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Scientific Investigations Report 2005–5279

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

Cover Photo Credit:

The photograph on the cover is a picture of the Little Niagara swimming area on Travertine Creek in the Chickasaw National Recreation Area. Photographer: Carol J. Becker, U.S. Geological Survey.

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By Carol J. Becker

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

U.S. Department of the Interior
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Conversion Factors and Datums

Multiply	By	To obtain
	Length	
inch (in.)	2.54	centimeter (cm)
mile (mi)	1.609	kilometer (km)
	Area	
square mile (mi^2)	2.590	square kilometer (km^2)
	Volume	
acre-foot (acre-ft)	1,233	cubic meter (m^3)
	Flow rate	
cubic foot per second (ft^3/s)	0.02832	cubic meter per second (m^3/s)
	Mass	
ton	0.9072	metric ton

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

$°F=(1.8\times°C)+32$

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

Altitude, as used in this report, refers to distance above the vertical datum.

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

Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L) or micrograms per liter (µg/L).

Water Quality and Possible Sources of Nitrogen and Bacteria to Rock and Travertine Creeks, Chickasaw National Recreation Area, Oklahoma, 2004

By Carol J. Becker

Abstract

During the summer months, thousands of visitors swim and wade in the waters along Rock Creek and Travertine Creek in the Chickasaw National Recreation Area. The National Park Service reports that since 2001, *Escherichia coli* bacteria counts exceeding the Oklahoma primary contact standard of 235 colonies per 100 milliliters have been measured at swimming areas on Rock Creek and Travertine Creek during periods of high use, after heavy rainfall events, and during periods of extended drought.

A better understanding of the potential sources of bacterial contamination to these streams would facilitate the development of strategies by the National Park Service officials to improve water quality. The U.S. Geological Survey, in cooperation with the National Park Service, conducted a study in the Upper Rock Creek basin in Murray County, Oklahoma during 2004. The objectives of the study were to identify the possible primary source(s) of *Escherichia coli* bacteria and related contaminants to surface water using: (1) analysis of nitrogen compounds and nitrogen-isotope ratios in nitrate nitrogen and ammonia nitrogen, (2) analysis of organic compounds associated with human wastewater, and (3) a genotypic method of bacterial source tracking.

In May 2004, stream discharge and water properties were measured in addition to counts of fecal coliform and *Escherichia coli* bacteria at sites on Rock Creek and Travertine Creek. Surface water was sampled an additional three times at these locations during August and October. During those three sampling occasions, water properties and stream discharge were measured and samples were analyzed for nitrogen compounds and the stable nitrogen isotopes ^{15}N and ^{14}N in nitrate and ammonia nitrogen, and organic wastewater compounds. Fecal indicator bacteria were enumerated and surface water was collected to culture and isolate *Escherichia coli* bacteria for a bacterial source tracking technique described as ribotyping.

The water quality of Rock Creek in the Chickasaw National Recreation Area seems to be affected most during periods of precipitation from surface-water runoff and sewage effluent. Fecal coliform and *Escherichia coli* bacteria counts in addition to ammonia nitrogen plus organic nitrogen and total nitrogen concentrations were highest in Rock Creek during the precipitation event on October 1. The increase of these constituents in surface water probably can be attributed in part to runoff containing fecal and organic material from non-point sources such as livestock grazing areas in the basin. The area of early runoff in the Rock Creek basin, downstream from the floodwater retarding structures and upstream from the Chickasaw National Recreation Area, may be an area of substantial non-point source contamination during precipitation events.

Swimmers and waders seem to be an intermittent source of bacteria to Travertine Creek during low-flow conditions. There was no strong evidence showing that surface-water runoff from residential areas in the subbasin or sewage effluent contributes bacteria or other contaminants to Travertine Creek. Two water samples had detections of wastewater organics; however, the chemicals detected are commonly used outdoors and could have been introduced by swimmers and waders upstream.

There are three concerns about the bacterial source tracking technique used for this study that should be considered when interpreting the results: the lack of representation of all the potential sources of bacteria in the source library, the small number of isolates in the source library, and the lack of reproducibility shown by the quality control isolates. The DNA fingerprints of *Escherichia coli* collected from Rock Creek were similar to those isolated from cattle and sewage on three surface-water collection dates. The DNA fingerprints of *Escherichia coli* collected from Travertine Creek were similar to those isolated from cattle and sewage on two collection dates and similar to those isolated from horses on one collection date.

Introduction

Rock Creek flows south southwest for 11 miles through rural Murray County and the City of Sulphur before entering the Chickasaw National Recreation Area (CNRA) (fig. 1). As Rock Creek enters the CNRA it gains fresh and mineralized water from flowing artesian wells and perennial springs. The mineralized springs discharge water with elevated concentrations of hydrogen sulfide, bromide, and other constituents that are considered by many people to have healing proper-

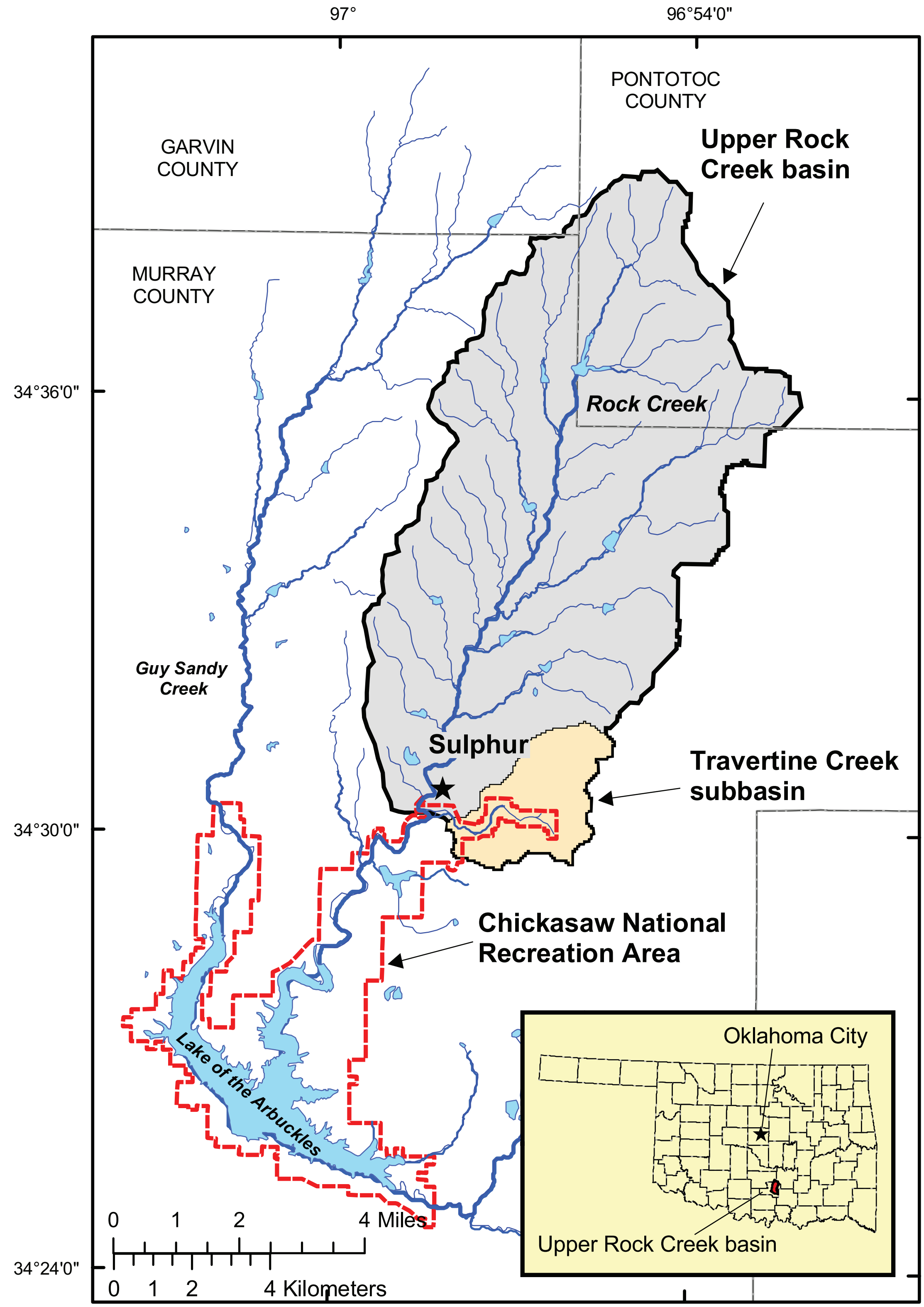

Figure 1. Location of the Upper Rock Creek basin and Travertine Creek subbasin, Chickasaw National Recreation Area, Oklahoma.

ties. Native Americans visited the springs for centuries and referred to the area as the "peaceful valley of rippling waters" (National Park Service, 2004b). In 1902, Native Americans deeded an area encompassing 27 springs to the U.S. Government. Platt National Park was created in 1906 to preserve and protect the springs from development (Gould and Schoff, 1939). Today the springs are part of CNRA and are managed by the National Park Service (NPS) as both recreational and natural resources. The two largest springs in the CNRA are Buffalo and Antelope Springs; both springs feed fresh water to Travertine Creek, a tributary of Rock Creek (fig. 2). During the summer months, thousands of visitors swim and wade in the waters along Travertine Creek and Rock Creek. The NPS monitors *Escherichia coli* (*E. coli*) levels at a number of popular swimming and wading areas along Rock Creek and Travertine Creek on a weekly basis during the summer. When *E. coli* levels exceed the Oklahoma primary contact standard of 235 colonies per 100 milliliters in individual samples or a five-sample geometric mean of 126 colonies per 100 milliliters over 30 days, warnings are posted notifying park visitors that bacteria levels are high and swimming and wading are prohibited (Oklahoma Water Resources Board, 2000) (Steve Burrough, National Park Service, written commun., 2004). The NPS reports that since 2001, *E. coli* counts exceeding the Oklahoma primary contact standard of 235 colonies per 100 milliliters have been measured at swimming areas on Rock Creek and Travertine Creek during periods of high use, after heavy rainfall events, and during periods of extended drought (National Park Service, 2004a). NPS officials are concerned about periodically elevated levels of fecal indicator bacteria in Rock Creek and Travertine Creek. High levels of fecal indicator bacteria have been correlated with fecal contamination and an increased risk for waterborne illnesses (U.S. Environmental Protection Agency, 2000a). A better understanding of the potential sources of bacterial contamination and nitrogen to these streams would facilitate the development of strategies by the NPS officials to improve water quality and address U.S. Geological Survey (USGS) goals of determining the effects of land use practices on surface water, evaluating the effectiveness of nonpoint source pollution management practices, and learning more about sources of contamination that may threaten the health of humans and other animals (Hirsch, 1999). As a result of this need, the USGS in cooperation with the NPS conducted a study in the Upper Rock Creek basin in Murray County during 2004. The objectives of the study were to identify the possible primary source(s) of *E. coli* bacteria and related contaminants to surface water using: (1) analysis of nitrogen compounds and nitrogen-isotope ratios in nitrate nitrogen and ammonia nitrogen, (2) analysis of organic compounds associated with human wastewater, and (3) a genotypic method of bacterial source tracking.

The highest potential for sources of nitrogen and bacteria are land use in the Rock Creek basin and Travertine Creek subbasin and sewer overflows to Rock Creek. Surface-water runoff from residential and urban areas in and around the City of Sulphur may contain fertilizers, pesticides, fecal material, and a host of other contaminants. Livestock grazing in the Upper Rock Creek basin is a potential non-point source of bacteria and possibly nitrogen, especially where runoff occurs during precipitation events.

Purpose and Scope

This report describes water quality and possible sources of nitrogen and bacteria to Rock and Travertine Creeks. Surface-water-quality data including counts of fecal coliform and E. coli bacteria from samples collected by the USGS at two sites, one on Rock Creek, and the other on Travertine Creek, on May 19, August 3, August 11, and October 1, 2004 are described (fig. 2). In addition, the results of a bacterial source-tracking investigation are discussed. The area of study encompasses 43 square miles in the Upper Rock Creek basin upstream from the CNRA and includes the Travertine Creek subbasin within the CNRA.

Description of Study Area

The Upper Rock Creek basin and Travertine Creek subbasin are in Murray County in south-central Oklahoma (fig. 1). This part of Murray County is in the Arbuckle Mountain geomorphic province and is characterized by gently rolling hills and plains developed on granites and gently dipping limestones (Curtis and Ham, 1979). This area is east of the Arbuckle Mountains in a region that has undergone extensive deformation and faulting. Detailed overviews of the geology and hydrogeology of the CNRA and surrounding area can be found in Fairchild and others (1990) and Hanson and Cates (1994).

The climate for this part of Oklahoma is subhumid, with most precipitation occurring as rainfall in the spring and fall. The average annual precipitation for a 30-year period (1961-90) for this area ranged from 32 to 34 inches (Johnson and Duchon, 1995). The average temperature for this area of the state is 61.0 degrees Fahrenheit for a 30-year period (1971-2000). The average low temperature of 26.3 degrees Fahrenheit occurs in January and the average high temperature of 93.6 degrees Fahrenheit occurs in August (National Weather Service, 2005).

The City of Sulphur abuts the northern boundary of the CNRA (fig. 1). Sulphur has a population of about 4,800 people (U.S. Census Bureau, 2004) and its economy is based on cattle ranching, oil-related industry, education, and medical services (Sulphur Chamber of Commerce, 2004). Tourism is a very important economic asset to the City of Sulphur; the City estimates that 3.6 million people visit the area yearly (Sulphur Chamber of Commerce, 2004).

Upper Rock Creek Basin

The headwaters of the Upper Rock Creek basin begin in northern Murray and southern Pontotoc Counties and flow

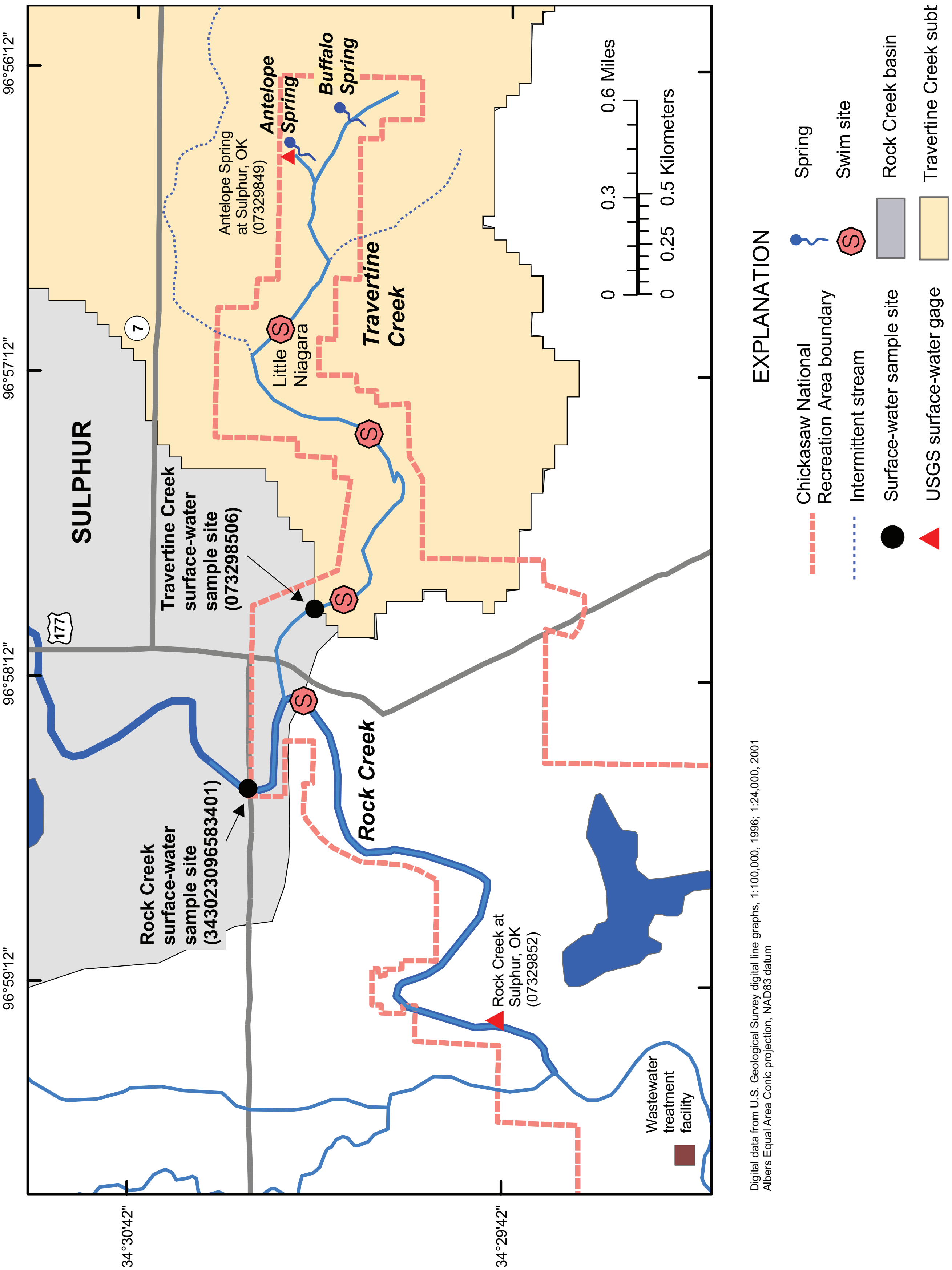

Figure 2. Location of the Rock Creek and Travertine Creek surface-water sampling sites and popular swimming areas in the Chickasaw National Recreation Area, Oklahoma.

southwest for about 10 miles before entering the City of Sulphur (fig. 1). The creek flows for another mile through the center of Sulphur and then continues south into the CNRA. Before entering the CNRA, Rock Creek receives water from small springs, ground-water discharge, and several unregulated flowing artesian wells. After entering the CNRA, Rock Creek gains water from an artesian well, several small springs, and Travertine Creek (fig. 2). The mean monthly discharge for Rock Creek, measured at the USGS surface-water gage Rock Creek at Sulphur (station number 07329852) (fig. 2) from calendar year 1990 to 2004, ranged from a low of 16.8 cubic feet per second (ft^3/s) in August to a high of 94.9 ft^3/s in April (U.S. Geological Survey, 2005).

The Upper Rock Creek basin above the CNRA is about 43 square miles (fig. 3). Land use information for 1992 shows the largest percent of land cover within the basin is grassland (57.5 percent) (U.S. Environmental Protection Agency, 2000b). Grassland, along with pasture and hay (8.8 percent), are used primarily for the grazing of beef and dairy cattle in Murray County (Bobby Cline, District Conservationist, U.S. Department of Agriculture, oral commun., 2004) (table 1). Animals are a potential source of fecal coliform bacteria within the study area; cattle are found throughout the basin and have access to Rock Creek and tributaries in many areas. It was estimated that about 4,270 cattle, 200 horses, and 100 sheep reside in the study area. The estimates for cattle were calculated from the percentage of the county area in the basin, and the livestock county estimates for January 1, 2003 (U.S. Department of Agriculture-National Agricultural Statistics Service, 2003). Other animals in the study area include deer, raccoon, migratory waterfowl, beaver, goats, bison, dogs, cats, and other small mammals.

Nine floodwater retarding structures were built by the Soil Conservation Service in the 1960s to avert flooding on Rock Creek (fig. 3). The floodwater retarding structures reduce peak flow on the main stem by catching and retaining surface water during periods of high flow and then releasing it at controlled discharges (Tortorelli and Bergman, 1985). These pond-like structures also may reduce the loading of nutrients and bacteria that reach Rock Creek from sources upstream from the floodwater retarding structures. Concentrations of nitrogen and phosphorus can be significantly decreased in ponds by natural processes that include burial from sedimentation, denitrification, and assimilation by macrophytes (Fairchild and others, 2005). Fecal coliform bacteria in water are sensitive to elevated temperature and die off quickly when exposed to sunlight (Burkhardt and others, 2000); (Whitman and others, 2004).

The nine floodwater retarding structures have a floodwater retention volume ranging from 190 to 1,911 acre feet with a total of 6,640 acre feet of water. The maximum discharge released from each structure ranges from 7 to 88 ft^3/s with a combined maximum discharge of 280 ft^3/s (Soil Conservation Service, 1959, table 3). These structures decrease the area of immediate runoff that Rock Creek receives upstream from the CNRA to less than 19 square miles (fig. 3) (Soil Conservation Service, 1959). This area of early runoff in the basin, downstream from the floodwater retarding structures and upstream from the CNRA, more than likely supplies the greatest percentage of non-point source contamination to Rock Creek (fig. 3).

During precipitation events, Rock Creek receives storm sewer runoff from the central portion of the City of Sulphur. Urban runoff is a potential source of nutrients, bacteria, and organic compounds such as pesticides and solvents. The central portion of Sulphur contains the downtown business district, small stores, gas stations, public schools, and residences.

The City of Sulphur provides sanitary sewage utilities within the city limits for residents, businesses, and CNRA park facilities. Sewage effluent flows by gravity to the wastewater treatment facility located in the CNRA where it is treated and discharged into nearby Guy Sandy Creek (figs. 1 and 2). There is concern by some NPS and city officials about the antiquated conduits for the City's sanitary sewage system. Sanitary sewage effluent has overflowed from two manholes into Rock Creek on a number of occasions during heavy rainfall events (Sue Braumiller, National Park Service, written commun., 2005). The volume of sanitary sewage flowing into the wastewater treatment facility also increases during periods of heavy precipitation, indicating that surface water or ground water is seeping into the system. Over a 2-week period in October and November 2004 the area received more than 4.5 inches of rain (Oklahoma Mesonet, 2005). During that period the volume of sewage received at the wastewater treatment facility increased three fold and stayed high for 2 weeks after precipitation stopped (Charles Johnson, City of Sulphur, oral commun., 2004). Outside of the Sulphur city limits, residences and businesses rely on septic tanks for sanitary wastewater disposal. Malfunctioning septic-tank systems can leak effluent and contaminate surface water, especially during precipitation events.

Travertine Creek Subbasin

Travertine Creek begins in the northeast corner of the CNRA and flows west for about 2.3 miles until it enters Rock Creek (fig. 2). Under normal low-flow conditions, Travertine Creek derives streamflow from Antelope Spring and Buffalo Spring, ground-water seepage along the creek bed, and overflow from several artesian wells in the City of Sulphur well field. During precipitation events, Travertine Creek receives runoff from about 3.45 square miles; only 0.6 square mile or 16.5 percent of the Travertine Creek subbasin is within the CNRA boundary (fig. 3). Land cover in this subbasin is predominately forested upland and grassland with small areas used for the cultivation of row crops and small grains (table 1). Livestock graze north and south of Highway 7 in this area (fig. 2). About 2 percent of the subbasin contains residential housing; the largest area being situated along the northwest CNRA boundary.

Travertine Creek is likely to have less nutrient and bacteria loading than Rock Creek because of the smaller drainage

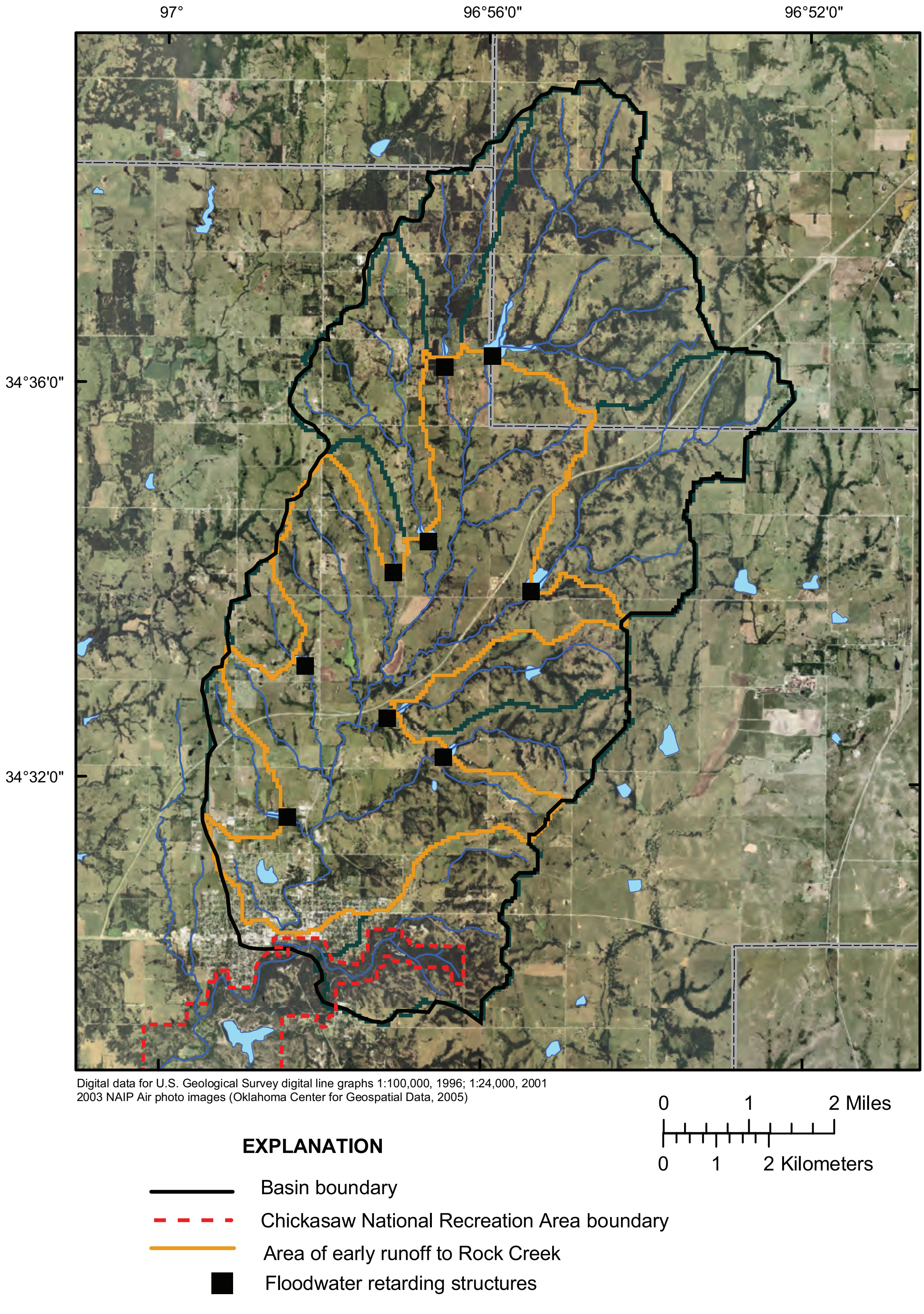

Figure 3. Location of floodwater retarding structures and area of early runoff to Rock Creek in the Chickasaw National Recreation Area, Oklahoma.

Table 1. Land use and cover in the Upper Rock Creek basin and Travertine Creek subbasin in 1992, Murray County, Oklahoma, (U.S. Environmental Protection Agency, 2000b)

Land use	Upper Rock Creek basin, percent of area	Travertine Creek subbasin, percent of area
Water	1.6	1.0
Residential/commercial	3.5	2.2
Forested	21.6	50.6
Shrubland	1.5	4.3
Grassland	57.5	35.5
Pasture/hay	8.8	1.4
Row crops/small grains	5.5	5.0

basin area, but has some of the same potential sources as Rock Creek from surface-water runoff during periods of precipitation. Those sources include feces from wildlife and livestock and runoff from residential areas. During the summer months, park visitors swim and wade in Travertine Creek throughout the week and especially on weekends. The swimming area at Little Niagara usually has a large concentration of visitors during summer days that can be a source of bacteria.

Methods

This section describes the methodology used to sample surface-water and collect material for the cultivation of *E. coli* bacteria for a bacterial source tracking technique. Information about the laboratory methods and bacterial source tracking also are provided.

Sampling Procedures and Water-Quality Analyses

In May, during base-flow conditions, stream discharge and the water properties—specific conductance, pH, water temperature, and dissolved oxygen concentration—were measured in addition to counts of fecal coliform and *E. coli* bacteria at sites on Rock Creek and Travertine Creek. Surface water was sampled an additional three times at these locations during August and October. During those three sampling occasions, water properties and stream discharge were measured. Samples also were analyzed for nitrite nitrogen, nitrite plus nitrate nitrogen, ammonia nitrogen, ammonia plus organic nitrogen, the stable nitrogen isotopes ^{15}N and ^{14}N in nitrate and ammonia nitrogen, and organic wastewater compounds. Fecal indicator bacteria were enumerated and surface water was collected to culture and isolate *E. coli* bacteria for a bacterial source tracking (BST) technique described as ribotyping. The BST technique characterized fragments from ribosome-encoding DNA (rDNA) in *E. coli* cultured from surface water and compared them to rDNA fragments from *E. coli* cultured from feces of cattle, horses, sheep and effluent from septic tanks and municipal sewage collected in the Upper Rock Creek basin.

Stream discharge was measured during each of the four water-quality sampling visits using procedures described by Buchanan and Somers (1968). Water properties consisting of specific conductance, pH, water temperature, and dissolved oxygen concentrations were measured using a YSI model 600XL multiprobe meter. The meter was calibrated on site before each use with standard specific conductance and pH solutions that bracketed the expected field values using methods described by Wilde and Radtke (1998). Surface-water samples were collected using isokinetic, equal-width sampling methods as described by Webb and others (1999). Water-quality samples were collected and processed as described in Wilde and others (1999, 2004).

Surface water for analysis of ammonia nitrogen, nitrate nitrogen, and nitrite plus nitrate nitrogen was filtered in the field through a 0.45-micron disposable capsule filter and collected in 125-milliliter (mL) brown polyethylene bottles that were prerinsed with deionized water and filtered sample water. Surface water for analysis of ammonia nitrogen plus organic nitrogen was collected in a 125-mL polyethylene bottle that was prerinsed with deionized water and sample water. The sample was preserved with 1 mL of 4.5 normal sulfuric acid and chilled on ice to 4 degrees Celsius (table 2).

Surface water for analysis of the stable nitrogen isotopes ^{15}N and ^{14}N in ammonia nitrogen and nitrate nitrogen was collected in a 1-liter polyethylene bottle that had been prerinsed with a solution of 95 percent deionized water and 5 percent trace-element grade hydrochloric acid. The samples

Table 2. Constituents analyzed, analyzing laboratories, laboratory methods, minimum reporting levels, and method references used for analysis of surface-water samples collected from Rock Creek and Travertine Creek, Chickasaw National Recreation Area, Oklahoma, 2004

[USGS, U.S. Geological Survey; ASF, automated-segmented flow; ID, identification number; m-FC, fecal coliform medium; TTC, 2,3,5-triphenyltetrazolium-chloride; °C, degrees Celsius; mg/L, milligrams per liter; --, not applicable]

Constituents	Analyzing laboratory	Laboratory method	Method reporting level	Method references
Ratio of the stable nitrogen isotopes in ammonia nitrogen nitrate nitrogen ^{15}N and ^{14}N	Water Sciences Laboratory, University of Nebraska, Lincoln, Nebraska	Steam distillation and isotope ratio mass spectrometry	--	Bremner and Keeney (1965), Gormly and Spalding (1979), Krietler (1975)
USGS schedule 1697 (1) Ammonia nitrogen, (filtered) (2) Ammonia nitrogen + organic nitrogen, (unfiltered) (3) Nitrite + nitrate nitrogen, (filtered) (4) Nitrite nitrogen, (filtered)	USGS National Water Quality Laboratory, Lakewood, Colorado	(1) Colorimetry, ASF, Salicylate-hypochlorite; method ID: I-2522-90 (2) Colorimetry, ASF, micro-Kjeldahl digestion; method ID: I-4515-91 (3) Colorimetry, ASF, cadmium reduction-diazotization, method ID: I-2545-90 (4) Colorimetry, ASF, method ID: I-2540-90	(1) 0.04 mg/L (2) 0.10 mg/L (3) 0.060 mg/L (4) 0.008 mg/L	(1,3,4,) Fishman (1993) (2) Patton and Truitt (2000)
USGS schedule 1433 Wastewater compounds	USGS National Water Quality Laboratory, Lakewood, Colorado	Polystyrene-divinylbenzene solid-phase extraction and capillary-column gas chromatography/mass spectrometry	See table 5	Zaugg and others (2002)
Enumeration of (1) fecal coliform and (2) *Escherichia coliform*	USGS Oklahoma Water Science Center, Oklahoma City, Oklahoma	(1) Membrane filtration, m-FC media, 24 hours at 44.5 °C (2) Membrane filtration, m-TEC, 2 hours at 35 °C, then 22 to 24 hours at 44.5 °C	One colony per 100 milliliters	Myers and Wilde (2003)
Escherichia coliform bacteria culture and isolation	Oklahoma Animal Disease Diagnostic Laboratory, Stillwater, Oklahoma	Swab inoculated Tergitol 7 agar with TTC incubated for 18 hours at 35 °C, confirmed by inoculation of Triple Sugar Iron Agar, Indole, Citrate, Urea, motility, Lysine Iron Agar, Methyl Red and Vogas-Proskauer reactions.	--	Clesceri and others (1998) Murray and others (1999)
Bacterial source tracking	Food and Agricultural Products Research and Technology Center, Oklahoma State University, Stillwater, Oklahoma	Double digestion with restriction enzymes: *EcoRI* and *PvuII* DuPont Qualicon, The RiboPrinter® Microbial Characterization System	--	Dupont Qualicon (2003)

were chilled to 4 degrees Celsius using ice until return to the USGS Oklahoma City office, then frozen until shipment to the laboratory. As described by Bremner and Keeney (1965) and Gormly and Spalding (1979), laboratory preparation for nitrogen isotope involves steam distillation and a quantitative conversion to nitrogen gas that is purified and collected on a high vacuum preparation system. Nitrogen isotope analysis was done using isotope ratio mass spectrometry (table 2). Water samples having concentrations of nitrate nitrogen or ammonia nitrogen less than 0.3 mg/L had insufficient concentrations for analysis and were not analyzed.

Surface-water samples for enumeration of fecal coliform and *E. coli* bacteria were collected in sterile 1-liter polyethylene bottles and chilled to 4 degrees Celsius using ice. The samples were transported to the laboratory at the USGS Oklahoma City office within 6 hours for culturing by membrane filtration and incubation, as described by Myers and Wilde (2003) (table 2).

Surface-water samples to be analyzed for organic wastewater compounds were collected in 1-liter baked amber glass bottles sealed with Teflon-lined caps and chilled to 4 degrees Celsius using ice. Samples were analyzed for 64 compounds commonly found in domestic and industrial wastewater. These compounds include the alkylphenol ethoxylate nonionic surfactants and degradates, food additives, antioxidants, flame retardants, disinfectants, solvents, plasticizers, fragrances, fecal sterols, polycyclic aromatic hydrocarbons, and commonly used domestic pesticides. As described by Zaugg and others (2002), the water sample is filtered at the laboratory and then extracted by vacuum through disposable solid-phase cartridges that contain polystyrene-divinylbenzene resin. The cartridges are dried with nitrogen gas and the sorbed compounds are eluted with dichloromethanediethyl ether (4:1) and measured by capillary-column gas chromatography/mass spectrometry (table 2).

Samples for analysis of nitrogen and organic wastewater compounds were shipped packed in ice to the USGS National Water-Quality Laboratory in Lakewood, Colorado, within 48 hours of sample collection. Nitrogen isotope samples were shipped frozen to the Nebraska Water Sciences Laboratory in Lincoln, Nebraska, within 4 weeks of sample collection (table 2).

Bacterial Source Tracking

Bacterial source tracking was performed to identify possible sources of *E. coli* bacteria to the creeks. Surface-water samples were collected in five sterile 1-liter polypropylene bottles, filled a minimum of 3 minutes apart. Bottles were filled by facing the bottle into the current and dipping into the stream at three to five equally spaced locations in the stream cross section. The objective was to isolate five *E. coli* bacteria colonies from each 1-liter bottle in order to have 25 bacteria colonies from each site on each sampling occasion. There were difficulties, however, in culturing viable *E. coli* on the growth plates because of interference caused by growth of non-target bacteria (Laura Dye, Oklahoma Animal Disease Diagnostic Laboratory, oral commun., 2004). Additional surface-water samples were collected at both sites on September 19 to supplement the small number of bacteria colonies isolated from the August samples.

A known-source library of 153 *E. coli* ribotypes was created from fecal samples collected from cattle, horses, and sheep and sewage and septic tank effluent located in the study area and are referred to as ‘knowns’. The *E. coli* bacteria were cultured from 62 cattle scattered throughout the watershed. A maximum of five fecal samples from individual cattle were collected at each location. Thirteen *E. coli* isolates were cultured from horses and 15 from sheep. A rectal swab was used for horses and sheep, whereas, fresh fecal material from cattle was collected in sterile whirl paks. One *E. coli* isolate was isolated from each animal. Sewage effluent was collected at the City of Sulphur wastewater treatment facility on four occasions. A total of 62 *E. coli* isolates were isolated from 10 separate sewage-effluent samples collected in sterile 250-mL polypropylene bottles and sterile whirl paks. Samples of effluent were collected at the inflow where untreated sewage enters the facility. The bacteria in sewage effluent may not always be from a human source even though most sanitary sewage originates from households and businesses. Accordingly, *E. coli* isolated from sewage effluent was not categorized as human in the known-source library. Four *E. coli* colonies were isolated from effluent collected from one septic tank located in the study area. These *E. coli* were assumed to be from humans. The effluent was collected inside the septic tank in two sterile 250-mL polypropylene bottles. An effort was made to locate septic tanks that serviced large numbers of people to ensure diversity of the bacterium. Only two such septic tanks were located in the study area and only one was available for sampling. All samples were chilled using ice to 4 degrees Celsius and delivered within 12 hours to the Oklahoma Animal Disease Diagnostic Laboratory, Stillwater, Oklahoma, for culturing, isolation, and verification of *E. coli* bacteria.

The known-source and surface-water *E. coli* isolates were ribotyped at the Food and Agricultural Products Research and Technology Center, at Oklahoma State University in Stillwater, Oklahoma. The DuPont Qualicon RiboPrinter® Microbial Characterization System (DuPont Qualicon, 2003) was used in combination with double digestion using the restriction enzymes *EcoRI* and *PvuII*. The resulting genotypic patterns of DNA fragments from ribosomal RNA genes in the *E. coli* isolates were compared and grouped based on similarities in band intensity and band position. *E. coli* having genotypic banding patterns more than 85 percent similar were considered to come from the same source category, that is, cattle, horse, human, or sewage.

Quality Assurance and Quality Control

Quality assurance practices were implemented to maintain consistency with sampling protocols throughout the study and to assure no contamination. One duplicate surface-water sample for nitrogen compounds, nitrogen isotopes, and organic wastewater compounds was collected. Additionally, one equipment blank sample was submitted for analysis of organic wastewater compounds. For BST, ten bacteria isolates from known sources were duplicated by the Oklahoma Animal Disease Diagnostic Laboratory and sent unlabeled to the Food and Agricultural Products Research and Technology Center for classification by ribotyping.

Acknowledgments

The author thanks the National Park Service for assistance provided during this study. Special thanks are extended to Steve Burrough and Sue Braumiller, National Park Service, for reviewing the text and making many useful suggestions; Charles Johnson, City of Sulphur; Laura Dye, Oklahoma State University Animal Disease Diagnostic Laboratory, Stillwater, Oklahoma; Siobhan Reilly and Jennifer Nangle, Oklahoma State University, Stillwater, Oklahoma; and to Dusty Perkins, Daniel 'Boone' Arms; and the many residents and livestock owners who graciously assisted with this project. Additionally, the author thanks Lee Ann Alf, Rick Hanlon, Jason Masoner, and Ernie Smith, U.S. Geological Survey.

Water Quality

Surface water was collected at two sites; one on the main stem of Rock Creek located downstream from the City of Sulphur just north of the CNRA boundary and another on Travertine Creek downstream from the swimming areas and about one third of a mile upstream from its confluence with Rock Creek (fig. 2). These sampling locations are referred to in this report as the Rock Creek and Travertine Creek sites, respectively. The sample site locations were chosen because of accessibility and position with regard to potential sources of contamination upstream in the basins.

The two sample sites were visited four times each between May and October 2004. During the first visit in May the water properties and discharge were measured along with fecal coliform and *E. coli* bacteria concentrations. During the next three visits in August and October, the water properties and discharge were measured and water samples were collected: nitrogen compounds in filtered and unfiltered water, the stable nitrogen isotopes ^{15}N and ^{14}N in nitrate and ammonia nitrogen, organic compounds commonly found in domestic and industrial wastewater, and enumeration of fecal coliform and *E. coli* bacteria. Surface water also was collected during these three visits for cultivation of *E. coli* bacteria for bacterial source tracking.

The hydrograph on figure 4 shows stream conditions on Rock Creek at the USGS surface-water gage Rock Creek at Sulphur, Oklahoma (07329852) when samples were collected and local rainfall amounts (Oklahoma Mesonet, 2005). The Rock Creek gage is about 2.1 miles downstream from the Rock Creek site and 1.8 miles downstream from where Travertine Creek enters Rock Creek. The August 3 and August 11 samples were collected during the recession of a high-flow event on Rock Creek. The samples collected on October 1 occurred while stream flow was increasing during a high-flow event at both sampling sites.

Water Properties

Measurements of specific conductance at Rock Creek ranged from 432 to 836 μS/cm and did not vary appreciably with stream flow (table 3). Specific conductance of 836 μS/cm was measured during low stream flow (0.77 ft^3/s) and probably is the result of water from upstream mineralized artesian wells composing a greater part of the flow. Specific conductance measured at Travertine Creek ranged from 419 to 612 μS/cm and did not show a relation to streamflow. The lowest and highest specific conductance measurements, 419 and 612 μS/cm, occurred when streamflow was 4.1 ft^3/s on August 3 and August 11.

The pH measured at Rock Creek ranged from 7.5 to 7.7 and dissolved oxygen ranged from 7.0 to 8.1 mg/L (table 3). The pH measured at Travertine Creek ranged from 7.5 to 8.1 and dissolved oxygen ranged from 8.5 to 10.3 mg/L. The higher dissolved oxygen at Travertine Creek is probably due to the colder water temperatures and turbulent flow. Water temperatures at Travertine Creek were about 0.5 to 3.1 degrees Celsius colder than Rock Creek.

Nitrogen Compounds

Nitrogen is a fundamental chemical compound used by plants and animals for biological processes and is present in many forms in the environment. When nitrogen becomes concentrated in a locality it can become a point source of contamination to surface water (or ground water). Point sources are usually discharges from large accumulations of animal waste such as septic tanks, sewage treatment facilities, animal feed lots, and cesspools. Non-point sources release nitrogen over large areas; examples include fields and lawns having nitrogen-based fertilizers or animal waste, decaying plant material, and soils. Precipitation also is a non-point source of nitrogen; nitrate nitrogen dissolved in rain water and snow is deposited at a rate of 1.7 to less than 2 tons per square mile a year in the study area (Mueller and Helsel, 1996). Studies have shown that non-point sources have the greatest effect on surface water during storms when source material, such as fecal material and fertilizer, are washed off, and point sources, such as sewage-treatment facilities, appear to have the greatest effect during low flow when little additional water is available for dilution of the effluents (Mueller and Helsel, 1996). Of the dissolved inorganic forms of nitrogen, nitrate nitrogen is the most chemically stable and the most mobile in water; whereas, ammonia and nitrite nitrogen are less stable and are most often found in oxygen-depleted environments close to the source

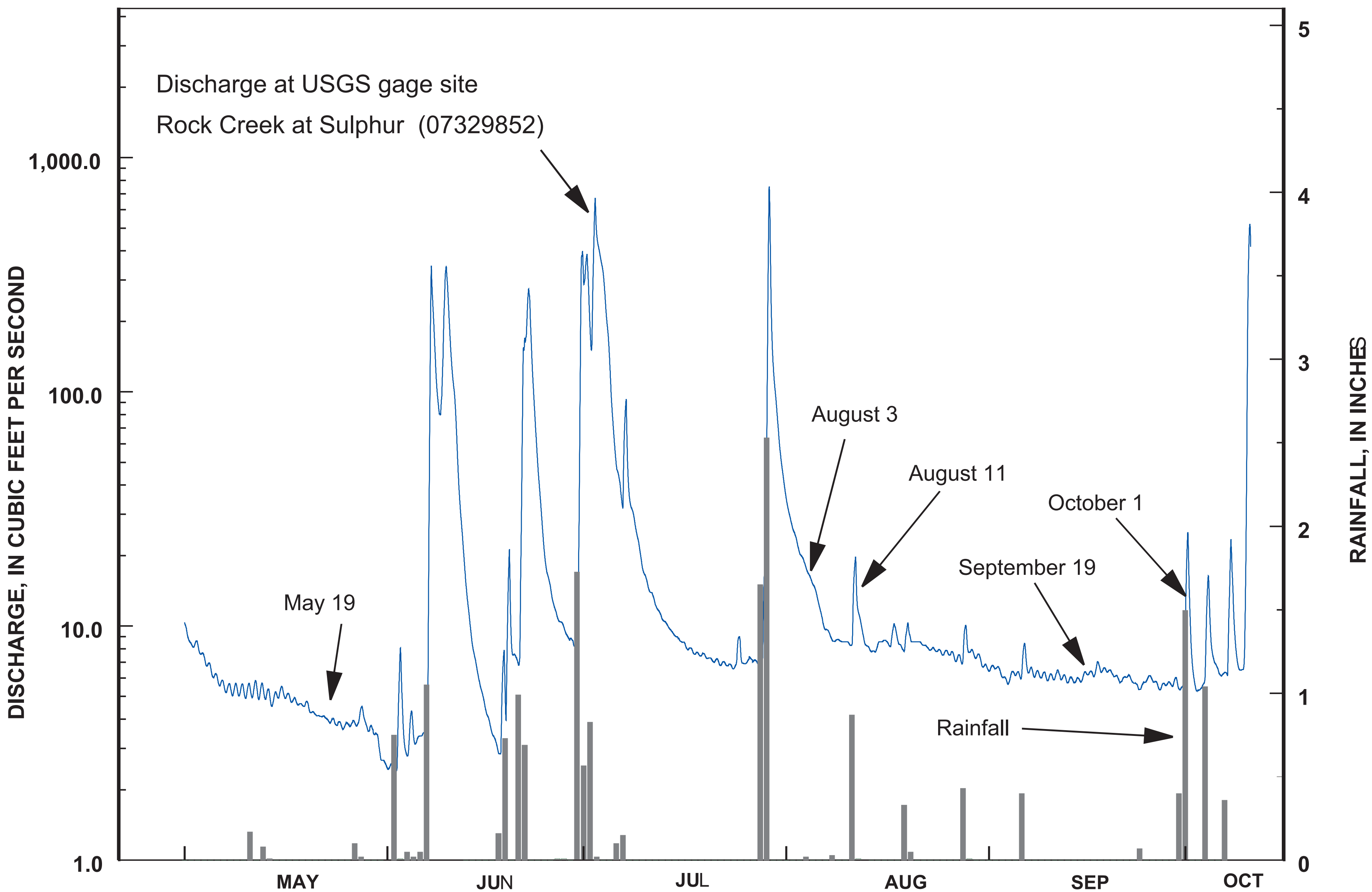

Figure 4. Stream conditions from May 1 to October 15, 2004, at the U.S. Geological Survey surface-water gage site Rock Creek at Sulphur, OK (07329852) and local rainfall measurements (Oklahoma Mesonet, 2005), Murray County, Oklahoma, 2004.

Table 3. Measured water properties, concentrations of nitrogen compounds and stable nitrogen isotopes, and bacteria concentrations in surface-water samples collected from Rock Creek and Travertine Creek, Chickasaw National Recreation Area, Oklahoma, 2004

[Q, discharge, instantaneous; ft^3/s, cubic feet per second; SC, specific conductance; µS/cm, microsiemens per centimeter at 25 degrees Celsius; Temp, temperature of water; °C, degrees Celsius; DO, dissolved oxygen; mg/L, milligrams per liter; NO^2-N, nitrite as nitrogen, dissolved; NO_2+NO_3-N, nitrite plus nitrate as nitrogen, dissolved; NO_3-N, nitrate as nitrogen; NH_3-N, ammonia as nitrogen, dissolved; NH_3+OrgN-N, ammonia plus organic nitrogen total; $\delta^{15}N$, Nitrogen-15/Nitrogen-14 ratio, unfiltered, per mil; mL, milliliters; *I*, interference from unknown growth; *K*, non-ideal count; <, less than; ≤, less than or equal to; >, greater than; E, estimated; --, no data; analytes detected below minimum reporting levels were censored to reporting level; USGS, U.S. Geological Survey; ID, identifier; NM, not measured]

Date	Time	Q ft^3/s	SC µS/cm	pH standard units	Temp °C	DO mg/L	NO_2–N mg/L dissolved	NO_2+ NO_3-N mg/L dissolved	NO_3-N mg/L calculated	NH_3 -N mg/L dissolved	NH_3+ Org N-N mg/L total	Total N mg/L calculated	$\delta^{15}N$-NO_3 per mil	$\delta^{15}N$-NH_4 per mil	Fecal coliform bacteria per 100 mL	*Escherichia coliform* bacteria per 100 mL
Rock Creek under Highway 7 bridge, USGS site ID 343023096583401																
05/19/2004	1300	0.77	836	7.5	24.5	7.5	--	--	--	--	--	--	--	--	86	7 *K*
08/03/2004	1030	8.5	489	7.7	26.8	7.1	< 0.008	E 0.05	≤ 0.05	E 0.02	0.68	E 0.73	NM	NM	670	150 *K*
08/11/2004	1300	4.5	432	7.6	23.7	7.0	0.058	0.43	0.37	< 0.04	0.95	1.38	NM	NM	>1 *I*	1 *I*
10/01/2004	0200	9.2	432	7.6	19.9	8.1	0.014	0.62	0.61	E 0.03	0.87	1.49	-2.93	NM	920	197
Travertine Creek near Sulphur, USGS site ID 073298506																
05/19/2004	1100	1.8	546	7.5	23.1	8.5	--	--	--	--	--	--	--	--	95	28 *K*
08/03/2004	1200	4.1	612	8.0	23.7	10.3	< 0.008	0.15	≤ 0.15	E 0.03	0.14	0.29	NM	NM	80	120 *K*
08/11/2004	1345	4.1	419	8.1	21.6	8.8	< 0.008	0.17	≤ 0.17	< 0.04	0.10	0.27	-3.94	NM	1,300	550 *K*
10/01/2004	0400	6.0	490	8.1	19.4	8.9	< 0.008	0.08	≤ 0.08	< 0.04	0.26	0.34	NM	NM	194	38
[a]10/01/2004	0401	--	--	--	--	--	< 0.008	0.08	--	< 0.04	0.22	--	NM	NM	--	--

[a] Duplicate sample

of contamination. Organic nitrogen is derived from plant and animal particulate material that consists of molecules containing nitrogen. Organic nitrogen is not as easily assimilated by plants as the dissolved forms of nitrogen.

Nitrate nitrogen was the predominate form of dissolved nitrogen in samples from Rock Creek and Travertine Creek (table 3). Nitrate nitrogen was not measured directly but was calculated by subtracting nitrite nitrogen from nitrite plus nitrate nitrogen concentrations. At the Rock Creek site, concentrations of nitrate nitrogen were less than or equal to 0.05 mg/L, 0.37 mg/L, and 0.61 mg/L (table 3). Nitrite nitrogen was measured at very small concentrations of 0.058 and 0.014 mg/L on August 11 and October 1. No nitrite nitrogen was measured in the August 3 sample. Ammonia was not detected in the August 11 sample but was detected at very low concentrations (estimated at 0.02 and 0.03 mg/L) in the August 3 and October 1 samples from Rock Creek, respectively.

Nitrate nitrogen in the three samples from the Travertine Creek sample site were 0.15, 0.17, and 0.08 mg/L. Nitrite nitrogen was not measured in surface-water samples from Travertine Creek; nor was ammonia, except for a very small concentration in the August 3 sample estimated to be 0.03 mg/L. Ammonia plus organic nitrogen concentrations were 3 to 9 times higher in the Rock Creek samples than the Travertine Creek samples. Concentrations of ammonia plus organic nitrogen from Rock Creek were 0.68, 0.95, and 0.87 mg/L. The highest concentration, 0.95 mg/L, was measured during the lowest streamflow of the three sampling occasions, 4.5 ft^3/s. Ammonia plus organic nitrogen measured at Travertine Creek ranged from 0.10 to 0.26 mg/L with the highest concentration measured during high streamflow on October 1, 2004.

Total nitrogen is the sum of all forms of nitrogen found in the water sample, consisting of dissolved and particulate organic forms. Calculated concentrations of total nitrogen in the Rock Creek samples ranged from an estimated 0.73 to 1.49 mg/L, 2 to 5 times greater than the Travertine Creek concentrations, which ranged from 0.27 to 0.34 mg/L. The larger total nitrogen concentrations in Rock Creek probably are the result of agricultural land uses including livestock and runoff from the City of Sulphur.

When the concentrations of total nitrogen and nitrite plus nitrate nitrogen from Rock Creek are compared to median concentrations measured at other surface-water sites in Oklahoma and part of Arkansas (excluding sites in the Ozark Highland and Ouachita Mountains) having similar stream characteristics (Haggard and others, 2003, table 7, stream category SS1), concentrations measured in Rock Creek are larger. The total nitrogen concentrations from Rock Creek range between the 50th and 75th percentiles (of 44 sites) and the August 11 and October 1 concentrations of nitrite plus nitrate nitrogen range between the 67th and 90th percentiles. When the concentrations of total nitrogen and nitrite plus nitrate nitrogen from Travertine Creek are compared to median concentrations measured at other surface-water sites in Oklahoma similarly, concentrations measured in Travertine Creek are smaller. Total nitrogen concentrations from Travertine Creek range between the 10th and 25th percentiles and concentrations of nitrite plus nitrate nitrogen range between the 33rd and 67th percentiles of other sites.

The concentrations of nitrate nitrogen (assuming nitrite nitrogen is negligible) and organic nitrogen in Rock Creek during 2004 were greater than historical water-quality data collected at the Rock Creek gage from 1993 to 1995. The August 11 and October 1 samples from the Rock Creek site had nitrite plus nitrate nitrogen concentrations (0.43 and 0.62 mg/L) that were four to six times greater than the highest historical concentration of 0.13 mg/L (12 samples collected at the Rock Creek gage from 1993 to 1995) (table 4). The August 3 and August 11 samples from the Travertine Creek site also had concentrations of nitrite plus nitrate nitrogen concentrations (0.15 and 0.17 mg/L) greater than the highest historical concentration of 0.13 mg/L collected at the Rock Creek gage. The three measurements of ammonia plus organic nitrogen (0.68, 0.95, and 0.87 mg/L) at the Rock Creek site are near or exceed the highest historical concentration of 0.7 mg/L (22 samples) and show an increase in plant and animal particulate material containing nitrogen carried downstream. Concentrations of the unstable nitrogen compounds, nitrite nitrogen and ammonia nitrogen, were relatively low or less than the reporting level at both sample sites when compared to historical data, except for the August 11 concentration of 0.058 mg/L of nitrite nitrogen at the Rock Creek site.

Nitrogen Isotopes

Ordinarily, an element has an equal number of protons and neutrons in the nucleus of atoms that define its structure. An isotope of an element is created when the number of neutrons in the nucleus increases or decreases, changing the atomic weight. The two (stable) isotopes of nitrogen are ^{15}N and ^{14}N and because the two have different masses, reaction rates are different. The difference in reaction rates can cause a disproportionate concentration of one isotope to another in the environment (Clark and Fritz, 1997). This process is referred to as fractionation and can be used to help determine sources of nitrogen in nitrogen compounds. The ^{14}N isotope is the lighter of the two isotopes and comprises over 99 percent of the nitrogen in the atmosphere (Junk and Svec, 1958). Animals take in nitrogen and tend to concentrate the heavier ^{15}N isotope in their wastes. Consequently, the ratio of ^{15}N to ^{14}N in nitrate nitrogen is higher in animal waste compared to other sources such as plants, atmospheric nitrogen, and synthetic fertilizer.

The units used to report nitrogen isotope ratios in a sample to those of a standard material are expressed as $\delta^{15}N$ values in parts per thousand, denoted as permil (‰). Enrichments or depletions of $\delta^{15}N$ are referenced to standard atmospheric nitrogen, from National Bureau of Standards, NBS-14 nitrogen gas (Fritz and Fontes, 1980, p. 16). Stable-isotopic ratios are computed as follows (Kendall and Aravena, 1999):

Table 4. Historical concentrations of nitrogen compounds measured in surface-water samples collected at the U.S. Geological Survey surface-water gage Rock Creek at Sulphur (station number 07329852), Chickasaw National Recreation Area, Oklahoma (modified from Andrews and Burrough, 2002)

[<, less than; NO_2 -N, nitrite nitrogen; NO_2+NO_3-N, nitrite plus nitrate nitrogen; NH_3-N, ammonia nitrogen; NH_3+OrgN-N, ammonia plus organic nitrogen]

Nitrogen compound	Number of measure-ments	Month year	Range (milligrams per liter)	Mean (milligrams per liter)	Median (milligrams per liter)
NO_2 –N, dissolved	10	May 1993 – August 1995	< 0.01 – 0.03	0.02	0.02
NO_2+ NO_3-N, dissolved	12	May 1993 – August 1995	< 0.05 – 0.13	0.10	0.09
NH_3 –N, dissolved	10	May 1993 – August 1995	< 0.01 – 0.03	0.02	0.02
NH_3+OrgN-N, total	22	April 1990 – August 1995	< 0.2 – 0.7	0.35	0.3

$$\delta^{15} = \left[\frac{\left(\frac{^{15}N}{^{14}N}\right) Sample}{\left(\frac{^{15}N}{^{14}N}\right) Standard} - 1 \right] X\ 1{,}000$$

where $\left(\frac{^{15}N}{^{14}N}\right)$ is the ratio of the heavier, less abundant isotope to the lighter isotope.

Concentrations of nitrate nitrogen were sufficient for analysis of δ^{15}N in only two samples (table 3). Concentrations of ammonia nitrogen were insufficient in all samples for analysis of δ^{15}N. The August 11 Travertine Creek sample had a δ^{15}N in nitrate nitrogen value of -3.94 ‰. The October 1 Rock Creek sample had a δ^{15}N in nitrate nitrogen value of -2.93 ‰. Potential sources having δ^{15}N in nitrate nitrogen values in that range include atmospheric nitrogen and synthetic fertilizers (Clark and Fritz, 1997).

Bacteria Enumeration

Enumeration of fecal coliform and *E. coli* bacteria colonies was performed four times from water collected at both sites (table 3). Two of three fecal coliform counts (August 3 and October 1) from Rock Creek exceeded the Oklahoma standard for primary contact of 235 colonies per 100 milliliters. Interference from nontarget bacterial growth prohibited enumeration of the August 11 sample. Too few samples were collected to determine a 30-day geometric mean, but some concentrations of *E. coli* bacteria exceeded the primary contact standard of 235 colonies per 100 mL for a single sample.

Bacteria concentrations from Travertine Creek were highest on August 11; fecal coliform were 1,300 colonies per 100 mL and *E. coli* were 550 colonies per 100 mL. That sample was collected at 1:45 p.m. on a Wednesday and swimmers were present upstream. Counts of both bacteria were below Oklahoma water-quality standards in samples collected on the other three occasions, including August 3, when swimmers probably were present upstream, and October 1 when streamflow was highest.

Wastewater Compounds

The analysis for wastewater compounds detects compounds that are commonly associated with domestic sewage and industrial wastewater. When multiple compounds are detected, the source may be contamination from sewage overflows and or septic tank effluent. The greatest number of wastewater compounds was detected in Rock Creek during the highest streamflow on October 1 (table 5). Thirty wastewater compounds of 64 analyzed were detected but only three had concentrations high enough to quantify; caffeine, phenol, and tris (2-butoxyethyl) phosphate. Caffeine is a stimulant found in soft drinks and coffee. Phenol, also known as carbolic acid, is commonly found in many man-made products such as disinfectants, plastics, and adhesives. The compound tris (2-butoxyethyl) phosphate is used as a flame retardant and a plasticizer in rubber and plastic (World Health Organization, 2000). Six wastewater compounds were detected in Travertine Creek during the same October 1 event, but concentrations of those compounds were too small to quantify and one compound was not detected in the duplicate sample. No compounds were detected on August 3 in samples from Rock Creek and Travertine Creek. Three compounds were detected

Table 5. Detections and concentrations of organic wastewater compounds measured in surface-water samples collected from Rock Creek and Travertine Creek, Chickasaw National Recreation Area, Oklahoma, 2004

[µg/L, micrograms per liter; <, less than; E, estimated; --, not detected; UV, ultraviolet]

Sample Dates										
Aug. 3, 2004		Aug. 11, 2004		Oct. 1, 2004		Quality assurance and quality control samples		Laboratory Report-ing Level (µg/L)	Wastewater compounds detected	Compound use
Rock Creek	Travertine Creek	Rock Creek	Travertine Creek	Rock Creek	Travertine Creek	Travertine Creek duplicate	Blank sample			
--	--	--	--	[a] M	--	--	--	2.0	3-beta-Coprostanol	Found in human and carnivorous animal feces
--	--	--	--	M	--	--	--	5	para-Nonylphenol	Nonionic detergent metabolite, termite control
--	--	--	--	[b] E 0.1	--	--	--	0.5	Anthraquinone	Used in the manufacturing of dyes, pigments, and paper. Bird repellent
--	--	--	--	E 0.2	--	--	--	0.5	Acetophenone	Food flavoring agent, in perfumes and tobacco, paint and varnish removers
--	--	--	--	E 1	--	--	--	2	beta-Sitosterol	Generally a plant sterol
--	--	--	--	E 1	--	--	--	2	beta-Stigmastanol	Generally a plant sterol
--	--	--	--	M	--	--	--	1	Bisphenol A	Manufacture of polycarbonate resins, flame retardants
--	--	0.5	E 0.1	2.9	M	--	--	0.5	Caffeine	Stimulant, found in coffee, soft drinks, and tea. Very mobile and biodegradable
--	--	--	--	E 0.1	M	M	--	0.5	Camphor	Used as a rubefacient, moth repellent, and as a preservative in drugs and cosmetics
--	--	M	--	--	--	--	--	1	Carbaryl	Insecticide for crop and garden uses, low environmental persistence
--	--	--	--	E 1	--	--	--	1	Cholesterol	Principal sterol found in humans and animals
--	--	--	--	E 0.22	--	--	--	1	Cotinine	The primary nicotine metabolite
--	--	E 0.1	1.6	E 0.3	M	M	--	0.5	N,N-diethyl-meta-toluamide (DEET)	Used in insect repellents
--	--	--	--	E 0.1	--	--	--	0.5	Diazinon	Insecticide for domestic and agricultural use. Restricted from domestic use 2005

Table 5. Detections and concentrations of organic wastewater compounds measured in surface-water samples collected from Rock Creek and Travertine Creek, Chickasaw National Recreation Area, Oklahoma, 2004

[µg/L, micrograms per liter; <, less than; E, estimated; --, not detected; UV, ultraviolet]

Sample Dates										
Aug. 3, 2004		Aug. 11, 2004		Oct. 1, 2004		Quality assurance and quality control samples		Labora-tory Report-ing Level (µg/L)	Wastewater com-pounds detected	Compound use
Rock Creek	Travertine Creek	Rock Creek	Travertine Creek	Rock Creek	Travertine Creek	Travertine Creek duplicate	Blank sample			
--	--	--	--	E 3	--	--	--	5	Diethoxynonylphenol	Nonionic detergent metabolite
--	--	--	--	M	--	--	--	1	Diethoxyoctylphenol	Nonionic detergent metabolite
--	--	--	--	M	--	--	--	1	Ethoxyoctylphenol	Nonionic detergent metabolite
--	--	--	--	M	--	--	--	0.5	Fluoranthene	Formed as a result of incomplete combustion of coal tar and asphalt (not gasoline/ diesel)
--	--	--	--	M	--	--	--	0.5	Indole	Pesticide inert, fragrance: coffee
--	--	--	--	M	--	--	--	0.5	Isophorone	Solvent for lacquers, plastics, oils, silicon, resins
--	--	--	--	E 0.1	--	--	--	0.5	Menthol	Found in cigarettes, cough drops, liniment, and mouthwash
--	--	--	--	E 0.1	M	M	--	0.5	methyl salicylate	Liniments, food, beverage, UV-absorbing lotions
--	--	--	--	M	M	--	--	1	p-Cresol	Wood preservative
--	--	--	--	M	--	--	--	2	Pentachlorophenol	Herbicide, fungicide, wood preservative
--	--	--	--	M	--	--	--	0.5	Phenanthrene	Manufacture of explosives, in tar, diesel, and crude (not gasoline)
--	--	--	E 0.4	2.9	0.9	E 0.2	--	0.5	Phenol	Disinfectants, mouthwashes, manufacture of resins, plastics, adhesives. Found in animal wastes and decomposing organic material
--	--	--	--	M	--	--	--	0.5	Pyrene	Common in coal tar and asphalt (not gasoline/diesel)

Table 5. Detections and concentrations of organic wastewater compounds measured in surface-water samples collected from Rock Creek and Travertine Creek, Chickasaw National Recreation Area, Oklahoma, 2004

[µg/L, micrograms per liter; <, less than; E, estimated; --, not detected; UV, ultraviolet]

Sample Dates								Laboratory Report-ing Level (µg/L)	Wastewater compounds detected	Compound use
Aug. 3, 2004		Aug. 11, 2004		Oct. 1, 2004		Quality assurance and quality control samples				
Rock Creek	Travertine Creek	Rock Creek	Travertine Creek	Rock Creek	Travertine Creek	Travertine Creek duplicate	Blank sample			
--	--	--	--	--	M	M	--	0.5	Tribromomethane	Used to make chemicals and drugs, and as an industrial solvent. By product of chlorination
--	--	--	--	E 0.4	--	--	--	0.5	Triphenyl phosphate	Plasticizer, resins, waxes, finishes, roofing paper, flame retardant
--	--	--	--	2	--	--	--	0.5	Tris (2-butoxyethyl) phosphate	Plasticizer, flame retardant
--	--	--	--	E 0.1	--	--	--	0.5	Tris (dichloroisopropyl) phosphate	Flame retardant
--	--	--	1.2	E 0.1	--	--	--	0.5	Tris (2-choroethyl) phosphate	Flame retardant in plastics

[a]Material has been detected and verified but concentration is very low and below the reporting level.

[b]Concentration values reported with an "E" remark code indicate an estimated concentration. Estimation occurs when a compound is detected in the region between the laboratory reporting level or lowest calibration standard, whichever is greater, and the long-term method detection level (Childress and others, 1999).

August 11 in Rock Creek, however, only caffeine was at a concentration high enough to measure at 0.5 micrograms per liter (µg/L). Four compounds were detected on August 11 in Travertine Creek, two at concentrations high enough to quantify: N, N-diethyl-meta-toluamide (DEET) (1.6 µg/L) and tris (2-choroethyl) phosphate (1.2 µg/L). The compound DEET is an insecticide commonly used in insect repellents and tris (2-choroethyl) phosphate is a flame retardant commonly used in plastics.

Bacterial Source Tracking

The BST technique used for this study is a genotypic method that relies on the assumption that the DNA fragments from ribosomal RNA genes of *E. coli* strains from one animal type, for example cattle, are more similar to one another than to those from other animal types or humans. This assumption remains under investigation and accordingly, the results should be characterized as experimental in nature.

The DuPont Qualicon RiboPrinter® Microbial Characterization System (DuPont Qualicon, 2003) was used in combination with the two restriction enzymes *EcoRI* and *PvuII* to create a banding sequence or fingerprint of selected DNA fragments. This BST technique requires the development of a source library containing DNA fingerprints of *E. coli* from known sources to compare to *E. coli* from surface water for possible matches. For this study, the source library contained 153 isolates for which DNA fingerprints were generated; 59 cattle, 15 sheep, 13 horse, 4 human, and 62 sewage (table 6). Although wildlife may be a significant source of bacteria to surface water in the study area, no fecal samples from wildlife were collected. All samples for the source library were collected in the Upper Rock Creek basin to decrease the potential for genetic variability that appears to occur with geographic distance (Scott and others, 2002); (Hartel and others, 2002).

There are three concerns about the BST technique used for this study, which should be considered when interpreting the results. The first is the lack of representation of all the potential sources of bacteria in the source library. Seventy-nine percent of the DNA fingerprints in the source library were from sewage and cattle; DNA fingerprints from humans, deer, waterfowl, cats, and other animals, which may be a significant source of bacteria, were not available or under represented. As a result, bacteria from other sources may have been present in surface water on collection dates but were not identified. The second concern is the small number of isolates in the source library. The 153 source isolates, realistically, is too small to represent the diversity of animals in the study area and the bacteria strains inhabiting each animal type. The source library size needed to accomplish this is uncertain but as a general rule is related to the size of the study area, the number of potential sources, and the BST methodology used (Stoeckel and others, 2004). In general, larger source libraries increase the possibility of discerning bacteria sources. The third concern is the lack of reproducibility shown by the quality-control isolates. Ten *E. coli* isolates from the source library were duplicated and submitted for BST, only one was matched to the correct source isolate. Reasons why the duplicates may not have matched the correct source isolates include the potential contamination of the duplicate isolates and or genetic mutations of the bacteria with time, as the duplicates were prepared and submitted at a date later than the original isolates. (S. Reilly, Food and Agricultural Products Research and Technology Center, Oklahoma State University, written commun., 2005).

In addition to the 153 DNA fingerprints generated for *E. coli* in the source library, there were fingerprints generated for 58 *E. coli* collected from Rock Creek and 54 *E. coli* collected from Travertine Creek surface-water samples. Comparisons and classification of the surface-water *E. coli* to *E. coli* in the source library were performed by analysis software associated with the DuPont Qualicon RiboPrinter® Microbial Characterization System (DuPont Qualicon, 2003) and were based on an 85 percent or greater similarity of band intensities and band sizes of the DNA fingerprints. The comparison produced 17 ribotype groups having similar DNA patterns composed of 29 surface-water *E. coli* from Rock Creek, 35 surface-water *E. coli* from Travertine Creek, and 36 *E. coli* from the source library. In seven groups, cattle was the sole source, sewage was the sole source in three groups, horse was the sole source in three groups, and five groups contained DNA patterns from multiple sources. Table 7 shows the number of *E. coli* bacteria isolates from surface water that were ribotyped and the number classified into ribotype groups based on similarities of DNA fingerprints. Results show that DNA fingerprints of *E. coli* collected from Rock Creek were similar to those isolated from cattle on the August 3 and 11, and September 19 collection dates and similar to those isolated from sewage on the August 11, September 19, and October 1 collection dates. The DNA fingerprints of *E. coli* collected from Travertine Creek were similar to those isolated from cattle and sewage on the September 19 and October 1 collection dates and similar to those isolated from horses on the September 19 collection date.

Possible Sources of Bacteria and Nitrogen to Rock and Travertine Creeks

The water quality of Rock Creek in the CNRA seems to be affected most during periods of precipitation from surface-water runoff and sewage effluent. Fecal coliform and *E. coli* bacteria counts in addition to ammonia nitrogen plus organic nitrogen and total nitrogen concentrations were highest in Rock Creek during the precipitation event on October 1. The increase of these constituents in surface water probably can be attributed in part to runoff containing fecal and organic material from non-point sources such as livestock grazing areas in the basin. The area of early runoff in the Rock Creek basin, downstream from the floodwater retarding structures

Table 6. Number of *Escherichia coli* bacteria isolates ribotyped from source-group material collected in the Upper Rock Creek basin and Travertine Creek subbasin for bacterial source tracking, Chickasaw National Recreation Area, Oklahoma. Bacterial source tracking was performed using the RiboPrinter® Microbial Characterization System (DuPont Qualicon, 2003) at the Food and Agricultural Products Research and Technology Center at Oklahoma State University, Stillwater, Oklahoma

Known-source groups (number of samples)	Number of *Escherichia coli* isolates ribotyped
Cattle (59)	59
Sheep (15)	15
Horse (13)	13
Human/septic tank (1)	4
Sewage (10)	62
Total	153 ribotyped

Table 7. Number of surface-water *Escherichia coli* bacteria isolates collected from Rock Creek and Travertine Creek that were ribotyped and similar to *Escherichia coli* in the source reference library, Chickasaw National Recreation Area, Oklahoma. *Escherichia coli* were ribotyped using the RiboPrinter® Microbial Characterization System (DuPont Qualicon, 2003) at the Food and Agricultural Products Research and Technology Center at Oklahoma State University in Stillwater, Oklahoma. Matching was based on an 85 percent similarity of band intensity and band size.

Date of Sample	Rock Creek		Travertine Creek	
	Number of surface-water *Escherichia coli* ribotyped	Number of surface-water *Escherichia coli* in ribotype groups	Number of surface-water *Escherichia coli* ribotyped	Number of surface-water *Escherichia coli* in ribotype group
August 3, 2004	25	5- cattle 1-horse 9-multiple	7	2- multiple
August 11, 2004	5	1-cattle 1-sewage 1-multiple	3	1-multiple
September 19, 2004	18	2-cattle 1-horse 1-sewage 2-multiple	20	4-cattle 1-horse 2-sewage 7-multiple
October 1, 2004	10	3-sewage 2-multiple	24	3-cattle 2-sewage 13-multiple

and upstream from the CNRA, may be an area of considerable non-point source contamination during precipitation events. The protection of this area from agricultural uses and urbanization may help prevent the degradation of water quality in Rock Creek.

The presence of bacteria and other contaminants in untreated sewage effluent also degrades the water quality of Rock Creek in the CNRA. The detection of 30 organic wastewater compounds on October 1 demonstrates the overflow of sewage effluent into the creek during periods of precipitation. The antiquated condition of the city's sanitary sewage conduits is an acknowledged problem indicated by the large increase in effluent that enters the wastewater treatment facility during periods of heavy rainfall. Sewage effluent also may be a source of nitrogen, but the degree of contribution is unknown. Septic tank effluent also may enter Rock Creek at locations upstream from the city of Sulphur; however, where septic tank effluent and or sewage effluent enters Rock Creek is unknown. The BST results show that *E coli* bacteria from sewage were present in Rock Creek samples collected August 11, September 19, and October 1 and *E. coli* bacteria from cattle were present August 3, August 11, and September 19. The accuracy of these data is questionable, however, because of the limitations associated with the BST technique and problems associated with the quality-control isolates.

The relatively high nitrogen compound concentrations compared to historical concentrations measured in Rock Creek suggest an increase of nitrogen sources in the basin upstream from the CNRA. More than half of the measured nitrogen compound concentrations either equaled or exceeded the highest historical concentration for that compound measured over a period of 2 to 5 years from 1990 to 1995. The nitrogen isotope values indicate that the source of nitrate nitrogen in Rock Creek and Travertine Creek is atmospheric and or synthetic fertilizers. These may be important sources to surface water in the basin; however, the measured concentrations of nitrate nitrogen were very small and only sufficient in two samples for analysis of nitrogen isotopes. Additional sampling is needed during various flow regimes at selected locations in the basin to better define sources of nitrogen to Rock Creek.

Swimmers and waders seem to be an intermittent source of bacteria to Travertine Creek during low-flow conditions. Fecal coliform and *E. coli* bacteria counts exceeded the standards in one of three samples collected from Travertine Creek. The water sample was collected August 11 in the afternoon during low-flow conditions with swimmers upstream. Additional samples upstream and downstream from the swimming areas would be needed to confirm that swimmers are a substantial source of bacteria during low-flow conditions. The DNA fingerprints of *E. coli* collected from Travertine Creek were similar to those isolated from cattle and sewage on the September 19 and October 1 collection dates and similar to horse on the September 19 collection date. As mentioned the accuracy of these results are questionable, however.

There was no strong evidence showing that surface-water runoff from residential areas in the subbasin or sewage effluent contributes bacteria or other contaminants to Travertine Creek. Two water samples (August 11 and October 1) had detections of four organic wastewater compounds and six organic wastewater compounds, respectively; however, the chemicals detected are commonly used outdoors and could have been introduced by swimmers and waders upstream. If sewage effluent was entering Travertine Creek, higher counts of fecal coliform and *E. coli* bacteria would be expected. Additional sampling is needed during various flow regimes to provide more information about possible sources of contamination to Travertine Creek.

There were no historical nitrogen compound concentrations for Travertine Creek for comparison to concentrations measured during this study. Nitrogen compound concentrations measured in Travertine Creek were relatively low compared to other streams having similar stream characteristics in Oklahoma.

Summary

The USGS in cooperation with the NPS conducted a study in the Upper Rock Creek basin in Murray County, Oklahoma during 2004 to identify the possible primary source(s) of *E. coli* bacteria and related contaminants to surface water using: (1) analysis of nitrogen compounds and nitrogen-isotope ratios in nitrate nitrogen and ammonia nitrogen, (2) analysis of organic compounds associated with human wastewater, and (3) a genotypic method of bacterial source tracking.

In May 2004, stream discharge and the water properties were measured in addition to counts of fecal coliform and *E. coli* bacteria at sites on Rock Creek and Travertine Creek. Surface water was sampled an additional three times at these locations during August and October. During those three sampling occasions, water properties and stream discharge were measured and samples were analyzed for nitrogen compounds and the stable nitrogen isotopes ^{15}N and ^{14}N in nitrate and ammonia nitrogen, and organic wastewater compounds. Fecal indicator bacteria were enumerated and surface water was collected to culture and isolate *E. coli* bacteria for a bacterial source-tracking technique described as ribotyping.

The water quality of Rock Creek in the CNRA seems to be affected most during periods of precipitation from surface-water runoff and sewage effluent. Fecal coliform and *E. coli* bacteria counts in addition to ammonia nitrogen plus organic nitrogen and total nitrogen concentrations were highest in Rock Creek during the precipitation event on October 1. The increase of these constituents in surface water probably can be attributed in part to runoff containing fecal and organic material from non-point sources such as livestock grazing areas in the basin. The area of early runoff in the Rock Creek basin, downstream from the floodwater retarding structures and upstream from the CNRA, may be an area of considerable non-point source contamination during precipitation events.

The presence of bacteria and other contaminants in untreated sewage effluent also degrade the water quality of Rock Creek in the CNRA. The detection of 30 organic wastewater compounds on October 1 demonstrates the possible overflow of sewage effluent into the creek during periods of precipitation.

The relatively high nitrogen compound concentrations compared to historical concentrations measured in Rock Creek suggest an increase of nitrogen sources in the basin upstream from the CNRA. More than half of the measured nitrogen compound concentrations either equaled or exceeded the highest historical concentration for that compound measured over a period of 2 to 5 years from 1990 to 1995. The nitrogen isotope values indicate that the source of nitrate nitrogen in Rock Creek and Travertine Creek is atmospheric and or synthetic fertilizers. These may be important sources of nitrogen to surface water in the basin; however the measured concentrations of nitrate nitrogen were very small and only sufficient in two samples for analysis of nitrogen isotopes. Additional sampling is needed during various flow regimes at selected locations in the basin to better define sources of nitrogen to the basin.

Swimmers and waders seem to be an intermittent source of bacteria to Travertine Creek during low-flow conditions. However, additional samples upstream and downstream of the swimming areas would be needed to confirm that swimmers are a substantial source of bacteria during low-flow conditions. There was no strong evidence showing that surface-water runoff from residential areas in the subbasin or sewage effluent contributes bacteria or other contaminants to Travertine Creek. The organic wastewater compounds detected are commonly used outdoors and could have been introduced by swimmers and waders upstream. If sewage effluent was entering Travertine Creek, higher counts of fecal coliform and *E. coli* bacteria would be expected. Additional sampling is needed during various flow regimes to provide more information about possible sources of contamination to Travertine Creek.

There were no historical nitrogen compound concentrations for Travertine Creek for comparison to concentrations measured for this study. However, nitrogen compound concentrations measured in Travertine Creek were relatively low compared to other streams having similar stream characteristics in Oklahoma.

The BST results show that *E coli* bacteria from sewage were present in Rock Creek samples collected August 11, September 19, and October 1 and *E. coli* bacteria from cattle were present August 3, August 11, and September 19. The accuracy of these data is questionable, however, because of the limitations associated with the BST technique and problems associated with the quality control isolates. There are three concerns about the bacterial source tracking technique used for this study that should be considered when interpreting the results: the lack of representation of all the potential sources of bacteria in the source library, the small number of isolates in the source library, and the lack of reproducibility shown by the quality control isolates. The DNA fingerprints of *E. coli* collected from Rock Creek were similar to those isolated from cattle and sewage on three surface-water collection dates. The DNA fingerprints of *E. coli* collected from Travertine Creek were similar to those isolated from cattle and sewage on two collection dates and similar to those isolated from horses on one collection date.

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