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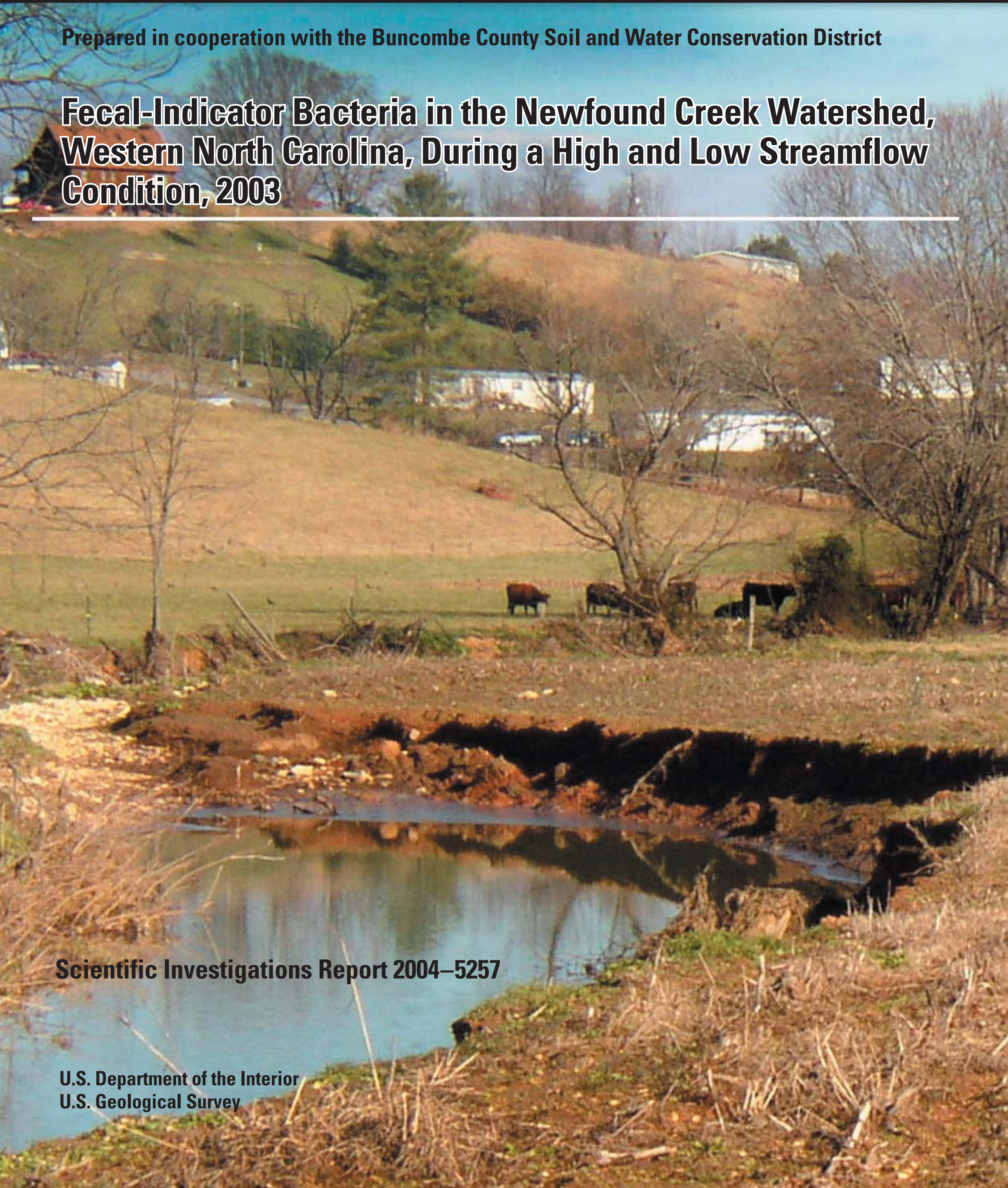
Fecal-Indicator Bacteria in the Newfound Creek Watershed, Western North Carolina, During a High and Low Streamflow Condition, 2003



Scientific Investigations Report 2004–5257

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

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By Elise M. Giddings and Carolyn J. Oblinger

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Gale A. Norton, Secretary

U.S. Geological Survey
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Inch/Pound to SI

Multiply	By	To obtain
	Flow rate	
foot per second (ft/s)	0.3048	meter per second (m/s)
million gallons per day (Mgal/d)	0.04381	cubic meter per second (m^3/s)

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

$$°F = (1.8 \times °C) + 32$$

Vertical coordinate information is referenced to the North American Vertical Datum of 1988 (NAVD 88).

Horizontal coordinate information is referenced to the North American Datum of 1983 (NAD 83).

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25 °C).

Except for measurements of streamflow, values given in this report are in metric units, which can be converted to standard international units by using the following conversion factors:

SI to Inch/Pound

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
kilometer (km)	0.6214	mile (mi)
	Area	
square kilometer (km^2)	0.3861	square mile (mi^2)
	Volume	
liter (L)	33.82	ounce, fluid (fl. oz)
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)

Abbreviations and Acronyms:

CFU/100 mL	colony-forming units per 100 milliliters
CFU/GDW	colony-forming units per gram of dry weight
E. coli	*Escherichia coli*
F+RNA	male-specific RNA coliphage
NC	North Carolina
NCDENR–DWQ	North Carolina Department of Environment and Natural Resources, Division of Water Quality
NPDES	National Pollutant Discharge Elimination System
PFU/L	plaque-forming units per liter
SWCD	Soil and Water Conservation District
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
VWIN	Volunteer Water Information Network

Fecal-Indicator Bacteria in the Newfound Creek Watershed, Western North Carolina, During a High and Low Streamflow Condition, 2003

By Elise M. Giddings and Carolyn J. Oblinger

Abstract

Water quality in the Newfound Creek watershed has been shown to be affected by bacteria, sediment, and nutrients. In this study, *Escherichia coli* (*E. coli)* bacteria were sampled at five sites in Newfound Creek and five tributary sites during low flow on May 28, 2003, and high flow on November 19, 2003. In addition, a subset of five sites was sampled for fecal coliform bacteria, *E. coli* bacteria in streambed sediments (low flow only), and coliphage virus for serotyping. Coliphage virus serotyping has been used to identify human and animal sources of bacterial contamination. A streamflow gage was installed and operated to support ongoing water-quality studies in the watershed.

Fecal coliform densities ranged from 92 to 27,000 colony-forming units per 100 milliliters of water for *E. coli* and 140 to an estimated 29,000 colony-forming units per 100 milliliters of water for fecal coliform during the two sampling visits. Ninety percent of the *E. coli* and fecal coliform samples exceeded corresponding U.S. Environmental Protection Agency or North Carolina water-quality criteria for recreational and ambient waters. During low flow, the middle part of the Newfound Creek watershed and the Dix Creek tributary had the highest densities of *E. coli* bacteria. During the high-flow sampling, all tributaries contained high densities of *E. coli* bacteria, although Dix Creek and Round Hill Branch were the largest contributors of these bacteria to Newfound Creek.

Coliphage virus serotyping results were inconclusive because most samples did not contain the male-specific RNA coliphage needed for serotyping. Positive results indicated, however, that during low flow, non-human sources of bacteria were present in Sluder Branch, and during high flow, human sources of bacteria were present in Round Hill Branch. Sampling of bacteria in streambed sediments during low flow indicated that sediments do not appear to be a substantial source of bacteria relative to the water column, with the exception of an area near the confluence of Sluder Branch and Newfound Creek.

Introduction

Newfound Creek, in the Blue Ridge Physiographic Province of western North Carolina (fig. 1), is listed by the North Carolina Department of Environment and Natural Resources, Division of Water Quality (NCDENR-DWQ), as impaired due to fecal bacteria contamination (North Carolina Division of Water Quality, 2003b). In previous assessments of water quality in Newfound Creek, stream impairment was noted, resulting from sediment, fecal-coliform bacteria, and nutrient enrichment (North Carolina Division of Water Quality, 2003a, 2003b).

North Carolina water-quality standards for bacteria state that fecal coliform densities are not to exceed a geometric mean of 200 colony-forming units per 100 milliliters (CFU/100 mL) based on at least five consecutive samples examined during any 30-day period, and are not to exceed 400 CFU/100 mL in more than 20 percent of the samples examined during the same period (North Carolina Division of Water Quality, 2003c). Periodic water-quality monitoring by volunteers in the Volunteer Water Information Network (VWIN), a program of the Environmental Quality Institute of The University of North Carolina at Asheville, and by Buncombe County staff detected fecal coliform counts that exceeded the criterion of 400 CFU/100 mL, although the sampling frequency was not great enough to determine violation of the water-quality standard (Maas and others, 1998; Kara Cassels, Buncombe County Soil and Water Conservation, written commun., 2004). These studies have increased awareness of fecal-origin bacteria exceedances and have increased interest in identifying source areas where efforts to establish best-management practices should be focused.

Activities in the watershed that may be potential sources of fecal coliform bacteria contamination are related to human and animal non-point sources, such as animal grazing in riparian areas, agriculture, and non-urban development, which includes failing or substandard suburban and rural residential septic systems (North Carolina Division of Water Quality, 2003b). Several dairy farmers in the watershed, with assistance from the

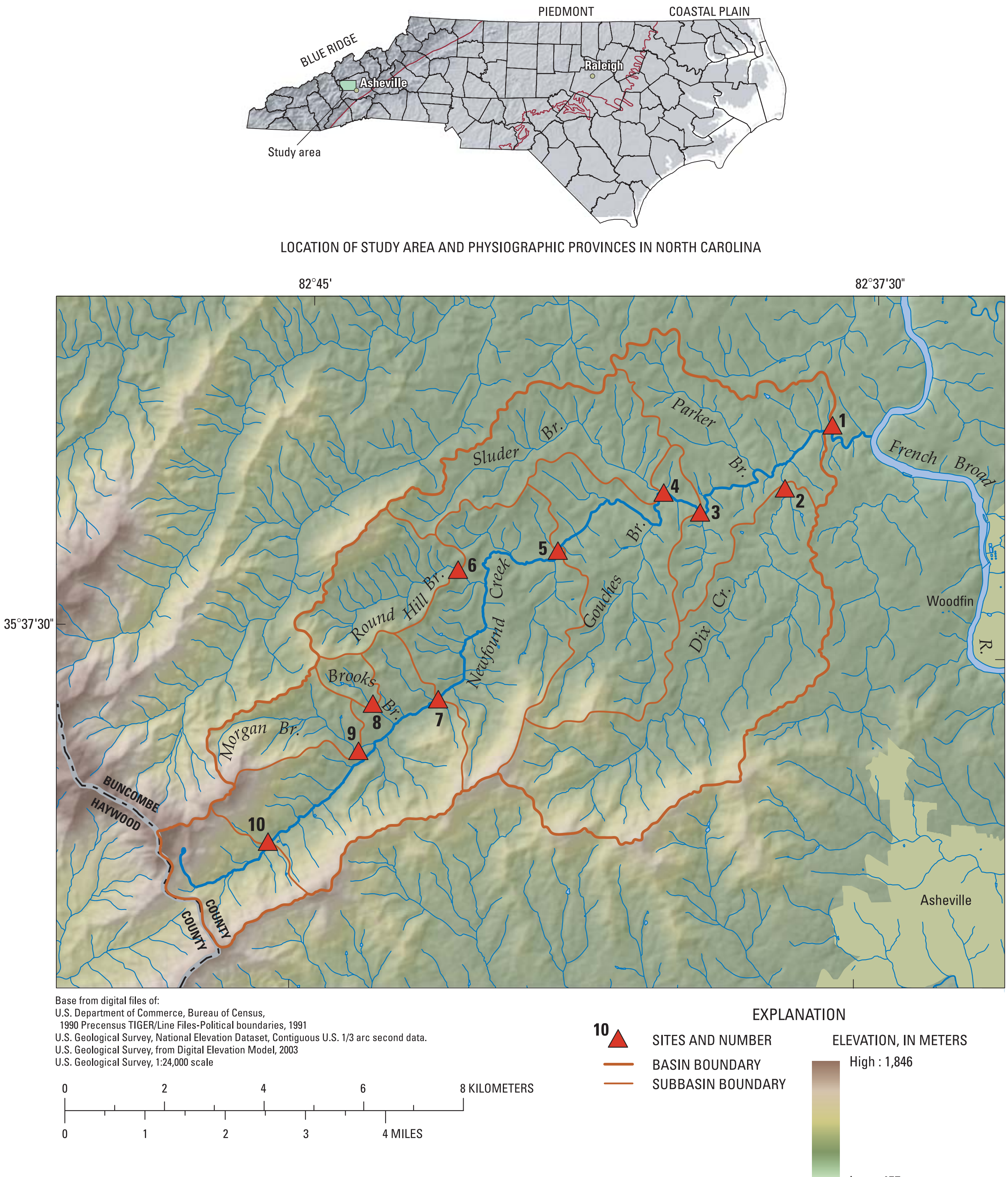

Figure 1. Locations of study sites in the Newfound Creek watershed, North Carolina (site names are in table 1).

Buncombe County Soil and Water Conservation District (SWCD), have voluntarily implemented or are in the process of implementing best-management practices to restrict animals from stream channels and to catch stormwater runoff from animal confinement areas. This has resulted in noticeable water-quality improvements since 1995 (North Carolina Division of Water Quality, 2003a). Elevated counts of fecal bacteria remain a problem, however, and at some locations, no clear source(s) for elevated fecal coliform densities can be identified.

Description of Study Area

Newfound Creek is a tributary to the French Broad River in the southern Appalachian Mountains of western North Carolina. The headwaters of Newfound Creek, which has a drainage area of 89.6 square kilometers, lie west of Asheville, in Buncombe County near the Buncombe-Haywood County line. The creek flows approximately 30 kilometers north and northeast into the French Broad River. Major tributaries to Newfound Creek include Gouches Branch, Dix Creek, Sluder Branch, and Parker Branch (fig. 1). Land cover in the Newfound Creek watershed is approximately 50 percent agricultural, 40 percent forested, and 10 percent residential/commercial (Chris Roessler, North Carolina Division of Water Quality, written commun., 1998; fig. 2). Pasture and agricultural land cover is present throughout the watershed, generally in close proximity to Newfound Creek and its tributaries. Residential development is primarily in the eastern part of the watershed.

The Newfound Creek watershed has only two point-source dischargers that require National Pollutant Discharge Elimination System (NPDES) permits (North Carolina Division of Water Quality, 2004). Both are minor dischargers (less than 1 million gallons per day) of domestic wastewater; one discharges to Sluder Branch and the other to Dix Creek.

Purpose and Scope

In October 2000, the U.S. Geological Survey (USGS) entered into an agreement with the Buncombe County SWCD to assess fecal-bacterial contamination in the Newfound Creek watershed. Identification of contaminant-source areas and types could assist Buncombe County SWCD in prioritizing areas in the watershed for restoration and implementation of best-management practices. The objectives of the cooperative investigation between the USGS and Buncombe County SWCD were to (1) measure and record streamflow near the mouth of Newfound Creek to support ongoing water-quality monitoring efforts in the watershed, (2) measure fecal coliform and *Escherichia coli* (*E. coli*) bacteria densities at 10 sites in the Newfound Creek watershed during a period of low flow and during a period of high flow to aid in the identification of source areas, and (3) attempt to distinguish between animal and human sources of fecal contamination by sampling and serotyping coliphage viruses. This report presents the results of the bacterial and coliphage sampling investigations.

Bacterial Contaminants Analyzed

E. coli bacteria are members (a subset) of the fecal coliform group; that is, all *E. coli* bacteria are fecal coliform bacteria but not all fecal coliform bacteria are *E. coli*. The presence of *E. coli* in water or sediment is direct evidence of fecal contamination from warm-blooded animals. In 1986, the U.S. Environmental Protection Agency (USEPA) recommended the use of *E. coli,* rather than fecal coliform, as the bacterial indicator for surface-water monitoring in recreational waters (U.S. Environmental Protection Agency, 1986), and established criteria ranging from 235 CFU/100 mL in a single sample from a designated beach area to 576 CFU/100 mL in a single sample from a water body that is infrequently used for full-body contact recreation. North Carolina continues to use fecal coliform as the indicator to determine bacterial water quality in ambient and recreational waters. As previously stated, North Carolina (NC) standards indicate that fecal coliform densities are not to exceed a geometric mean of 200 CFU/100 mL based on at least five consecutive samples examined during any 30-day period, and are not to exceed 400 CFU/100 mL in more than 20 percent of the samples examined during the same period (North Carolina Division of Water Quality, 2003c). Fecal coliform bacteria data have been collected in the Newfound Creek watershed by the VWIN and NCDENR-DWQ, but *E. coli* bacteria have not been collected.

Fecal-indicator bacteria can survive for relatively long periods in stream and lake sediments. Bacteria attach to sediment particles and survive in the nutrient-rich environment of the streambed where they are deposited (Gerba and McLeod, 1976; Burton and others, 1987). Lake and streambed sediments can contain densities of fecal-indicator bacteria several times those of the overlying water column (Bromel and others, 1978; Tunnicliff and Brickler, 1984). Streambed sediments in Newfound Creek may provide a reservoir of fecal-indicator bacteria that are deposited from point or non-point sources and then resuspended by physical disturbances, such as high flow.

Coliphage viruses are used sometimes to aid in the identification of sources of fecal contamination. They almost always come from fecal material and are found in high numbers in sewage. Coliphage viruses are considered to be reliable indicators of sewage contamination (International Association on Water Pollution Research and Control, Study Group on Health Related Water Microbiology, 1991). Serotyping of certain coliphage groups, that is male-specific RNA coliphage (F+RNA), has been used successfully to distinguish human and non-human sources of bacterial contamination (Hsu and others, 1997). There are four groups of F+RNA coliphage. Group I is commonly associated with non-human sources of *E. coli*; group II is associated with fecal material from humans and pigs; group III is strongly associated with human sources of *E. coli*; and

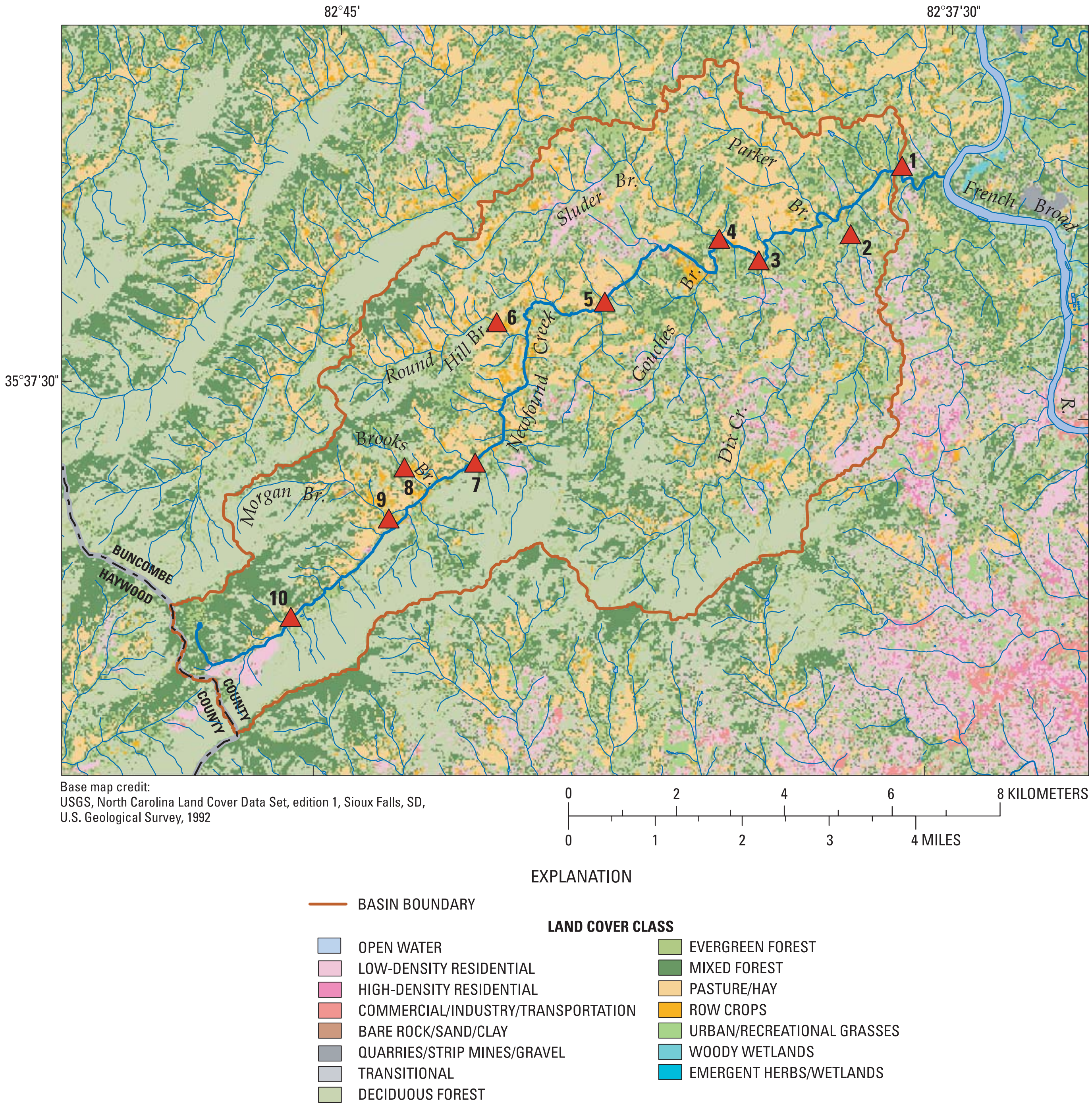

Figure 2. Land cover in the Newfound Creek watershed, North Carolina.

group IV is associated predominantly with animal sources (Simpson and others, 2002). Serogroups I and IV are the most common F+RNA coliphage isolated from cattle and other bovines, and high proportions of these serotypes generally can be used to distinguish animal fecal contamination sources from municipal wastewater sources (Cole and others, 2003).

Methods

A multifaceted approach was taken to assess fecal contamination in the Newfound Creek watershed. Ten sites were selected in the watershed—five in the main stem of Newfound Creek (sites 1, 3, 5, 7, and 10) and five in major tributaries (sites 2, 4, 6, 8, and 9, fig. 1; table 1). Samples were collected and streamflow measurements were made during two different hydrologic conditions—a low-flow condition and a high-flow (storm runoff) condition. The low-flow samples were collected on May 28, 2003, and the high-flow samples were collected on November 19, 2003.

Streamflow and Physical Measurements

A streamflow gage was installed at site 1 (Newfound Creek at Jenkins Valley Road) in December 2000 and has been operating continuously since that time. The daily discharge and summary statistics are published annually in the USGS annual data reports for water years 2000 through 2003[1] (Ragland and others, 2004) and are available online at *http://water.usgs.gov/pubs/wdr/wdr_nc/* . Real-time data also are available online at *http://nc.waterdata.usgs.gov/nwis/uv/* . The purpose of the gaging station and streamflow measurement is to support ongoing water-quality monitoring programs, but those results are not discussed here.

[1]Water year is the period October 1 through September 30 and is identified by the year in which the period ends.

Table 1. Water-quality data-collection sites in the Newfound Creek watershed, North Carolina.

[USGS, U.S.Geological Survey; *E. coli, Escherichia coli* bacteria; SR, secondary road; shaded sites are on tributary streams and unshaded sites are on the main stem of Newfound Creek]

Site (fig. 1)	USGS station number [a]	Site name	Latitude	Longitude	Samples collected and types of analyses			
					Water			Sediment [b]
					E. coli	Fecal coliforms	Coliphage viruses	*E. coli*
1	03451690	Newfound Creek near Alexander (at Jenkins Valley Road)	35° 39'58.4"	82° 38'03.3"	x	x		x
2	0345168045	Dix Creek at SR1622 (old N.C. 20) near Juno	35° 39'16.2"	82° 38'39.3"	x		x	
3	03451662	Newfound Creek at SR1617 (Sluder Branch Road) near Leicester	35° 38'58.5"	82° 39'46.2"	x			x
4	03451661	Sluder Branch at mouth near Leicester	35° 39'10.6"	82° 40'15.9"	x		x	
5	03451658	Newfound Creek at SR1378 (Old Newfound Road) near Leicester	35° 38'30.2"	82° 41'38.5"	x	x	x[c]	x
6	0345165645	Round Hill Branch at SR 1382 (Rabbit Ham Road) near Leicester	35° 38'15.5"	82° 42'57.4"	x	x	x	
7	03451656	Newfound Creek at Browntown Road	35° 36'50.7"	82° 43'09.4"	x	x	x	x
8	0345165593	Brooks Branch above mouth near Newfound	35° 36'46.2"	82° 44'01.4"	x			
9	0345165570	Morgan Branch at SR1220 (Morgan Branch Rd) at Newfound	35° 36'15.2"	82° 44'11.4"	x			
10	0345165540	Newfound Creek at Haylandy Drive near Newfound Gap	35° 35'13.9"	82° 45'20.8"	x	x	x[c]	x

[a]Station number is assigned by the USGS based on geographic location and downstream order.
[b]Sediment samples were collected only at low flow.
[c]Coliphage viruses were analyzed from samples collected at site 5 during low-flow sampling and site 10 during high-flow sampling.

Stream discharge and physical properties of water were measured at each site during the sampling visits. Physical properties, including water temperature, specific conductance, pH, and dissolved oxygen concentration, were measured in situ using a multisensor instrument. Stream discharge was measured using standard USGS protocols (Rantz and others, 1982).

E. coli and Fecal Coliform Bacteria Collection

Water samples were collected from each site by hand dipping a sterile polypropylene bottle or, when streamflow was sufficient, by using a DH-81 sampler to collect a depth- and width- integrated sample in a sterile 1-liter polypropylene bottle (Wilde and others, 1999). Samples were immediately placed on ice in a cooler. Samples were analyzed onsite using membrane filtration methods within 6 hours of collection (Myers and Wilde, 2003). Samples from each site were analyzed for *E. coli* by plating on mTEC media and incubating for 22 to 24 hours, followed by 20 minutes of exposure to urea-phenol substrate broth (Myers and Wilde, 2003). At five sites, samples also were analyzed for fecal coliform by plating on mFC media and incubating for 22 to 24 hours. These sites were selected on the basis of their proximity to locations previously sampled by NCDENR-DWQ and VWIN personnel to enable comparison with previously collected fecal coliform data.

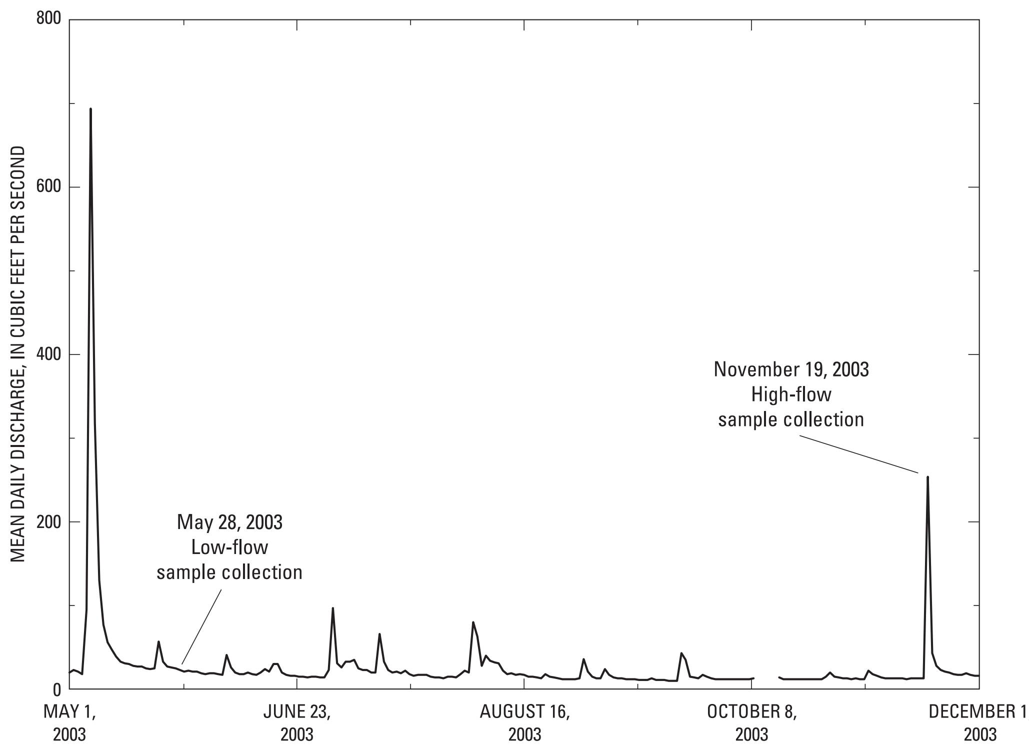

Figure 3. Mean daily streamflow at Newfound Creek near Alexander, North Carolina (site 1), from May 1 to December 1, 2003.

During low flow, streambed-sediment samples were collected from the five Newfound Creek sites (table 1) for enumeration of *E. coli*. Samples of the top 2 centimeters of sediment were collected aseptically from three sediment depositional areas at each site. At each depositional area, at least 50 grams of material was collected in three separate sterile jars, chilled on ice in a cooler, and shipped overnight to the USGS Ohio District Microbiology Laboratory in Columbus, Ohio. Samples from each site were composited and analyzed within 24 hours of collection at the Ohio District Microbiology Laboratory using methods described in Francy and Darner (1998). *E. coli* were incubated on mTEC media and reported as colony-forming units per gram of dry weight (CFU/GDW) of sediment. The results from the bacteria and water-quality samples were published in the USGS annual data report for water year 2003 (Ragland and others, 2004).

The high-flow condition selected for sampling followed a long period of dry weather in which no substantial high-flow events had occurred during the previous 55 days (fig. 3). During the storm, streamflow at site 1 increased from 22 cubic feet per second (ft^3/s) to greater than 915 ft^3/s (fig. 4). Samples were collected after the storm peak as discharge was receding. Sample collection began at the most upstream site and proceeded downstream to enable sampling as near to peak discharge as possible (table 2).

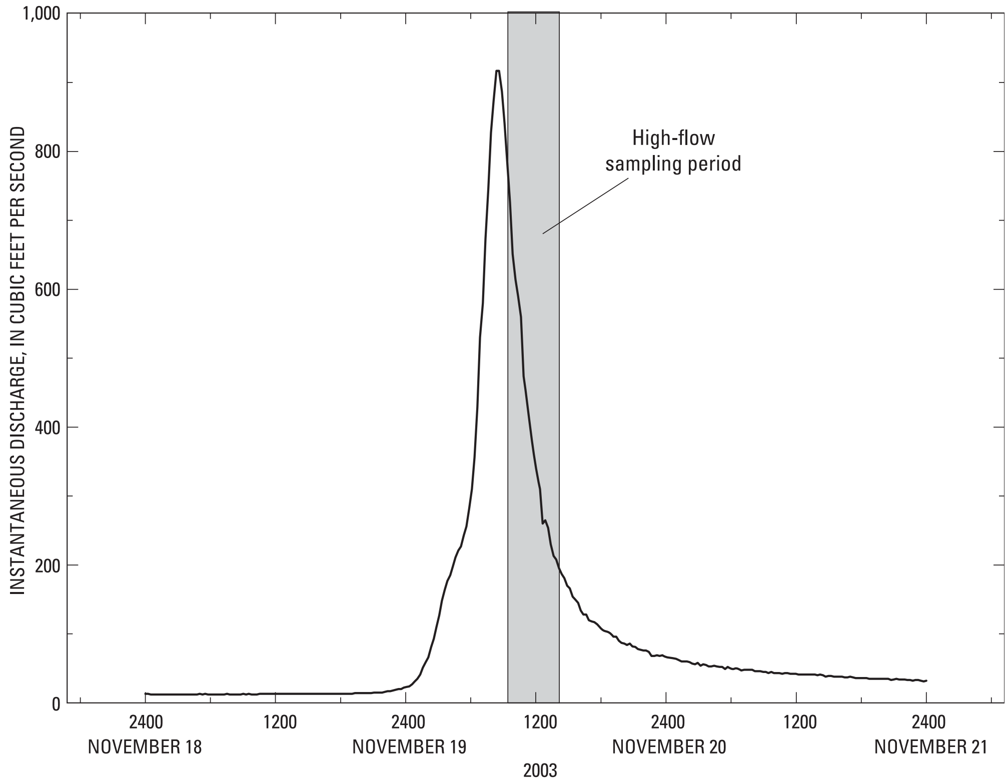

Figure 4. Streamflow discharge during a storm on November 19, 2003, at U.S. Geological Survey stream-gaging station on Newfound Creek near Alexander at Jenkins Valley Road, North Carolina (site 1).

Table 2. Physical properties of water collected at low flow on May 28, 2003, and high flow on November 19, 2003, from sites on Newfound Creek, North Carolina.

[µS/cm, microsiemens per centimeter at 25º Celsius; ºC, degrees Celsius; mg/L, milligrams per liter; shaded sites are on tributary streams and unshaded sites are on the main stem of Newfound Creek]

Site (fig. 1)	Site name	Sample-collection time		Specific conductance (µS/cm)		Water temperature (ºC)		pH (standard units)		Dissolved oxygen (mg/L)	
		Low flow	High flow	Low flow	High flow	Low flow	High flow	Low flow	High flow	Low flow	High flow
1	Newfound Creek at Jenkins Valley Road	0840	1410	77	105	14.3	14.8	7.2	7.9	9.7	8.2
2	Dix Creek at old N.C. 20	0945	1355	68	66	13.8	14.9	7.2	8.6	10.0	8.6
3	Newfound Creek at Sluder Branch Road	1035	1320	98	110	15.3	15.0	7.4	6.2	10.5	8.2
4	Sluder Branch at mouth	1100	1252	114	117	15.2	15.0	7.4	6.5	10.1	8.1
5	Newfound Creek at Old Newfound Road	1145	1152	86	111	15.7	14.4	7.4	6.6	10.3	8.5
6	Round Hill Branch at Rabbit Ham Road	1215	1230	153	135	18.1	14.8	7.6	6.8	10.2	8.4
7	Newfound Creek at Browntown Road	1240	1102	66	85	15.8	13.9	7.1	6.8	10.1	8.6
8	Brooks Branch above mouth	1315	1040	103	94	16.3	13.8	7.2	6.7	9.5	8.7
9	Morgan Branch at Morgan Branch Road	1325	1020	82	116	16.7	13.7	7.2	6.7	9.5	8.8
10	Newfound Creek at Haylandy Drive	1345	0955	54	80	15.0	13.1	7.0	6.4	10.1	9.2
Range				54–153	66–135	13.8–18.1	13.1–15.0	7.0–7.6	6.2–8.6	9.5–10.5	8.1–9.2
Median				84	107	15.5	14.6	7.2	6.7	10.1	8.6

Coliphage Virus Collection

Samples for analysis of coliphage viruses in water were collected at five sites during each sampling visit (table 1). Samples were collected in 4-liter sterile bottles and immediately chilled on ice in coolers. Coolers were delivered to the Environmental Virology Laboratory at The University of North Carolina at Chapel Hill within 24 hours of sample collection. At the laboratory, water samples were concentrated by membrane filtration-elution (Sobsey and others 1990) and analyzed for the presence of F+RNA coliphage by using the single-agar layer USEPA method 1602 (U.S. Environmental Protection Agency, 2001). Up to 10 of the isolated F+RNA coliphage were then serotyped as described by Hsu and others (1995). Serotyping of 10 coliphage isolates from each sample was expected to provide a reasonable selection of the variety of coliphage serotypes present in a given sample. Previous samples analyzed by the Environmental Virology Laboratory indicated that in the majority of cases, all of the isolates from a single sample were of the same serogroup (Douglas Wait, The University of North Carolina, Environmental Virology Laboratory, written commun., 2003).

Streamflow and Physical Water-Quality Properties

Measurements of temperature, pH, and dissolved oxygen in streams in the Newfound Creek basin (table 2) were typical of these same measurements in other streams in the western part of North Carolina. Specific conductance values were elevated over background conditions typical of Appalachian streams, which generally are below 20 microsiemens per centimeter (µS/cm) at 25 degrees Celsius. Water-quality measurements collected by the NCDENR-DWQ in summer 2002 indicated that specific conductance in Newfound Creek was higher than in other streams in the French Broad River basin (North Carolina Division of Water Quality, 2003a), and Newfound Creek was in the highest 20 percent of the specific conductance values at sites sampled by the VWIN program (Maas and others, 1998). Specific conductance is a general water-quality indicator, and high levels may be a result of sediments, nutrients, or other dissolved constituents. Because sediments and nutrients have been implicated as potential causes of degradation in the Newfound Creek watershed (Maas and others, 1998), the high specific conductance values observed support the case for further investigation of contaminant sources in the Newfound Creek watershed.

Significant differences were observed in temperature, pH, and dissolved oxygen measurements between the two sampling periods (Wilcoxon rank sum test; $p<0.05$). For temperature and dissolved oxygen, the differences likely are a result of the different seasonal conditions. For pH, the difference likely was a result of the lower pH in precipitation in the runoff samples collected in November 2003.

Fecal-Indicator Bacteria in Streamwater

Fecal coliform densities ranged from 92 to 27,000 CFU/100 mL for *E. coli* and from 140 to an estimated 29,000 CFU/100 mL for fecal coliform in stream samples from the Newfound Creek watershed (table 3). At low flow, *E. coli* densities were highest at the mouth of Dix Creek (site 2), an

Table 3. Fecal-indicator bacteria densities and stream discharge collected at low flow on May 28, 2003, and high flow on November 19, 2003, in the Newfound Creek watershed, North Carolina.

[*E. coli, Escherichia coli* bacteria; CFU/100 mL, colony-forming units per 100 milliliters; ft^3/s, cubic feet per second; —, not collected; e, estimated; >, greater than; shaded sites are on tributary streams and unshaded sites are on the main stem of Newfound Creek]

Site (fig. 1)	Site name	*E. coli* (CFU/100 mL)		Fecal coliform (CFU/100 mL)		Discharge (ft^3/s)	
		Low flow	High flow	Low flow	High flow	Low flow	High flow
1	Newfound Creek at Jenkins Valley Road	1,300	27,000	930	24,000	22.0	195
2	Dix Creek at old N.C. 20	3,100	11,000	—	—	6.21	35.8
3	Newfound Creek at Sluder Branch Road	820	14,000	—	—	19.7	118
4	Sluder Branch at mouth	400	9,100	—	—	1.81	21.1
5	Newfound Creek at Old Newfound Road	1,100	22,000	1,400	29,000e	13.8	88.4
6	Round Hill Branch at Rabbit Ham Road	130	20,000	140	>6,000	1.16	15.0
7	Newfound Creek at Browntown Road	2,400	18,000	8,700	11,000	9.17	51.1
8	Brooks Branch above mouth	92	4,500	—	—	0.16	2.78
9	Morgan Branch at Morgan Branch Road	1,800	16,000	—	—	1.29	9.79
10	Newfound Creek at Haylandy Drive	670	4,700	1,300	2,900	2.70	13.5

area dominated by residential development, and at Newfound Creek at Browntown Road (site 7), which is downstream from a pasture area with heavy animal use (table 3; fig. 5). Morgan Branch (site 9) also had high *E. coli* densities even though samples were collected upstream from a known confined animal area. The tributaries of Brooks Branch (site 8), Round Hill Branch (site 6), and Sluder Branch (site 4) had relatively low densities of *E. coli* at low flow. At all sites except these three, *E. coli* densities exceeded the USEPA single-sample criterion for *E. coli* of 576 CFU/100 mL for a single sample taken from a waterbody used infrequently for full-body contact recreation (U.S. Environmental Protection Agency, 1986). Of the samples analyzed for fecal coliform, all but Round Hill Branch (site 6) had densities much greater than the NC fecal-coliform criterion of 400 CFU/100 mL[2] (table 3). Because the NC water-quality standard for fecal coliform densities is based on a minimum of five samples collected during a 30-day period, these samples by themselves do not indicate an exceedance of the standard. Exceedance of both the USEPA *E. coli* single-sample criterion and the NC fecal coliform criterion, however, is an indication of bacterial contamination.

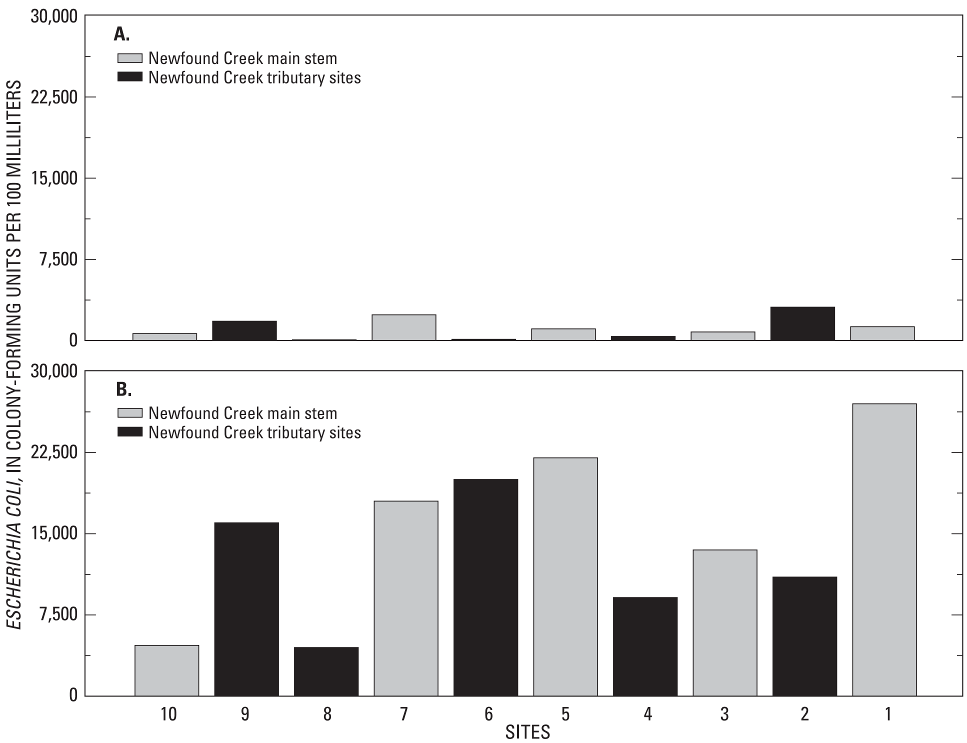

Figure 5. *E. coli* densities during (A) low flow (May 28, 2003) and (B) high flow (November 19, 2003) at Newfound Creek and tributary sites, North Carolina.

During storm runoff, densities of *E. coli* bacteria were one to two orders of magnitude greater than during low flow (table 3; fig. 5). The highest densities were in Newfound Creek at Jenkins Valley Road (site 1), the most downstream site sampled in the watershed, and at Old Newfound Road (site 5). Densities also were high in Newfound Creek at Browntown Road (site 7). All of the tributaries contributed high densities of *E. coli* to the main stem during high flow—most notably Round Hill Branch (site 6), Morgan Branch (site 9), and Dix Creek (site 2). Round Hill Branch had much higher *E. coli* densities during high flow than during low flow. Morgan Branch and Dix Creek had relatively high densities at both low and high flows. Brooks Branch (site 8) had the lowest densities. *E. coli* densities in all of the high-flow samples exceeded the USEPA criterion by more than an order of magnitude.

Fecal-indicator concentrations were adjusted by the amount of flow at each site to assess the relative contributions of sections of the watershed to bacteria transport (table 4). To obtain the transport numbers, the concentration of *E. coli* (CFU/100 mL) at each site was multiplied by the instantaneous discharge, in cubic feet per second, at the site times a volume conversion from milliliter to cubic feet. This resulted in the number of CFU per second passing each site—a flow-weighted comparison of *E. coli* concentration. The number of *E. coli* passing each site per second generally increased in a downstream direction as the flow of Newfound Creek increased (fig. 6). During the low-flow sampling, Dix Creek (site 2) had high densities of *E. coli* relative to the other tributaries sampled (table 4). Dix Creek transported almost six times more *E. coli* to Newfound Creek than all other sampled tributaries combined, although the streamflow in Dix Creek was 1.5 times greater than the combined streamflow (table 3). Morgan Branch (site 9) contributed the second greatest number of *E. coli,* although much less than Dix Creek, as a result of high bacteria densities but low discharge.

During high flow, Dix Creek again contributed the highest number of *E. coli* of the tributary sites, and *E. coli* transport in Round Hill Branch (site 6) was similar to Dix Creek (table 4). Round Hill Branch had high densities of *E. coli* but had lower discharge than Dix Creek (table 3). Sluder Branch (site 4) and Morgan Branch (site 9) contributed similar

[2]North Carolina criterion for class C waters requires that no more than 20 percent of samples in a 30-day period may exceed 400 CFU/100 mL. The State recognizes that violations are likely to occur in stormwater runoff.

Table 4. Total transport of *E. coli* at low flow and high flow from sites in the Newfound Creek watershed, North Carolina.

[*E. coli, Escherichia coli* bacteria; CFU/s, colony-forming units per second; shaded sites are on tributary streams and unshaded sites are on the main stem of Newfound Creek]

Site (fig. 1)	Site name	*E. coli*, in 1,000 CFU/s	
		Low flow	High flow
1	Newfound Creek at Jenkins Valley Road	8,100	1,500,000
2	Dix Creek at old N.C. 20	5,400	110,000
3	Newfound Creek at Sluder Branch Road	4,600	450,000
4	Sluder Branch at mouth	200	54,000
5	Newfound Creek at Old Newfound Road	4,300	550,000
6	Round Hill Branch at Rabbit Ham Road	43	85,000
7	Newfound Creek at Browntown Road	6,200	260,000
8	Brooks Branch above mouth	4	3,500
9	Morgan Branch at Morgan Branch Road	660	44,000
10	Newfound Creek at Haylandy Drive	510	18,000

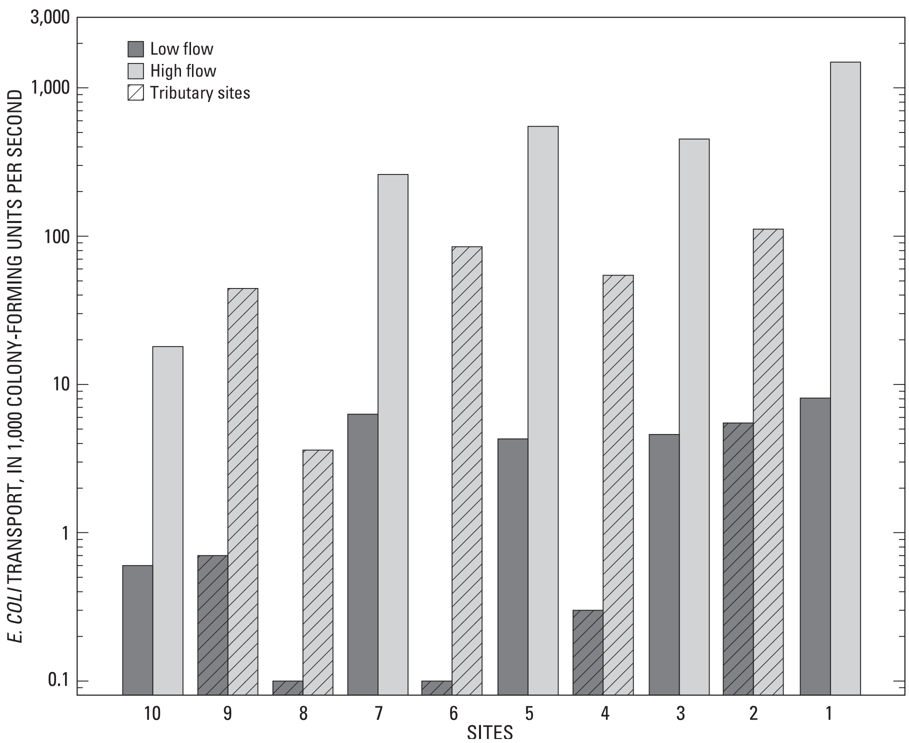

Figure 6. Transport of *E. coli* bacteria in the Newfound Creek watershed, North Carolina, during low-flow and high-flow conditions.

numbers of *E. coli* to Newfound Creek, about half the amounts of Round Hill Branch and Dix Creek. At both low and high flow, Brooks Branch (site 8) contributed the lowest number of *E. coli* to Newfound Creek, because of the very low discharge and relatively low concentrations of *E. coli* in this tributary. Fecal-indicator bacteria concentrations may vary widely through time; therefore, these relative observations may or may not be representative of all low- or high-flow conditions. *E. coli* results during low and high flow correlated well with fecal coliform results (R^2= 0.92; fig. 7), which indicates that current results based on *E. coli* generally can be compared with previous results for fecal coliform.

Fecal coliform data collected from Newfound Creek during 13 sampling trips from April 2003 to December 2003 by Buncombe County SWCD personnel and analyzed at the laboratory at The University of North Carolina at Asheville were compared to data from this study. Two USGS sites are located within one-third of a kilometer (km) of sites sampled by Buncombe County. USGS site 10 is located close to Buncombe County SWCD site 1B, and site 6 is near Buncombe County SWCD site 3B. Fecal coliform bacterial densities in samples collected by the USGS on May 28, 2003, at these two sites were at the low end of the range collected by the Buncombe County SWCD during the rest of 2003 (table 5; Kara Cassels, Buncombe County Soil and Water Conservation District, written commun., August 2004). Fecal coliform densities in samples collected by the USGS on November 19, 2003, were lower for site 10 and similar for site 6 to samples collected by the Buncombe County SWCD on the same day.

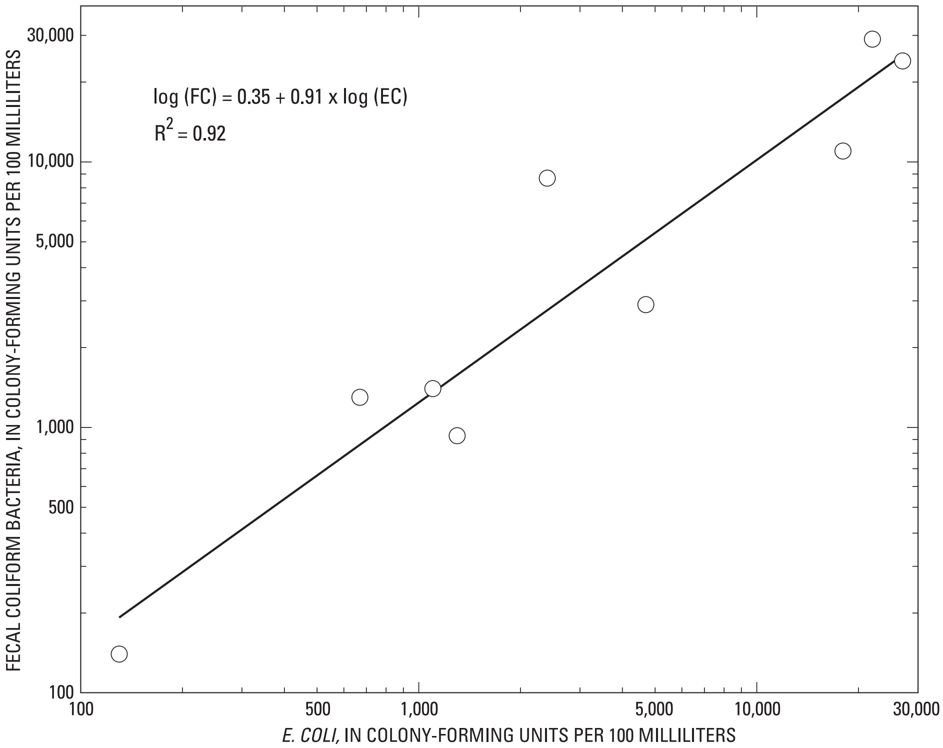

Figure 7. Relation of *E. coli* and fecal coliform density (in colony-forming units per 100 milliliters) in the Newfound Creek watershed, North Carolina.

Table 5. Fecal coliform data collected at comparative sites in the Newfound Creek watershed by the U.S.Geological Survey and Buncombe County Soil and Water Conservation District in 2003.

[USGS, U.S. Geological Survey; ft^3/s, cubic feet per second; CFU/100 mL, colony-forming units per 100 milliliters; SWCD, Buncombe County Soil and Water Conservation District; >, greater than]

Date	Flow at USGS site 1 (ft^3/s)	Fecal coliform (CFU/100 mL)			
		SWCD site 1B	USGS site 10	SWCD site 3B	USGS site 6
April 28	20	1,100		138	
May 28	22		1,300		140
June 12	21	2,017		560	
July 2	75	>11,600		5,500	
July 24	18	1,517		260	
August 6	28	2,100		900	
August 20	14	3,400		270	
September 3	19	1,080		460	
September 15	14	>6,800		1,283	
October 1	13	1,275		235	
October 15	14	7,900		420	
November 5	15	>47,000		>6,000	
November 19	590 *	4,067		>12,000	
November 19	195 *		2,900		>6,000
December 10	39	28,000		10,900	

*Differences in discharge reflect different times of collection on the same day, as discharge rapidly changes during high-flow conditions such as occurred on this day.

Bacteria in Streambed Sediments

Fecal-indicator bacteria can survive for relatively long periods in stream and lake sediments by adsorbing to suspended sediments that are deposited into the stream or lake bed (Burton and others, 1987). Streambed sediments in Newfound Creek may provide a short-term reservoir of fecal-indicator bacteria from point or non-point sources. Resuspension of these sediments and the associated fecal bacteria can occur during physical disturbances, such as high flows caused by stormwater runoff.

The density of *E. coli* in sediments ranged from 390 to 12,000 CFU/GDW and was greater in the middle and lower reaches of Newfound Creek compared with the upper reaches (fig. 8). The high *E. coli* density at Newfound Creek at Sluder Branch Road (site 3), which is in the middle part of the watershed, may be a result of the many animal operations and home sites upstream and the particularly large depositional area at this site where sediments accumulate from upstream sources. The presence of high concentrations of bacteria in sediments indicates that, at least for some period of time, the sediments could be acting as a source of bacteria to the water column during high flows.

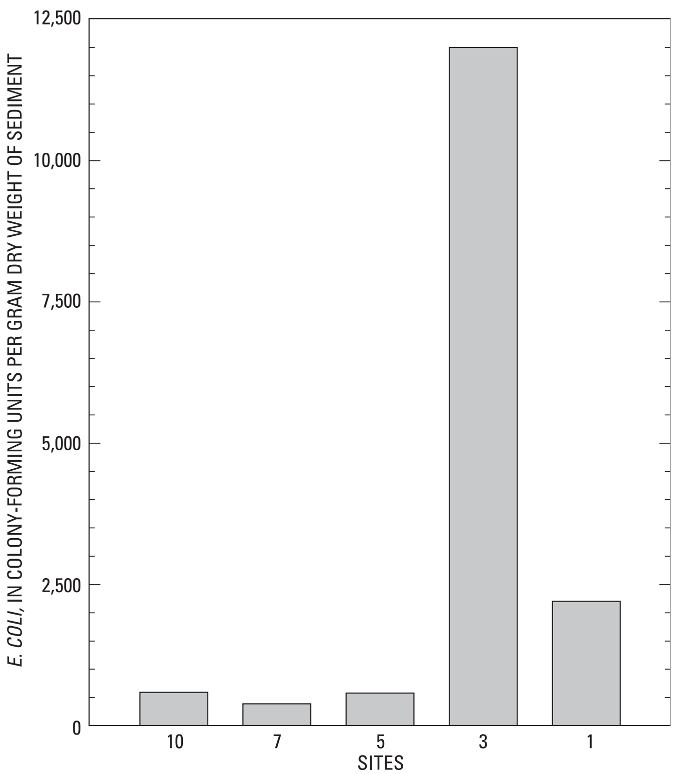

Figure 8. Density of *E. coli* in bed-sediment samples from Newfound Creek, North Carolina.

Coliphage Results

The sites selected for collection of samples for coliphage enumeration and serotyping were located in areas where the contributing sources of bacteria from animals and humans were most uncertain. Coliphage densities ranged from less than 0.5 to 2,168 plaque-forming units per liter (PFU/L; table 6). Densities generally were one to two orders of magnitude greater in the high-flow sample compared to those in the low-flow sample.

Most of the coliphage detected in samples were DNA coliphage. This type of coliphage uses DNA instead of RNA to preserve their genetic code. Little is known about the ecology and serology of DNA coliphage, which also are present in fecal-contaminated water but have not been used to distinguish animal and human sources (Douglas Wait, The University of North Carolina, Environmental Virology Laboratory, written commun., 2003). Due to the absence of F+RNA coliphage, serotyping could only be performed at site 4 during low flow and at site 6 during high flow (table 6). During low flow, group I coliphage, which generally are associated with non-human sources, were identified in Sluder Branch (site 4). During high flow, group III coliphage, which predominately are from human sources, were identified in Round Hill Branch (site 6). Although these results indicate that site 4 had contamination from non-human sources and site 6 had contamination from human sources, these results cannot be generalized to time periods other than the sampling periods because of the small amount of data.

Summary

Exceedances of North Carolina water-quality criteria for fecal-indicator bacteria have long been noted in the Newfound Creek basin. Possible sources of these bacteria are from dairy and other agricultural practices or from failing or substandard septic systems. This study was designed to provide continuous streamflow measurements and information on fecal and *E. coli* bacteria concentrations, and to determine whether an experimental method for serotyping coliphage could be used to determine the primary source (animal or human) of fecal contamination in the basin and subbasins.

Ten sites in the Newfound Creek basin, including five sites on the main stem and five sites on tributaries, were sampled during low-flow conditions on May 28, 2003, and during high-flow conditions on November 19, 2003. In-situ measurements were made of streamflow, pH, water temperature, specific conductance, and dissolved oxygen. Samples were collected for analysis of *E. coli* bacteria at all 10 sites and at 5 sites for analysis of fecal coliform bacteria and coliphage virus

Table 6. Results of coliphage virus analysis (serotyping) of water samples collected at low flow on May 28, 2003, and at high flow on November 19, 2003, from the Newfound Creek watershed, North Carolina.

[PFU/L, plaque-forming units per liter; F+RNA, male-specific RNA coliphage]

Site (fig. 1)	Site name	Coliphage			
		Number of plaques isolated	Density (PFU/L)	Number of isolates identified as DNA coliphage	Number of isolates identified as F+RNA coliphage and associated serotype
Low-flow sample					
2	Dix Creek at old NC 20	38	19	6	0
4	Sluder Branch at mouth	4	2.0	1	3 Group I
5	Newfound Creek at Old Newfound Rd	14	7.0	8	0
6	Round Hill Branch at Rabbit Ham Rd	0	<0.5	0	0
7	Newfound Creek at Browntown Rd	1	1.0	1	0
High-flow sample					
2	Dix Creek at old NC 20	209	279	10	0
4	Sluder Branch at mouth	722	1,719	10	0
6	Round Hill Branch at Rabbit Ham Rd	1,301	2,168	4	6 Group III
7	Newfound Creek at Browntown Rd	919	1,021	9	0
10	Newfound Creek at Haylandy Drive	217	181	4	0

serotyping. In addition, *E. coli* was measured in bed sediment at five selected sites.

During low flow, the highest densities of *E. coli* bacteria were found in the middle part of the Newfound Creek watershed at Newfound Creek at Browntown Road and the Dix Creek tributary. When densities were adjusted by the amount of streamflow in each tributary, Dix Creek (site 2) contributed the greatest number of bacteria to Newfound Creek during low flow. Because low-flow conditions occur frequently, a continual contribution of fecal-indicator bacteria from Dix Creek at the measured concentration could be substantial over the period of a year.

During the sampled high-flow condition, all tributaries contained high densities of *E. coli*, but Dix Creek (site 2) and Round Hill Branch (site 6) were the largest contributors. Round Hill Branch had the lowest concentration of *E. coli* and fecal coliform at low flows, which made its large contribution in the November 2003 sample surprising. Samples collected at a nearby site by Buncombe County SWCD indicated a large increase in density of fecal coliform at this site beginning at the end of October 2003. Serotyping of F+RNA coliphage at this site during high flow indicated that at least some of the coliphage were associated with human waste.

Sampling of bacteria in sediments during low flow indicated that sediments do not appear to be a substantial source relative to the water column, with the exception of an area near the confluence of Sluder Branch and Newfound Creek. Samples of fecal coliform bacteria collected concurrently with *E. coli* samples had similar densities.

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