Water Quality in the Upper Shoal Creek Basin, Southwestern Missouri, 1999–2000

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Abstract

Results of a water-quality investigation of the upper Shoal Creek Basin in southwestern Missouri indicate that concentrations of total nitrite plus nitrate as nitrogen (NO_{2t}+NO_{3t}) in water samples from Shoal Creek were unusually large [mean of 2.90 mg/L (milligrams per liter), n (sample size)=60] compared to other Missouri streams (mean of 1.02 mg/L, n=1,340). A comparison of instantaneous base-flow loads of NO_{2t}+NO_{3t} indicates that at base-flow conditions, most NO_{2t}+NO_{3t} discharged by Shoal Creek is from nonpoint sources. Nearly all the base-flow instantaneous load of total phosphorus as P (P_t) discharged by Shoal Creek can be attributed to effluent from a municipal wastewater treatment plant. Samples collected from a single runoff event indicate that substantial quantities of P_t can be transported during runoff events compared to base-flow transport. Only minor quantities of NO_{2t}+NO_{3t} are transported during runoff events compared to base-flow transport.

Fecal coliform bacteria densities at several locations exceed the Missouri Department of Natural Resources (MDNR) standard of 200 col/100 mL (colonies per 100 milliliters) for whole-body contact recreation. During 13 months of monitoring at 13 stream sites, fecal coliform densities (median of 277 and 400 col/100 mL) at two sites (sites 2 and 3) on Shoal Creek exceeded the MDNR standard at base-flow conditions. The maximum fecal coliform density of 120,000 col/100 mL was detected at site 3 (MDNR monitoring site) during a runoff event in April 1999 at a peak discharge of 1,150 ft^3/s (cubic feet per second). Fecal coliform densities also exceeded the MDNR standard in three tributaries with the largest densities (median of 580 col/100 mL) detected in Pogue Creek.

Results of ribopattern analyses indicate that most *Escherichia coli* (*E. coli*) bacteria in water samples from the study area probably are from nonhuman sources. The study area contains about 25,000 cattle, and has an estimated annual production of 33 million broilers and 300,000 turkeys. Probable nonhuman sources included turkeys, horses, chickens, and cattle; however, wildlife sources such as deer, raccoon, muskrat, and opossum were not evaluated. Human waste was an important source of *E. coli* in water samples collected at the MDNR monitoring site (site 3) on Shoal Creek and at two tributary sites (Joyce Creek and Woodward Creek). In general, the detection of human ribopatterns was consistent with the detection of organic compounds commonly associated with human wastewater such as caffeine, triclosan, or phenol, and the fecal indicators cholesterol and 3*B*-coprostanol. Ribopattern analysis indicate that horses were an important source of *E. coli* in Woodward Creek, which was consistent with horses being pastured immediately upstream from the sampling site on this creek. Pogue Creek contains a large density of turkey barns and five of eight *E. coli* isolates from one sample from Pogue Creek were matched to turkeys. Water samples from Pogue Creek generally did not contain detectable concentrations of

human wastewater compounds, but one sample did contain detectable quantities of the antibiotics tylosin and lincomycin (widely used in the animal industry), and sulfamethoxazole (human use only). Although promising, the ability of ribopattern analyses to positively identify the source of a particular isolate is uncertain because of the small sample size, possible differences between animal source patterns in the study area and database used, lack of native wildlife source patterns, and variation in results depending on the number of possible animal host considered.

Results of this study indicate that a trend of increasing fecal coliform densities with increasing time detected by the MDNR is, in part, caused by trends in annual precipitation and stream discharge, and not necessarily changes in land use or densities of animal operations. A multiple linear regression (MLR) model using specific conductance and water temperature explained 65 percent of the variability in the logarithm of fecal coliform densities in water samples collected from the MDNR sampling site since 1992. The model correctly predicted fecal coliform densities above the MDNR standard of 200 col/100 mL 83 percent of the time (45 of 54 samples). Although the trend of increasing fecal coliform densities with time at the MDNR sampling site may not be related to changes in animal production in the basin, the large fecal coliform densities at sites 2 and 3 on Shoal Creek and in several tributaries are, in part, probably related to the high density of animals in the basin. Screening of 13 water samples from 8 sites in the study area detected the presence of the human pathogen *E. coli* O157:H7 in one sample from the MDNR sampling site.

INTRODUCTION

Shoal Creek is located within the Springfield Plateau of the Ozark Plateaus physiographic province in southwestern Missouri. From its headwaters in Barry County, Missouri, Shoal Creek flows about 70 mi (miles) through mostly rural agricultural areas until it reaches the Missouri-Kansas state line about 8 mi west of Joplin, Missouri (fig. 1). Shoal Creek is an important source of drinking water supply for the cities of Joplin, Missouri (population about 150,000) and Neosho, Missouri (population about 10,000) (U.S. Census Bureau, 2000). The lower 49-mi reach of Shoal Creek from the Missouri state line to about 0.5 mi downstream from the mouth of Capps Creek has eight designated beneficial uses (protection of warm-water aquatic life and human-health fish consumption, boating and canoeing, cold water fishery, drinking water supply, industrial water supply, irrigation, livestock and wildlife watering, and whole-body contact recreation)—more than any other stream in the State except the Missouri River (Missouri Department of Natural Resources, 1996). Whole-body contact recreation and boating and canoeing are among the six designated beneficial uses along a 13.5-mi reach from the mouth of Capps Creek to about 1 mi upstream from the mouth of Woodward Creek (fig. 1). Livestock and wildlife watering and protection of warm-water aquatic life are the two designated beneficial uses for the 5-mi reach of Shoal Creek from 1 mi upstream from the mouth of Woodward Creek to near its headwaters.

Densities of fecal coliform bacteria above the Missouri Standard of 200 col/100 mL (colonies per 100 milliliters) for whole-body contact recreation (Missouri Department of Natural Resources, 1996) have been routinely detected by the Missouri Department of Natural Resources (MDNR) at a monitoring site on the upper reach of Shoal Creek near Fairview, Missouri (fig. 1). The exceedences of fecal coliform bacteria at this site have resulted in a part of Shoal Creek upstream from Capps Creek (fig. 2) being included on the 1998 Clean Water Action Plan (CWP) 303(d) list of impaired water bodies by the MDNR (Missouri Department of Natural Resources, 2000). In addition, concentrations of total nitrite plus nitrate as nitrogen (NO_{2t}+NO_{3t}) in grab samples collected by the Natural Resources Conservation Service (NRCS) from the upper reaches of Shoal Creek and its tributaries since 1995 are larger than most other streams in Missouri (Missouri Department of Natural Resources, January 2000). There is a need to better understand the water quality in the upper Shoal Creek Basin as related to various land uses and activities within the basin. Because of this need, the MDNR, Division of Environmental Quality, Water Pollution Control Program and U.S. Environmental Protection Agency (USEPA) entered into a cooperative agreement with the U.S. Geological Survey (USGS) to investigate water quality in the basin, with an emphasis on the distribution and possible sources of nutrients and fecal coliform bacteria.

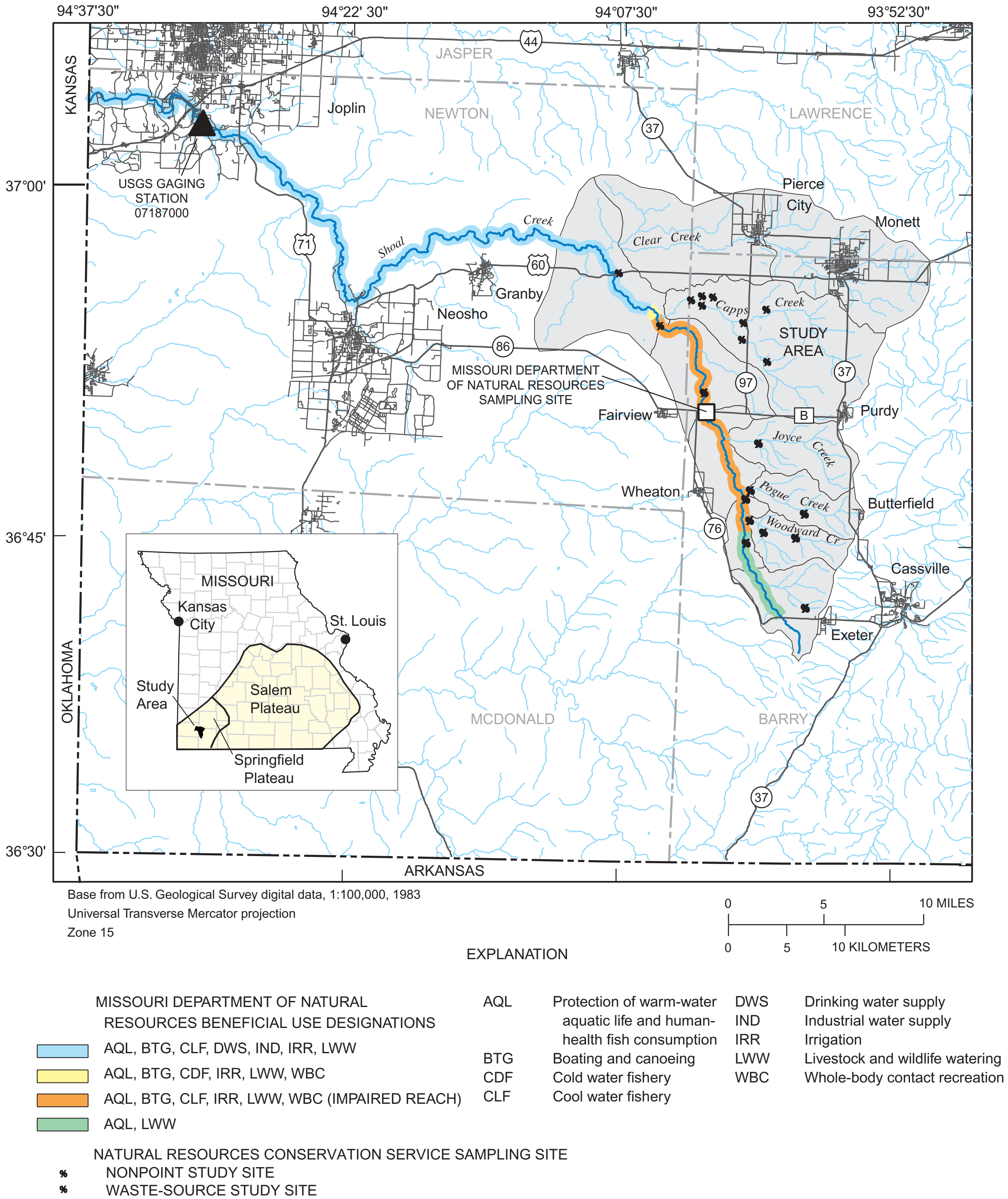


Figure 1. Location of the upper Shoal Creek Basin study area and Missouri Department of Natural Resources beneficial use designations for Shoal Creek.

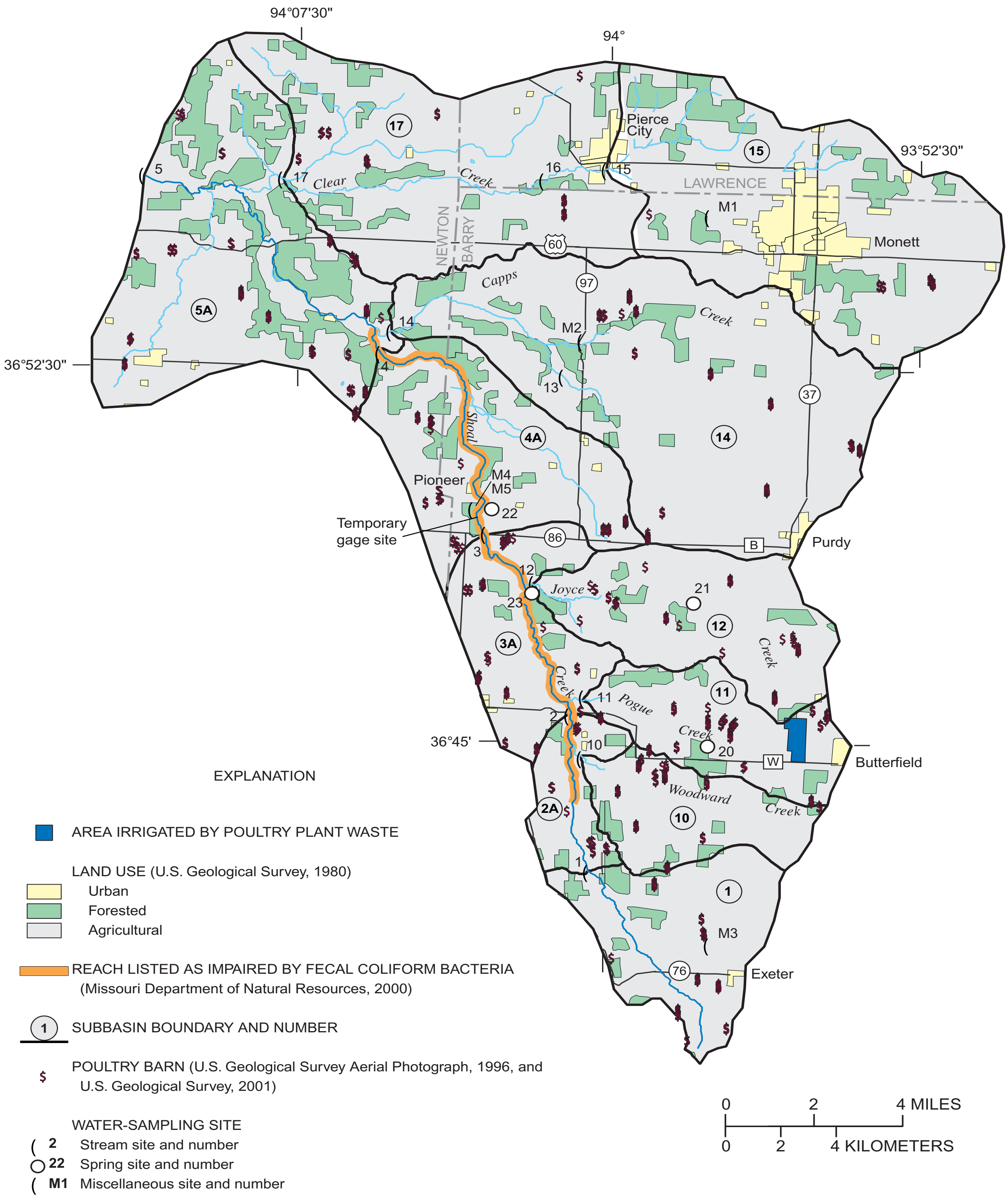


Figure 2. Location of U.S. Geological Survey sampling sites, land use, and distribution of poultry barns in the study area.

Purpose and Scope

This report describes water quality in the upper Shoal Creek Basin, with an emphasis on distribution and possible sources of nutrients and fecal coliform bacteria. Data also are presented on the distribution of major ions, selected trace elements, and organic compounds commonly associated with municipal and domestic wastewater, and the presence of the known human pathogen *Escherichia coli* (*E. coli*) O157:H7. The possible origin of fecal coliform bacteria in several water samples was investigated using molecular genetic techniques being researched at the University of Missouri at Columbia (UMC) College of Veterinary Medicine.

More than 170 water samples were collected from a network of 17 sites during a 13-month study period between April 1, 1999, and May 1, 2000. Water samples also were collected from three sites on the main stream of Shoal Creek during an April 1999 runoff event. Water samples were submitted for various analyses including total nutrients, indicator bacteria densities, and optical brighteners. Selected samples were analyzed for major ions and selected trace elements, a suite of 48 organic compounds, and pharmaceutical compounds. A temporary continuously recording gaging station to monitor stage, specific conductance, and water temperature was installed on Shoal Creek about 0.5 mi downstream from the MDNR sampling site at the State Highway 97 bridge (fig. 2).

Previous Investigations

The MDNR began ambient monitoring at two sites in the upper Shoal Creek Basin during 1992. One site was located on the main stem of Shoal Creek at State Highway 97 (site 3) and the other site was near the mouth of Capps Creek (site 14), a major tributary in the upper Shoal Creek Basin (fig. 2). Water samples generally were collected monthly during 1992 and 1993 and analyzed for fecal coliform and fecal streptococcus densities, total phosphorus (P_t), NO_{2t}+NO_{3t}, total ammonia as nitrogen (NH_{3t}), total Kjehdahl nitrogen as N (TKN), and chloride (Cl). Field measurements were made of discharge, water temperature, specific conductance, and dissolved oxygen (DO) concentrations. Monitoring was suspended during 1994 and resumed on a monthly cycle only at the Shoal Creek site during 1995; however, discharge was not measured and samples were not analyzed for Cl concentrations. Between 1992 and 1998, densities of fecal coliform bacteria at the Shoal Creek site averaged 13,140 col/100 mL with a median of 320 col/100 mL and a range of less than 1 to 400,000 col/100 mL (data supplied by the MDNR, Water Pollution Control Program). Concentrations of NO_{2t}+NO_{3t} and P_t in these samples averaged 3.15 and 0.17 mg/L (milligrams per liter), respectively.

During 1995, the USEPA and MDNR initiated a 5-year nonpoint-source study focusing on reducing nutrient concentrations in the upper Shoal Creek Basin. This study included the collection of monthly grab samples from six stream sites and four spring sites (fig. 1). The study also included technical assistance from the NRCS to work with area poultry producers and farmers to develop nutrient management plans and implement best management practices (BMPs). Beginning in 1998, the MDNR sponsored a Special Area Land Treatment (SALT) project in the upper Shoal Creek Basin. The 5-year SALT project is a locally led watershed project that focuses on reducing agricultural nonpoint-source pollution. This project is a cooperative effort between the MDNR, NRCS, Missouri Department of Conservation (MDOC), University of Missouri Extension Office, local volunteers, farmers, and poultry producers. The project includes monthly nutrient sampling at six stream sites and four spring sites on or near demonstration farms, predominantly in the Capps Creek Basin (fig. 1).

Acknowledgments

The author is indebted to the many local landowners for granting permission to sample streams or springs on their property. Special acknowledgment is given to Dan Philbrick and Kari Rhoades of the Natural Resources Conservation Service, Barry County office for their technical assistance and for providing historical data on farming practices in the region. The author also is grateful to Joseph Renkoski for allowing the installation of a temporary stream-gaging and water-quality sampling station on his property.

DESCRIPTION OF THE STUDY AREA

The study area encompasses the upper 233 mi^2 (square miles) of the Shoal Creek Basin in parts of Barry, Newton, and Lawrence Counties in southwestern Missouri (fig. 2). This area was selected for study because it contains the largest density of poultry operations in the Shoal Creek Basin, is sparsely populated, and encompasses the reach of Shoal Creek listed by the MDNR as impaired by fecal coliform bacteria. There are five major tributaries to Shoal Creek in the study area (Woodward Creek, Pogue Creek, Joyce Creek, Capps Creek, and Clear Creek) that have drainage areas ranging from 9.6 to 69.3 mi^2.

Climate

The upper Shoal Creek Basin is characterized by temperate climate with warm, humid summers, and cool, wet winters. The National Oceanic and Atmospheric Administration (NOAA) operates two climatological stations in the area—one in Monett Missouri, with 29 years of record, and the other about 15 mi south in Cassville, Missouri, with 60 years of record (National Oceanic and Atmospheric Administration, 1999, 2000). The mean annual temperature measured at Monett is about 55 ^{o}F (degrees Fahrenheit). The temperature record at the Cassville station is incomplete. During the 13-month sampling period between April 1, 1999, and May 1, 2000, the recorded temperature extremes at Monett were a minimum of -1 ^{o}F (January 4, 2000) and a maximum of 102 ^{o}F (August 13, 1999). The mean annual precipitation measured at Cassville is 43.18 in. (inches) with about 70 percent of the precipitation falling between February and November. Rainfall recorded at Cassville (42.38 in.) during the 13-month sampling period was 7.16 in. below normal. Rainfall during the first 3 months of the study (18.10 in.) was 4.24 in. above the normal of 13.86 in. (fig. 3). The study area experienced drought conditions during the final 10 months of sample collection activities with only 24.28 in. of rainfall recorded at Monett compared to the normal rainfall of 35.68 in.

Topography and Geohydrology

Land-surface elevations in the study area range from 1,060 ft (feet) at the downstream site (site 5) on Shoal Creek in the northwest part of the study area to more than 1,570 ft in the southeast. The eastern part of the study area is gently to moderately sloping with slopes of less than 10 percent. This area is part of a larger plateau with elevations exceeding 1,500 ft that extends across much of central Barry County, Missouri (Aldrich and Meinert, 1994).

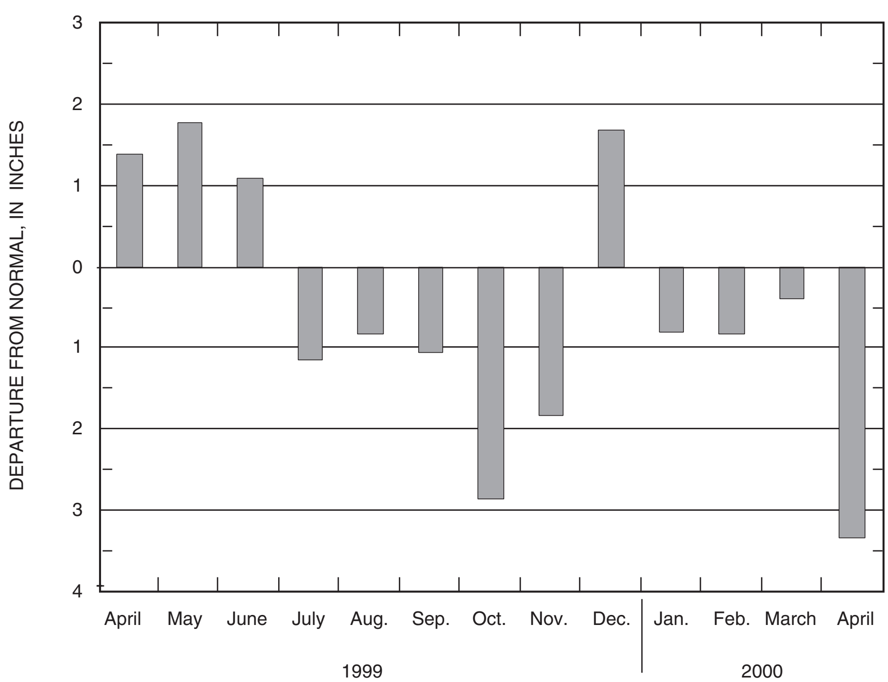


Figure 3. Departure from average monthly rainfall at Cassville, Missouri, April 1999 through April 2000 (data from the National Oceanic and Atmospheric Administration, 1999–2000).

The study area is underlain by Mississippian-age limestones that are, in decreasing age, the undifferentiated Reeds Spring and Elsey Formations, Grand Falls Chert, and the undifferentiated Burlington-Keokuk Limestone. Usage of geologic names in this report conforms to usage by the MDNR, Division of Geology and Land Survey (DGLS). Pennsylvanian-age shales crop out in isolated areas in the eastern part of the study area. Rocks in the study area can contain up to 70 percent chert. For example, the discontinuous Grand Falls Chert (0 to 40 ft thick), described as a chert-rich facies of several Mississippian-age units, consists of massive layers of chert as much as 6 ft thick (Thompson, 1986). The unit is exposed in the bed of Shoal Creek in the southern part of the study area where it forms shoals, riffles, and small waterfalls. Numerous wet-weather seeps and small springs appear in fields with little soil cover that are underlain by the Grand Falls Chert.

The Mississippian-age rocks beneath the study area are part of the Springfield Plateau aquifer system (Imes and Smith, 1990). The aquifer is about 200 ft thick in the study area. The aquifer is adequate for most domestic uses with well yields of 5 to 20 gal/min (gallons per minute) and low dissolved solids concentrations (less than 200 mg/L) where exposed at the surface. Karst topography developed in the Mississippian-age rocks at the land surface makes the aquifer vulnerable to contamination from surficial sources. Within the study area, numerous small [less than 1 ft^3/s (cubic foot per second)] springs indicate the presence of a karst system; however, features characteristic of more intense karst development, such as sinkholes and losing streams, are infrequent and limited to the northern part of the study area within the Clear Creek and Capps Creek Basins (fig. 2).

Soils

There are three major soil associations in the study area (fig. 4). The Scholten-Tonti association are very deep, moderately well drained, silty and gravelly-silty soils that have a fragipan (Aldrich and Meinert, 1994). Many of these soils developed from weathering of the Burlington-Keokuk Limestone and have chert nodules and dark red clay that is predominantly kaolinite. Typical soils developed from the Burlington-Keokuk Limestone include Noark and Clarksville, and Scholten soils of the Scholten-Tonti association that are widely distributed across the uplands in the eastern and southern part of the study area (fig. 4). The Clarksville-Noark-Nixa association generally is found in the northwest part of the study area and is characterized by very deep, well drained to excessively drained, very gravelly, silty soils without a fragipan. Soils of the Secesh-Claiborne association are well drained, silty soils, and commonly found on stream terraces and at the foot of slopes. Soils in the study area naturally have small soil-test phosphorus contents [4 to 30 lbs/acre (pounds per acre)]; however, soils in fields where poultry litter has been land applied for nearly 10 years have average soil test phosphorus values of 257 lbs/acre (Kari Rhoades, Natural Resources Conservation Service, written commun., December 2000).

Soils within the study area have a wide rage of physical and hydrologic properties. Runoff from low to moderate intensity rainfall within the study area is limited because of the moderate to high vertical permeability in the upper 1 ft of the soils. Soils covering 93 percent of the study area have average vertical permeabilities equal to or exceeding 1.3 in/hr (inches per hour), and soils covering 36 percent of the study area have average vertical permeabilities exceeding 4 in/hr.

Land Use and Population

Land use in the study area is predominantly agricultural (84 percent) with deciduous forest (13 percent) and minor (about 3 percent) urban areas (fig. 2). Nearly all of the agricultural land is used for pasture or hay production. Most forested areas are concentrated in the northwest part of the study area on steep slopes unsuitable for agricultural uses or in lowland areas along streams. Land use in the region changed dramatically during the 20^{th} century. In 1902, more than 120,000 acres of Barry County (25 percent) were under cultivation, and about 10,000 acres were used for hay production (Dan Philbrick, Natural Resources Conservation Service, written commun., January 2000). In 1999, less than 2,000 acres in Barry County were cultivated for row crops and 60,000 acres were used for hay production (Missouri Agricultural Statistics Service, September 2000).

The population in the study area was estimated to be about 13,518, of which about 78 percent live within towns. The study area population was derived by summing the estimated 1998 population (U.S. Census Bureau, 2000) of the incorporated towns of Monett

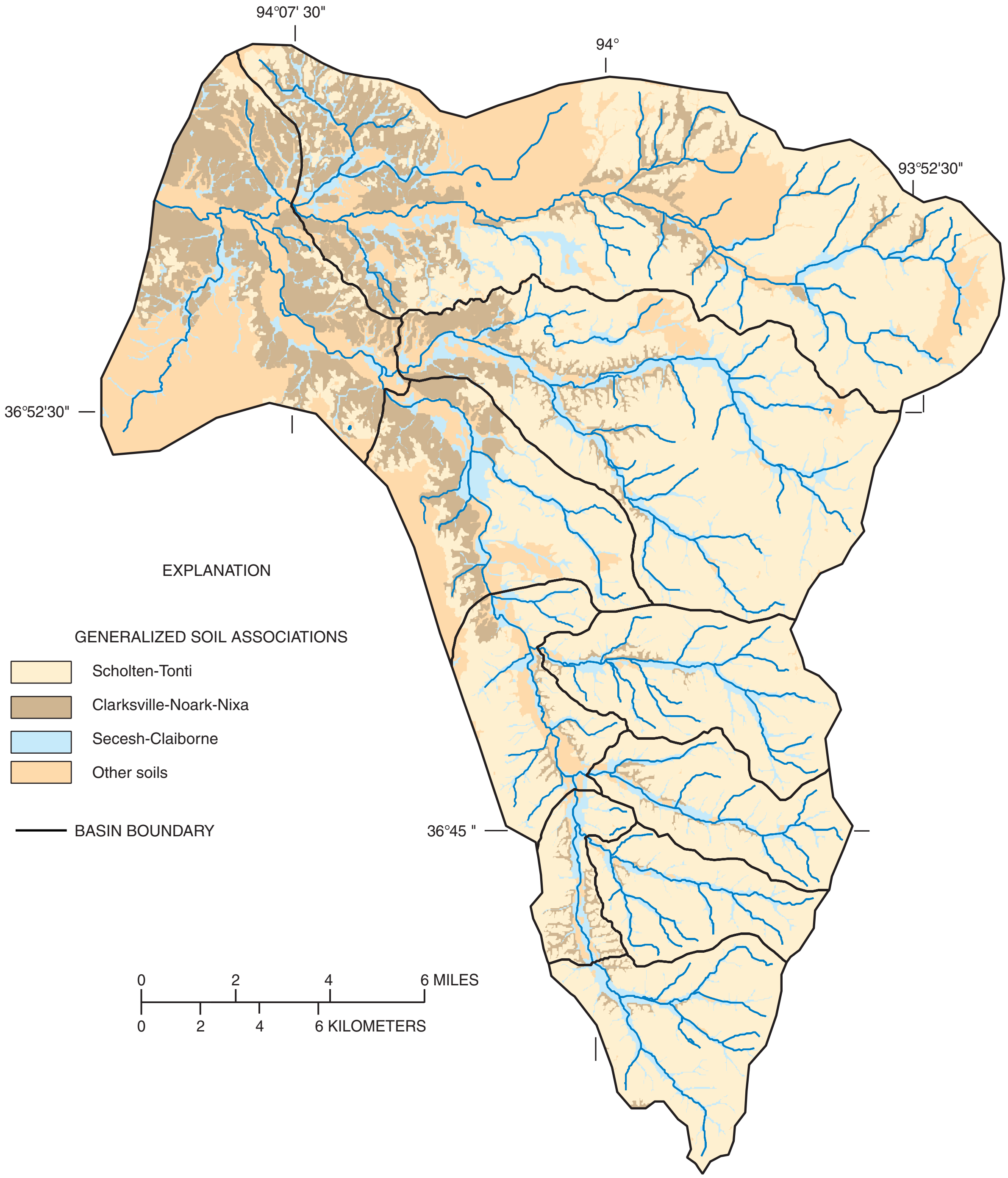


Figure 4. Distribution of predominant soil associations in the study area (soils data modified from Aldrich and Meinert, 1994).

(7,431), Pierce City (1,537), Purdy (1,168), Butterfield (315), and Pioneer (40) and estimates of the rural population. The rural population of 3,027 was estimated by multiplying the number of rural homes identified on 1996 aerial photography (1,121) by the average of 2.7 persons per household estimated by the U.S. Bureau of the Census for rural Missouri.

Distribution of Animal Feeding Operations and Nutrient Loadings

The MDNR has identified 99 animal feeding operations (AFOs) in the study area by permit or by letter submission of manure management plans having an estimated 63,600 animal units (AUs). Eighty-nine of these facilities are commercial poultry operations and 10 are dairy operations with a total of 1,670 AUs (data provided by John Ford, Missouri Department of Natural Resources, Water Pollution Control Program, 1999). One AU is defined as the manure production equivalent to one 1,000-lb (pound) beef cow. The MDNR list is not comprehensive because only facilities exceeding 1,000 AUs are required to be permitted.

Since 1982, the number of commercial broilers produced in Barry County, Missouri, has increased about tenfold with a substantial part of the production occurring within the upper Shoal Creek Basin (Missouri Agricultural Statistics Service, September 2000). An inventory of commercial poultry operations using 1996 aerial photography and data from the NRCS indicated that in April 2000 there were 377 active poultry barns within the study area (fig. 2). About 75 percent of the poultry barns are used in the production of broilers where chicks are raised to about 4-1/2 lbs. One poultry barn holds approximately 21,000 broilers with 5 to 6 flocks raised per year, resulting in an estimated 33 million broilers produced annually within the study area. About 300,000 turkeys also are produced annually within the study area. The distribution of commercial poultry operations in the various subbasins in the study area is listed in table 1. Cattle are pastured throughout the study area and their numbers are difficult to estimate; however, the NRCS estimates there are about 25,000 cattle within the study area (Dan Philbrick, Natural Resources Conservation Service, written commun., January 2000). Several hobby horse farms are located in the Shoal Creek Basin upstream from site 2, along Woodward Creek immediately upstream from site 10, and in the Joyce Creek Basin.

The large numbers of animals represent a significant quantity of nutrient loading from the animal waste generated annually within the study area. About 45,240 tons of poultry litter waste is produced within the study area each year (table 1). This poultry litter is land applied to pastures within a few miles of the barns as a rich source of nutrients. Nutrients in poultry litter are derived from feed grains grown far outside the study area resulting in a net import of millions of pounds of nutrients into the upper Shoal Creek Basin annually. The NRCS determined that poultry litter in the study area contains an average of 59 lbs of phosphorus (as P_2O_5) per ton and 59.5 lbs of nitrogen (nitrogen as N) per ton (Kari Rhoades, Natural Resources Conservation Service, written commun., January 2000). Using these values, an estimated 2.7 Mlbs (million pounds) of P_2O_5 and 2.7 Mlbs of N in the form of poultry litter are applied annually to fields within the study area (loadings in fig. 5). If evenly distributed across all available agricultural land in the study area, the resulting annual application rates would be 21.3 lbs of P_2O_5 and 21.5 lbs of N per acre. Theoretical application rates are as large as 46.4 and 54.4 lbs/acre of P_2O_5 for the Pogue Creek and Woodward Creek Basins (table 1).

Waste from the estimated 25,000 cattle and the more than 13,000 people within the study area also is a significant source of nutrient loadings in the study area (fig. 5). Assuming all cattle are beef cows (65 pounds of manure per day), about 274,000 tons of cattle manure is generated annually within the study area. Given an average P_2O_5 and N contents in cattle manure of 7 and 14 lbs/ton (pounds per ton), about 1.9 Mlbs of P_2O_5 and 3.8 Mlbs of N are derived from cattle manure each year. Nutrient contents in cattle manure were provided by Dan Philbrick, Natural Resources Conservation Service, written commun., January 2000. Unlike poultry litter, most of the nutrients in pastured cattle manure are recycled within the study area. Routine monitoring of nutrients in the effluent from the Monett and Pierce City wastewater treatment plants (WWTPs) is not done, and the annual nutrient loading from these facilities (about 189,000 lbs of N and 313,000 lbs of P_2O_5) was estimated using the median nutrient concentrations and discharge from site 16 downstream from Pierce City (fig. 2). As will be discussed later, concentrations of P in Clear Creek can be attributed mostly to effluent from the city of Monett WWTP. About 35 percent of the estimated 13,518 residents in the study area depend on septic tanks for treatment and disposal of

Table 1. Characteristics of subbasins in the upper Shoal Creek study area

[P_2O_5, phosphoric oxide; --, no data]

Site or subbasin (fig. 2)		Total area (square miles)	Percent urban land use	Percent agricultural land use	Percent forested land use	Percent of basin with greater than 10 degree slope	Estimated rural population	Total number of homes	Density of homes per square mile	Number of homes within 250 feet of a stream
1	Shoal Creek near Ridgley	14.0	1	91	9	22	178	66	4.7	4
2	Shoal Creek at Highway W	28.9	0	90	10	27	410	152	5.3	8
3	Shoal Creek at State Highway 97	68.1	1	90	9	27	1,067	395	5.8	21
4	Shoal Creek at Jolly	87.3	1	88	11	28	1,337	495	5.7	25
5	Shoal Creek at Ritchey	233.4	3	84	13	26	3,270	1,211	5.2	69
10	Woodward Creek near mouth	9.6	0	89	11	27	203	75	7.8	2
11	Pogue Creek near mouth	11.1	2	86	12	29	194	72	6.5	[a]6
12	Joyce Creek near mouth	16.1	0	92	8	19	267	99	6.2	3
14	Capps Creek at Jolly	44.6	1	91	8	23	743	275	6.2	19
17	Clear Creek near mouth	69.3	8	80	12	22	834	309	4.5	17
Minor subbasins upstream from main stem sampling sites										
2A	--	5.3	1	86	13	43	30	11	2.1	2
3A	--	12.1	1	92	7	37	194	72	5.9	4
4A	--	19.2	1	84	16	29	270	100	5.2	4
5A	--	32.1	1	71	28	33	356	132	4.1	8

Table 1. Characteristics of subbasins in the upper Shoal Creek study area—Continued

[P_2O_5, phosphoric oxide; --, no data]

Site or subbasin (fig. 2)		Number of active poultry barns (April 2000)	Density of poultry barns per square mile	Density of poultry barns per square mile of agricultural land use	Number of poultry barns within 500 feet of a stream	Estimated annual tons of poultry litter produced[b]	Estimated annual pounds of P_2O_5 from poultry litter[c]	Estimated annual pounds of P_2O_5 from poultry litter per acre of agricultural land	Estimated annual pounds of nitrogen from poultry litter[d]	Estimated annual pounds of nitrogen from poultry litter per acre of agricultural land	Percent of tributaries without riparian buffer
1	Shoal Creek near Ridgley	33	2	2.6	3	3,960	233,700	28.7	235,620	28.9	61
2	Shoal Creek at Highway W	92	3	3.6	5	11,040	651,360	40.5	656,880	40.8	--
3	Shoal Creek at State Highway 97	210	3	3.4	23	25,200	1,486,800	37.0	1,499,400	37.3	--
4	Shoal Creek at Jolly	248	3	3.2	23	29,760	1,755,840	32.0	1,770,720	32.2	64
5	Shoal Creek at Ritchey	377	2	1.9	31	45,240	2,669,160	21.3	2,691,780	21.5	62
10	Woodward Creek near mouth	42	4	4.9	2	5,040	297,360	54.4	299,880	54.8	67
11	Pogue Creek near mouth	40	4	4.2	6	4,800	283,200	46.4	285,600	46.7	67
12	Joyce Creek near mouth	37	2	2.5	5	4,440	262,000	27.6	264,180	27.9	53
14	Capps Creek at Jolly	51	1	1.3	5	6,120	361,080	13.9	364,140	14.0	51
17	Clear Creek near mouth	32	0	.6	0	3,840	226,560	6.4	228,480	6.4	32
Minor subbasins upstream from main stem sampling sites											
2A	--	17	3	3.7	0	2,040	120,365	40.7	121,382	41.0	69
3A	--	41	3	3.7	7	4,920	290,292	40.9	292,745	41.3	60
4A	--	38	2	2.4	0	4,560	269,051	26.1	271,325	26.4	57
5A	--	46	1	2.0	3	5,520	325,694	22.3	328,446	22.5	--

[a] There are an additional 83 homes within the town of Butterfield that are on septic tanks in this basin.

[b] Assuming 120 tons of litter produced annually per poultry barn (Kari Rhoades, Natural Resources Conservation Service, written commun., January 2001).

[c] Assuming an average of 59 pounds (dry weight) of phosphorus (as P_2O_5) per ton of poultry litter and 120 tons of litter generated annually per poultry barn (Kari Rhoades, Natural Resources Conservation Service, written commun., January 2001).

[d] Assuming 59.5 pounds (dry weight) of nitrogen (as N) per ton of poultry litter and 120 tons of litter generated annually per poultry barn (Kari Rhoades, Natural Resources Conservation Service, written commun., January 2001).

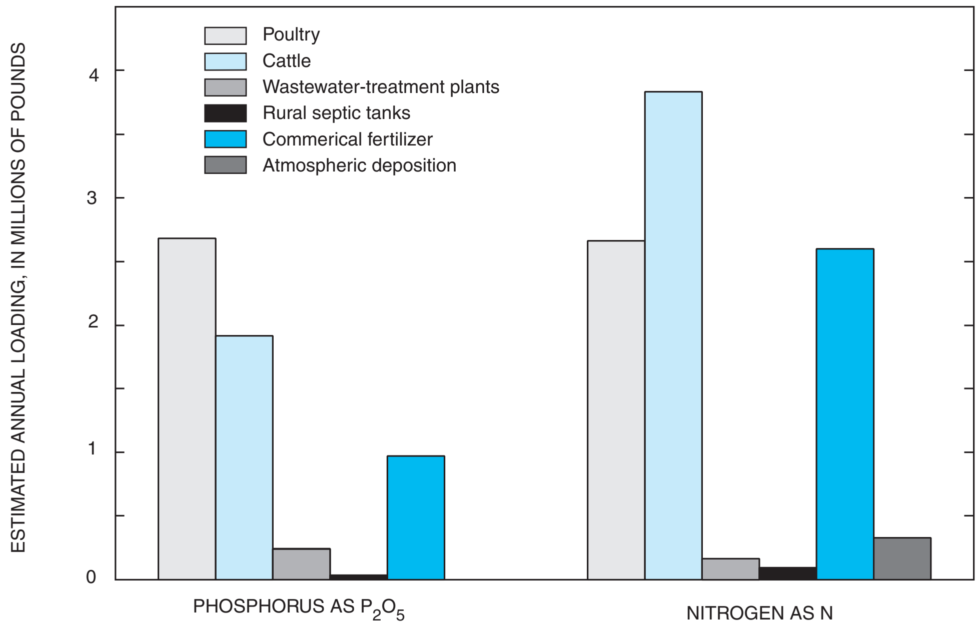


Figure 5. Estimated annual nutrient loadings from various sources within the study area.

domestic wastes. Using average P and N concentrations of 8 and 50 mg/L, respectively, in domestic wastewater, and about 75 gal (gallons) of wastewater produced per day per person (Randy Clarkson, Missouri Department of Natural Resources, written commun., July 2000), the annual nutrient loading from rural septic systems in the study area is estimated to be about 20,000 lbs of P_2O_5 and 54,500 lbs of N. Compared to nutrient loadings from poultry and cattle waste, atmospheric deposition was a minor source of N and P loading in the study area.

Commercial fertilizer use in Barry County has decreased since about 1980, while the number of acres in hay crop, total hay production, and hay yield have risen (fig. 6). The decrease in fertilizer use coincides with a depressed farm economy during the early 1980's and the expansion of the poultry industry in the region. Despite the decrease in commercial fertilizer use since the 1980's, nutrient loading from this source represents about 17 percent of the P_2O_5 loading and 26 percent of the N loading in the study area. A comparison of the estimated nutrient loadings indicates that poultry litter represents about 50 percent of the P_2O_5, and 30 percent of the N loading within the study area (fig. 5).

METHODS OF STUDY

To meet the study objectives, a network of monthly and quarterly sampling sites was established in the study area, and a temporary gaging and water-quality monitoring station was established. Water samples were collected from these sites and analyzed for indicator bacteria densities and a variety of inorganic and organic constituents.

Monitoring Network and Sample Collection

Following a reconnaissance of the study area in March 1999, a network of 10 monthly and 7 quarterly sampling sites was established in the study area (fig. 2). Site numbers less than 6 are main-stem sites, site numbers between 9 and 19 are tributary sites, and site numbers greater than 19 are springs. Sampling sites were selected based on accessibility to the stream, adequate channel morphology and water velocity for the collection of water-quality samples and the measurement of discharge at low and high flows, proximity of point- and nonpoint-pollution sources, and adjacent land use. Monthly sampling sites were located along the main stem of Shoal Creek and near the mouths of principal

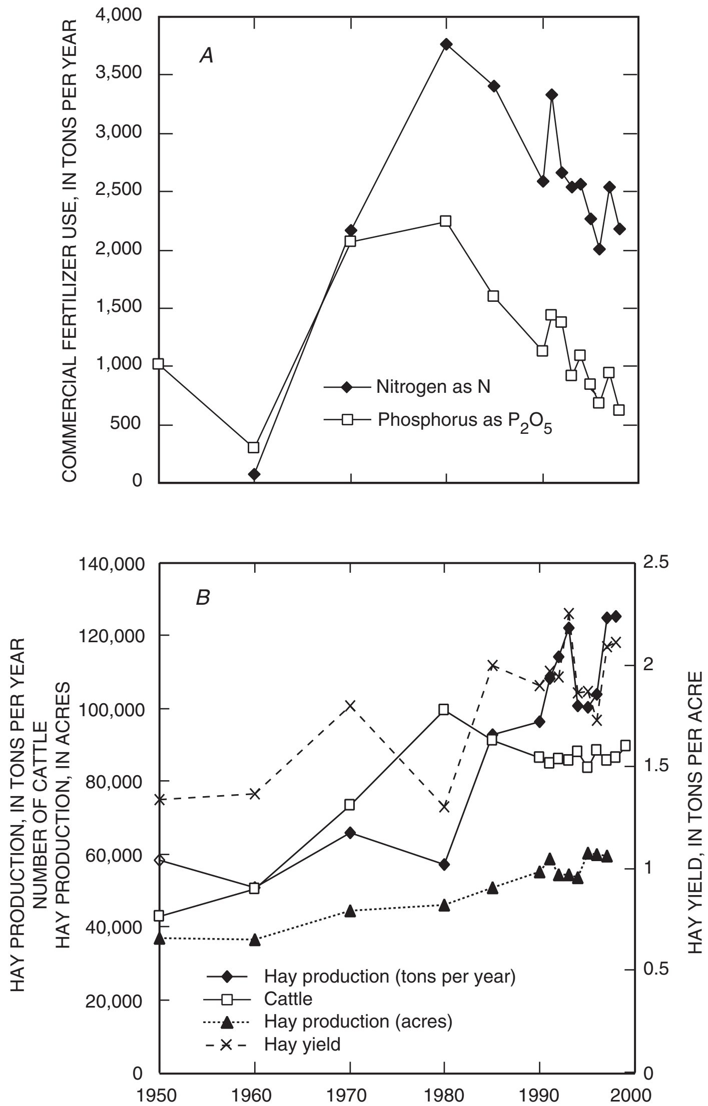


Figure 6. Estimated annual commercial fertilizer use (A) and annual hay production, number of cattle, and hay yield (B) in Barry County, Missouri, 1950–2000 (data from Missouri Agricultural Statistics Service, 2000).

tributaries to provide data on the distribution of nutrients and bacteria within the study area. Site 3 on Shoal Creek and site 14 on Capps Creek coincide with the MDNR sampling sites (fig. 2). Two stream sites to be sampled quarterly were selected at upstream locations on Clear Creek (sites 15 and 16) to provide additional information on the effects of municipal WWTP discharges from Monett and Pierce City on the water quality of Clear Creek. A quarterly sampling site (site 13) also was located at an upstream location on Capps Creek. Quarterly sampling also was done at four springs.

To facilitate an understanding of hydrologic conditions within the upper Shoal Creek Basin preceding and during water-quality sampling trips to the study area, a continuously-recording stream-gaging and water-quality-monitoring station was installed on the main stem of Shoal Creek immediately downstream from site 3 (fig. 2). The station was equipped with a submersible pressure transducer to record stage and a specific conductance and temperature probe. Measurements were recorded every 15 minutes. The gage was installed on May 11, 1999, and was operated through June 2000 without any loss of record. Instantaneous discharge measurements made during routine sampling trips and a runoff event on June 17, 2000, provided enough data for a stage-discharge rating to be developed.

At each monthly sampling site, measurements of discharge, water temperature, specific conductance, DO, and pH were made and water samples were collected and analyzed for indicator bacteria, total (unfiltered) nutrients, and optical brighteners. Indicator bacteria included fecal coliform, fecal streptococcus, and *E. coli*. Nutrient analyses included NO_{2t}+NO_{3t}, nitrite (NO_{2t}), NH_{3t}, P_t, and orthophosphorus (PO_{4t}). Quarterly sampling included all monthly sampling sites and the seven additional sites. Water-quality samples collected during quarterly sampling were analyzed for the suite of constituents listed above, and additional samples were collected and analyzed for dissolved major cations and anions including calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), sulfate (SO_4), Cl, fluoride (F), bicarbonate (HCO_3), and the trace elements boron (B) and strontium (Sr). Selected sites were sampled for wastewater organic compounds, several of which are suspected endocrine disrupters (table 2). Additional samples were collected from sites 11 and 16 and analyzed for a suite of pharmaceutical compounds (table 3) as part of a nationwide reconnaissance of pharmaceutical compounds in streams being conducted by the USGS Toxic Substances Hydrology Program.

Water samples for the analysis of chemical constituents were collected according to the general protocols described in Shelton (1994). Depth integrated, equal-width samples were collected from streams and springs using a hand-held USGS DH-81 isokinetic Teflon sampler. A minimum of five individual subsamples were collected at equal-width intervals across the stream or spring channel and composited in 1- or 3-L (liter) Teflon containers. Where depths were less than 1 ft and velocities were less than 1 ft/s (foot per second), grab samples were collected by filling a 1-L Teflon bottle near the center of flow.

Samples for the determination of nitrogen species and orthophosphorus were placed in 125-mL (milliliter) amber polyethylene bottles and chilled to 4 ^{o}C (degree Celsius). Samples for the determination of P_t were placed in 125-mL clear polyethylene bottles, and preserved to pH less than 2 with sulfuric acid (H_2SO_4) before chilling to 4 ^{o}C. Samples for the determination of dissolved inorganic constituents were filtered through a 0.45-μm (micrometer) pore-size disposable capsule filter using a peristaltic pump as the pressure source, and placed in 250-mL polyethylene bottles. Samples for major and trace cations were placed in acid washed 250-mL polyethylene bottles and acidified to pH less than 2 using nitric acid (HNO_3). Samples for the determination of organic constituents were placed in baked 1-L amber glass bottles (wastewater compounds and antibiotics) or 40-mL amber vials (optical brighteners). Blanks for inorganic constituents and nutrients were prepared and processed during every quarterly sampling event in the field using inorganic-free water prepared by the USGS laboratory in Denver, Colorado. Concentrations of inorganic constituents and nutrients in the blank samples were at or below reporting limits, except for one detection of P_t (0.05 mg/L) and one detection of B [4 μg/L (microgram per liter)].

Concentrations of inorganic constituents and nutrients were determined at the USGS laboratory in Ocala, Florida, using published USEPA or USGS methods. Concentrations of wastewater organic compounds were determined by gas chromatography-mass spectrometry (GCMS) at the USGS laboratory in Denver, Colorado. Concentrations of antibiotic compounds were determined using liquid chromatography-mass spectrometry (LCMS) at the USGS laboratory in Ocala, Florida. Determinations of optical brighteners was done using spectrofluoroscopy at the USGS laboratory in Rolla, Missouri.

Bacteria Methods

Bacteria samples were collected in sterilized 500-mL polyethylene bottles. The bottles were filled by plunging them neck downward beneath the water surface at three equal width intervals across the stream. After collection, the samples were placed on ice, processed within 6 hours, and enumerated using the membrane filter technique according to methods described in Wilde and Radtke (1998). Daily blanks were prepared by filtering 100 mL of sterile buffer water through the appropriate filters and incubating with the samples. No fecal coliform, fecal streptococcus, or *E. coli* colonies were detected in any of the blank samples.

Table 2. List of wastewater organic compounds analyzed in selected water samples

[EDC, suspected endocrine disrupting compound; --, no data; Y, yes]

Compound	EDC	Compound	EDC
Non-ionic detergent metabolites		**Stimulants and metabolites**	
Nonylphenol (total)	--	Caffeine	--
NPEO1 (Nonylphenol monoethoxylate)	Y	Codeine (analgesic)	--
NPEO2 (Nonylphenol diethoxylate)	Y	Cotinine (nicotine metabolite)	--
OPEO1 (otcylphenol, monoethoxylate)	Y		
OPEO2 (otcylphenol, diethoxylate)	Y	**Plasticizers and polymer precursors**	
para-nonylphenol (total)	Y	bis(2-ethylhexyl) adipate	--
		bisphenol A (polymer manufacture)	Y
Disinfectants		bis(2-ethylhexyl) phthalate	Y
Phenol	--	Diethylphthalate	Y
Triclosan (antimicrobial)	--	Ethanol, 2-butoxy-, phosphate	--
		Phthalic anhydride (plastic manufacture)	--
Fecal indicator and hormones		Triphenyl phosphate	--
3*B*-coprostanol (carnivores)	--		
Cholesterol	--	**Polynuclear Aromatic Hydrocarbons (PAHs)**	
17-*beta*-estradiol (estrogen metabolite)	Y	Anthracene	--
Stigmastanol (plant sterol)	--	Benzo(a)pyrene	Y
		Fluoranthene	--
Fire Retardants		Naphthalene	--
tri (2-chloroethyl) phosphate	--	Phenanthrene	--
tri (dichloroisopropyl) phosphate	--	Pyrene	--
Flavoring agent		**Food and other preservatives**	
Benzaldehyde	--	5-methyl-1H-benzotriazole (industrial use)	--
		2,6-di-tert-butylphenol	--
Fragrance		2,6-di-tert-para-benzoquinone	--
Acetophenone	--	Butylated hydroxyanisole (BHA)	Y
		Butylated hydroxytoluene (BHT)	--
Fumigants		para-cresol (wood preservative)	--
1,2-dichlorobenzene	--		
1,3-dichlorobenzene	--	**Solvents and gasoline addatives**	
1,4-dichlorobenzene	--	Tetrachloroethelene	--
Pesticides		**Ethene**	
Carbaryl	Y	1,2,4-Trimethylbenzene	--
Chlorpyrifos	Y		
cis-chlordane	Y		
Dieldrin	Y		
Diazinon	Y		
Lindane	--		
methyl parathion	Y		
N,N-diethyltoluamide (DEET)	--		

Table 3. List of pharmaceutical compounds analyzed in selected water samples

Human drugs	Antibiotics
Acetaminophin	Carbodox
Cimetidine	Chlorotetracycline
Cotinine	Erthromycin-H_2O
Dehydronifedipine	Lincomycin
Digoxigenin	Oxytetracycline
Diltiazem	Roxithromycin
17-Dimethylxanthine	Sulfachloropyridazine
Enalaprilat	Sulfadimethoxine
Fluoxetine	Sulfadimethoxine
Gemfibrozil	Sulfamerazine
Ibuprofen	Sulfamerazine
Paroxetine metabolite	Sulfamethazine
Ranitidine	Sulfamethizole
Salbutamol	Sulfamethoxazole
Sulfamethoxazole	Sulfathiazole
Trimethoprim	Tetracyclinem
Warfarin	Trimethoprim
	Tylosin
	Virginiamycin

Samples to determine the presence of the human pathogen *E. coli* O157:H7 were processed at the College of Veterinary Medicine, UMC. Water samples were transferred to the laboratory within 6 hours of collection where a multi-step incubation was done. The initial step (A) involved membrane filtration of sample aliquots using sterile 0.45-µm pore-size filters and incubating at 37 ^{o}C for 12 to 16 hours on mEndo-LES agar. Between 20 and 30 shiny-metallic colonies on the filters from (A) were transferred to a second set of mEndo-LES plates (B1) and to mFC plates (B2) using sterile toothpicks. The mEndo plates were incubated at 37 ^{o}C and the mFC plates were incubated at 44.5 ^{o}C for 24 hours. Only colonies that were positive on plates from B1 and B2 were further tested. The third step (C) involved transferring positive colonies from step B1 to MacConkey Sorbitol plus MUG agar and incubating for 4 to 6 hours at 37 ^{o}C. Presumptive *E. coli* O157:H7 colonies (white) on the MacConkey Sorbitol plus MUG plates were confirmed by serology using Remel RIM *E. coli* O157:H7 latex test for presumptive identification of *E. coli* serogroup O157.

Microbial source tracking (MST), the comparison of DNA "fingerprints" of bacteria isolates from an environmental sample to isolate groups from known sources, was used in this study to help identify the primary sources of *E. coli* in stream water samples. A form of MST called "ribotyping" has recently been shown to be useful in discriminating between human and nonhuman sources of *E. coli* in water samples from a Florida estuary (Parveen and others, 1999) and between various animal sources (Schlottmann and others, 2000). Recently, Carson and others (2001) determined that analysis of ribopatterns was useful in discriminating between *E. coli* isolated from human and various animal hosts (cattle, chicken, turkey, and horse) in Missouri. Ribopattern analysis is a complicated technique that involves the extraction, restriction, hydridization, and comparison of fragmentation patterns of DNA-extracted *E. coli* isolates from selected water and source samples.

Water samples from five stream sites (sites 2, 3, 10, 11, and 12) and composite samples from potential animal waste sources within the study area (5 poultry litter and 1 dairy and 5 beef cattle manure) were collected during October 1999 and August 2000 and submitted to the UMC College of Veterinary Medicine for ribopattern analysis according to the methods described in Carson and others (2001). Water samples for ribotyping were collected in an identical manner to samples for indicator bacteria. Additional animal source patterns were obtained from the University of Missouri farms in central Missouri. Human source patterns were obtained from staff and students at the UMC. Samples of wild deer droppings were collected from several locations, but were not of sufficient quality to yield *E. coli* growth. Source samples of poultry litter were collected with the assistance of personnel from the NRCS. Source samples were a composite of 20 to 30 subsamples from a single poultry barn. Samples were collected from the upper few inches of litter and from individual droppings. One poultry barn per farm was sampled. Composite beef and dairy cattle samples consisted of subsamples from 10 to 20 individual manure piles from each field or lot.

Data Analysis

Statistical tests on water quality were done using the computer software SYSTAT (SPSS Inc., 1998). Summary statistics for each sampling site were computed using all data except the four runoff samples collected from sites 1, 3, and 4 during April 26 and 27, 1999. Data from the August 2000 samples submitted for ribotyping were not included in the calculations of summary statistics. Censored data included values reported as not detected at the laboratory reporting limit (less-than values), estimated values ("e" values) for some measurements or chemical constituents detected below the reporting limit, and non-ideal plate counts (K values) for indicator bacteria. Because the less-than values and estimated values represented a small fraction of the data set, they were converted to numerical values by removing the remark code. Censored indicator bacteria densities, such as non-ideal plate counts, also were used in statistical calculations by removing the remark code.

Data analysis also included hypothesis testing using ANOVA (analysis of variance) and student's t-tests procedures. A significance (alpha) level of 0.05 was used for hypothesis testing. Hypothesis testing was limited to comparisons between similar sites; for example, main-stem sites along Shoal Creek (sites 1 through 5) were compared as a group, and sites at the mouths of tributaries (sites 10, 11, 12, 14, and 17) were compared as a group. Before hypothesis tests, the data sets for individual field measurements and chemical constituents were tested for normal distribution using a two-tailed Lilliefor's routine in SYSTAT (SPSS Inc., 1998). Results of Lilliefor's tests indicated most field properties and concentrations of chemical constituents were not normally distributed (probability, or p values, less than 0.05) and the data were transformed before hypothesis testing. Three transformations of the raw data were done— natural logarithm, base-10 logarithm, and joint ranking. In general, the rank transformation provided the best approximation to the normal distribution (largest p values) and all ANOVA and student's t-tests were done using ranked data sets.

Multiple comparison tests were done where ANOVA results were significant (p value less than 0.05) to evaluate differences among mean constituent ranks from various sites. To control the overall error rate resulting from making multiple comparisons, a Tukey post-hoc test was done following the ANOVA. The mean constituent rank between two sites was determined to be significantly different if the p value was less than 0.05.

BASE-FLOW WATER QUALITY

Routine samples were collected from 10 sites at approximately monthly intervals between April 1999 and April 2000, and from 7 sites sampled at quarterly intervals (fig. 2; table 4, at the back of this report). Data from five miscellaneous sites (fig. 2) sampled only once during the reconnaissance of the study area are listed in table 5.

Discharge

The drought conditions that existed during the last 10 months of the study period affected stream flows throughout the Shoal Creek Basin. Except for occasional runoff events, the discharge of Shoal Creek at site 3 generally decreased from May 12, 1999, to May 1, 2000 (fig. 7). Between January 12 and February 16, 2000, the daily mean discharge at the gaging station at site 3 was equal to or less than the 7-day mean min-

Table 5. Water-quality data for miscellaneous sites sampled during March 1999

[Q, discharge, in cubic feet per second; Temp, temperature, in degrees Celsius; SC, specific conductance, in microsiemens per centimeter at 25 degrees Celsius; DO, dissolved oxygen, in milligrams per liter; pH, in standard units; FC, fecal coliform density, in colonies per 100 milliliters; FS, fecal streptococcus density, in colonies per 100 milliliters; *E. coli*, *Escherichia coli* density, in colonies per 100 milliliters; K, non-ideal count; e, estimated; <, less than; --, no data]

Site (fig. 2)	Site name	Date	Time	Q	Temp	SC	DO	pH	FC	FS	*E. coli*
M1	Clear Creek below Monett	03/01/99	1200	5.3	13.6	2,170	15.8	8.19	150	K55	175
M2	Hudson Creek near Pulaskifield	03/02/99	1310	1.96	10.7	352	11.3	7.77	K20	K15	K38
M3	Talbert Spring	03/03/99	0935	1.0e	13.8	302	6.74	6.98	K7	<2	56
M4	Pioneer Spring	03/02/99	1740	1.5e	13.8	317	--	7.12	K35	K31	K15
M5	Shoal Creek at Pioneer	03/02/99	1720	42.4	11.3	306	10.8	8.1	72	K35	92

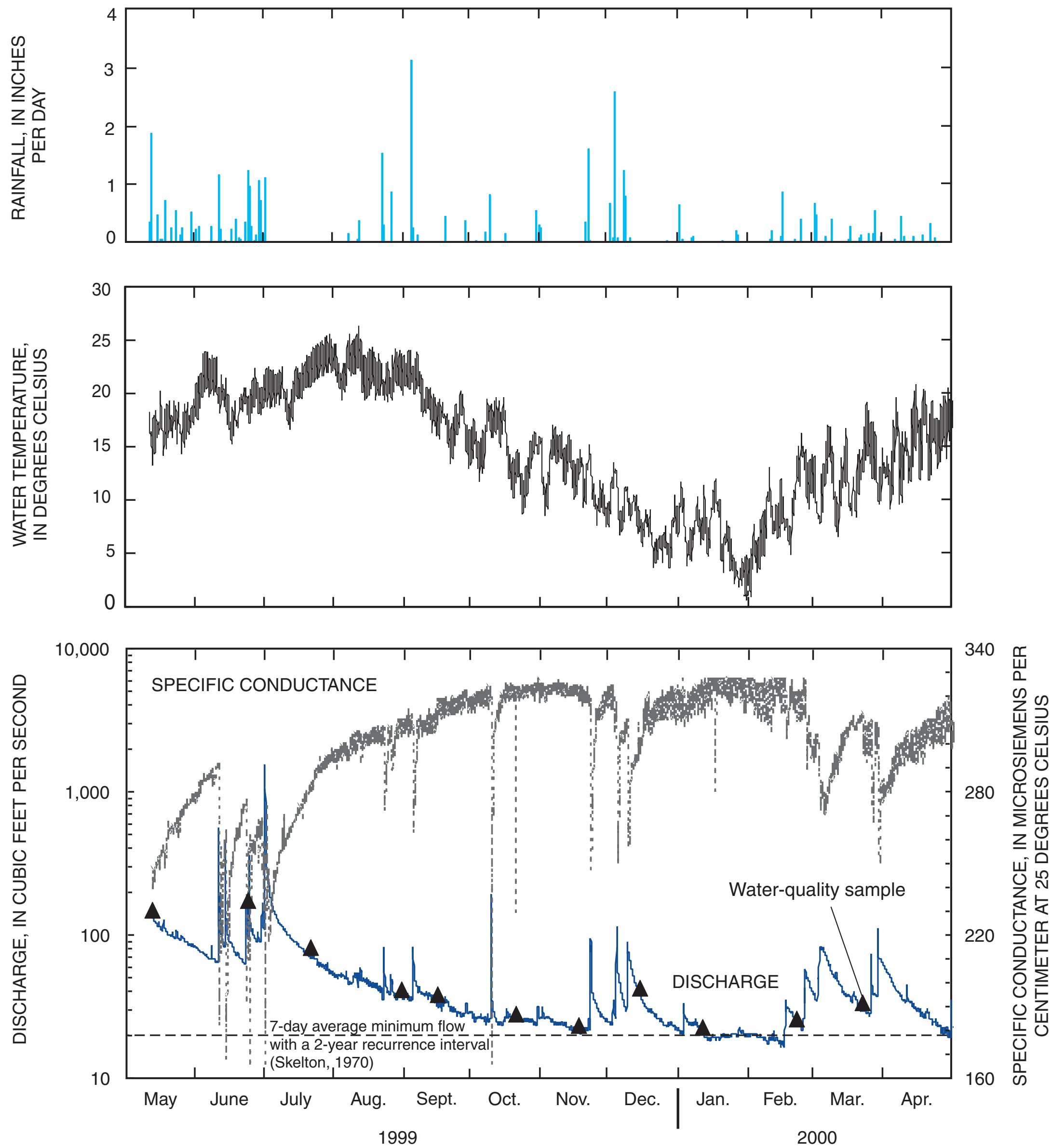


Figure 7. Precipitation at Monett, Missouri, and instantaneous discharge, specific conductance, and temperature at the temporary gaging station on Shoal Creek near site 3, May 12, 1999, to May 1, 2000.

imum flow with a recurrence interval of 2 years (7-day Q_2) of 20 ft^3/s calculated by Skelton (1970). The minimum daily discharge of 17 ft^3/s occurred on February 15 and 16, 2000 (fig. 7). The annual mean discharge for the 354-day period (May 12, 1999, to May 1, 2000) that the temporary gaging station near site 3 was operated was 43.9 ft^3/s. The maximum recorded instantaneous discharge of 1,550 ft^3/s occurred at 12:15 p.m. on July 1, 1999. The maximum recorded daily discharge of 636 ft^3/s also occurred on July 1, 1999.

Shoal Creek is a gaining stream; the discharge increased from a mean of 4.46 ft^3/s at site 1 to 173 ft^3/s at site 5 (table 6, at the back of this report). The increase in discharge is consistent with results of discharge measurements made by the USGS along Shoal Creek during drought conditions in 1964 (U.S. Geological Survey, 1964), and indicates that Shoal Creek gains flow from ground-water sources throughout the study area. A comparison of discharge measurements made on Shoal Creek and its tributaries during base-flow conditions in January 2000, indicates that about 60 percent of the increase in discharge between sites 1 and 2 (0.59 to 7.39 ft^3/s) is from ground-water inflow. Ground-water inflow accounts for about 38 percent of the increase in discharge between sites 2 and 3 (7.39 to 22.5 ft^3/s), all of the increase in discharge between sites 3 and 4 (22.5 and 28.9 ft^3/s), and 17 percent of the increase in discharge between sites 4 and 5 (28.9 and 97.7 ft^3/s).

Mean discharges of the various tributaries were variable, ranging from 6.47 ft^3/s in Woodward Creek (site 10) to 59.2 ft^3/s in Capps Creek (site 14, fig. 2). The large mean discharge in Capps Creek is unusual because its drainage area (44.6 mi^2) is considerably smaller than that of the Clear Creek (69.3 mi^2). A comparison of discharge per unit drainage area using base-flow measurements made during January 2000 indicates that Capps Creek at site 14 has an anomalously large discharge yield [about 0.7 $ft^3/s/mi^2$ (cubic foot per second per square mile)] compared to other sites (fig. 8). During the fall and winter, nearly all the flow in Capps Creek can be attributed to several large (discharge more than a few cubic feet per second) springs about 1.5 mi upstream from site 14. Kiner and others (1997) classified Clear Creek and its tributaries upstream from Pierce City as losing streams, and it is possible that a subsurface connection may exist between losing reaches of Clear Creek and the larger springs along Capps Creek. The small discharge per unit drainage area of Joyce Creek (about 0.07 ft^3/s) probably is caused by spring 23, located less than 0.2 mi south of the mouth of Joyce Creek, pirating water from the Joyce Creek subbasin (fig. 2). Dye-trace tests in the lower reach of Joyce Creek could confirm a subsurface connection between Joyce Creek and spring 23.

Physical Properties and Inorganic Constituents

Except for site 1, stream samples generally had slightly alkaline pH values (7.33 to 8.54) and DO concentrations larger than 6.0 mg/L (table 4). Samples from site 1 had smaller pH values (6.86 to 7.33), and generally smaller DO concentrations (3.5 to 8.6 mg/L), and were more comparable to spring samples (table 4). The similarity of field measurements at site 1 to field

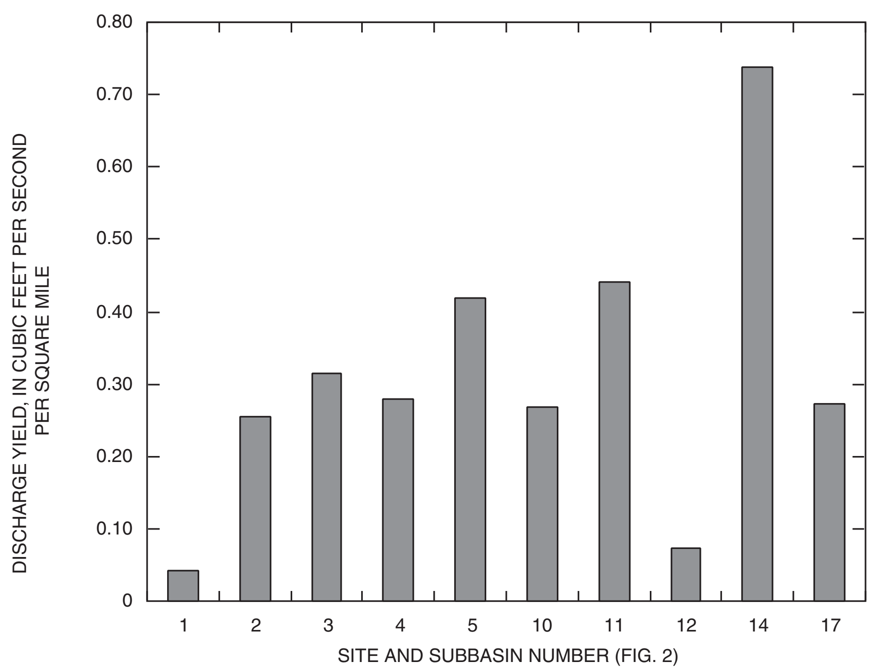


Figure 8. Measured discharge yield from selected subbasins during January 2000.

measurements from springs is expected because during base-flow conditions, a small spring beneath the low-water crossing at site 1 appears to supply most of the flow. Most stream and spring samples were calcium-magnesium-bicarbonate (Ca-Mg-HCO_3) type waters with specific conductance values generally less than 350 μS/cm (microsiemens per centimeter at 25 degrees Celsius). Spring samples generally had larger specific conductance values and larger concentrations of inorganic constituents than stream samples.

The specific conductance and concentrations of inorganic constituents in Shoal Creek generally increased with increasing distance downstream (fig. 9). Median specific conductance values increased from 290 and 291 μS/cm at sites 1 and 2, to 311, 307, and 340 μS/cm at sites 3, 4, and 5 (table 6). The largest increase in specific conductance and constituent concentrations was between sites 4 and 5. Median concentrations of Mg (2.2 and 3.3 mg/L), Na (5.2 and 8.6 mg/L), K (1.8 and 3.3 mg/L), Cl (10.3 and 12.0 mg/L; fig. 9), and SO_4 (3.5 and 8.0 mg/L) increased significantly (p value less than 0.05) between sites 4 and 5 (table 6). The increase in specific conductance values and constituent concentrations is the result of inflow from Clear Creek, which accounts for about 20 percent of the discharge of Shoal Creek at site 5 during base-flow conditions.

Water samples collected from Clear Creek (sites 15, 16, and 17, fig. 2) generally contained the largest specific conductance values (340 to 1,280 μS/cm) and concentrations of inorganic constituents such as Na (24 to 180 mg/L), Mg (3.6 to 8.8 mg/L), K (9.2 to 77 mg/L), Cl (21 to 140 mg/L), and SO_4 (19 to 160 mg/L) detected (table 6). Results of analysis variance and multiple comparison tests indicate that the specific conductance (fig. 10) and concentrations of Na, SO_4, Mg, K, and B (not shown on figure) at the downstream site on Clear Creek (site 17) were significantly larger (p values less than 0.05) than all other tributaries to Shoal Creek. During the four quarterly sampling events, Clear Creek (site 17) accounted for about 60 percent of the instantaneous Na load and about 40 percent of the instantaneous Cl load discharged by Shoal Creek at site 5.

The large specific conductance values and constituent concentrations in Clear Creek are the result of effluent from the Monett WWTP. During summer base-flow conditions, effluent from this WWTP comprises most of the flow in the upper reaches of Clear Creek. Effluent from municipal WWTPs commonly contains increased concentrations of Na, K, Cl, SO_4, relative to Ca, Mg, and HCO_3. Most stream and spring samples collected during this study were Ca-Mg-HCO_3 type water; however, samples from Clear Creek were sodium-sulfate and sodium-chloride type waters (fig. 11). Samples from sites 15, 16, and 17 plot along a line trending toward the Ca-Mg-HCO_3 vertex on the trilinear diagram, indicating that water from site 15 is being increasingly diluted with water having background major ion ratios. Specific conductance values and concentrations of inorganic constituents at site 16 were smaller than those at site 15, and the mean instantaneous loads of Na and Cl at site 15 [27.1 and 23.7 g/s (grams per second)] and 16 (26.2 and 24.0 g/s) were nearly the same indicating no measurable effect from the Pierce City WWTP.

Samples from Pogue Creek (site 11) and spring 20 in the upper part of the Pogue Creek subbasin also plotted outside the cluster of samples at the Ca-Mg-HCO_3 vertex on the trilinear diagram, indicating probable anthropogenic effects (fig. 11). Excluding samples from sites on Clear Creek (sites 15, 16, and 17), specific conductance values (240 to 372 μS/cm), and concentrations of Na (9 to 11 mg/L) and Cl (17 to 26 mg/L) in samples from Pogue Creek were among the largest detected in stream samples (table 6, fig. 10). Pogue Creek has six homes within 250 ft of the main channel that presumably use septic tanks—more per unit area than any other subbasin. Liquid waste from a poultry processing plant is used to irrigate 300 acres of fields in the upper part of the Pogue Creek subbasin east of spring 20 (fig. 2). This plant has a design capacity of 1.6 Mgal/d (million gallons per day). The large Na and Cl concentrations in Pogue Creek may be related to liquid waste from the poultry processing plant or septic tanks.

Samples from spring 20 contained the largest concentrations of Na (11 to 19 mg/L) and Cl (20 to 43 mg/L) detected in spring samples. Concentrations were variable with Na and Cl concentrations in the December 1999 sample being nearly twice as large as those in the September 1999 sample. Possible sources for the large Na and Cl concentrations in spring 20 include two homes on septic tanks within 750 ft of the spring, liquid waste from the poultry processing plant east of the spring, and septic tanks in the town of Butterfield (fig. 2). There are several springs that have not been sampled that emerge in fields along Pogue Creek about

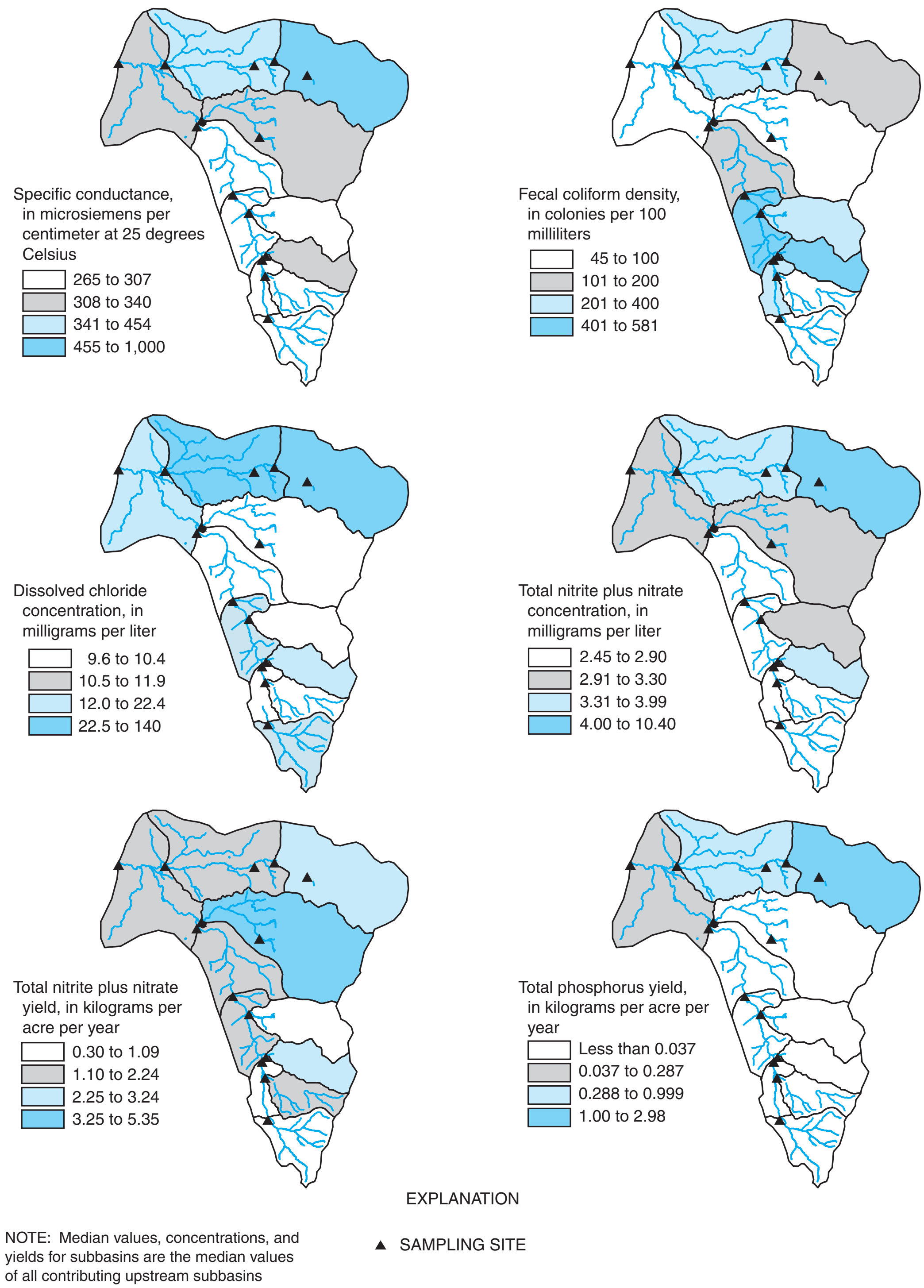
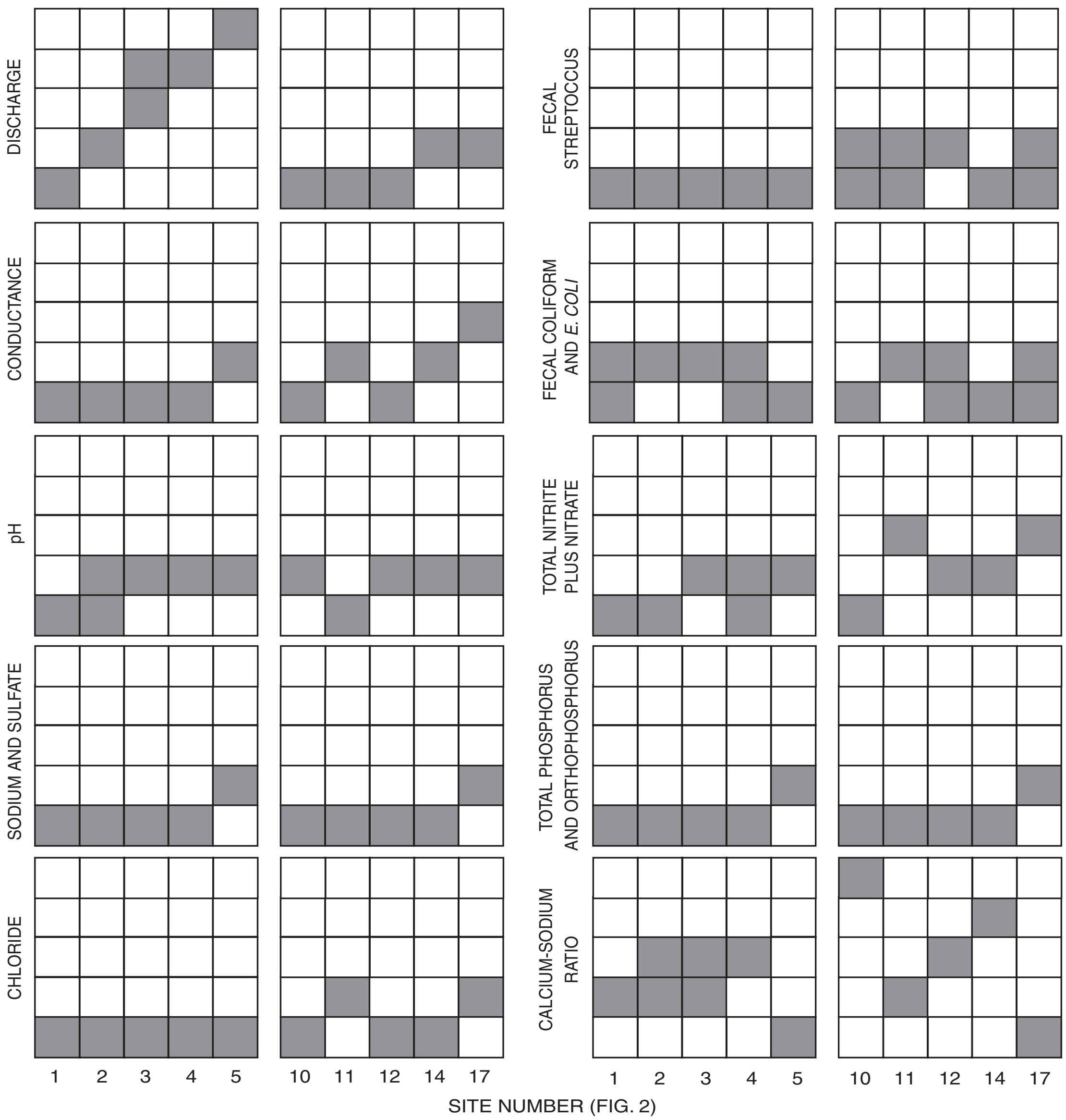


Figure 9. Geographic distribution of median values of specific conductance, fecal coliform bacteria densities, concentrations of selected constituents, and estimated base-flow nutrient yields in the study area.



EXPLANATION

Shaded boxes in each column represent the relative mean rank for selected physical properties, bacterial densities, or chemical constituents for each site. Main-stem sites (1, 2, 3, 4, and 5) and tributary sites (10, 11, 12, 14, and 17) were compared separately. Sites with shaded boxes in the lowermost rows have among the smallest mean ranks, whereas sites with shaded boxes in the uppermost rows have among the largest mean ranks. Two or more sites with boxes shaded in the same row have mean ranks that are not significantly different at an alpha level of 0.05. For example, the mean rank of discharge at site 1 (Shoal Creek near Ridgley) is significantly smaller than all other main-stem sites (sites 2, 3, 4, and 5) on Shoal Creek. The mean ranks of discharge at sites 2 and 3 are significantly different from each other and larger than at site 1. However, the mean ranks of discharge at sites 3 and 4, while significantly larger than mean ranks of discharge at sites 1 and 2 and significantly smaller than the mean rank of discharge at site 5, are not significantly different.

Figure 10. Graphical representation of analysis of variance and multiple comparison tests for selected physical properties and chemical constituents.

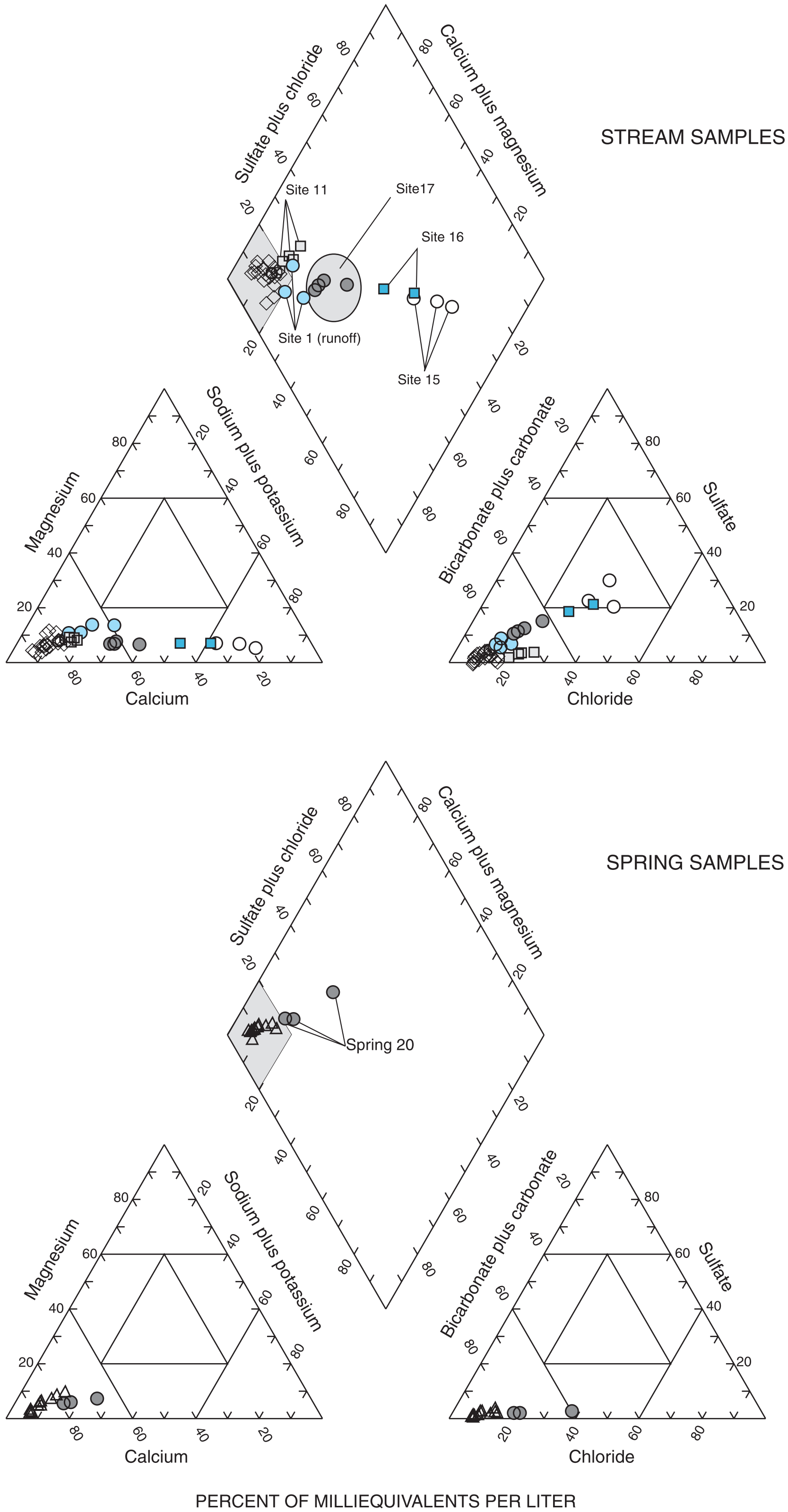


Figure 11. Trilinear diagram of major ions in stream and spring samples.

1,000 ft east and at a slightly higher elevation than spring 20. Pogue Creek is generally dry upstream from these springs. Additional sampling of spring 20 and the springs east of spring 20 could contribute toward a better understanding of the sources of the Na and Cl detected in spring 20.

Nutrients

Analysis of stream samples from this study indicate that base-flow concentrations of NO_{2t}+NO_{3t} in Shoal Creek (mean and median of 2.90 mg/L, range of 2.20 to 4.40 mg/L) are significantly larger (p value less than 0.001; ranked t test) compared to base-flow NO_{2t}+NO_{3t} concentrations (mean of 1.02 and median of 0.52 mg/L) in other Missouri streams (fig. 12). Data from other Missouri streams were summarized from 1,340 base-flow water-quality samples (combination of total and dissolved nitrite plus nitrate values) collected from 20 stream sites throughout the state for various USGS programs between 1960 and 2000. No statistical difference was detected between total and dissolved nitrite plus nitrate values; therefore, these values were combined for the comparisons used in this report. Base-flow samples at the other stream sites were those samples collected at discharge values not exceeding the 80^{th} percentile of measured discharges for the respective site. There was no significant difference in base-flow P_t concentrations between the upper Shoal Creek Basin and other Missouri streams. Base-flow yields (concentration times discharge divided by the contributing drainage area) of NO_{2t}+NO_{3t} in the upper Shoal Creek Basin (fig. 9) are significantly larger (p values less than 0.001; ranked t test) than base-flow NO_{2t}+NO_{3t} yields from other Missouri streams. The mean base-flow yield of NO_{2t}+NO_{3t} in main-stem sites in the upper Shoal Creek Basin was 2.46 kg/acre/yr (kilograms per acre per year) with a median of 1.59 kg/acre/yr, compared to a mean base-flow NO_{2t}+NO_{3t} yield of 0.93 kg/acre/yr (median of 0.32 kg/acre/yr) for other Missouri streams. The comparison of NO_{2t}+NO_{3t} yields normalizes the effect of drainage area and indicates that NO_{2t}+NO_{3t} concentrations in the upper Shoal Creek Basin are anomalous and cannot be explained by variations in contributing drainage area or discharge.

The geographic distribution of nutrient concentrations in the upper Shoal Creek Basin indicates that tributaries tend to have larger concentrations and yields of NO_{2t}+NO_{3t} and P_t than main-stem sites, and that NO_{2t}+NO_{3t} and P_t concentrations in the main stem of Shoal Creek tend to increase with increasing distance downstream (fig. 9). Concentrations of NO_{2t}+NO_{3t} in samples from the main stem of Shoal Creek ranged from 2.0 to 4.4 mg/L and concentrations of P_t ranged from less than 0.02 to 0.7 mg/L (table 6). Ammonia concentrations (NH_{3t}) were less than 0.1 mg/L at all main-stem and tributary sites except for sites 15 and 16 on Clear Creek. Increases in NO_{2t}+NO_{3t} concentrations were gradual suggesting nonpoint sources, whereas concentrations of P_t and PO_{4t} were similar at sites 1 through 4 (mean of 0.03 and 0.02 mg/L) but increased dramatically between sites 4 and 5 (mean increase of 0.35 mg/L) as a result of inflow from Clear Creek. Mean ranks of P_t and PO_{4t} at site 5 were significantly larger (p value less than 0.05) than at other main-stem sites (fig. 10).

Samples from Clear Creek (sites 15, 16, and 17) and Pogue Creek (site 11) contained the largest nutrient concentrations detected in stream samples. The large concentrations of NO_{2t}+NO_{3t} (3.4 to 13.0 mg/L), P_t, and PO_{4t} (0.97 to 11.0 mg/L) in Clear Creek are the result of point-source effects from the Monett WWTP. Although nutrient concentrations in Clear Creek decreased with increasing distance downstream from Monett, P_t and PO_{4t} concentrations and mean ranks of P_t and PO_{4t} at site 17 remained significantly larger than at other tributary sites (fig.10). Mean ranks of NO_{2t}+NO_{3t} were significantly larger than in all other tributaries except for Pogue Creek (fig. 10). Base-flow yields of P_t in Clear Creek at site 17 generally were more than 10 times those in other tributary sites (fig. 9). Although Clear Creek and Pogue Creek contained the largest NO_{2t}+NO_{3t} concentrations, Capps Creek (site 14) had the largest NO_{2t}+NO_{3t} yields (fig. 9). As previously discussed, Capps Creek has anomalously large discharge yields (fig. 8). The large NO_{2t}+NO_{3t} yield and large discharge yield from Capps Creek may be related, and may indicate that NO_{2t}+NO_{3t}-rich water is being pirated from the Clear Creek Basin. A series of dye tests in losing reaches of Clear Creek may help resolve this issue. Within measurement error, Clear Creek (site 17) accounted for essentially 100 percent of the instantaneous P_t load, but only about 20 percent of instantaneous NO_{2t}+NO_{3t} load, discharged by Shoal Creek at site 5. Capps Creek is a major source of NO_{2t}+NO_{3t} in the upper Shoal Creek Basin, accounting for about 40 percent of the instantaneous NO_{2t}+NO_{3t} loads at site 5.

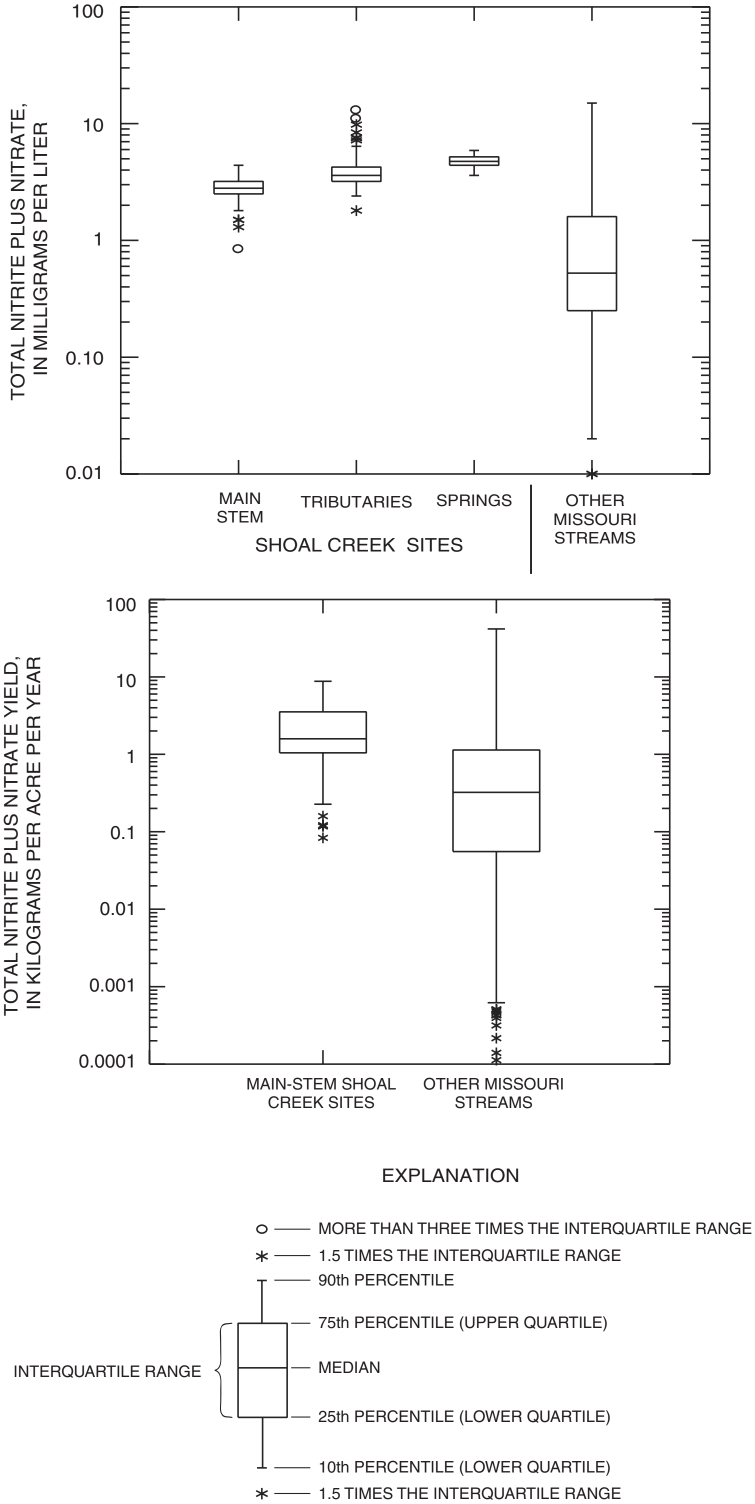


Figure 12. Concentrations and estimated base-flow yields of total nitrite plus nitrate in the upper Shoal Creek Basin and other streams in Missouri sampled by the U.S. Geological Survey.

Similar to inorganic constituents, concentrations of NO_{2t}+NO_{3t} (3.4 to 5.0 mg/L) in Pogue Creek (site 11) were among the largest detected in stream samples. The large concentrations of NO_{2t}+NO_{3t} and inorganic constituents in Pogue Creek probably are related to the proximity of homes with septic tanks and a poultry processing plant to the Creek, and to nonpoint sources within the subbasin. Compared to other subbasins, the Pogue Creek subbasin has the largest percent of contributing drainage area with greater than 10 percent slopes (29 percent), among the largest density of poultry barns (4.2 per mi^2 of agricultural land use), and much of Pogue Creek has almost no riparian buffer (table 1). Of the poultry barns in the Pogue Creek subbasin, 24 of 40 are turkey barns, and 14 of the 20 barns within 1,000 ft of the creek are turkey barns. Although the land application of poultry plant wastewater may contribute to increased concentrations of inorganic constituents in samples from Pogue Creek and spring 20, this potential source does not appear to affect NO_{2t}+NO_{3t} concentrations in samples from spring 20 which are similar to other springs (table 4). Despite having among the largest NO_{2t}+NO_{3t} concentrations, NO_{2t}+NO_{3t} discharged from Pogue Creek at site 11 represents only about 5 percent of the instantaneous NO_{2t}+NO_{3t} load at site 5.

Shoal Creek is a gaining stream throughout the study area, and ground-water inflow is an important source of NO_{2t}+NO_{3t} in Shoal Creek. Concentrations of NO_{2t}+NO_{3t} in water samples collected from springs (mean of 4.62 mg/L) were significantly larger (p value less than 0.05) than NO_{2t}+NO_{3t} concentrations in samples collected from main-stem sites (2.86 mg/L). Unlike specific conductance values that decreased with increasing discharge in December 1999, concentrations of NO_{2t}+NO_{3t} at all sites except site 17 increased with increasing discharge during December 1999. The increase in NO_{2t}+NO_{3t} concentrations in December 1999 suggests that, in addition to ground-water inflow, NO_{2t}+NO_{3t} concentrations in the streams may be affected by the leaching or flushing of NO_{2t}+NO_{3t} from surface or near-surface soils or other sources during wet periods.

Bacteria

The density of fecal indicator bacteria is one indicator used to determine if water is free from disease-causing organisms and is safe for human recreation and consumption. The fecal indicator bacteria measured generally do not cause disease, but are used as proxies for the presence of human pathogens that generally are much more difficult to measure. The fecal indicator bacteria measured in the study originate in the intestinal tracts of warm-blooded animals, and include the fecal coliform and fecal streptococcus groups and *E. coli*. The fecal coliform bacteria test, however, is not strictly specific to fecal coliform bacteria (those originating in the intestinal tracts of warm-blooded animals); as much as 7 percent of bacteria enumerated may be non-fecal bacteria such as *Klebsiella* (Eaton and others, 1995). *E. coli* is a member of the fecal coliform group, and exists only in the intestinal tracts of warm-blooded animals. The presence of *E. coli* in a water sample is evidence of fecal contamination from warm-blooded animals and the possible presence of human pathogens (Eaton and others, 1995).

Results of this study are consistent with the MDNR including part of Shoal Creek being included on the 1998 Clean Water Action Plan (CWP) 303(d) list of impaired water bodies for fecal coliform bacteria. Fecal coliform densities in samples from site 3 exceeded the MDNR standard of 200 col/100mL for whole-body contact recreation in 12 of the 13 samples and ranged from 43 to 33,000 col/100 mL (median of 2,860 col/100 mL). Densities of fecal coliform also exceeded the MDNR standard upstream at site 2 in 8 of 13 samples and ranged from 25 to 9,200 col/100 mL (median of 277 col/100 mL). Densities of fecal coliform generally were less than the MDNR standard at sites 1, 4, and 5. Except for site 5, the largest fecal coliform densities at each of the above sites were from samples collected during a small runoff event that occurred during a wet period in June 1999 (fig. 7). Currently, there is no MDNR standard for *E. coli* densities in water samples; however, *E. coli* densities were similar to those of fecal coliform and ranged from 40 to 42,000 col/100 mL at site 3 (table 6).

Densities of fecal coliform bacteria also exceeded the MDNR standard of 200 col/100 mL for whole-body contact recreation in samples from three of the five tributaries sampled (fig. 9). Densities of fecal coliform bacteria exceeded the MDNR standard at site 11 on Pogue Creek in 12 of 13 samples (median of 580 col/100 mL), site 12 on Joyce Creek (9 of 12 samples, median of 340 col/100 mL), and in samples from Clear Creek at site 17 (9 of 13 samples, median of 320 col/100 mL). Fecal coliform densities generally were less than the MDNR standard in samples from Woodward Creek (site 10) and the downstream Capps Creek site (site 14). The mean ranks of fecal coliform and *E. coli* densities in samples from Pogue Creek were significantly larger (p value less than 0.05) than fecal coliform and *E. coli* densities in samples from sites 10 and 14 (fig. 10). The large densities of fecal coliform bacteria in Pogue Creek are unusual. A large complex of turkey barns (20 barns), a poultry processing plant, and more than 80 homes on septic tanks in the town of Butterfield are located in the headwaters of Pogue Creek (fig. 2). All of these potential sources are upslope and possibly lie within the recharge area of spring 20; however, spring 20 does not have elevated fecal coliform densities compared to other springs sampled. Flow in Pogue Creek actually begins about 1,000 ft upstream from spring 20 at another spring that was not sampled during this study. The small fecal coliform densities in spring 20 and large fecal coliform densities in samples collected from Pogue Creek at site 11 indicate that the source of the large fecal bacteria densities in Pogue Creek at site 11 probably is between spring 20 and site 11, or possibly the spring upstream from spring 20.

Although the increased specific conductance values and concentrations of major ions and nutrients in Clear Creek at base flow could be attributed to effluent from the Monett WWTP, this effluent does not appear to affect indicator bacteria densities. Unlike specific conductance values and concentrations of most major ions and nutrients which decreased from site 15 to site 17, densities of fecal coliform and *E. coli* generally tended to increase between these sites, indicating sources of fecal coliform and *E. coli*, in the lower reaches of Clear Creek other than the Monett WWTP.

Organic Compounds

Water samples from several sites contained trace quantities of organic compounds commonly associated with municipal or domestic sewage effluent (table 7). However, several of the organic compounds detected can have sources other than municipal or domestic sewage effluent. For example, polynuclear aromatic hydrocarbons (PAHs) can be associated with asphalt roads, concrete waterproofing on foundations, and asphalt roof singles, to mention a few. Plasticizers are nearly ubiquitous in an industrialized society, are found in a myriad of products, and also are commonly reported laboratory contaminants. Because of their ubiquitous use and propensity to be identified as laboratory contaminants, plasticizers were not used in interpretations in this report. Cholesterol and 3*B*-coprostanol are indicators of fecal contamination, and may be associated with a variety of waste sources other than municipal or domestic sewage effluent. The wood preservative paracresol may be contained in treated lumber commonly used on farms and unless detected in combination with other compounds more specific to human wastes, its detection is equivocal. Of the organic compounds detected in the water samples from the Shoal Creek Basin, the most reliable indicators of municipal or domestic sewage effluent are caffeine, the disinfectants phenol and triclosan, nonylphenol (detergent metabolite), and the fire retardant tri (2-chloroethyl) phosphate. These five compounds and paracresol are hereinafter referred to as human wastewater compounds and their detection may indicate affects from municipal or domestic wastewater sources. Phenol is also used as a disinfectant in the poultry industry. Because the frequency of sampling varied among sites, the number of detections of human wastewater compounds was normalized by dividing the number of detections at a particular site by the number of samples from that site to give a human wastewater "score". None of the water samples had detectable concentrations of optical brighteners, which are indicators of laundry detergents.

Site 15 on Clear Creek had the largest human wastewater score of 3.0 (6 detections in two samples) indicating the largest impact from human wastes (fig. 13). The large human wastewater score at site 15 is consistent with the large concentrations of major ions and nutrients attributed to effluent from the Monett WWTP. Organic compounds detected at site 15 included caffeine, triclosan, tri (2-chloroethyl) phosphate, the PAH fluoranthene, and the fecal indicators cholesterol and 3*B*-coprostanol (table 7). The relatively low human wastewater score of 0.7 at site 17 compared to site 15 (3.0) indicates dilution of municipal wastewater effluent with increasing distance downstream in Clear Creek.

Shoal Creek at site 3, Joyce Creek (site 12), and spring 20 had human wastewater scores of 1.0 or larger (fig. 13). Three of the four samples from Shoal Creek at site 3 contained at least one of the human wastewater compounds caffeine, triclosan, or phenol, and two of the samples contained the fecal indicators cholesterol or 3*B*-coprostanol. One of the two samples from Joyce Creek (site 12) contained triclosan, and the other sam-

Table 7. Concentrations of wastewater organic compounds detected in selected water samples

[All concentrations in micrograms per liter; bold number indicates compound detected; <, less than; e, estimated; --, no data]

			Human wastewater compounds						Polyaromatic hydrocarbons			Plasticizers						
Site (fig. 2)	Date	Time	Caffeine	Triclosan	Phenol	tri (2-chloroeth yl) phosphate	Nonyl-p henol (total)	para-c resol	Fluoran-thene	Pyrene	Benzo(a)-pyrene	Phthalic anhy-dri de	Ethanol, 2-butoxy-, phosphate	bis(2-ethyl hexyl) phthalate	Triphenyl phos-phat e	3*B*-copr os-tanol	Choles-t erol	Stigmas-tanol
1	06/23/99	1300	<0.08	<0.004	<0.08	<0.04	<0.5	<0.03	<0.03	<0.03	<0.05	<0.15	<0.10	<0.2	**0.01e**	**0.2e**	**0.5e**	--
1	02/22/00	1335	<.06	<.04	<.08	<.04	<.5	<.03	<.03	<.20	<.05	<.15	<.07	<.2	<.07	<.60	<1.0	<2
2	06/23/99	0915	<.08	<.04	<.08	<.04	<.5	<.03	<.03	<.03	<.05	<.15	<.10	<.2	<.10	<.60	**.4e**	--
2	01/11/00	1330	**.03e**	<.04	<.08	<.04	<.5	<.03	<.03	<.20	<.05	<.15	<.07	<.2	**.06e**	<.60	**.4**	<2
3	06/23/99	1015	**.01e**	**.02e**	<.08	<.04	<.5	<.03	**.01e**	**.01e**	<.05	<.15	<.10	<.2	<.10	**.2e**	**.9e**	--
3	12/14/99	1040	<.06	<.04	<.15	<.04	<.5	<.15	<.03	<.20	<.05	<.20	**.05e**	**5**	<.07	<.60	<1.0	--
3	01/11/00	1525	<.03	<.04	**.8**	<.04	<.5	<.03	<.03	<.20	<.05	<.15	<.07	<.2	<.07	<.60	<1.0	<2
3	02/22/00	1745	**.03e**	<.04	<.08	<.04	<.5	<.03	<.03	<.20	<.05	<.15	<.07	<.2	<.07	<.60	**.4**	<2
4	06/22/99	1330	<.08	<.04	<.08	<.04	<.5	<.03	<.03	<.03	<.05	<.15	<.10	<.2	<.10	<.60	<1.0	--
5	06/22/99	1130	<.08	<.04	<.08	<.04	<.5	<.03	<.03	<.03	<.05	<.15	<.10	<.2	<.10	<.60	<1.0	--
10	06/23/99	0740	<.08	<.04	<.08	<.04	<.5	<.03	<.03	<.03	<.05	<.15	<.10	<.2	<.10	<.60	<1.0	--
10	09/14/99	1315	**.01e**	<.04	<.15	<.04	<.5	**.03e**	<.03	<.20	<.05	<.20	<.10	<.2	<.07	<.60	<1.0	--
11	06/23/99	0830	<.08	<.04	<.08	<.04	<.5	<.03	<.03	<.03	<.05	<.15	<.10	<.2	<.10	<.60	<1.0	--
11	09/14/99	1415	<.06	<.04	<.15	<.04	<.5	<.15	<.03	<.20	<.05	<.20	<.10	<.2	<.07	<.60	<1.0	--
11	12/13/99	1525	<.06	<.04	<.15	<.04	<.5	<.15	<.03	<.20	<.05	<.20	<.10	<.2	<.07	<.60	<1.0	--
11	01/11/00	1240	<.06	<.08	<.2	<.08	<.5	<.03	**.08**	**.09**	**.03e**	<.3	<.07	**2.75**	**.13**	<.60	<1.0	<2
11	02/22/00	1655	**.02e**	<.04	<.08	<.04	<.5	<.03	<.03	<.20	<.05	**.2**	<.07	<.2	<.07	**.1e**	**.4e**	**E .2**
11	03/22/00	1200	<.06	<.04	<.08	<.04	<.5	<.03	<.03	<.20	<.05	<.15	<.07	<.2	<.07	<.60	<1.0	<2
12	06/23/99	1450	<.08	**.02e**	<.08	<.04	<.5	<.03	**.01e**	**.01e**	<.05	<.15	<.10	<.2	<.10	**.2e**	**.8e**	<2
12	02/22/00	1920	**.03e**	<.04	<.08	<.04	<.5	<.03	<.03	<.20	<.05	<.15	<.07	<.2	<.07	<.60	**.44e**	<2
13	06/22/99	1610	<.08	<.04	<.08	<.04	<.5	<.03	<.03	<.03	<.05	<.15	<.10	<.2	<.10	<.60	<1.0	--
14	06/22/99	1445	<.08	<.04	<.08	<.04	<.5	<.03	<.03	<.03	<.05	<.15	<.10	<.2	<.10	<.60	<1.0	--
15	06/22/99	0740	**.03e**	**.03e**	<.08	**.03e**	<.5	<.03	**.02e**	<.03	<.05	**.19**	<.10	<.2	<.10	**.4e**	**.7e**	--
15	12/15/99	0945	**.03e**	**.07**	<.15	**.03e**	<.5	<.15	<.03	<.20	<.05	<.20	<.10	<.2	<.07	<.60	<1.0	
17	06/22/99	0945	<.08	<.04	<.08	<.04	<.5	<.03	<.03	<.03	<.05	<.15	<.10	<.2	<.10	<.60	<1.0	--
17	01/12/00	1235	**.08**	<.08	<.2	<.08	<.5	<.03	**.08**	**.09**	**.03e**	<.3	<.07	**1.6**	**.09e**	<.60	<1.0	<2
17	02/22/00	1415	**.03e**	<.04	<.08	<.04	<.5	<.03	<.03	<.20	<.05	<.15	<.07	**5.7**	**.05e**	<.60	**.5e**	<2
20	06/21/99	1415	**.01e**	<.04	<.08	<.04	<.5	<.03	**.01e**	**.01e**	<.05	<.15	<.10	<.2	<.10	<.60	<1.0	--
20	09/14/99	1630	<.06	<.04	**.41**	<.04	<.5	<.15	<.03	<.20	<.05	<.20	<.10	<.2	<.07	<.60	<1.0	--
20	12/13/99	1705	<.08	**.06**	<.08	<.04	<.5	<.03	<.03	<.20	<.05	<.15	<.10	<.2	<.10	<.60	**.15e**	--
20	09/12/00	1150	**.93**	<.04	<.08	<.04	**.65**	<.03	<.03	<.20	<.05	<.15	<.10	<.2	<.10	<.60	<1.0	
21	06/21/99	1600	<.08	<.04	<.08	<.04	<.5	<.03	<.03	<.03	<.05	<.15	<.10	<.2	<.10	<.60	<1.0	--
22	06/21/99	1700	<.08	<.04	<.08	<.04	<.5	<.03	<.03	<.03	<.05	<.15	<.10	<.2	<.10	<.60	<1.0	--

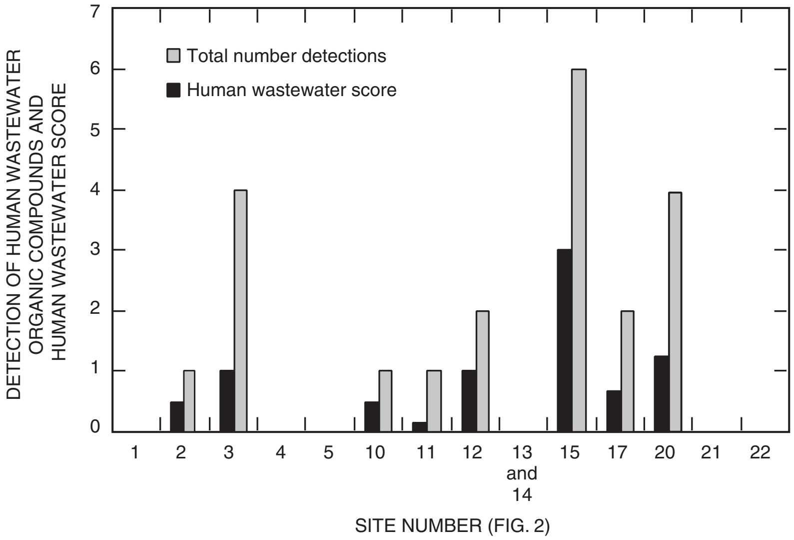


Figure 13. Number of detections of human wastewater organic compounds and human wastewater score at selected sites.

ple contained caffeine. Both samples from site 12 contained the fecal indicator cholesterol, and one of the samples contained 3*B*-coprostanol (table 7). The detection of human wastewater indicators at sites 3 and 12 suggests possible affects from septic tanks, but is unexpected because of the small human population upstream from these sites. The detection of cholesterol and 3*B*-coprostanol at these two sites, however, is consistent with these sites having among the largest indicator bacteria densities. The basins upstream from these sites also have large concentrations of pastured and confined animals.

Spring 20 (human wastewater score of 1.25) was the only one of the three sampled springs that contained organic compounds. All four samples from spring 20 contained at least one organic compound, such as caffeine, phenol, or triclosan, and one sample contained cholesterol (table 7). The larger than expected concentrations of Na and Cl compared to other springs, and the detection of human wastewater compounds, indicate a source of human wastewater in the contributing recharge area of spring 20. Possible sources include several nearby homes on septic systems, homes on septic systems in the town of Butterfield, or runoff from fields where liquid waste from a poultry processing plant is applied. Although samples from Pogue Creek at site 11 downstream from spring 20 contained among the largest densities of indicator bacteria and concentrations of major ions and nutrients of stream samples in the study, only one human wastewater compound (caffeine) was detected in the six samples from this site (table 7). The small detection frequency suggests little effect from human wastewater sources in the lower reach of Pogue Creek. It is possible that organic compounds from potential human sources may be diluted, or that chemical processes within the stream may degrade these compounds.

Water samples collected from Pogue Creek (site 11) and Clear Creek downstream from Pierce City (site 16) contained several pharmaceutical compounds. The analytical methods used to determine these compounds are experimental, and reported results should be considered tentative. False detections are possible, although a sample collected from a reference site in a wilderness area about 120 mi east of the study area (Paddy Creek near Slabtown, Missouri) and a sample collected about 40 mi southwest of the study area (Elk River near Tiff City, Missouri) did not contain detectable concentrations of pharmaceutical compounds. The sample from site 11 contained antibiotics used to treat human and animal infections, such as sulfamethoxazole and lincomycin, and the veterinary antibiotic tylosin (table 8). The sample from site 16 also contained lincomycin and sulfamethoxazole, in addition to erythromycin-H_2O and trimethoprim. Lincomycin and tylosin are widely used in the animal industry without veterinary prescription. Lincomycin is widely used as a poultry feed additive at rates of about 100 to 200 g/ton (grams per ton) in the prevention and treatment of respiratory and intestinal infections (Pennsylvania State

Table 8. Pharmaceutical compounds tentatively identified in selected water samples from the upper Shoal Creek Basin and selected reference sites in Missouri

[All concentrations in micrograms per liter; <, less than; D, detected but not quantified; e, estimated]

Antibiotic compound	Shoal Creek Basin		Paddy Creek near Slabtown, Missouri[c]	Elk River near Tiff City, Missouri[d]
	Site 11[a]	Site 16[b]		
Erythromycin-H_2O	<0.05	0.10	<0.05	<0.05
Lincomycin	.16	.28	<.05	<.05
Sulfadimethoxine	<.05	<.05	<.05	<.05
Sulfamethoxazole	D	D	<.1	<.1
Trimethoprim	<.03	.03	<.03	<.03
Tylosin	.02e	<.05	<.05	<.05

[a] Sampled 04/05/99.
[b] Sampled 04/07/99.
[c] Background reference site in the Mark Twain National Forest in south-central Missouri. Sampled 05/15/99.
[d] Stream in southwestern Missouri listed by the Department of Natural Resources as affected by nutrients from unknown sources. Sampled 04/07/99 (Missouri Department of Natural Resources, 1996).

College of Agricultural Sciences, January 2000). During 2000, respiratory infections were reportedly particularly severe in turkey operations in the study area. Lincomycin generally is used in humans to treat infections in persons allergic to penicillin (Physician’s Desk Reference Nurse’s Drug Handbook, 1999). Tylosin is used only in animals; a soluble form commonly is added to poultry water at rates of 2 to 1,000 mg/L for several days to treat respiratory infections (Pennsylvania State College of Agricultural Sciences, January 2000). Erythromycin is used to treat infections in humans and animals. Sulfamethoxazole and trimethoprim are widely used together to treat urinary tract infections in humans; there are no known uses of sulfamethoxazole and trimethoprim in the poultry or cattle industries. The detection of erythromycin-H_2O, lincomycin, and sulfamethoxazole in samples from site 16 is consistent with the detection of human wastewater organic compounds and large concentrations of inorganic constituents and nutrients, indicating effects from the Monett or Pierce City WWTPs. The detection of lincomycin and tylosin in the sample from site 11, and the near absence of human wastewater organic compounds in the six samples from this site suggests that human waste is not a major source of the nutrients and fecal coliform densities observed in Pogue Creek at site 11. However, the detection of sulfamethoxazole in the Pogue Creek sample also may indicate additional effects from human wastewater, considering that this compound is used exclusively in humans.

Bacterial Sources and Pathogens

Isolates of *E. coli* were obtained from water samples collected at site 2 (2 samples), site 3 (2 samples), site 10 (1 sample), site 11 (3 samples), and site 12 (1 sample) and submitted to the UMC for ribopattern analysis. This technique has been used, with mixed results, in attempts to identify sources of fecal coliform and *E. coli* contamination in water bodies (Parveen and others, 1999; Schlottmann and others, 2000). Carson and others (2001) used the technique to discriminate between various types of *E. coli* isolated from animal waste. Whereas most efforts to determine the source of bacteria in streams rely on indirect measures of inorganic and organic constituents, ribotyping has the potential of directly linking the bacteria to their source using DNA ribopatterns. The technique relies on the assumption that ribopatterns of *E. coli* from various animal species will be unique. However, little is known about the temporal and geographic variability of ribopatterns within a single animal group or the potential sharing of ribotypes between various animals. For example, Sargeant and others (1999) showed that wild deer foraging in fields where dairy cattle were pastured became infected with the identical strain of *E. coli* O157:H7 carried by the cattle. The transfer of *E. coli* O157:H7 to sea gulls foraging at a landfill also has been noted (Wallace and others, 1997). However, Kariuki and others (1999) downplay the crossover between various animal species. In addition, ribopattern analysis involves the use of multivariate statistical methods to compare patterns in large data sets. The method compares the degree of similarity of the patterns from unknown samples to known patterns in a database—not the rigorous hypothesis testing that commonly is done with water-quality data. As additional patterns are added to the database of "known" patterns, the apparent degree of similarity between unknown and known patterns changes. Given the large degree of uncertainty in the methods, results of the method should be treated as experimental, and interpretations should be made only in conjunction with other data.

Ribopatterns were obtained from 120 isolates from nine water samples and compared to more than 80 isolates obtained from potential animal sources within the study area and several hundred isolates obtained from animal sources at the UMC Veterinary Medicine farms. Initially, a two-class analysis was made to differentiate the patterns into human and nonhuman groups. Results of the initial matching using discriminate analysis indicated that 85 of the 120 isolates (71 percent) matched the nonhuman group and 22 isolates (18 percent) matched the human group with a probability of 0.80 or larger (table 9). Thirteen of the isolate patterns (11 percent) were not matched to either the human or nonhuman group. Overall, the smaller abundance of human patterns is consistent with the relatively small human population in the study area. Human waste appeared to be an important source of the *E. coli* in the October 1999 water sample from site 3 (8 of 13 isolates), site 10 (5 of 16 isolates), and site 12 (6 of 12 isolates). The detection of human patterns at sites 3 and 12 is consistent with human wastewater organic compounds including the fecal indicators cholesterol and 3*B*-coprostanol being detected in samples from these sites (table 7), although no obvious source of human wastewater was apparent upstream from these sites. Site 10 is located less than 150 ft downstream from a farm with a septic tank, and effluent from this tank may explain the detection of human isolates in this sample. A trace quantity of the human wastewater compound caffeine was detected in one sample from site 10 (table 7). The association of isolates identified as human with the detection of organic compounds associated with human wastewater is tenuous because laboratory schedules did not permit the collection of samples for ribopattern analysis at the same time samples for wastewater organic compounds were collected.

Results of a second level of discriminate analysis comparing the 120 isolates obtained from water samples to five potential animal sources (human, horse, cattle, turkey, and chicken) generally were consistent with the first-level analysis, indicating that human waste appeared to be an important source of *E. coli* in the October 1999 samples from sites 3, 10, and 12 (table 9). During the second-level analysis, 72 of the 120 *E. coli* isolates (about 60 percent) were matched to one of the five possible source groups at a probability of 0.80 or larger (table 9). The smaller number of water isolates matched to animal sources is not surprising given the relatively small number of source samples obtained during this study and the increased number of possible sources, which tends to lower individual probabilities. Of the isolates that matched a possible animal host, human and turkey were the most frequently identified (19 isolates each), followed by horse (15 isolates), chickens (10 isolates), and cattle (9 isolates). The percent of isolates matched with possible animal sources ranged from 36 percent (5 of 14 isolates) in the February 2000 sample from site 2 to 81 percent (13 of 16 isolates) in the October 1999 sample from site 10. Except for two isolates in the sample from site 10, all isolates previously identified as matching human in the first-level analysis also were identified as matching human in the five-class second-level analysis. One of the human isolates from site 10 was matched to horse in the second-level analysis and the other was not matched to any source at the 0.80 probability level.

There were notable differences in the possible animal sources of the *E. coli* isolated from the various sites. For example, horses appeared to be an important source (6 of 13 isolates identified) of *E. coli* isolated from the sample from site 10 (table 9). The presence of horse ribopatterns is consistent with the presence of stabled and pastured horses immediately upstream from site 10. Horse manure occasionally was observed along the stream banks upstream from site 10. Turkeys appeared to be an important source of *E. coli* in the March 2000 (5 of 8 isolates identified) sample from site 11 and in the August 2000 sample (5 of 7 isolates identified) from site 3. The predominance of turkey ribopatterns in the March 2000 sample from site 11 is consistent with the large number of turkey barns upstream from this site, and the fact that poultry litter was being applied to fields about the time of sample collection. Although there are several turkey barns immediately upstream from site 3, the predominance of

Table 9. Number of *Escherichia coli* isolates in water samples assigned to various sources by ribopattern analysis

Site (fig. 2)	Date	Number of isolates	Possible source using two-class analysis (probability of 0.80 or larger)			Possible source using five-class analysis (probability of 0.80 or larger)						
			Human	Nonhuman	Unknown	Percent identified	Human	Horse	Cattle	Turkey	Chicken	Unknown
2	02/23/00	14	0	11	3	36	0	2	3	0	0	9
	08/08/00	13	0	13	0	54	0	2	1	1	3	6
3	10/20/99	13	8	4	1	69	7	0	0	2	0	4
	08/08/00	10	0	9	1	70	0	0	0	5	2	3
10	10/20/99	16	5	9	2	81	3	6	2	1	1	3
11	10/20/99	15	2	11	2	40	2	1	0	2	1	9
	03/22/00	13	1	12	0	62	1	1	1	5	0	5
	08/08/00	14	0	12	2	57	0	2	1	3	2	6
12	10/20/99	12	6	4	2	75	6	1	1	0	1	3
	Total	120	22	85	13	60	19	15	9	19	10	48

turkey ribopatterns in the August 2000 sample from this site seems unusual because litter generally is not applied to land during the summer.

The small number of *E. coli* isolates that matched cattle in the samples is unusual given that cattle have access to nearly all streams in the study area, and cattle waste was observed along stream banks in some areas. Little is known about the geographic variability in ribopatterns in animal species, and it is possible that cattle in the study area may have different ribopatterns than cattle from the UMC farms in central Missouri. In addition, cattle in the study area often graze on fields where poultry litter is applied, and it is not known what affect this may have on ribopatterns obtained from cattle in the study area. To test the possibility that cattle in the study area have unique ribopatterns, ribopatterns from cattle manure collected from the study area were removed from the database, and tested as unknowns with five possible sources (UMC cattle, chicken, horse, human, and turkey). Results of the comparison indicate that of the 23 cattle isolates obtained from the study area, only 7 isolates (30 percent) were correctly matched to cattle at the 0.80 probability level. Two of the 23 isolates were incorrectly matched to horse, and one each was incorrectly matched to chicken and turkey. The small number of isolates correctly identified as cattle is problematic, and suggests that cattle from the study area have unique ribopatterns compared to ribopatterns obtained from cattle on UMC farms about 150 mi from the study area. The small number of cattle isolates matched to chicken or turkey isolates suggests that cattle in the study area have not acquired a "poultry" signature as a result of land application of poultry litter onto fields where cattle graze.

Although ribopattern results generally are consistent with land-use patterns, such as the identification of horse patterns near hobby horse farms, there is uncertainty in the data. A particular concern is the variation in percentages of unknown water isolates matching various nonhuman animal sources as the number of potential sources is changed. For example, in the October 1999 sample from site 10, six isolates were matched to the horse, three were matched to human, two were matched to cattle, and one each was matched to turkey and chicken with probabilities exceeding 0.80 (table 9). This comparison was based on five possible source groups in the database (chicken, cattle, horse, turkey, and human) selected to represent the major potential sources thought to exist in the basin. When the number of possible source groups was increased to eight by adding dogs, geese, and pigs, four of the six horse isolates were reassigned to geese and the probability of the remaining two horse isolates dropped to less than 0.20. Geese rarely are seen in the study area, and the case could be made that the removal of them from the database for the study area is valid. Four of the five isolates matched to turkey in the March 2000 sample from site 11 became unknown when the number of possible source groups was increased to eight. Increasing the number of possible source groups did not appear to have a dramatic affect on the identification of human isolates. For example, of the seven isolates matched to human in the October 1999 sample from site 3, six of the isolates remained matched to human, and all six of the isolates matched to human in the sample from site 12 also were matched to human as the number of source groups was increased to eight. Given the effect that adding known source patterns in the database can have on the results, uncertainty exists in the ability of the method to be used to unequivocally identify the animal host of a particular isolate.

The large number of isolates not assigned to any of the five animal sources considered (40 percent), and the relatively large percent of isolates matched to turkeys or chickens, may be caused by the water samples containing isolates from animal sources not in the database, such as wild deer, raccoons, and opossum. Depending on the degree of similarity, it is possible that patterns from these wild animal sources could be matched to one of the five animal sources. Turkeys have among the simplest ribopatterns and it is possible that there is less error in assigning a particular pattern to turkeys than to more complicated patterns such as human. In addition, uncertainty exists in the ability of the multivariate analysis used to differentiate between the known animal source patterns in the database. When examining only known source patterns, the method was able to correctly identify patterns from horse and turkey isolates only 65 and 77 percent of the time, respectively. The method was much more reliable in correctly identifying patterns from human (95 percent), chicken (96 percent), and cattle isolates (87 percent). The database consists of less than 300 known source patterns, and may be of insufficient size or quality to reliably discriminate between various animal groups other than between human and nonhuman sources. Some of the uncertainty may be related to the relatively small number of known animal patterns in the database, incomplete wildlife source sampling, and the non-rigorous nature of the statistical methods used in pattern analysis.

All nine water samples submitted for ribotyping, and five additional water samples collected from sites 1, 2, 10, 11, and 12 during February 2000 were tested for the human pathogen *E. coli* O157:H7. A minimum of 20 isolates were tested for the pathogen in each water sample using the methods previously described. Only one sample (from site 3, collected October 1999) was positive for the presence of *E. col*i O157:H7. Four out of more than 40 isolates from this sample were confirmed as *E. coli* O157:H7. The presence of this organism in only 1 of 14 water samples suggests that its distribution is episodic; however, because rigorous screening for the pathogen was not done in all water samples, the actual abundance of the organism is unknown. Although *E. coli* O157:H7 can be shed by a variety of animals including wild deer, cattle are the predominant reservoir for this organism (Sargeant and others, 1999). Fisher and others (2001) detected *E. coli* O157:H7 in only 3 of 469 free ranging deer fecal samples collected from animals in the southeastern United States during 1997. The presence of *E. coli* O157:H7 in the October 1999 water sample from site 3 strongly suggests a cattle-derived source. This conclusion appears to be inconsistent with the results of the ribopattern analyses that indicated no cattle patterns in this particular water sample, and a predominance of human patterns; however, the isolates ribotyped were not the same isolates screened for *E. coli* O157:H7.

RUNOFF WATER QUALITY

Water samples were collected at three sites along Shoal Creek (sites 1, 3, and 4) during a runoff event on April 26 and 27, 1999. A total of 12 samples were collected during this event and analyzed for major ions, selected trace elements, nutrients, and indicator bacteria. Data from these samples were used to examine the importance of runoff events in nutrient and bacteria transport. Sample results from different runoff events may vary from those described below.

Discharge and Inorganic Constituents

During the morning of April 26, 1999, more than 1.9 in. of rainfall occurred at Monett within about a 1-hour period (National Oceanic and Atmospheric Administration, 1999). An additional 0.67 in. of rainfall occurred during the afternoon of April 26. Soils were saturated and substantial runoff occurred. Four sets of discharge measurements and water-quality samples were obtained at sites 1, 3, and 4 during a 24-hour period beginning at 3:00 p.m. on April 26. Based on discharge measurements made during this event, estimated peak discharges at sites 1, 3, and 4 were 352, 1,150, and 892 ft^3/s, respectively (table 10). The first set of samples was collected just after the stream began to rise, the second set was collected at or across the peak discharge, and the third and fourth sets were collected on the recession limb (table 10). Discharge measurements and water-quality samples generally were collected by wading the stream. Water depths (more than 4 ft) and velocities (more than 4 ft/s) during the second and third sampling events at sites 3 and 4 exceeded the safety margin for wading, so discharge measurements were made from bridges. A boom-operated water-quality sampler was not available; water-quality samples were collected from two vertical sections by wading approximately one-third the distance across the stream from the left and right banks.

Specific conductance values and concentrations of most inorganic constituents were inversely related to discharge and decreased with increasing discharge at each site (fig. 14). However, concentrations of K tended to increase with increasing discharge (table 10, fig. 14). The increase in K concentrations with increasing flow at all three sites indicates a runoff-derived source for these constituents. An increase in K concentrations during high discharge has been attributed to leaching of K from soils and decaying organic matter (Hem, 1992). It is probable that the increase in K concentrations with increasing discharge is caused by leaching of these elements from soils and from organic matter in fields.

Nutrients and Bacteria

Concentrations of NO_{2t}+NO_{3t} decreased with increasing discharge at all sites during the April 1999 runoff event; however, concentrations of NH_{3t}, P_t, and PO_{4t} increased with increasing discharge (table 10, fig. 14). The decrease in NO_{2t}+NO_{3t} concentrations and increase in K, NH_{3t}, P_t, and PO_{4t} concentrations indicates different sources or transport mechanisms for these nutrients during runoff events. Phosphorus is less soluble in water than NO_{2t}+NO_{3t} and solubility and uptake by biota generally limit its concentrations in

ABBREVIATIONS AND REPORTING UNITS FOR CHEMICAL CONSTITUENTS AND NOTATIONS USED IN TABLE 10

Abbreviation	Description
Q	Discharge, in cubic feet per second
Temp	Temperature, in degrees Celsius
SC	Specific conductance, in microsiemens per centimeter at 25 degrees Celsius
SS	Suspended solids, in milligrams per liter
pH	pH, in standard units
$NO_{2t}+NO_{3t}$	Total nitrite plus nitrate as N, in milligrams per liter
NO_{2t}	Total nitrite as N, in milligrams per liter
NH_{3t}	Total ammonia as N, in milligrams per liter
P_t	Total phosphorus as P, in milligrams per liter
PO_{4t}	Total orthophosphorus as P, in milligrams per liter
FC	Fecal coliform density, in colonies per 100 milliliters
FS	Fecal streptococcus density, in colonies per 100 milliliters
E. coli	*Escherichia coli* density, in colonies per 100 milliliters
Ca	Calcium, dissolved, in milligrams per liter
Mg	Magnesium, dissolved, in milligrams per liter
Na	Sodium, dissolved, in milligrams per liter
K	Potassium, dissolved, in milligrams per liter
Cl	Chloride, dissolved, in milligrams per liter
SO_4	Sulfate, dissolved, in milligrams per liter
$Alk_{(ep)}$	Total acid neutralizing capacity, endpoint titration to pH 4.5, in milligrams per liter
$Alk_{(it)}$	Total acid neutralizing capacity, incremental titration, in milligrams per liter
HCO_3	Bicarbonate, total, incremental titration, in milligrams per liter
B	Boron, dissolved, in micrograms per liter
Sr	Strontium, dissolved, in micrograms per liter
<	Less than

Table 10. Water-quality data for the April 26 and 27, 1999, runoff event

Date	Time	Q	Temp	SC	SS	pH	NO_{2t}+ NO_{3t}	NO_{2t}	NH_{3t}	P_t	PO_{4t}	FC	FS	*E. coli*	Ca	Mg	Na	K	Cl	SO_4	$Alk_{(ep)}$	$Alk_{(it)}$	HCO_3	B	Sr
Site 1, Shoal Creek near Ridgley																									
04/26/99	1445	69	15.5	139	45	7.36	2.3	0.02	0.11	0.39	0.32	22,000	12,600	46,000	20	1.8	2.8	5	5.7	3.8	44	42	51	20	32
04/26/99	1800	352	18.4	85	947	7.29	.84	.03	.38	1.3	.71	90,000	29,800	58,000	10	1.4	1.4	6.6	2.5	2.5	28	25	31	21	19
04/27/99	0025	114	15	128	63	7.25	2.2	.03	.14	.62	.52	23,600	7,600	19,200	17	2.1	2.3	6.1	4.8	<.2	41	40	49	20	31
04/27/99	1200	65	14.4	162	26	7.33	3.5	.02	.04	.17	.16	3,000	550	4,100	24	2	3	4.2	6.5	4.9	72	70	85	17	38
Site 3, Shoal Creek at State Highway 97																									
04/26/99	1530	190	15.9	197	92	7.84	2.4	0.02	0.15	0.31	0.22	28,000	9,800	29,000	34	1.9	3.7	3.6	7.2	3.3	72	71	86	19	34
04/26/99	1940	1,150	17.2	135	1,560	7.54	1.3	.02	.32	1.1	.34	120,000	12,000	44,000	21	1.4	2.1	5.1	4.2	2.4	49	47	57	19	22
04/27/99	0150	491	15.9	146	269	7.48	1.8	.03	.2	.7	.5	24,000	12,000	39,000	22	1.8	2.8	5.6	5.3	3.4	85	86	105	22	27
04/27/99	1330	218	15.3	202	73	7.75	3.3	.02	.06	.22	.16	12,400	3,400	12,200	33	2.1	3.7	3.6	7.4	4.7	113	112	137	18	38
Site 4, Shoal Creek at Jolly																									
04/26/99	1645	265	15.6	237	76	7.96	2.7	0.02	0.11	0.21	0.15	28,000	9,800	36,000	42	2	4.1	3.1	8	3.3	90	90	110	14	39
04/26/99	2300	892	16.8	145	1,314	7.6	1.5	.03	.28	.96	.3	70,000	22,000	55,000	24	1.3	2.2	4.8	4.4	2.6	52	52	63	20	23
04/27/99	0250	576	16.4	146	503	7.52	1.5	.03	.25	.78	.47	42,000	13,000	43,000	23	1.5	2.6	5.3	4.8	<.2	88	87	106	20	25
04/27/99	1500	262	15.6	204	91	7.72	2.8	.02	.07	.26	.2	14,000	960	17,000	34	2	3.4	3.7	6.8	4.4	117	114	139	20	37

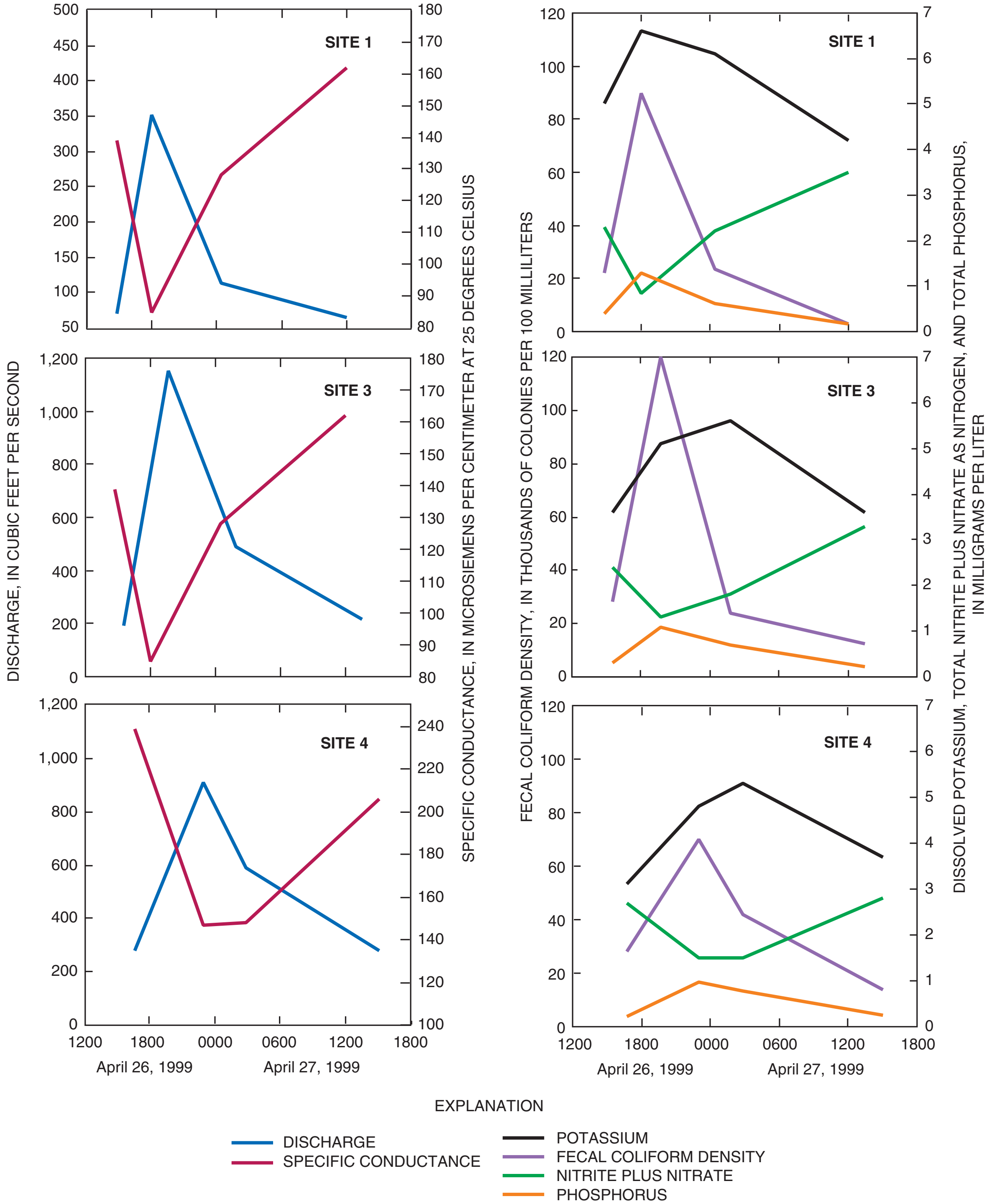


Figure 14. Instantaneous discharge, specific conductance, and concentrations of selected chemical constituents in runoff water samples from sites 1, 3, and 4 collected on April 26 and 27, 1999.

natural waters to less than a few tenths of milligrams per liter (Hem, 1992). The increase in P_t and PO_{4t} during runoff is likely related to these constituents being sorbed to and transported with particulate matter. Concentrations of suspended sediment increased dramatically with increasing discharge at each site (table 10). Nitrogen species are soluble in water, and the decrease in NO_{2t}+NO_{3t} with increasing discharge suggests that much of the NO_{2t}+NO_{3t} is derived from ground-water sources and that substantial quantities were not leached from soils or fields during this runoff event. Concentrations of NO_{2t}+NO_{3t} in the final runoff sample from sites 1 and 2 were more than 30 percent larger than those in the initial runoff samples (fig. 14).

During the 24 hours that samples were collected during the runoff event, a substantial quantity of P_t was transported compared to the estimated P_t mass transported annually during base-flow conditions. The total P_t transported during the April 1999 runoff event at site 3 [estimated at 860 kg (kilograms)] was about 14 percent of the annual quantity of P_t transported (estimated at 6,100 kg) during base-flow conditions. The annual base-flow P_t and NO_{2t}+NO_{3t} transported was estimated using the measured discharges and nutrient concentrations in the monthly base-flow samples collected at site 3 (table 4). The quantity of NO_{2t}+NO_{3t} transported during the 24-hour sampling period (2,400 kg) at site 3 represented less than 1 percent of the annual NO_{2t}+NO_{3t} transported (320,000 kg) at site 3 during base-flow conditions. The data indicate that substantial quantities of P_t can be transported during a single runoff event, and that annual quantities of P_t transported from the basin cannot be accurately determined without monitoring all runoff events.

During the April 1999 runoff event, concentrations of indicator bacteria increased with increasing discharge at each site (table 10). Concentrations of fecal coliform bacteria increased 200 to 400 percent as discharge peaked at each site, with the largest density (120,000 col/100 mL) detected at site 3 (fig. 14). Increases in *E. coli* densities were more subdued, increasing about 50 percent or less as discharge peaked at each site (table 10). Densities of fecal streptococcus increased less than 250 percent between the initial and peak samples at each site, with the smallest increases (about 20 percent) occurring at site 3 (table 10). The dramatic increase in fecal coliform densities compared to *E. coli* and fecal streptococcus densities may possibly be related to the fecal coliform test including other non-fecal bacteria such as *Klebsiella* (Eaton and others, 1995) washed into the stream by runoff or resuspended from bottom sediments.

BACTERIA TRENDS

Densities of fecal coliform and *E. coli* in stream samples were variable but, similar to discharge, tended to decrease with time at most sites during the 13-month study period (table 4). Exceptions to this general trend were sites 3 and 4, where fecal coliform and *E. coli* densities remained about the same during the 13-month study period. Densities of fecal streptococcus bacteria generally were less variable than fecal coliform or *E. coli* densities and except for site 3, decreased sharply at all main-stem sites during the last two months of the study (table 4). The decreases in indicator bacteria densities probably are related to decreasing stream discharges caused by the drought conditions during the latter part of the study.

Since 1995, the MDNR has detected a trend of increasing fecal coliform densities with increasing time at site 3 (Missouri Department of Natural Resources, 2000). A plot of MDNR data (1992–2000) and USGS data (1999–2000) illustrates a trend of increasing fecal coliform densities between water years[a] 1995 and 1999 (fig. 15). During this same period, however, the specific conductance values and NO_{2t}+NO_{3t} concentrations (not shown) in water samples collected from site 3 tended to decrease and annual precipitation recorded at the NOAA station at Monett increased (fig. 15). The trends of fecal coliform densities, specific conductance values, and NO_{2t}+NO_{3t} concentrations between water years 1995 and 1999 probably are related, in part, to a general trend of increasing discharge in Shoal Creek in response to precipitation trends, and not necessarily to land-use changes or changes in the number of animal operations in the study area. Between water years 1996 and 1999, the annual mean discharge at the downstream gaging station on Shoal Creek near Joplin, Missouri (fig. 1), increased more than 60 percent from 309 ft^3/s in water year 1996 to about 514 ft^3/s in water year 1999 (fig 15). The annual 7-day minimum discharge more than doubled from 64 ft^3/s in water year 1996 to 137 ft^3/s in water year 1999. Because precipitation and runoff usually have small specific conductance values

[a]Water year is defined as the period from October 1 through September 30.

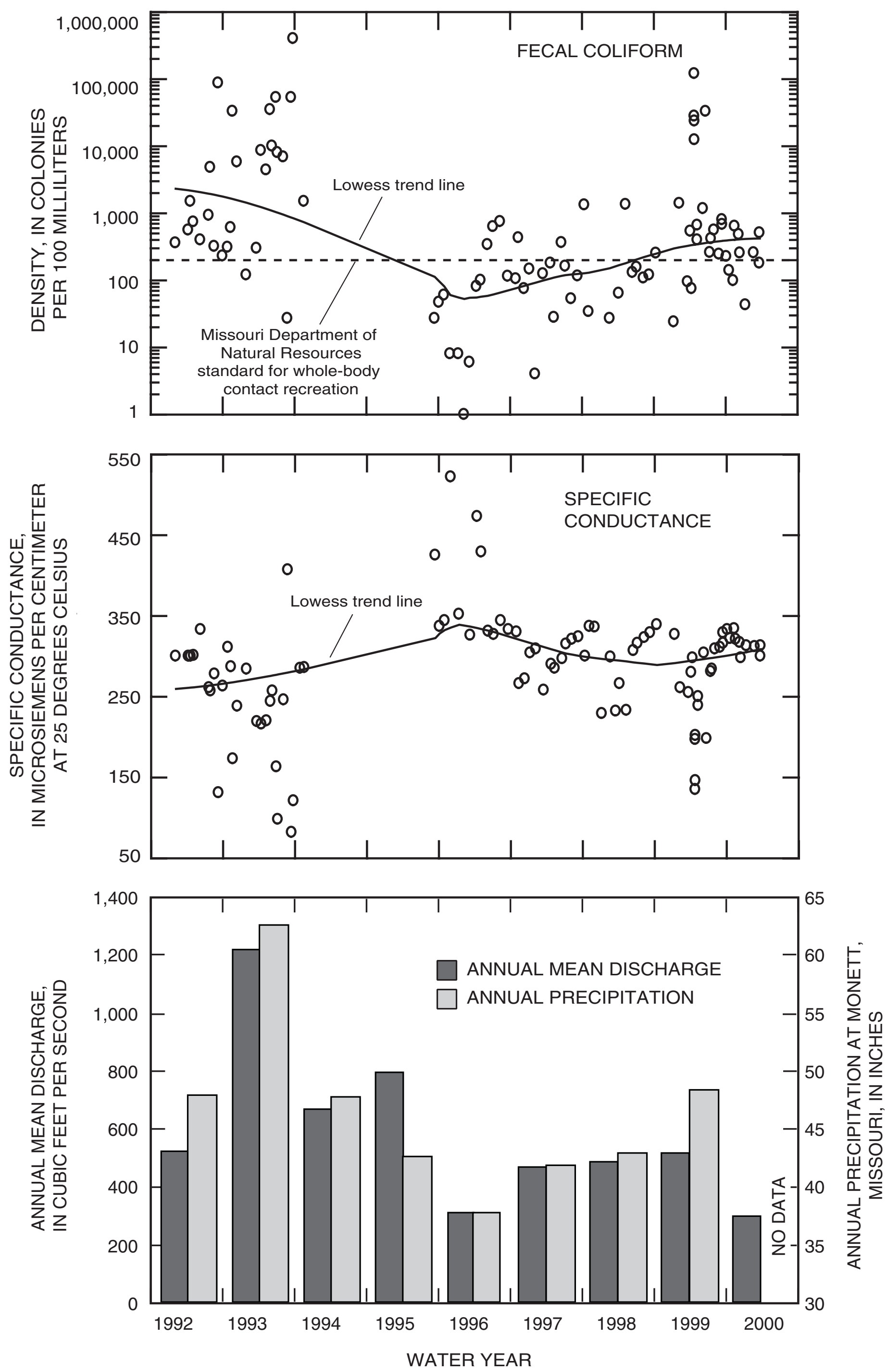


Figure 15. Fecal coliform density and specific conductance in water samples from site 3 on Shoal Creek, annual mean discharge at the U.S. Geological Survey gaging station on Shoal Creek near Joplin, Missouri, and annual precipitation at Monett, Missouri, water years 1992 through 2000 (data from the Missouri Department of Natural Resources, U.S. Geological Survey, and National Atmospheric and Oceanic Administration).

Table 11. Spearman's ranked correlation coefficients between discharge and selected water-quality measurements at site 3, Shoal Creek at State Highway 97, made between 1992 and 2000

	Discharge	Temperature	Specific conductance	Fecal coliform	Total nitrite plus nitrate	Total phosphorus
Discharge	1.00					
Temperature	.14	1.00				
Specific conductance	-.75	-.24	1.00			
Fecal coliform	.71	.42	-.76	1.00		
Total nitrite plus nitrate	.25	-.28	-.26	.03	1.00	
Total phosphorus	.75	.16	-.83	.65	.29	1.00

compared to the base-flow component of streamflow, which consists largely of ground-water inflow, the trend of decreasing specific conductance values with increasing annual mean discharge is reasonable. The fact that fecal coliform densities and NO_{2t}+NO_{3t} concentrations vary inversely during this period suggests that they have different mechanisms of transport to the stream. Spearman's ranked correlation coefficients were calculated for the combined MDNR and USGS data set at site 3 to assess the monotonic relations between fecal coliform densities and other water-quality measurements (table 11). Fecal coliform densities were negatively correlated with specific conductance (-0.76) and positively correlated with discharge (0.71), temperature (0.42), and P_t (0.65). Concentrations of NO_{2t}+NO_{3t} were weakly correlated with discharge (0.25).

The time trend of increasing fecal coliform bacteria densities at the MDNR sampling site was evaluated using data from the MDNR and from this study. Environmental variables have been used to develop statistical models that predict densities of indicator bacteria more readily than plate-culture methods currently used (Francy and Darner, 1998; Meyers and others, 1998). The construction of these models often has been driven by the need for public health officials to make rapid decisions about the safety of recreational activities, such as swimming, in waters that may contain pathogens. Using the limited amount and type of available data, a multiple linear regression (MLR) model was developed to predict fecal coliform densities at site 3. Variables evaluated for inclusion in the model included discharge, rainfall, temperature, specific conductance, pH, and seasonality. Discharge was not available for most of the MDNR samples and therefore was not included in the MLR. Rainfall values from the NOAA weather stations at Monett and Cassville were used to compute the 24-, 48-, and 72-hour rainfall amounts preceding sample collection. Seasonal affects were evaluated using a sine and cosine function of the sample date. Variables were added to the model in various combinations, and retained only if their coefficients were significantly different from zero at an alpha level of 0.05. The chosen MLR model contained only two explanatory variables (temperature and specific conductance) and explained 65 percent of the variability (r^2 equals 0.65) in the $\log_{10}$ (base-10 logarithm) of fecal coliform densities (fig. 16). The final regression equation was:

$$\log_{10}(\text{fecal coliform density}) = 0.0431\,Temp - 0.0115\,SC + 5.2847,$$

where *Temp* is the measured temperature in degrees Celsius and *SC* is the measured specific conductance in microsiemens per centimeter at 25 °C. The standard error of the estimate was 0.60. Using the measured values of temperature and specific conductance, the model correctly predicted fecal coliform densities above the MDNR standard of 200 col/100 mL 83 percent of the time (45 of 54 samples). The model was only 49 percent correct predicting when fecal coliform densities were less than the MDNR standard (17 of 35 samples).

Although the trend of increasing fecal coliform densities with time at the MDNR sampling site may not be related to changes in animal production in the basin, the large number of animals and small human population upstream from sites 2 and 3 on Shoal Creek and in the Pogue Creek (site 11) and Joyce Creek (site 12)

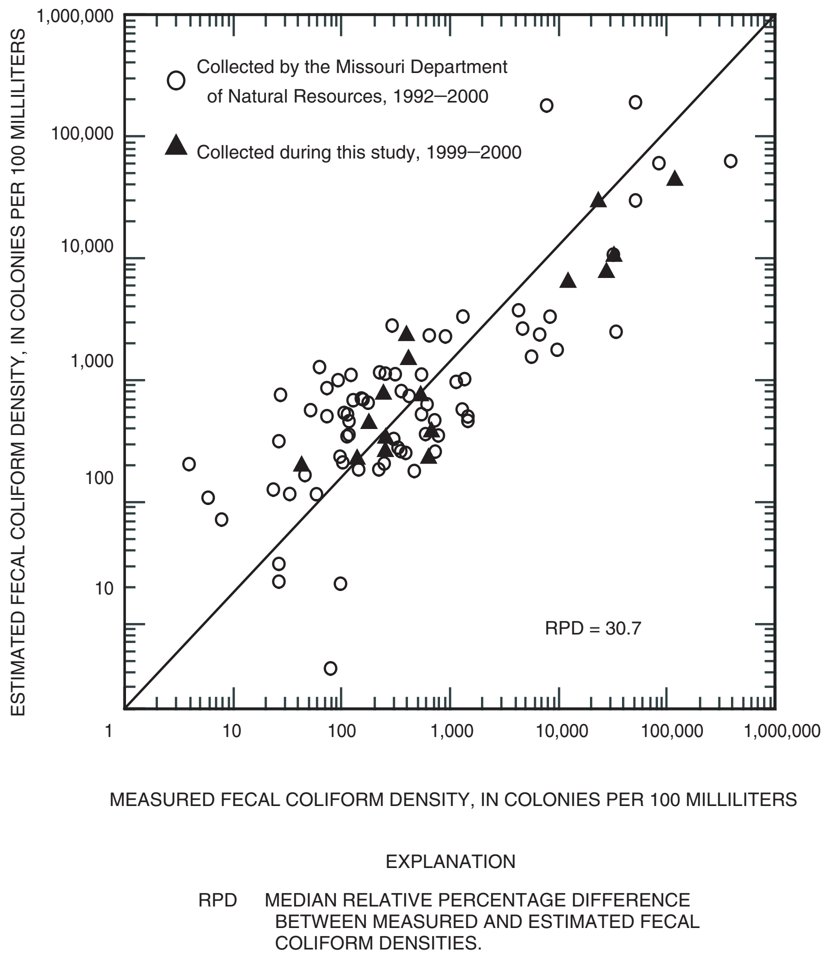


Figure 16. Comparison of measured and estimated fecal coliform density at site 3 in Shoal Creek, 1992 to 2000 (data from the Missouri Department of Natural Resources and the U.S. Geological Survey).

subbasins suggest an animal source for the large fecal coliform densities detected. This conclusion is supported by the predominance of ribopatterns from *E. coli* isolated from water samples in the study area (85 of 120 isolates) matched to nonhuman sources. The detection of small concentrations of organic compounds associated with human wastewater also suggests a mixture of human and animal sources that generally is consistent with ribopattern analysis, which indicated that 22 of the 120 *E. coli* isolates probably were of human origin. Perhaps the most notable indication of an animal-derived source is the increase in fecal coliform densities and decrease in concentrations of organic compounds associated with human wastewater with increasing distance downstream as Clear Creek flows through agricultural areas downstream from Monett and Pierce City.

SUMMARY AND CONCLUSIONS

During 1999 and 2000 a water-quality investigation of the upper 233 mi^2 (square miles) of the Shoal Creek Basin in southwestern Missouri was conducted by the U.S. Geological Survey (USGS) in cooperation with the Missouri Department of Natural Resources (MDNR) and the U.S. Environmental Protection Agency (USEPA). A 13.5-mile reach of Shoal Creek in Barry County, Missouri, was placed on the USEPA

303(d) list of impaired waters because of fecal coliform densities above the MDNR standard of 200 col/100 mL (colonies per 100 milliliters). This water-quality investigation focused on the distribution and possible sources of nutrients and fecal coliform bacteria in the upper Shoal Creek Basin. More than 170 water-quality samples were collected between April 1999 and April 2000 from a network of 13 stream and 4 spring sites and analyzed for nutrients, the indicator bacteria fecal coliform, fecal streptococcus, and *Escherichia coli* (*E. coli*), major ions, and selected trace elements. Water samples also were collected from three sites on the main stream of Shoal Creek during an April 1999 runoff event.

An estimated 33 million broilers and 300,000 turkeys are produced annually within the study area, which also contains about 25,000 cattle. Poultry litter and cattle manure represent the majority of the phosphorus (as P_2O_5) and N (nitrogen as N) loading in the study area. An estimated 2.7 Mlbs (million pounds) of phosphorus as P_2O_5 (almost 50 percent of the total P_2O_5 loading in the study area) and N are applied to fields in the form of poultry litter each year in the study area. Cattle manure represents about 1.9 Mlbs of P_2O_5 and 3.8 Mlbs of N loading, most of which are recycled nutrients from within the basin. Commercial fertilizer use represents about 17 percent of the P_2O_5 loading and 26 percent of the N loading in the study area. Municipal and domestic wastewater are minor sources of P_2O_5 and N loading in the basin.

Stream flow and water quality in the study area were affected by drought conditions that existed during the study. During the final 10 months of the 13-month study period, rainfall recorded at a National Oceanic and Atmospheric Administration station near the study area was 7.16 inches below normal, and between January 12 and February 16, 2000, the discharge of Shoal Creek at the temporary gaging station near site 3 was less than or equal to the 7-day mean minimum discharge, with a 2-year recurrence interval (7-day Q_2) of 20 ft^3/s (cubic feet per second). The annual mean discharge at the temporary gage (May 12, 1999, to May 1, 2000) was 43.9 ft^3/s. Shoal Creek gains flow from ground-water sources throughout the study area, increasing from a mean discharge of 4.46 ft^3/s at the upstream site (site 1) to 173 ft^3/s at the downstream site (site 5).

Specific conductance values and concentrations of inorganic constituents and nutrients in Shoal Creek at base-flow conditions increased with increasing distance downstream in the study area. Increases in total nitrite plus nitrate as nitrogen (NO_{2t}+NO_{3t}) concentrations were gradual suggesting nonpoint sources, whereas the large increases in concentrations of magnesium (Mg), sodium (Na), potassium (K), chloride (Cl), sulfate (SO_4), total phosphorus as P (P_t), and total orthophosphorus as P (PO_{4t}) detected between sites 4 and 5 on Shoal Creek suggest point sources in the Clear Creek subbasin (tributary between sites 4 and 5). The water quality in Clear Creek is affected by effluent from a wastewater treatment plant (WWTP) serving the city of Monett. Water samples collected from Clear Creek (sites 15, 16, and 17) generally contained the largest specific conductance values [340 to 1,280 μS/cm (microsiemens per centimeter at 25 degrees Celsius)] and concentrations of inorganic constituents such as Na [24 to 180 mg/L (milligrams per liter)], Mg (3.6 to 8.8 mg/L), K (9.2 to 77 mg/L), Cl (21 to 140 mg/L), SO_4 (19 to 160 mg/L), NO_{2t}+NO_{3t} (3.4 to 13.0 mg/L), and P_t (0.97 to 11.0 mg/L) detected. During four quarterly sampling events, Clear Creek accounted for about 60 percent of instantaneous Na load, about 40 percent of the instantaneous Cl, and nearly 100 percent of the instantaneous P_t load discharged by Shoal Creek at the downstream sampling site (site 5).

Water samples from Pogue Creek at site 11, a tributary in the central part of the study area, also contained increased specific conductance values (240 to 372 μS/cm) and concentrations of inorganic constituents such as Na (9 to 11 mg/L) and Cl (17 to 26 mg/L). Pogue Creek is unique in that its basin has among the largest percentage of drainage area with a slope greater than 10 percent (29 percent), the largest number of septic tanks (6) within 250 ft of the stream, among the largest density of poultry barns (4.2 per mi^2 of agricultural land use), and little riparian corridor. In addition, liquid waste from a poultry processing plant is sprayed onto 300 acres of fields in the upper part of the Pogue Creek Basin, and more than 80 homes on septic tanks in the town of Butterfield are located within the Pogue Creek Basin. A spring (spring 20) downslope of the poultry processing plant and the town of Butterfield contained increased concentrations of Na (11 to 19 mg/L) and Cl (20 to 43 mg/L) compared to the other springs sampled.

Compared to other streams in Missouri sampled by the USGS, Shoal Creek has anomalously large concentrations and yields of NO_{2t}+NO_{3t}. Base-flow concentrations of NO_{2t}+NO_{3t} in the five main-stem sampling sites on Shoal Creek (2.0 to 4.40 mg/L, mean of 2.90 mg/L) were significantly larger than base-flow

$NO_{2t}+NO_{3t}$ concentrations in 1,340 base-flow water-quality samples collected from 20 other Missouri streams (mean concentrations of 1.02 mg/L) by the USGS between 1960 and 2000. The mean base-flow yield of $NO_{2t}+NO_{3t}$ in main-stem sites on Shoal Creek was 2.46 kg/acre/yr (kilograms per acre per year) compared to the mean base-flow $NO_{2t}+NO_{3t}$ yields of 0.93 kg/acre/yr for other Missouri streams sampled by the USGS. Gradual increases in $NO_{2t}+NO_{3t}$ concentrations with increasing distance downstream suggest most $NO_{2t}+NO_{3t}$ in Shoal Creek is from non-point sources.

Results of this study are consistent with the MDNR finding that fecal coliform densities in water samples from Shoal Creek exceeded the Missouri standard of 200 col/100 mL. Fecal coliform densities in samples from the MDNR sampling site (site 3) exceeded the MDNR standard in 12 of the 13 samples collected and ranged from 43 to 33,000 col/100 mL (median of 400 col/100 mL). Densities of fecal coliform bacteria also exceeded the MDNR standard upstream from site 3 at site 2 in 8 of 13 samples and ranged from 25 to 9,200 col/100 mL (median of 277 col/100 mL). Densities of fecal coliform bacteria also exceeded the MDNR standard in three of the five tributaries sampled. The largest frequency of exceedences (12 of 13 samples) was at site 11 on Pogue Creek (median density of 580 col/100 mL). Fecal coliform densities above the MDNR standard also were detected at site 12 on Joyce Creek (9 of 12 samples, median of 340 col/100 mL) and at site 17 on Clear Creek (9 of 13 samples, median of 320 col/100 mL). Effluent discharged into Clear Creek from the Monett and Pierce City WWTPs did not appear to affect fecal coliform densities in Clear Creek, which actually increased with increasing distance downstream from the WWTPs.

Organic compounds commonly associated with human wastewater were detected in samples from Shoal Creek at the MDNR sampling site (site 3), several tributary sites, and one spring. The largest number of organic compounds (6 detections in 2 samples) were detected in samples from Clear Creek downstream from the Monett WWTP. These samples contained caffeine, triclosan (antimicrobial agent in many liquid soaps), or tri (2-chloroethyl) phosphate (fire retardant), and the fecal indicators cholesterol and 3*B*-coprostanol, among others. The detection of the antibiotics erythromycin-H_2O, sulfamethoxazole, lincomycin, and trimethoprim in a sample collected from Clear Creek downstream from Monett and Pierce City (16) also indicates effects from human wastewater. Three of four samples from Shoal Creek at site 3 contained at least one organic compound associated with human wastewater including caffeine, triclosan, or phenol (disinfectant), and the fecal indicators cholesterol or 3*B*-coprostanol. All four samples from spring 20 in the upper part of the Pogue Creek Basin contained one or more organic compounds associated with human wastewater including caffeine, phenol, or triclosan. The sources of these organic compounds probably are wastewater from a poultry processing plant, nearby farm septic tanks, or septic tanks in the town of Butterfield. The detection of the tylosin and lincomycin (both commonly used in the poultry industry) in addition to the human antibiotic sulfamethoxazole in a sample from Pogue Creek, and the near absence of human wastewater organic compounds, suggests that human waste probably is not a major source of nutrients and fecal coliform bacteria in Pogue Creek at site 11.

Results of ribopattern analysis indicate that 85 (71 percent) of the 120 isolates of *E. coli* extracted from nine water samples probably were from nonhuman sources. Twenty-two isolates were matched to human, and 13 isolates were unknown. Human waste appeared to be an important source of *E. coli* in the October 1999 water samples from Shoal Creek at site 3 (8 of 13 isolates), Woodward Creek at site 10 (5 of 16 isolates), and Joyce Creek at site 12 (6 of 12 isolates). The detection of human patterns at sites 3 and 12 is consistent with human wastewater organic compounds including the fecal indicators cholesterol and 3*B*-coprostanol being detected in samples from these sites, although no obvious source of human wastewater upstream from these sites is apparent. A septic tank a short distance upstream from site 10 may be the source of the human isolates identified in the water sample from this site. Further analysis compared the ribopatterns in the water samples to patterns obtained from five groups of animals (human, horse, cattle, turkey, and chicken). Results of this second level of analysis indicate that of the 72 isolates matched to a possible source, human and turkey were the most frequent (19 isolates each), followed by horse (15 isolates), chickens (10 isolates), and cattle (9 isolates). Results of the second level of analysis generally were consistent with the first-level analysis. In addition to humans, horses appeared to be an important source (6 of 13 isolates identified) of *E. coli* in a sample from site 10. The presence of horse ribopatterns is consistent with the location of stabled and pastured horses immediately upstream from site 10, and horse manure was observed along the stream banks upstream from site 10. Turkeys appeared to be an important source of *E. coli* in the March 2000 (5 of 8 isolates identified) sample from site 11, and in an August 2000 sample from site 3 (5 of 7 isolates identified). The presence of turkey ribopatterns in the March 2000 sample from site 11 is consistent with a large density of turkey barns upstream from this site.

Uncertainty exists in the ability of ribotyping to unequivocally identify the animal host of a particular isolate. When cattle source patterns from the study area were treated as unknowns and compared to five known sources (human, horse, cattle, turkey, and chicken) in the database, only 30 percent of the isolates were correctly matched to cattle, suggesting that cattle from the source area may have different ribopatterns than those in the database. The large number of *E. coli* isolates in the study not assigned to any of the five animal sources considered (48 of 120 total isolates), and the relatively large percent of isolates matched to poultry, may be caused by the water samples containing isolates from animal sources such as wild deer, raccoon, and opossum that were not contained in the database of known patterns. In addition, the probability of unknown water isolates matching a particular animal source may change as the number of potential sources is changed. However, increasing the number of possible source groups to eight did not appear to have a dramatic affect on the identification of human isolates. Knowing the affect that adding additional known source patterns in the database can have on the results, uncertainty exists in the ability of the method used to unequivocally identify the animal host of a particular isolate.

The human pathogen *E. coli* O157:H7 was identified in 1 of 13 water samples (site 3, October 1999) collected from six sites that were tested for this organism. The small occurrence of this organism suggests that the distribution of this organism is episodic; however, because rigorous screening for the pathogen was not done in all water samples, the actual abundance of the organism is unknown. The presence of *E. coli* O157:H7 in the water sample from site 3 strongly suggests a cattle-derived source.

Analysis of water samples collected from three sites on Shoal Creek during a runoff event in April 1999 indicated that specific conductance values and concentrations of most inorganic constituents and $NO_{2t}+NO_{3t}$ were inversely related to discharge and decreased with increasing discharge at each site. However, concentrations of K, P_t, suspended sediment, and indicator bacteria densities increased with increasing discharge. A significant mass of P_t [860 kg (kilograms)], representing 14 percent of the estimated annual base-flow P_t load of 6,100 kg at the downstream site (site 5) was transported at site 3 during this single event. The total mass of $NO_{2t}+NO_{3t}$ transported (2,400 kg) during the April 1999 runoff event was less than 1 percent of the estimated annual base-flow $NO_{2t}+NO_{3t}$ load of 320,000 kg at site 5. Densities of indicator bacteria were positively correlated with discharge during the runoff event. Concentrations of fecal coliform bacteria increased 200 to 400 percent, as discharge peaked at each site with the largest densities (120,000 col/100 mL) detected during the discharge peak (1,150 ft^3/s) at the MDNR sampling site (site 3). Densities of *E. coli* bacteria were less variable during the runoff event, increasing only 50 percent or less as discharge peaked at all sites.

The apparent trend of increasing fecal coliform densities with increasing time at the MDNR sampling site (site 3) on Shoal Creek probably is, in part, related to a general trend of increasing discharge in Shoal Creek in response to an increase in annual precipitation, and not necessarily land-use changes or changes in the number of animal operations in the basin. Using a combination of MDNR and USGS data collected at site 3 from 1992 to 2000, a multiple linear regression (MLR) model was developed that was able to explain 65 percent of the variability in fecal coliform densities at site 3. Using the measured values of specific conductance and temperature, the model correctly predicted fecal coliform densities above the MDNR standard of 200 col/100 mL 83 percent of the time (45 of 54 samples). The model was only 49 percent correct in predicting when fecal coliform densities were less than the MDNR standard (17 of 35 samples). Although the increasing of trend in fecal coliform densities with time at the MDNR sampling site may not be related to changes in animal production in the basin, the large fecal coliform densities at sites 2 and 3 on Shoal Creek and in Pogue Creek (site 11), Joyce Creek (site 12), and the lower part of Clear Creek (site 17) are, in part, probably related to the high density of animals in the basin. This conclusion is supported by the predominance of ribopatterns of *E. coli* isolated from water samples in the study area matching nonhuman sources. Perhaps the most dramatic example of an animal derived source is the increase in fecal coliform densities and decrease in concentrations of organic compounds associated with human wastewater with increasing distance downstream as Clear Creek flows through agricultural areas downstream from Monett and Pierce City.

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