/00	1215	WBR126	30234	Dog	E
05/10/00	1215	WBR126	30232	–	n.a.
05/10/00	1215	WBR126B	30235	–	n.a.
05/10/00	1215	WBR126B	30236	–	n.a.
05/10/00	1215	WBR126B	30237	–	n.a.
05/10/00	1215	WBR126C	30239	Dog	E
05/10/00	1215	WBR126C	30238	–	n.a.
05/10/00	1215	WBR126C	30240	–	n.a.
06/14/00	1245	WBR127	31788	Avian	E
06/14/00	1245	WBR127	31786	Human	V
06/14/00	1245	WBR127	31787	–	n.a.
06/14/00	1250	WBR128	31789	Bovine	E
06/14/00	1250	WBR128	31790	Human	E
06/14/00	1250	WBR128	31791	Human	V
06/14/00	1255	WBR129	31792	Bovine	E
06/14/00	1255	WBR129	31793	Bovine	E
06/14/00	1255	WBR129	31794	Bovine	E
06/14/00	1255	WBR129B	31796	Human	E
06/14/00	1255	WBR129B	31797	Turkey	V
06/14/00	1255	WBR129B	31795	–	n.a.
06/14/00	1255	WBR129C	31799	Bovine	E, V
06/14/00	1255	WBR129C	31800	Goose	E
06/14/00	1255	WBR129C	31798	–	n.a.
06/14/00	1900	WBR130	31878	Dog	E
06/14/00	1900	WBR130	31877	–	n.a.
06/14/00	1900	WBR130	31879	–	n.a.
06/14/00	1957	WBR131	31880	Avian	n.r.
06/14/00	1957	WBR131	31882	Cat	E
06/14/00	1957	WBR131	31881	–	n.a.
06/14/00	2051	WBR132	31885	Poultry	E, V
06/14/00	2051	WBR132	31883	–	n.a.
06/14/00	2051	WBR132	31884	–	n.a.
06/14/00	2122	WBR133	31886	Canine	E
06/14/00	2122	WBR133	31887	–	n.a.
06/14/00	2122	WBR133	31888	–	n.a.
06/14/00	2143	WBR134	31889	Avian	E
06/14/00	2143	WBR134	31890	Avian	E
06/14/00	2143	WBR134	31891	–	n.a.
06/14/00	2215	WBR135	31894	Deer	E
06/14/00	2215	WBR135	31892	–	n.a.
06/14/00	2215	WBR135	31893	–	n.a.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
06/14/00	2235	WBR136	31895	Chicken	V
06/14/00	2235	WBR136	31896	–	n.a.
06/14/00	2235	WBR136	31897	–	n.a.
06/14/00	2345	WBR137	31898	Avian	E
06/14/00	2345	WBR137	31899	–	n.a.
06/14/00	2345	WBR137	31900	–	n.a.
06/15/00	220	WBR138	31903	Bovine	E
06/15/00	220	WBR138	31901	Rodent	E
06/15/00	220	WBR138	31902	–	n.a.
06/15/00	720	WBR139	31904	–	n.a.
07/18/00	1540	WBR140	33732	Raccoon	E, V
07/18/00	1540	WBR140	33733	Raccoon	E, V
07/18/00	1540	WBR140	33734	–	n.a.
07/18/00	1545	WBR141	33735	Bovine	V
07/18/00	1545	WBR141	33737	Feline	E
07/18/00	1545	WBR141	33736	Raccoon	E, V
07/18/00	1550	WBR142	33738	Bovine	E
07/18/00	1550	WBR142	33740	Raccoon	E, V
07/18/00	1550	WBR142	33739	–	n.a.
07/18/00	1550	WBR142B	33742	Avian	E
07/18/00	1550	WBR142B	33743	Raccoon	E, V
07/18/00	1550	WBR142B	33741	–	n.a.
07/18/00	1550	WBR142C	33744	–	n.a.
07/18/00	1550	WBR142C	33745	–	n.a.
07/18/00	1550	WBR142C	33746	–	n.a.
08/17/00	1135	WBR148	34950	Sludge	E
08/17/00	1135	WBR148	34949	–	n.a.
08/17/00	1135	WBR148	34951	–	n.a.
08/17/00	1140	WBR149	34952	Bovine	E
08/17/00	1140	WBR149	34954	Poultry	E, V
08/17/00	1140	WBR149	34953	–	n.a.
08/17/00	1140	WBR149B	34957	Human	E
08/17/00	1140	WBR149B	34955	Rabbit	E
08/17/00	1140	WBR149B	34956	–	n.a.
08/17/00	1145	WBR150	34958	Cat	E, V
08/17/00	1145	WBR150	34959	–	n.a.
08/17/00	1145	WBR150	34960	–	n.a.
08/17/00	1150	WBR151	34961	Goose	E
08/17/00	1150	WBR151	34962	–	n.a.
08/17/00	1150	WBR151	34963	–	n.a.
08/17/00	1150	WBR151B	34964	Avian	E, V

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
08/17/00	1150	WBR151B	34965	–	n.a.
08/17/00	1150	WBR151B	34966	–	n.a.
			Christians Creek		
5/5/99	910	WCC07	23597	Bovine	E
5/5/99	910	WCC07	23598	Sea Gull	n.r.
5/5/99	915	WCC08	23600	Bovine	E
5/5/99	915	WCC08	23599	Bovine	E, V
5/5/99	920	WCC09	23601	–	n.a.
5/5/99	920	WCC09	23602	–	n.a.
5/5/99	925	WCC10	23603	Bovine	V
5/5/99	925	WCC10	23604	–	n.a.
5/5/99	930	WCC11	23606	Feline	E, V
5/5/99	930	WCC11	23605	–	n.a.
5/5/99	935	WCC12	23608	Deer	E
5/5/99	935	WCC12	23607	Dog	E
5/5/99	940	WCC13	23610	–	n.a.
5/5/99	940	WCC13	23609	–	n.a.
5/5/99	945	WCC14	23612	Dog	E
5/5/99	945	WCC14	23611	Feline	E
6/16/99	1515	WCC16	24238	Rodent	E
6/16/99	1515	WCC16	24239	–	n.a.
6/16/99	715	WCC17	24240	–	n.a.
6/16/99	715	WCC17	24241	–	n.a.
6/16/99	830	WCC18	24243	Human	E
6/16/99	830	WCC18	24242	–	n.a.
6/16/99	935	WCC19	24245	Bovine	E
6/16/99	935	WCC19	24244	–	n.a.
6/16/99	945	WCC20	24246	Bovine	E, V
6/16/99	945	WCC20	24247	Bovine	E, V
6/16/99	1000	WCC21	24249	Bovine	E, V
6/16/99	1000	WCC21	24248	Bovine	E, V
6/16/99	1005	WCC22	24251	Bovine	E, V
6/16/99	1005	WCC22	24250	Bovine	E, V
6/16/99	1010	WCC23	24252	Bovine	E, V
6/16/99	1010	WCC23	24253	–	n.a.
7/28/99	845	WCC30	24684	Duck	E
7/28/99	845	WCC30	24685	Bovine	E, V
7/28/99	850	WCC31	24686	Horse	E
7/28/99	850	WCC31	24687	–	n.a.
7/28/99	855	WCC32	24688	–	n.a.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
7/28/99	855	WCC32	24689	Bovine	V
7/28/99	900	WCC33	24690	Human	E
7/28/99	900	WCC33	24691	Feline	E
7/28/99	905	WCC34	24692	Bovine	V
7/28/99	905	WCC34	24693	–	n.a.
7/28/99	910	WCC35	24694	–	n.a.
7/28/99	910	WCC35	24695	Human	E
7/28/99	915	WCC36	24696	Human	E
7/28/99	915	WCC36	24697	Poultry	V
7/28/99	920	WCC37	24698	Human	E, V
7/28/99	920	WCC37	24699	Avian	E
9/6/99	410	WCC39	25169	Dog	E, V
9/6/99	410	WCC39	25168	Turkey	E, V
9/6/99	410	WCC39	25167	Turkey	E, V
9/6/99	410	WCC39	25166	–	n.a.
9/6/99	825	WCC40	25172	Bovine	E
9/6/99	825	WCC40	25173	Bovine	V
9/6/99	825	WCC40	25170	Bovine	E
9/6/99	825	WCC40	25171	Duck	V
9/6/99	1220	WCC41	25174	Bovine	E
9/6/99	1220	WCC41	25176	Bovine	E, V
9/6/99	1220	WCC41	25177	Poultry	V
9/6/99	1220	WCC41	25175	–	n.a.
9/6/99	1650	WCC42	25178	Feline	E
9/6/99	1650	WCC42	25180	Horse	E
9/6/99	1650	WCC42	25181	Human	V
9/6/99	1650	WCC42	25179	–	n.a.
9/6/99	1815	WCC43	25183	Bovine	E
9/6/99	1815	WCC43	25182	Bovine	V
9/6/99	1815	WCC43	25184	Bovine	E, V
9/6/99	1900	WCC44	25186	Bovine	E
9/6/99	1900	WCC44	25185	Dog	V
9/6/99	1900	WCC44	25187	–	n.a.
9/6/99	1900	WCC44	25188	–	n.a.
9/6/99	1940	WCC45	25192	Avian	E
9/6/99	1940	WCC45	25191	Dog	E
9/6/99	1940	WCC45	25189	Human	E
9/6/99	1940	WCC45	25190	–	n.a.
9/6/99	2215	WCC46	25193	Avian	E
9/6/99	2215	WCC46	25194	Avian	E
9/6/99	2215	WCC46	25196	Poultry	n.r.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
9/6/99	2215	WCC46	25195	–	n.a.
9/6/99	50	WCC47	25197	Avian	E
9/6/99	50	WCC47	25199	Human	E
9/6/99	50	WCC47	25198	Sea Gull	E
9/6/99	50	WCC47	25200	–	n.a.
9/6/99	835	WCC48	25203	Bovine	E
9/6/99	835	WCC48	25204	Bovine	E
9/6/99	835	WCC48	25201	Bovine	E, V
9/6/99	835	WCC48	25202	Human	E
9/14/99	1110	WCC54	25336	–	n.a.
9/14/99	1110	WCC54	25337	–	n.a.
9/14/99	1110	WCC54	25335	–	n.a.
9/14/99	1110	WCC54	25334	–	n.a.
9/14/99	1115	WCC55	25338	Human	V
9/14/99	1115	WCC55	25339	–	n.a.
9/14/99	1115	WCC55	25340	–	n.a.
9/14/99	1115	WCC55	25341	–	n.a.
9/14/99	1120	WCC56	25343	Goose	n.r.
9/14/99	1120	WCC56	25345	–	n.a.
9/14/99	1120	WCC56	25342	–	n.a.
9/14/99	1120	WCC56	25344	–	n.a.
9/14/99	1125	WCC57	25346	Turkey	V
9/14/99	1125	WCC57	25348	–	n.a.
9/14/99	1125	WCC57	25349	–	n.a.
9/14/99	1125	WCC57	25347	–	n.a.
9/14/99	1130	WCC58	25350	–	n.a.
9/14/99	1130	WCC58	25351	–	n.a.
9/14/99	1130	WCC58	25352	–	n.a.
9/14/99	1130	WCC58	25353	–	n.a.
9/14/99	1135	WCC59	25356	Turkey	E, V
9/14/99	1135	WCC59	25355	–	n.a.
9/14/99	1135	WCC59	25354	–	n.a.
9/14/99	1135	WCC59	25357	–	n.a.
9/14/99	1140	WCC60	25359	Human	E
9/14/99	1140	WCC60	25360	–	n.a.
9/14/99	1140	WCC60	25361	–	n.a.
9/14/99	1140	WCC60	25358	–	n.a.
9/14/99	1145	WCC61	25363	Avian	E
9/14/99	1145	WCC61	25365	–	n.a.
9/14/99	1145	WCC61	25362	–	n.a.
9/14/99	1145	WCC61	25364	–	n.a.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
10/25/99	1010	WCC62	26318	Avian	n.r.
10/25/99	1010	WCC62	26321	Coyote	E
10/25/99	1010	WCC62	26319	Human	E
10/25/99	1010	WCC62	26320	Turkey	V
10/25/99	1015	WCC63	26324	Avian	n.r.
10/25/99	1015	WCC63	26323	Human	V
10/25/99	1015	WCC63	26322	–	n.a.
10/25/99	1015	WCC63	26325	–	n.a.
10/25/99	1020	WCC64	26328	Avian	n.r.
10/25/99	1020	WCC64	26327	Avian	n.r.
10/25/99	1020	WCC64	26329	Chicken	V
10/25/99	1020	WCC64	26326	–	n.a.
10/25/99	1025	WCC65	26333	Dog	E
10/25/99	1025	WCC65	26332	Human	E, V
10/25/99	1025	WCC65	26330	–	n.a.
10/25/99	1025	WCC65	26331	–	n.a.
10/25/99	1030	WCC66	26335	Avian	E
10/25/99	1030	WCC66	26336	Turkey	V
10/25/99	1030	WCC66	26337	–	n.a.
10/25/99	1030	WCC66	26334	–	n.a.
10/25/99	1035	WCC67	26338	Avian	n.r.
10/25/99	1035	WCC67	26339	Deer	E
10/25/99	1035	WCC67	26340	Opossum	E
10/25/99	1035	WCC67	26341	–	n.a.
10/25/99	1040	WCC68	26343	Avian	n.r.
10/25/99	1040	WCC68	26344	Dog	E
10/25/99	1040	WCC68	26342	Human	E, V
10/25/99	1040	WCC68	26345	Human	E
10/25/99	1045	WCC69A	26346	Turkey	E, V
10/25/99	1045	WCC69A	26349	–	n.a.
10/25/99	1045	WCC69A	26348	–	n.a.
10/25/99	1045	WCC69A	26347	–	n.a.
10/25/99	1045	WCC69B	26352	Avian	n.r.
10/25/99	1045	WCC69B	26351	Dog	E
10/25/99	1045	WCC69B	26353	Poultry	E
10/25/99	1045	WCC69B	26350	Skunk	V
10/25/99	1045	WCC69C	26357	Dog	E, V
10/25/99	1045	WCC69C	26354	Horse	E
10/25/99	1045	WCC69C	26356	Turkey	E, V
10/25/99	1045	WCC69C	26355	–	n.a.
11/1/99	45	WCC71	26366	Horse	E

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
11/1/99	45	WCC71	26365	Horse	E
11/1/99	45	WCC71	26367	Human	V
11/1/99	45	WCC71	26368	–	n.a.
11/1/99	900	WCC72	26371	Dog	E
11/1/99	900	WCC72	26370	Duck	V
11/1/99	900	WCC72	26372	Poultry	E
11/1/99	900	WCC72	26369	–	n.a.
11/1/99	1230	WCC73	26374	Deer	E
11/1/99	1230	WCC73	26373	Deer	E
11/1/99	1230	WCC73	26375	Human	E
11/1/99	1230	WCC73	26376	Human	V
11/1/99	1530	WCC74	26378	Horse	E
11/1/99	1530	WCC74	26380	Horse	E
11/1/99	1530	WCC74	26377	Poultry	E
11/1/99	1530	WCC74	26379	Raccoon	V
11/1/99	1630	WCC75	26384	Bovine	E
11/1/99	1630	WCC75	26383	Human	E
11/1/99	1630	WCC75	26381	–	n.a.
11/1/99	1630	WCC75	26382	–	n.a.
11/1/99	1700	WCC76	26386	Bovine	E, V
11/1/99	1700	WCC76	26385	Horse	E
11/1/99	1700	WCC76	26387	Poultry	V
11/1/99	1700	WCC76	26388	–	n.a.
11/1/99	1725	WCC77	26390	Avian	E
11/1/99	1725	WCC77	26391	Bovine	E
11/1/99	1725	WCC77	26389	Dog	V
11/1/99	1725	WCC77	26392	–	n.a.
11/1/99	1800	WCC79	26394	Avian	E
11/1/99	1800	WCC79	26396	Dog	V
11/1/99	1800	WCC79	26395	Human	E
11/1/99	1800	WCC79	26393	–	n.a.
11/1/99	1745	WCC80	26399	Avian	E
11/1/99	1745	WCC80	26397	Avian	E
11/1/99	1745	WCC80	26398	Dog	E
11/1/99	1745	WCC80	26400	–	n.a.
11/1/99	2015	WCC81	26403	Avian	n.r.
11/1/99	2015	WCC81	26401	Human	E
11/1/99	2015	WCC81	26404	Raccoon	E
11/1/99	2015	WCC81	26402	–	n.a.
11/1/99	920	WCC82	26408	Poultry	V
11/1/99	920	WCC82	26406	Poultry	V

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
11/1/99	920	WCC82	26407	Sea Gull	n.r.
11/1/99	920	WCC82	26405	–	n.a.
11/1/99	1400	WCC83	26409	Bovine	E, V
11/1/99	1400	WCC83	26412	Bovine	E, V
11/1/99	1400	WCC83	26410	Dog	E
11/1/99	1400	WCC83	26411	Human	E
11/1/99	1200	WCC84	26436	Avian	E
11/1/99	1200	WCC84	26433	Avian	E
11/1/99	1200	WCC84	26435	Bovine	E, V
11/1/99	1200	WCC84	26434	Dog	E
11/1/99	1225	WCC85	26437	Avian	E
11/1/99	1225	WCC85	26440	Deer	E
11/1/99	1225	WCC85	26438	Human	E
11/1/99	1225	WCC85	26439	Human	E
12/6/99	1045	WCC88	26753	Avian	n.r.
12/6/99	1045	WCC88	26756	Bovine	E
12/6/99	1045	WCC88	26755	Goose	E
12/6/99	1045	WCC88	26754	–	n.a.
12/6/99	1050	WCC89	26758	Avian	E
12/6/99	1050	WCC89	26759	Bovine	n.r.
12/6/99	1050	WCC89	26760	–	n.a.
12/6/99	1050	WCC89	26757	–	n.a.
12/6/99	1055	WCC90	26763	Avian	E
12/6/99	1055	WCC90	26762	Bovine	E, V
12/6/99	1055	WCC90	26761	Horse	E
12/6/99	1055	WCC90	26764	Horse	E
12/6/99	1100	WCC91	26765	Avian	E
12/6/99	1100	WCC91	26768	Bovine	E, V
12/6/99	1100	WCC91	26766	Bovine	E, V
12/6/99	1100	WCC91	26767	Deer	E
12/6/99	1105	WCC92	26769	Human	E
12/6/99	1105	WCC92	26770	–	n.a.
12/6/99	1105	WCC92	26771	–	n.a.
12/6/99	1105	WCC92	26772	–	n.a.
12/6/99	1110	WCC93	26773	Bovine	E
12/6/99	1110	WCC93	26774	Deer	E
12/6/99	1110	WCC93	26775	Feline	E
12/6/99	1110	WCC93	26776	–	n.a.
12/6/99	1115	WCC94	26777	Dog	E
12/6/99	1115	WCC94	26780	Feline	E
12/6/99	1115	WCC94	26779	Sludge	n.r.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
12/6/99	1115	WCC94	26778	–	n.a.
12/6/99	1120	WCC95	26784	Bovine	E, V
12/6/99	1120	WCC95	26783	Bovine	E, V
12/6/99	1120	WCC95	26782	Poultry	V
12/6/99	1120	WCC95	26781	–	n.a.
12/6/99	1120	WCC95B	26787	Bovine	E, V
12/6/99	1120	WCC95B	26786	Bovine	E, V
12/6/99	1120	WCC95B	26788	Feline	E
12/6/99	1120	WCC95B	26785	Poultry	V
12/6/99	1120	WCC95C	26789	Avian	n.r.
12/6/99	1120	WCC95C	26791	Bovine	E, V
12/6/99	1120	WCC95C	26792	Bovine	E, V
12/6/99	1120	WCC95C	26790	–	n.a.
1/18/00	1100	WCC96	27132	Turkey	E, V
1/18/00	1100	WCC96	27133	Avian	E
1/18/00	1100	WCC96	27134	Human	E
1/18/00	1100	WCC96	27135	Dog	n.r.
1/18/00	1105	WCC97	27136	Avian	E
1/18/00	1105	WCC97	27137	–	n.a.
1/18/00	1105	WCC97	27138	Bovine	E
1/18/00	1105	WCC97	27139	Avian	E
1/18/00	1110	WCC98	27140	–	n.a.
1/18/00	1110	WCC98	27141	–	n.a.
1/18/00	1110	WCC98	27142	Avian	E
1/18/00	1110	WCC98	27143	–	n.a.
1/18/00	1115	WCC99	27144	–	n.a.
1/18/00	1115	WCC99	27145	–	n.a.
1/18/00	1115	WCC99	27146	–	n.a.
1/18/00	1115	WCC99	27147	Human	n.r.
1/18/00	1120	WCC100	27148	Beaver	V
1/18/00	1120	WCC100	27149	–	n.a.
1/18/00	1120	WCC100	27150	–	n.a.
1/18/00	1120	WCC100	27151	–	n.a.
1/18/00	1125	WCC101	27152	–	n.a.
1/18/00	1125	WCC101	27153	–	n.a.
1/18/00	1125	WCC101	27154	–	n.a.
1/18/00	1125	WCC101	27155	–	n.a.
1/18/00	1130	WCC102	27156	–	n.a.
1/18/00	1130	WCC102	27157	–	n.a.
1/18/00	1130	WCC102	27158	Horse	E
1/18/00	1130	WCC102	27159	Avian	n.r.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
1/18/00	1135	WCC103	27160	–	n.a.
1/18/00	1135	WCC103	27161	–	n.a.
1/18/00	1135	WCC103	27162	–	n.a.
1/18/00	1135	WCC103	27163	–	n.a.
3/20/00	1530	WCC105	27893	Avian	n.r.
3/20/00	1530	WCC105	27894	Raccoon	E
3/20/00	1530	WCC105	27895	–	n.a.
3/20/00	2010	WCC106	27898	Avian	n.r.
3/20/00	2010	WCC106	27897	Poultry	E, V
3/20/00	2010	WCC106	27896	–	n.a.
3/21/00	600	WCC107	27900	Feline	E, V
3/21/00	600	WCC107	27899	Raccoon	E
3/21/00	600	WCC107	27901	Turkey	V
3/21/00	800	WCC108	27903	Avian	n.r.
3/21/00	800	WCC108	27902	Bovine	E
3/21/00	800	WCC108	27904	–	n.a.
3/21/00	950	WCC109	27907	Avian	E
3/21/00	950	WCC109	27905	Horse	E, V
3/21/00	950	WCC109	27906	–	n.a.
3/21/00	1100	WCC110	27908	Human	n.r.
3/21/00	1100	WCC110	27910	–	n.a.
3/21/00	1100	WCC110	27909	–	n.a.
3/21/00	1115	WCC111	27912	Horse	E
3/21/00	1115	WCC111	27913	Human	E
3/21/00	1115	WCC111	27911	–	n.a.
3/21/00	1140	WCC112	27914	Human	n.r.
3/21/00	1140	WCC112	27915	–	n.a.
3/21/00	1140	WCC112	27916	–	n.a.
3/21/00	1335	WCC113	27919	Avian	E
3/21/00	1335	WCC113	27917	Human	n.r.
3/21/00	1335	WCC113	27918	–	n.a.
3/21/00	1815	WCC114	27922	Avian	E
3/21/00	1815	WCC114	27920	Avian	E
3/21/00	1815	WCC114	27921	Feline	E
3/30/00	940	WCC127	28006	Avian	E
3/30/00	940	WCC127	28005	–	n.a.
3/30/00	945	WCC128	28007	Avian	E
3/30/00	945	WCC128	28008	–	n.a.
3/30/00	945	WCC128	28009	–	n.a.
3/30/00	950	WCC129	28011	Avian	E
3/30/00	950	WCC129	28010	Horse	n.r.

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
3/30/00	950	WCC129	28012	–	n.a.
3/30/00	955	WCC130	28014	Deer	E
3/30/00	955	WCC130	28013	–	n.a.
3/30/00	955	WCC130	28015	–	n.a.
3/30/00	955	WCC130B	28019	Chicken	E
3/30/00	955	WCC130B	28016	Dog	E
3/30/00	955	WCC130B	28017	Dog	E
3/30/00	955	WCC130C	28020	Deer	E
3/30/00	955	WCC130C	28022	Dog	E
3/30/00	955	WCC130C	28021	–	n.a.
4/24/00	2115	WCC131	29466	Avian	E
4/24/00	2115	WCC131	29467	Dog	E, V
4/24/00	2115	WCC131	29468	–	n.a.
4/25/00	800	WCC134	29471	–	n.a.
4/25/00	800	WCC134	29470	–	n.a.
4/25/00	800	WCC134	29469	–	n.a.
4/25/00	1310	WCC136	29474	Avian	E
4/25/00	1310	WCC136	29473	Septage	n.r.
4/25/00	1310	WCC136	29472	Septage	n.r.
4/25/00	1400	WCC138	29475	Turkey	E
4/25/00	1400	WCC138	29477	–	n.a.
4/25/00	1400	WCC138	29476	–	n.a.
4/25/00	1430	WCC139	29479	Bovine	E, V
4/25/00	1430	WCC139	29480	Bovine	E, V
4/25/00	1430	WCC139	29478	–	n.a.
4/25/00	1500	WCC140	29482	Avian	E
4/25/00	1500	WCC140	29481	Human	V
4/25/00	1500	WCC140	29483	Turkey	V
4/25/00	1530	WCC143	29485	Dog	E
4/25/00	1530	WCC143	29486	Turkey	V
4/25/00	1530	WCC143	29484	–	n.a.
4/25/00	2200	WCC144	29489	Feline	E
4/25/00	2200	WCC144	29488	Poultry	E, V
4/25/00	2200	WCC144	29487	Turkey	E, V
4/25/00	900	WCC145	29490	Raccoon	E
4/25/00	900	WCC145	29491	–	n.a.
4/25/00	900	WCC145	29492	–	n.a.
4/25/00	1230	WCC148	29493	Human	E
4/25/00	1230	WCC148	29495	Septage	n.r.
4/25/00	1230	WCC148	29494	–	n.a.
5/10/00	945	WCC151	30211	Bovine	E, V

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
5/10/00	945	WCC151	30213	Goose	E
5/10/00	945	WCC151	30212	–	n.a.
5/10/00	950	WCC152	30214	–	n.a.
5/10/00	950	WCC152	30215	–	n.a.
5/10/00	950	WCC152	30216	–	n.a.
5/10/00	955	WCC153	30217	Crow	E
5/10/00	955	WCC153	30218	–	n.a.
5/10/00	955	WCC153	30219	–	n.a.
5/10/00	955	WCC153B	30222	Bovine	E
5/10/00	955	WCC153B	30220	Deer	E
5/10/00	955	WCC153B	30221	–	n.a.
5/10/00	955	WCC153C	30225	Bovine	E
5/10/00	955	WCC153C	30224	–	n.a.
5/10/00	955	WCC153C	30223	–	n.a.
6/20/00	1135	WCC156	32012	Bovine	V
6/20/00	1135	WCC156	32011	Dog	E
6/20/00	1135	WCC156	32010	Duck	E
6/20/00	1140	WCC157	32014	Avian	E, V
6/20/00	1140	WCC157	32013	Crow	n.r.
6/20/00	1145	WCC158	32015	Avian	E
6/20/00	1145	WCC158	32016	Human	E
6/20/00	1145	WCC158B	32018	Human	E
6/20/00	1145	WCC158B	32017	–	n.a.
6/20/00	1145	WCC158B	32019	–	n.a.
6/20/00	1145	WCC158C	32021	Avian	E
6/20/00	1145	WCC158C	32022	Feline	E
6/20/00	1145	WCC158C	32020	–	n.a.
6/27/00	1625	WCC160	33106	–	n.a.
6/27/00	1625	WCC160	33104	–	n.a.
6/27/00	1625	WCC160	33105	–	n.a.
6/27/00	852	WCC162	33107	–	n.a.
6/27/00	915	WCC163	33108	Fox	n.r.
6/27/00	915	WCC163	33109	Fox	n.r.
6/27/00	2020	WCC165	33110	Bovine	E
6/27/00	2020	WCC165	33111	Goat	E
6/27/00	2020	WCC165	33112	Human	n.r.
6/27/00	2130	WCC166	33114	Deer	E
6/27/00	2130	WCC166	33113	–	n.a.
6/27/00	2215	WCC167	33115	Avian	E
6/27/00	2215	WCC167	33116	–	n.a.
6/27/00	30	WCC169	33117	Dog	V

Appendix 1. Bacterial source tracking data from streamwater samples collected March 1999 through October 2000 at the stream gages on Accotink Creek, Blacks Run, and Christians Creek, Virginia—Continued

[Stream-gage locations are in figures 2-4. For each water sample, 1-5 *E. coli* isolates were ribotyped]

Source library used: E, University of Washington Microbial Source Tracking Laboratory's large available source library; V, Virginia-specific source library, which included source isolates collected during this study; n.r., source library used was not recorded; n.a., not applicable.

Source: For purposes of data analysis, feline was considered to be cats; canine was considered to be dogs; septage was considered to be human, whereas sludge, digested sludge, combined sewer overflow, and sanitary sewer were treated as individual categories. Transient, isolates that have been observed in more than one animal species; –, isolate that could not be matched to a specific source

Sample date	Sample time	Sample identifier	*E. coli* isolate number	Source	Source library used
6/27/00	30	WCC169	33118	Human	E, V
6/27/00	130	WCC170	33119	Bovine	E
6/27/00	130	WCC170	33121	Human	n.r.
6/27/00	130	WCC170	33120	–	n.a.
6/27/00	430	WCC171	33122	Horse	E
8/3/00	820	WCC183	34511	Deer	E
8/3/00	820	WCC183	34509	–	n.a.
8/3/00	820	WCC183	34510	–	n.a.
8/3/00	825	WCC184	34512	Deer	E
8/3/00	825	WCC184	34514	Deer	E
8/3/00	825	WCC184	34513	Deer	E
8/3/00	825	WCC185	34517	Avian	E
8/3/00	825	WCC185	34516	–	n.a.
8/3/00	825	WCC185	34515	–	n.a.
8/3/00	830	WCC186	34520	Horse	E
8/3/00	830	WCC186	34518	–	n.a.
8/3/00	830	WCC186	34519	–	n.a.
8/3/00	835	WCC187	34521	Avian	E
8/3/00	835	WCC187	34523	Bovine	E, V
8/3/00	835	WCC187	34522	–	n.a.
8/3/00	835	WCC188	34524	Avian	E
8/3/00	835	WCC188	34526	Human	E
8/3/00	835	WCC188	34527	Raccoon	n.r.
8/3/00	835	WCC188	34525	Raccoon	n.r.