Survival, Transport, and Sources of Fecal Bacteria in Streams and Survival in Land-Applied Poultry Litter in the Upper Shoal Creek Basin, Southwestern Missouri, 2001–2002

by John G. Schumacher

Abstract

Densities of fecal coliform bacteria along a 5.7-mi (mile) reach of Shoal Creek extending upstream from State Highway 97 (site 3) to State Highway W (site 2) and in two tributaries along this reach exceeded the Missouri Department of Natural Resources (MDNR) standard of 200 col/100 mL (colonies per 100 milliliters) for whole-body contact recreation. A combination of techniques was used in this report to provide information on the source, transport, and survival of fecal bacteria along this reach of Shoal Creek. Results of water-quality samples collected during dye-trace and seepage studies indicated that at summer low base-flow conditions, pastured cattle likely were a substantial source of fecal bacteria in Shoal Creek at the MDNR monitoring site (site 3) at State Highway 97. Using repeat element Polymerase Chain Reaction (rep-PCR), cattle were the presumptive source of about 50 percent of the *Escherichia coli* (*E. coli*) isolates in water samples from site 3. Cattle, horses, and humans were the most common presumptive source of *E. coli* isolates at sites further upstream. Poultry was identified by rep-PCR as a major source of *E. coli* in Pogue Creek, a tributary in the upper part of the study area. Results of the rep-PCR were in general agreement with the detection and distribution of trace concentrations of organic compounds commonly associated with human wastewater, such as caffeine, the antimicrobial agent triclosan, and the pharmaceutical compounds acetaminophen and thiabendazole (a common cattle anthelmintic).

Significant inputs of fecal bacteria to Shoal Creek occurred along a 1.6-mi reach of Shoal Creek immediately upstream from site 3. During a 36-hour period in July 2001, average densities of fecal coliform and *E. coli* bacteria increased from less than or equal to 500 col/100 mL upstream from this stream reach (sample site 2c) to 2,100 and 1,400 col/100 mL, respectively, at the MDNR sampling site. Fecal bacteria densities exhibited diurnal variability at all five sampling sites along the 5.7-mi study reach of Shoal Creek, but the trends at successive downstream sites were out of phase and could not be explained by simple advection and dispersion. At base-flow conditions, the travel time of bacteria in Shoal Creek along the 5.7-mi reach between State Highway W (site 2) and the MDNR sampling site (site 3) was about 26 hours. Substantial dispersion and dilution occurs along the upper 4.1 mi of this reach because of inflows from a number of springs and tributaries and the presence of several long pools and channel meanders. Minimal dispersion and dilution occurs along the 1.6-mi reach immediately upstream from the MDNR sampling site. Measurements of fecal bacteria decay in Shoal Creek during July 2001 indicated that about 8 percent of fecal coliform and *E. coli* bacteria decay each hour with an average first-order decay constant of 0.084 h^{-1} (per hour).

Results of field test plots indicated that substantial numbers of fecal bacteria present in poul-

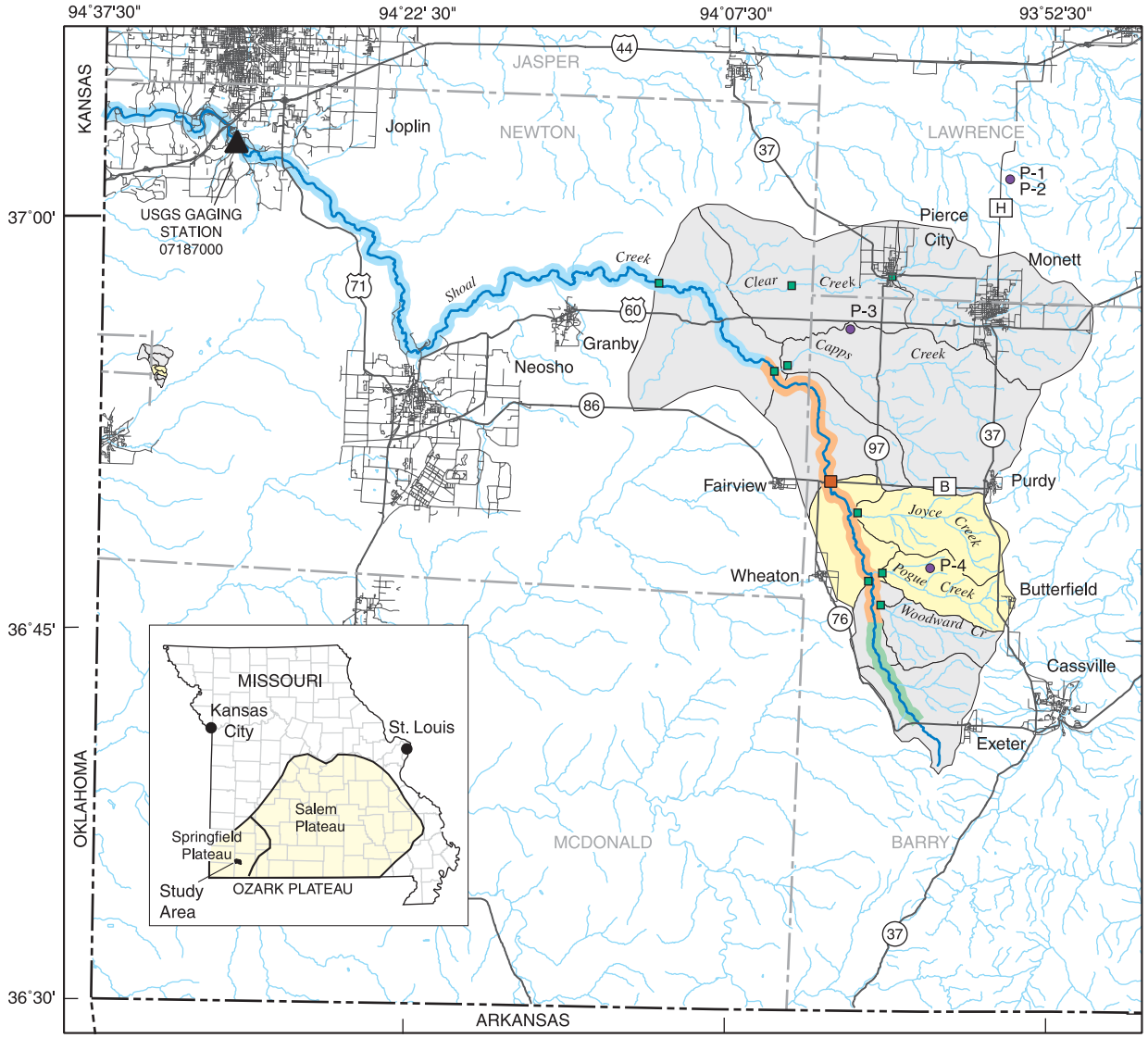
try litter can survive in fields for as much as 8 weeks after the application of the litter to the land surface. Median densities of fecal coliform and *E. coli* in slurry-water samples collected from fields increased from less than 60 col/100 mL before the application of turkey and broiler litter, to as large as 420,000 and 290,000 col/100 mL after the application of litter. Bacteria densities in the test plots generally decreased in a exponential manner over time with decay rates ranging from 0.085 to 0.185 d^{-1} (per day) for fecal coliform to between 0.100 and 0.250 d^{-1} for *E. coli*. The apparent survival of significant numbers of fecal bacteria on fields where poultry litter has been applied indicates that runoff from these fields is a potential source of fecal bacteria to vicinity streams for many weeks following litter application.

INTRODUCTION

Shoal Creek (fig. 1) is located within the Springfield Plateau of the Ozark Plateaus physiographic province in southwestern Missouri. From its headwaters in Barry County, 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 the city of Joplin. Shoal Creek is an important source of drinking-water supply for the cities of Joplin (population about 150,000) and Neosho (population about 10,000) (U.S. Bureau of the Census, 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 (irrigation, livestock and wildlife watering, protection of warm-water aquatic life and human-health fish consumption, cool-water fishery, whole-body contact recreation, boating and canoeing, drinking-water supply, and industrial-water supply)—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 stated beneficial uses along a 13.5-mi reach immediately downstream from the mouth of Capps Creek to about 1 mi upstream from the mouth of Woodward Creek (fig. 1).

The detection of fecal coliform densities greater than the Missouri standard of 200 col/100 mL (colonies per 100 milliliters) for whole-body contact recreation (Missouri Department of Natural Resources, 1996) at the Missouri Department of Natural Resources (MDNR) monitoring site at State Highway 97 (site 3, fig. 2) resulted in part of Shoal Creek being included on the 1998 Clean Water Action Plan (CWP) 303(d) list of impaired water bodies by the MDNR. During a 1999–2000 study of the water quality of the upper 233 mi^2 (square miles) of the Shoal Creek Basin, the U.S. Geological Survey (USGS) determined that densities of fecal coliform bacteria in Shoal Creek exceeded the MDNR standard along a 5.7-mi reach upstream from State Highway 97 and in several upstream tributaries (Schumacher, 2001). Fecal coliform densities decreased significantly downstream from the MDNR site. Based on the results of limited deoxyribonucleic acid (DNA) fingerprinting of *Escherichia coli* (*E. coli*) isolated from water samples, the USGS study suggested that most of the *E. coli* bacteria in water samples collected from Shoal Creek probably were from non-human sources and that poultry may be an important contributor. Schumacher (2001) estimated that more than 45,000 tons of poultry litter is generated annually within the upper Shoal Creek Basin. This litter is applied to the land surface as a source of nutrients within a few miles of the poultry barns. Little is known about the quantities of fecal bacteria within the poultry litter or the survival of the bacteria after the litter is applied to the land surface.

During 2001, the Food and Agricultural Policy Research Institute (FAPRI) initiated a study to construct a fecal coliform transport model for the Shoal Creek Basin upstream from the MDNR sampling site. Before accurate models can be developed and remedial measures implemented to reduce the levels of fecal bacteria at the MDNR sampling site, the host source of the bacteria must be identified, and the stream reaches located where the bacteria are entering the stream. Although the results of the 1999–2000 USGS study indicated that non-human sources are an important source of fecal bacteria at the MDNR sampling site, the specific reach where these bacteria are entering Shoal Creek and transport times along this reach were outside the scope of that study. Because the specific area where the fecal bacteria detected at the MDNR sampling site are entering the stream has not been identified, the U.S. Environmental Protection Agency (USEPA) and MDNR entered into a cooperative agreement with the USGS to investigate the survival, transport, and source of fecal bacteria in Shoal Creek upstream from the MDNR sampling site and to determine if viable fecal



EXPLANATION

MISSOURI DEPARTMENT OF NATURAL RESOURCES BENEFICIAL-USE DESIGNATIONS

- AQL, BTG, CDF, DWS, IND, IRR, LWW, WBC
- AQL, BTG, CLF, IRR, LWW, (IMPAIRED REACH)
- AQL, LWW

AQL	Protection of warm-water aquatic life and human-health fish consumption
BTG	Boating and canoeing
CDF	Cold-water fishery
CLF	Cool-water fishery
DWS	Drinking-water supply
IND	Industrial-water supply
IRR	Irrigation
LWW	Livestock and wildlife watering
WBC	Whole-body contact recreation

- CURRENT STUDY AREA
- STUDY AREA FROM 1999–2000 U.S. GEOLOGICAL SURVEY STUDY
- MISSOURI DEPARTMENT OF NATURAL RESOURCES SAMPLING SITE
- SURFACE-WATER SAMPLING SITE FOR 1999–2000 U.S. GEOLOGICAL SURVEY STUDY
- P-1 POULTRY LITTER FIELD TEST PLOT AND NUMBER

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

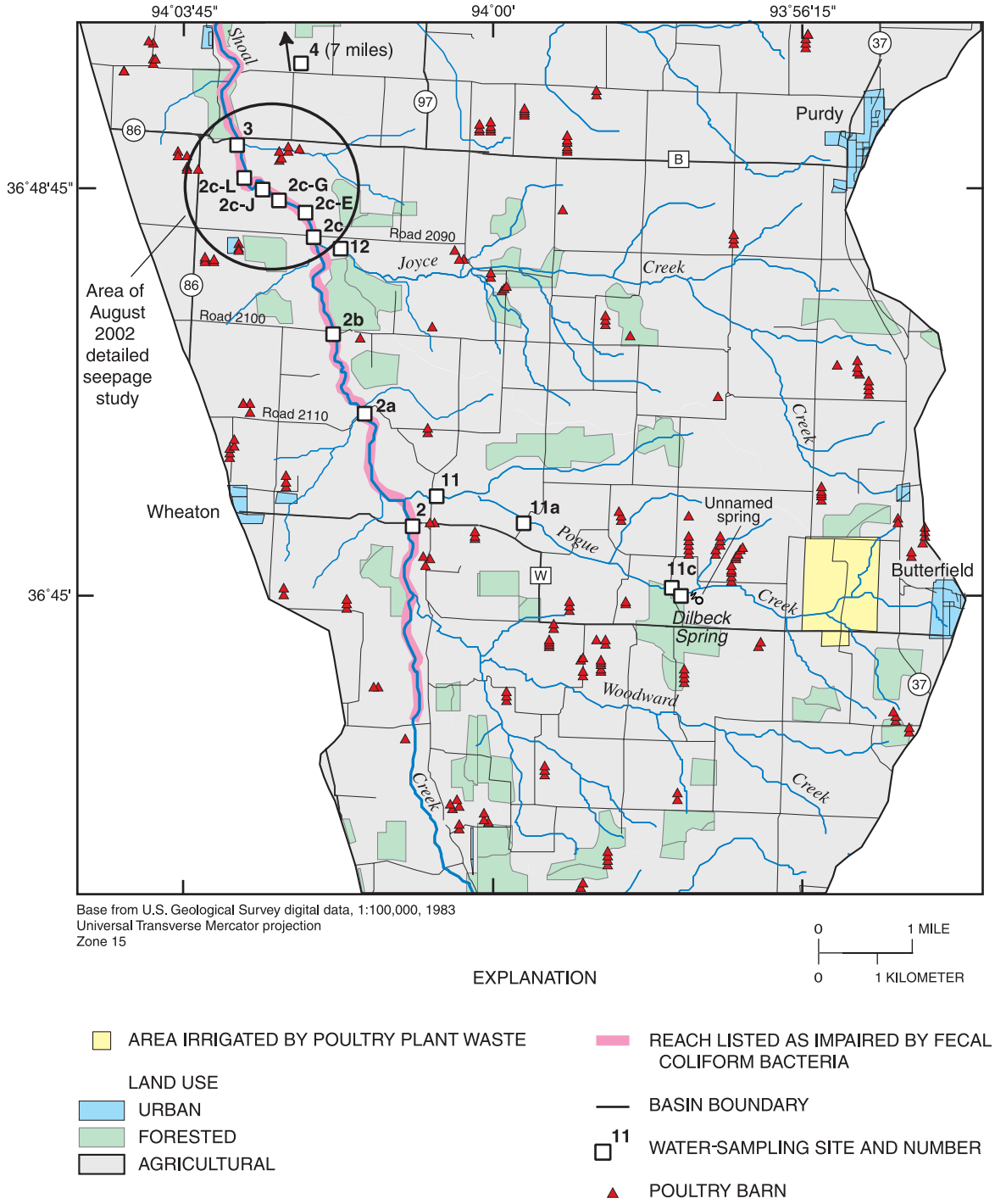


Figure 2. Location of U.S. Geological Survey sampling sites, land use, and distribution of poultry barns in the study area.

bacteria are present in poultry litter that is applied to the land surface in the area.

Purpose and Scope

This report describes information on the survival, transport, and source of fecal bacteria in Shoal Creek. The information is based on results of dye-trace and seepage studies conducted during base-flow conditions along a 5.7-mi reach of Shoal Creek upstream from the MDNR sampling site on State Highway 97 (fig. 1). Concentrations of fecal coliform and *E. coli* bacteria that were measured along Shoal Creek during the 2-day period of the dye-trace study are presented. In addition, water-quality samples were collected as the dye peak passed selected sampling sites. Because large quantities of poultry litter are applied to fields in the Shoal Creek Basin, preliminary data also are presented on the concentration and survival of fecal bacteria in poultry litter that is applied to the land surface.

During the dye-trace study conducted in July 2001, 68 water-quality samples were collected from 10 sites along Shoal Creek and 2 tributaries. Samples were analyzed for fecal coliform and *E. coli* bacteria, and a subset of samples were analyzed for total nutrients and a suite of organic compounds commonly associated with human wastewater and selected pharmaceutical compounds. The rate of fecal coliform and *E. coli* decay in the stream was measured during the dye-trace study using in-situ diffusion chambers. Water-quality samples were collected and discharge measurements were made along a 1.6-mi reach of Shoal Creek immediately upstream from the MDNR sampling site (site 3, fig. 2) during a seepage study in August 2002. During the summer of 2001 and spring and summer of 2002, four test plots were monitored for the survival of fecal coliform and *E. coli* bacteria in fields where poultry litter or poultry litter compost had been applied to the land surface. Relative densities of fecal coliform and *E. coli* bacteria were measured at 7 to 10 locations in each of the 4 test plots for up to 8 weeks following the application of poultry litter.

Previous Investigations

The MDNR began ambient monitoring on Shoal Creek at the State Highway 97 bridge (site 3) during 1992. 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 ranged from less than 1 to 400,000 col/100 mL (data supplied by the Missouri Department of Natural Resources, Water Pollution Control Program). Concentrations of total nitrite plus nitrate as nitrogen (NO_{23t}) and total phosphorus (P_t) in these samples averaged 3.15 and 0.17 mg/L (milligrams per liter).

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 5-year study included the collection of monthly grab samples from six stream and four spring sites. The study also included technical assistance for the Natural Resources Conservation Service (NRCS) to work with area poultry producers and farmers to develop nutrient management plans and to implement Best Management Practices (BMPs). During 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 project that focuses on reducing agricultural nonpoint-source pollution. The upper Shoal Creek SALT 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 included nutrient sampling at six stream sites and four springs on or near demonstration farms downstream from the MDNR sampling site.

From 1999 to 2000, the USGS conducted a study of the water quality of Shoal Creek and its principal tributaries in the upper 233 mi^2 of the Shoal Creek Basin (Schumacher, 2001). More than 170 water samples were collected during 13 months from a network of 12 stream and 5 spring sites. Analyses of the samples indicated that fecal coliform concentrations in Shoal Creek exceeded the MDNR standard of 200 col/100 mL at site 3 (fig. 2), the MDNR sampling site (median density of 400 col/100 mL and a range of 43 to 33,000 col/100 mL), and at site 2 (fig. 2) about 5.7 mi upstream at State Highway W (median density of 277 col/100 mL and a range of 25 to 9,200 col/100 mL). Fecal coliform densities also exceeded the 200 col/100 mL standard in two tributaries upstream from the MDNR sampling site—Pogue Creek (median density of 580 col/100 mL) and Joyce Creek (median density of 340 col/100 mL). Results of ribopattern analysis of *E. coli* isolates from a small number of water samples indicated that 85 percent of the *E. coli* isolates extracted were from non-human sources. Humans and poultry appeared to be a major source of *E. coli* bacteria at the MDNR sampling site; however, the results were

suspect because known cattle isolates from the study area frequently were misidentified by the ribopattern analysis. Schumacher (2001) postulated that cattle from the study area have unique ribopatterns compared to the known cattle patterns used in the ribopattern database, which were mostly from central Missouri. The study also detected the human pathogen *E. coli* O157:H7 (commonly associated with cattle) in a water sample collected upstream from the MDNR sampling site. Historical data on fecal coliform densities at the MDNR sampling site also were examined, and the report concluded that an apparent increasing trend of fecal coliform bacteria with time at the MDNR sampling site was, in part, related to a general trend of increasing stream discharge in response to an increase in annual precipitation and not necessarily land-use changes or increases in poultry production in the basin.

During 2000, the USGS conducted a ground-water study in the upper Shoal Creek Basin to determine if fecal coliform and nutrient concentrations in shallow ground water were increasing as a result of poultry operations in the region (Mugel, 2002). Analysis of water samples collected from 47 wells and 8 springs indicated that ground water in proximity to poultry operations did not have statistically larger densities of fecal bacteria or nutrient concentrations than water from wells in non-poultry producing areas.

In 2001, the FAPRI began a study to develop a transport model for fecal coliform in the upper Shoal Creek Basin. This study includes the collection of weekly fecal coliform and *E. coli* samples from several sites on Shoal Creek including the MDNR sampling site (site 3) and several tributaries upstream. Preliminary data from DNA-fingerprinting of these samples indicated that most *E. coli* patterns probably are from cattle, humans, and poultry, and that cattle patterns appeared more frequently during the summer months (Claire Baffaut, Food and Agricultural Policy Research Institute, written commun., 2002).

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 Kari Rhoades and Dan Philbrick of the Barry County NRCS and to local poultry producers for providing access and assistance during the field test plots to measure the survival of fecal bacteria in land-applied poultry litter. The author also is grateful to the family of Mr. Joseph Renkoski for allowing the continued operation of a stream-gaging and water-quality sampling station on their property.

DESCRIPTION OF THE STUDY AREA

The study area focused on a 5.7-mi reach of Shoal Creek from the MDNR sampling site (site 3) upstream to State Highway W (site 2) in Barry County (fig. 2). This reach of Shoal Creek was selected because it is part of the reach listed as impaired by fecal coliform bacteria by the MDNR (Missouri Department of Natural Resources, 2000), and it was identified by the USGS as containing fecal coliform levels above the MDNR standard of 200 col/100 mL (Schumacher, 2001). Samples also were collected from a site several miles downstream from this reach (site 4) to verify that fecal coliform densities above the MDNR standard were confined to the reach previously identified as impaired.

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 a climatological station at Monett (fig. 1) with 29 years of record. The average annual temperature measured at Monett is about 55 °F (degrees Fahrenheit) and the average annual precipitation is 42.01 in. (inches). During the study period from May 2001 through August 2002, the minimum temperature recorded at Monett was 0 °F on March 3, 2002, and the maximum recorded temperature was 99 °F on July 25, 2002 (National Oceanic and Atmospheric Administration, 2001; 2002). The maximum daily precipitation was 2.57 in. recorded on May 8, 2002. Southwest Missouri experienced drought conditions that began during the 1999–2000 USGS water-quality study (Schumacher, 2001). Overall, drought conditions persisted throughout the current study period with 59.73 in. of precipitation recorded at Monett from May 2001 through August 2002. Although only 0.85 in. below normal for this period, almost 20 percent of this total (12.07 in.) fell during May 2002, which was more than 7 in. above normal for the month. Excluding May 2002, precipitation was about 8 in. below normal for the study period.

Topography and Geology

Topography in the upper Shoal Creek Basin is characterized by gently rolling hills, except near streams where local relief can be steep. Elevations in the study area range from about 1,200 ft (feet) [National Geodetic Vertical Datum of 1929 (NGVD 29)] at site 3 at State Highway 97 to about 1,570 ft in the headwaters of Pogue Creek (fig. 1) on the east side of the study area.

Mississippian-age limestones underlie the study area. A massive chert bed known as the Grand Falls Chert underlies most of the study reach between sites 2 and 3 (Schumacher, 2001). The Grand Falls Chert is a discontinuous unit (0 to 40 ft thick) consisting of massive layers of chert up to 6 ft thick. The chert has a gnarled and knotted structure that produces an uneven surface where the unit is exposed (Thompson, 1986). Where the Grand Falls Chert is exposed in the bed of Shoal Creek, it forms shoals, riffles, and small waterfalls. Numerous wet-weather seeps and small springs appear in fields along this reach of Shoal Creek where the Grand Falls Chert is overlain by a thin veneer of soil.

Land Use and Population

The study reach of Shoal Creek drains about 42 mi^2 (this area excludes the drainage area of Shoal Creek upstream from site 2). Land use in this area is about 91 percent agricultural, 8 percent forested, and 1 percent urban (fig. 2). Nearly all the agricultural land use is pasture, hay crop, or poultry barns. An inventory conducted in 2001 (Schumacher, 2001) indicated there were 118 active poultry barns in this area, producing an estimated 14,200 tons of waste litter annually. Thirty-nine barns are used for the production of turkeys, 24 of which are located in the Pogue Creek Basin. Liquid waste from a poultry processing plant is sprayed onto more than 400 acres of fields in the upper part of the Pogue Creek Basin (fig. 2). This plant has a design capacity of more than 1.6 Mgal/d (million gallons per day) (Missouri Department of Natural Resources, Water Pollution Control Program, written commun., 2002). No reliable estimate of the number of cattle is available for the study area. During the 2001 study, an estimated 25,000 cattle were present within the 233 mi^2 study area (Schumacher, 2001), and several thousand cattle are assumed to be within the drainage area of the study reach of Shoal Creek.

The population of the study area was estimated at 1,640. This estimate was calculated by multiplying the 2000 population of the incorporated towns of Butterfield (397), Purdy (1,103), and Wheaton (721) by their respective percent areas contained within the Shoal Creek study area (100, 50, and 5 percent), and adding this total to the estimated rural population. The rural population was estimated by multiplying the number of rural homes (243) identified on 1996 aerial photography by the average of 2.7 persons per household estimated by the U.S. Bureau of the Census for rural Missouri (U.S. Bureau of the Census, 2000).

METHODS OF STUDY

Three main phases of field activity were conducted to address the purpose of this report. The first phase was a dye-trace study conducted during July 2001 along a 5.7-mi reach of Shoal Creek to determine the survival rates, transport characteristics, and temporal variation of fecal coliform and *E. coli* bacteria in the stream. The dye-trace study included the collection of water-quality and dye-trace samples. Presumptive sources of fecal bacteria were identified by DNA-fingerprinting of *E. coli* isolates in selected water samples. The second phase involved a detailed seepage study in August 2002 (fig. 2) focusing on the 1.6-mi reach of Shoal Creek upstream from site 3, which was part of the reach investigated during the dye-trace study. The purpose of the detailed seepage study was to locate sources of fecal bacteria input into Shoal Creek immediately upstream from the MDNR sampling site (site 3, fig. 2). Both the dye-trace and detailed seepage studies were conducted during low base-flow conditions. The third phase of field activity was conducted during 2001 and 2002 using a series of field test plots to provide preliminary data on the survival of fecal coliform and *E. coli* bacteria in poultry litter commonly applied to the land surface throughout the Shoal Creek Basin.

Stream Monitoring Network and Water-Quality Sample Collection

To determine the survival rates, transport time, and temporal variability of fecal bacteria along the 5.7-mi reach of Shoal Creek previously identified as having fecal coliform densities above the MDNR standard of 200 col/100 mL, a network of monitoring sites was established along Shoal Creek and on Joyce Creek

and Pogue Creek (fig. 2). The collection of water-quality samples from this network coincided with measurements of fecal bacterial survival rates and the injection of fluorescent dye at site 2 to evaluate transport characteristics of fecal bacteria along this stream reach. An evaluation of fecal bacteria transport using this combined effort is referred to as the July 2001 dye-trace study. During a 36-hr (hour) period beginning on July 23, 2001, seven to nine water-quality samples were collected and analyzed for fecal bacteria densities at five sites along the main stem of Shoal Creek (sites 2, 2a, 2b, 2c, and 3; fig. 2) and at two sites near the mouths of Pogue Creek and Joyce Creek (sites 11 and 12, fig. 2). As the dye peak passed each main-stem site, a suite of additional water-quality samples were collected and analyzed for nutrients, wastewater organic compounds, and pharmaceutical compounds; and an additional fecal bacterial sample was collected and submitted for DNA-fingerprinting. These peak samples would provide information on how densities of fecal bacteria and concentrations of selected chemical constituents changed as the same parcel of water moved through the stream system between sites 2 and 3.

An additional surface-water site (site 4, fig. 2) located on Shoal Creek about 6.7 mi downstream from State Highway 97 and two sites in the upper part of the Pogue Creek Basin (sites 11a and 11c, fig. 2) were sampled less frequently. Samples from sites 11a and 11c were analyzed only for fecal bacteria densities and nutrients and samples from site 4 were analyzed only for fecal bacteria densities. Because the 1999–2000 USGS study indicated that water samples from Pogue Creek at site 11 contained among the largest fecal coliform densities in the upper Shoal Creek Basin, bacteria samples were collected from sites 11a and 11c to determine if large densities of fecal coliform also were present in the upstream reaches of Pogue Creek. Several fecal bacteria samples were collected at site 4 to confirm results of the 1999–2000 USGS study, which indicated that fecal coliform densities downstream from State Highway 97 were less than the MDNR standard of 200 col/100 mL (Schumacher, 2001).

Based on results from the 2001 dye-trace study, additional sampling was conducted during a detailed seepage study along a 1.6-mi reach between site 2c and site 3 during August 2002 (fig. 2). During this detailed seepage study, the stream channel was waded along this reach, and the discharge of all inflows was measured. Water-quality measurements were made and samples for fecal bacteria analyses were collected at several intervals along the reach and from inflows to identify specific areas where fecal bacteria were entering the creek. Environmental factors such as the general channel characteristics (stream depth and width, bottom composition, riparian corridor, and height and steepness of the stream bank) of the reach were noted, and cultural features such as the location of fences, density and type of livestock, and proximity of homes, farm buildings, poultry houses, and livestock feeding areas to the stream channel were recorded. Water-quality samples also were collected from the main stem of Shoal Creek at sites 2, 2c, and 3, from sites 11 and 12 on Pogue and Joyce Creeks, and from Dilbeck Spring along the upper reach of Pogue Creek (fig. 2) at the time of the detailed seepage study.

Hydrologic conditions within the upper Shoal Creek Basin preceding and during the detailed seepage study and dye-trace study were determined using data from a continuously recording streamflow-gaging and water-quality monitoring station that was installed on Shoal Creek about 0.2 mi downstream from site 3 during the 1999–2000 USGS study (Schumacher, 2001). The station has been in operation since May 1999 and is equipped with a submersible pressure transducer to record stage and a probe that measures specific conductance and temperature. Measurements are recorded every 15 min (minutes). The FAPRI installed an automatic water-quality sampler at the station in 2001 as part of their data collection efforts to model fecal coliform transport.

During the dye-trace and detailed seepage studies, measurements of discharge, water temperature, specific conductance, dissolved oxygen, and pH were made, and water samples were collected and analyzed for fecal coliform and *E. coli* bacteria. One set of samples was collected from selected sites during both studies and analyzed for total (unfiltered) nutrients, wastewater organic compounds, and pharmaceutical compounds. Nutrient analyses included NO_{23t}, total nitrite as nitrogen (NO_{2t}), total ammonia as nitrogen (NH_{3t}), P_t, and total orthophosphorus as phosphorus (PO_{4t}).

Water samples for the analysis of chemical constituents were collected according to the general protocols described in Wilde and Radtke (1998). 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 was 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 container near the center of flow. No replicate samples were collected, and one field blank was collected during the dye-trace study and one during the detailed seepage study.

Samples for the determination of nitrogen (N) species and PO_{4t} were placed in 125-mL (milliliter) amber polyethylene bottles and chilled to 4 °C (degrees 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 before chilling to 4 °C. Selected water samples were analyzed for human wastewater organic compounds and pharmaceutical compounds. These samples were placed in baked 1-L amber glass bottles and chilled to 4 °C. The wastewater organic compounds were determined in unfiltered water samples by continuous liquid-liquid extraction with methylene chloride and by capillary-column gas chromatography/mass spectrometry (Zaugg and others, 2002). Pharmaceutical compounds were determined on filtered samples by high performance liquid chromatography/mass spectrometry (Koplin and others, 2002). Rigorous method detection limits have not been established for many compounds for these research methods, which are extremely sensitive to the detection of their respective target analytes, and the reported concentrations of many compounds are listed as estimated. General information on the usage of the wastewater organic compounds is discussed in Zaugg and others (2002).

Bacteria Sampling and Analyses

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 until processing and bacteria were 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 or *E. coli* colonies were detected in any of the blank samples. Water samples from five surface-water sites (sites 2, 2a, 2b, 3, and 11) collected during the July 2001 dye-trace study were submitted to the University of Missouri College of Veterinary Medicine for DNA-fingerprinting using the repeat element Polymerase Chain Reaction (rep-PCR) method modified from Dombek and others (2000). Although DNA-fingerprinting was done to identify presumptive sources of fecal bacteria, the identification of the major sources of fecal bacteria in Shoal Creek was made using a combination of data from the dye-trace study, bacterial survival rates, detailed seepage study, DNA-fingerprinting, and presence of wastewater organic and pharmaceutical compounds.

Streambed-sediment samples were collected from sites 2 and 3 and analyzed to determine if fecal bacteria were accumulating in the streambed of Shoal Creek. Sample locations were biased toward areas of fine-grained sediment deposition, such as behind large rocks or in eddies and small pools. Approximately 1 kg (kilogram) of sediment was collected from the top 2 to 3 cm (centimeters) of the streambed using a stainless steel scoop and placed into sterile 1-L wide-mouth, polyethylene containers. The containers were chilled to 4 °C and transported within 24 hrs to the USGS laboratory in Rolla, Missouri. The containers consisted of coarse-grained sediment ranging from sand to small cobbles and 3 to 4 cm of overlying water. Because of the coarse-grained nature of the sediments, the original plan of placing 2 to 3 g (grams) of a subsample from each container into sterile buffer water was abandoned, and the entire container was shaken violently for 2 min and allowed to settle for 2 min. After three cycles of shaking and settling, a sterile pipette was used to remove a 5-mL sample from the upper 1 cm of the overlying water in the containers. The 5-mL sample was placed in a sterile flask with 45 mL of phosphate buffer solution and fecal coliform and *E. coli* density were determined on serial dilutions according to the procedures previously described. The mass of sediment in the containers was determined by drying at 105 °C for 48 hrs. Data were reported as colonies per gram as dry weight. Because 1 mL of water is approximately 1 g, the bacteria densities from the streambed sediment and the overlying water could be directly compared.

Bacteria Survival in Shoal Creek

Fecal indicator bacteria such as fecal coliform and *E. coli* normally inhabit the intestinal tracts of warm-blooded animals and humans. As these bacteria are introduced to the surface-water environment, they are exposed to environmental conditions that stress the organisms, such as changes in temperature, solution

salinity, pH, nutrient concentrations, and the effects of solar radiation and predation (Rozak and Colwell, 1987; Davis and others, 1995; Mezrioui and others, 1995; Ozkanca and Flint, 1997; Sinton and others, 1999). These stresses cause a reduction of indicator bacteria densities in streams as a result of injury and death. According to Sinton and others (1999), exposure to solar radiation and predation are among the most important factors. Fecal bacteria are thought to survive from several hours to a few days in surface water and from several weeks to months in streambed sediments (Rozak and Colwell, 1987; Doyle and others, 1992). An understanding of how quickly fecal bacteria decay in the stream can provide important information on the magnitude and location of potential sources.

Decay rates of fecal coliform and *E. coli* were estimated by fitting an exponential equation to bacterial densities measured at various time intervals in diffusion chambers placed at sites 2 and 3 during the July 2001 dye-trace study. Because of the design of the diffusion chambers, the changes in densities of fecal coliform and *E. coli* with increasing time are attributed to the sum of natural processes that occur within the stream. The equations were expressed as first order decay in the general form of:

$$C_t = C_o * e^{(-kt)},$$

where C_t = the bacteria density at elapsed time t, in colonies per 100 milliliters;
C_o = the initial bacteria density in colonies per 100 milliliters;
k = the decay constant in hours^{-1}; and
t = the elapsed time in hours.

The initial bacteria density (C_o) was set to the bacteria density measured at the time the diffusion chambers were deployed.

The survival of fecal bacteria in Shoal Creek was investigated using diffusion chambers constructed of polycarbonate cylinders that were fitted with 0.2-µm (micrometer) pore-size filters at each end to form a chamber (fig. 3). Except for the polycarbonate cylinders, which were obtained from a plastics manufacturer, and the filters, which were obtained from Gelman Sciences, all materials used to construct the chambers were available at local hardware stores. The 0.2-µm pore-size filters served to isolate bacteria inside the chamber, while allowing for the diffusion of water and dissolved nutrients into and out of the chambers. Polycarbonate was selected so that results could be generally compared to those obtained in the Cuyahoga River by Myers and others (1998). Polycarbonate transmits more than 80 percent of visible solar radiation, but blocks most ultraviolet (UV) radiation; however, Sinton and others (1999) reported that fecal coliform bacteria are sensitive to a wide range of sunlight radiation outside the UV range.

The survival of fecal coliform and *E. coli* was measured at sites 2 and 3 during the July 2001 dye-trace study. Diffusion chambers were deployed at each site at the beginning of the dye-trace study on July 23, 2001. The chambers were filled with native water from the stream and submerged near the middle of the stream channel at each location. The chambers were suspended with nylon cable ties to bricks so they were submerged parallel to the water surface and at about one-half the average water depth (about 8 in. below the surface) at each location. Two chambers were removed immediately; two more at elapsed times of about 25 and 40 hrs. At each time interval about 150 mL of water was removed from each chamber and placed into separate, sterile 250-mL bottles. Fecal coliform and *E. coli* bacteria densities were then enumerated using standard membrane-filtration methods previously described.

Bacteria Transport in Shoal Creek

Fecal bacteria densities and concentrations of chemical constituents in streams are strongly affected by dilution, dispersion, and the areal distribution, timing, and magnitude of input sources as they are transported downstream with the advective flow of the water. Dilution and dispersion are important physical factors that attenuate or decrease the densities of fecal bacteria that enter the stream from specific sources. The magnitude of other factors, such as bacteria survival, depends on the elapsed time between the input of bacteria into the stream and the collection of water-quality samples.

Transport characteristics of fecal bacteria in Shoal Creek were evaluated during the July 2001 dye-trace study using a fluorescent dye to determine the time-of-travel and dilution and dispersion characteristics during low base-flow conditions between sites 2 and 3. A series of stream discharge measurements and background dye concentration measurements were made at sites 2, 2a, 2b, 2c, 3, 11, and 12 (fig. 2). At 1828 hrs on July 23, 2001, 2.5 L of rhodamine-WT dye solution was injected into a riffle immediately upstream from the State Highway W bridge at site 2

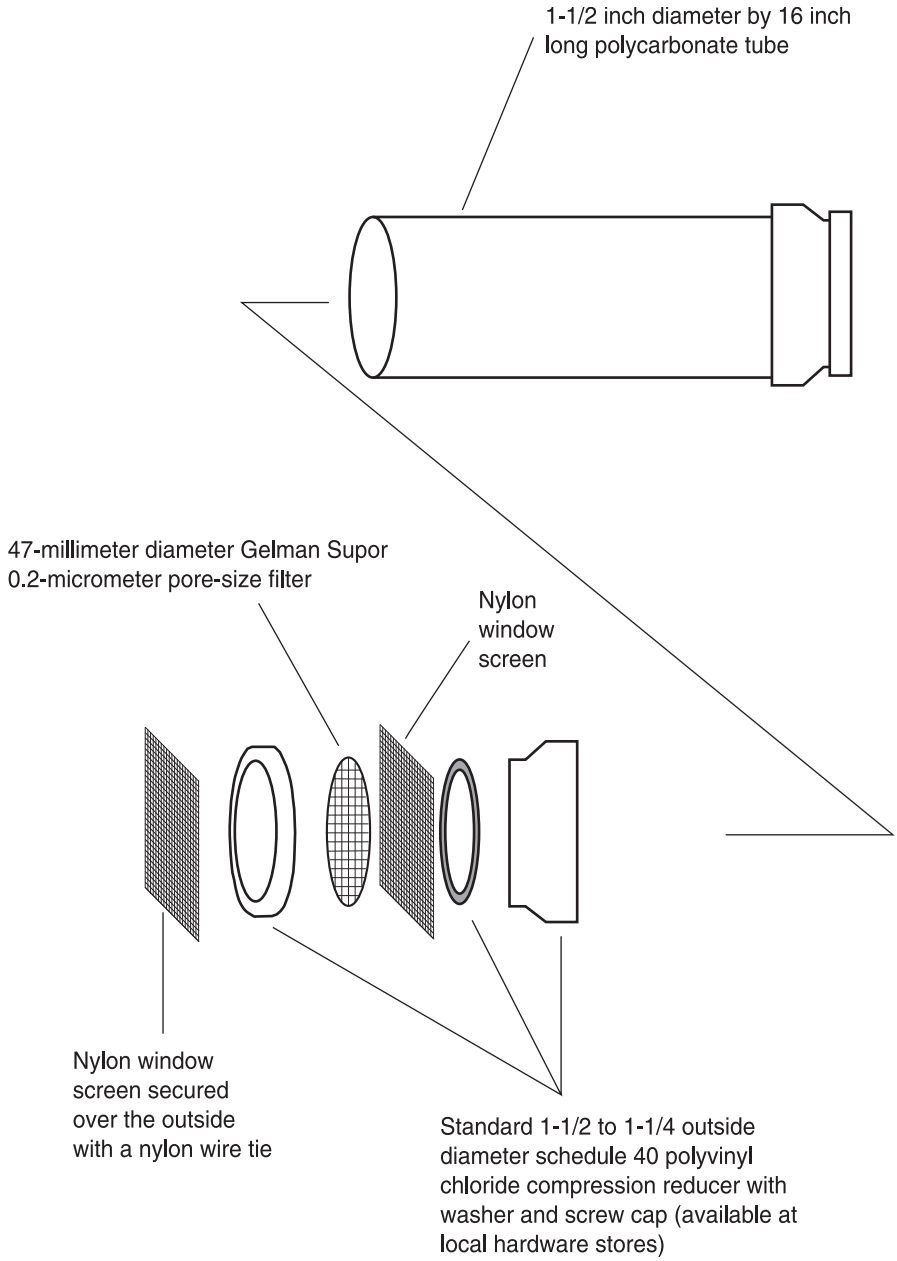


Figure 3. Schematic diagram of a diffusion chamber used to measure bacteria survival.

(fig. 4). Concentrations of dye were measured at sites 2, 2a, 2b, 2c, 3, and 4 during the next 2 1/2 days to follow the dye peak as it moved downstream. Water samples also were collected periodically from each site to determine the temporal variability of fecal coliform and *E. coli* densities along this reach of Shoal Creek, as described in the methods of study section of this report.

Dye concentrations were measured only occasionally at site 4. As the dye peak passed each successive downstream site, an additional set of water samples was collected and analyzed for fecal coliform and *E. coli* densities, nutrients, wastewater organic compounds, and pharmaceutical compounds and DNA-fingerprinting of *E. coli*.

Concentrations of rhodamine-WT dye at sites 2a, 2b, 2c, 3, and 4 were measured using a Turner Designs Model 10 fluorometer according to published USGS methods (Wilson and others, 1986). The fluorometer was calibrated directly using standards at concentrations of 0.1, 1.0, 10.0, 100, and 1,000 µg/L (micrograms per liter). Dye concentrations at site 2 (fig. 5) were measured with a Turner Designs Aquafluor hand-held fluorometer every 2 to 5 min after the dye was injected until the dye peak had passed, after which the measurement frequency was decreased. Results of the individual analyses are listed in Appendix A, at the back of this report. The Aquafluor fluorometer was calibrated using a 100 µg/L standard obtained from the manufacturer, and calibrations were checked using dye standards prepared for the Model 10 fluorometer. After the dye was injected, dye concentrations were measured downstream at site 2a approximately every 10 to 30 min until dye was detected, then about every 5 to 10 min until the dye peak passed, then less frequently thereafter. As

Figure 4. Shoal Creek upstream from State Highway W at site 2 immediately after the injection of fluorescent dye at 1828 hours on July 23, 2001. Arrow points to dye injection site.

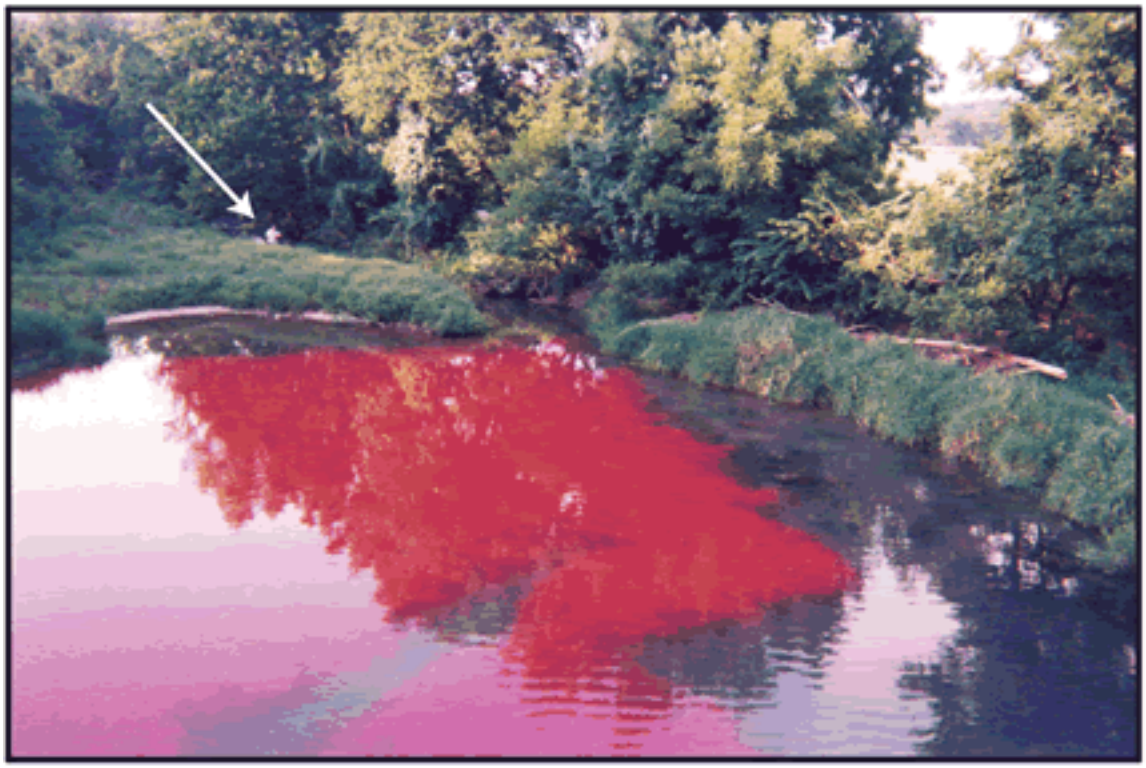


Figure 5. Fluorescent dye mixing in pool downstream from the State Highway W bridge (site 2) at 1840 hours on July 23, 2001. Arrow points to U.S. Geological Survey technician collecting samples.

soon as dye was detected at site 2a, measurements were started at the next site downstream (site 2b) following the same protocol. In this manner, the dye peak was followed downstream. As the dye peak broadened with increasing distance downstream from the injection point, the measurement interval was adjusted. Calibration of the fluorometer was checked every 4 to 6 hrs using a 100 µg/L standard.

Bacteria Survival in Land-Applied Poultry Litter

Field tests were conducted to determine the concentrations of fecal bacteria in poultry litter generated within the upper Shoal Creek Basin and to provide an estimate of the survival of these bacteria following the land application of poultry litter. The tests were conducted with the assistance of the Barry County NRCS and several poultry producers in the area. The conceptual design of the tests was to measure the density of fecal coliform and *E. coli* bacteria in fields before and after the land application of poultry litter. Fields selected for the tests were hay fields (fescue) where domestic livestock had been excluded from grazing for a least 2 months. Test plots ranging from 5,000 to 23,000 ft^2 (square feet) were marked in each of four fields (fig. 6). The test plots were scattered across the upper Shoal Creek Basin (fig. 1). Bacteria were "washed" from the surface at seven locations within each test plot and at one to three control points located upslope and upwind of each field test plot using a steel cylinder, bacteria-free well water, and a battery powered paint mixer. All equipment was rinsed with methanol and flame sterilized before sample collection. The steel cylinder was tapped about 1 in. into the soil surface and filled with about 10 L of bacteria-free well water (pH 7 to 7.3). A flat-bottomed paint mixer was placed just below the water surface and the solution was agitated at 1,200 rpm (revolutions per minute) for 25 seconds (fig. 7). After mixing, a sterile 250-mL bottle was used to collect a sample of the slurry water from inside the steel cylinder. Fecal coliform and *E. coli* were enumerated using the membrane-filter method previously described.

Samples were collected at each test plot immediately before and after the poultry litter application and weekly for as many as 8 weeks. Poultry litter was applied using a truck-mounted broadcast spreader (fig. 8) or a manure spreader. To avoid leaching the same spot at each sample location, the cylinder placement was rotated clockwise for each sampling trip (fig. 6). Litter application rates at each test plot were measured by weighing the amount of litter collected on polyethylene mats placed at two or three locations within each test plot before litter application (fig. 6). The application rate was calculated as the total weight of litter collected on the mats divided by the total surface area of the mats. Densities of fecal coliform and *E. coli* bacteria in the initial litter spread at each test plot were measured by leaching a composite litter sample collected from the mats. The litter collected on each mat was transferred into one or two 8-L plastic bags that were weighed to the nearest gram and mixed thoroughly with a sterilized stainless steel spatula. A 20- to 25-g composite litter sample was made by removing subsamples weighing less than 1 g from the plastic bags (alternating between each bag) and placing them into a sterile 500-mL glass mixing jar. After the net weight of litter in the jar was recorded, 250 mL of sterile-phosphate (PO_4) water was added, and the solution was mixed at about 600 rpm on a stationary mixer for 1 min, then allowed to settle for 10 min. After settling, the solution was remixed for 30 seconds and allowed to settle for 10 min. After the solution was allowed to settle the second time, a sterile pipette was used to transfer 5 mL of the solution (collected from just beneath the surface) to a sterile bottle, where it was diluted with PO_4 buffer to a total volume of 200 mL. Densities of fecal coliform and *E. coli* bacteria were determined using the membrane-filter method on serial dilutions of this final solution.

SURVIVAL AND TRANSPORT OF FECAL BACTERIA IN SHOAL CREEK

The study described in this report focused on a 5.7-mi reach of Shoal Creek between State Highway 97 (site 3) and State Highway W (site 2) that was determined to have fecal coliform densities exceeding the MDNR standard of 200 col/100 mL for whole-body contact recreation. A network of five sites on Shoal Creek (sites 2, 2a, 2b, 2c, and 3) were monitored during this study, in addition to a main stem site further downstream (site 4) and two tributaries—Pogue Creek (sites 11, 11a, and 11c) and Joyce Creek (site 12)—and a spring (Dilbeck Spring). A combination of techniques was used to determine the sources of the increased fecal coliform densities along this reach of Shoal Creek including a determination of decay or "die-off" rates of fecal coliform and *E. coli* in the stream, a dye-trace

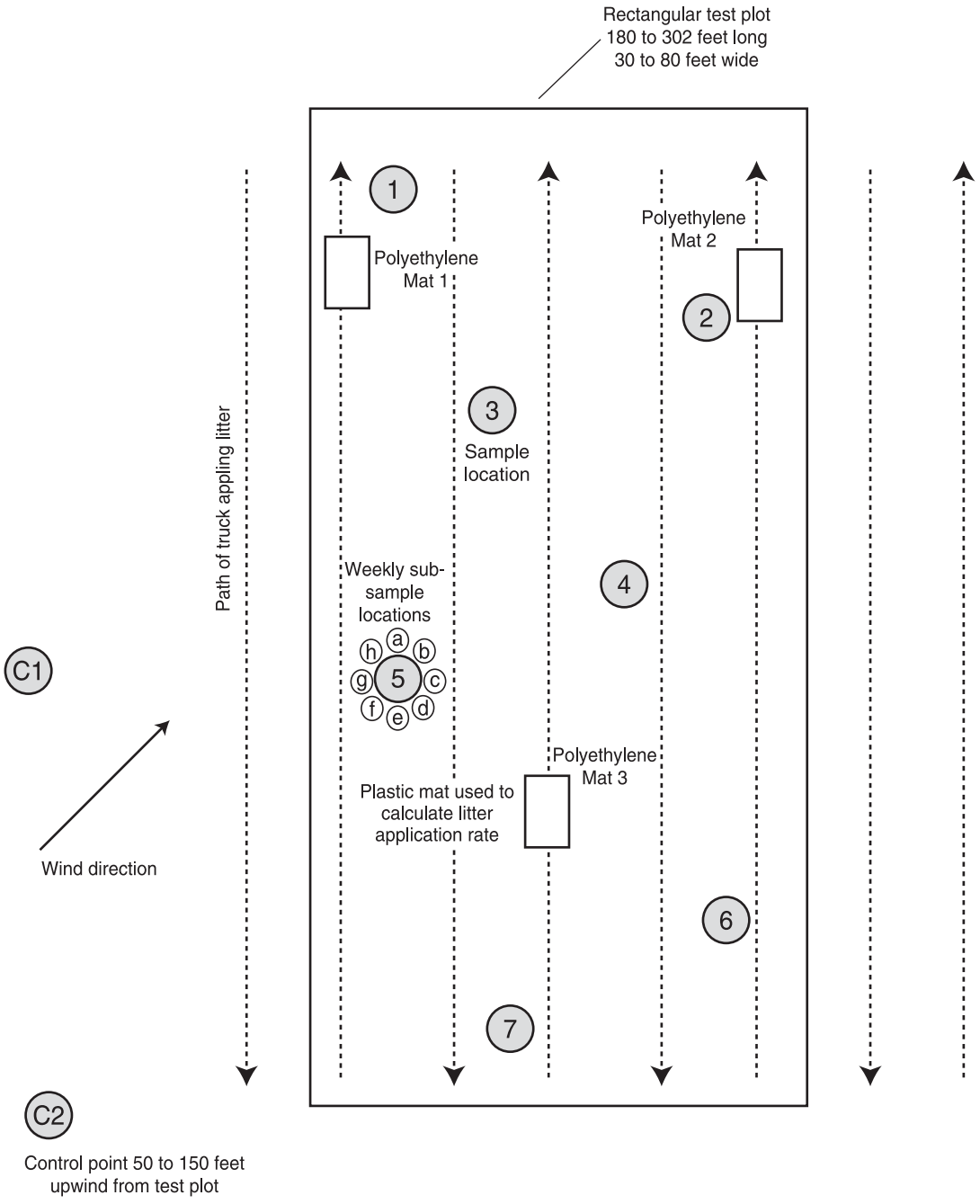


Figure 6. Generalized layout of a poultry litter field test plot.

Figure 7. Steel cylinder used to collect slurry-water samples from a test plot immediately after the land application of poultry litter.

Figure 8. Poultry litter being applied at field test plot P-2, May 7, 2002.

study to determine travel times and dispersion characteristics along this stream reach, a detailed seepage study and water-quality sampling to locate specific sources of fecal bacteria input to the stream, and DNA-fingerprinting of *E. coli* isolates from stream samples to identify presumptive sources of fecal bacteria.

Bacterial Survival

During the 42 hrs that the diffusion chambers were left in the stream, fecal coliform and *E. coli* densities decreased (fig. 9) more than 90 percent from initial densities (table 1). Decay constants ranged from a minimum of 0.070 h^{-1} (per hour) for fecal coliform at site 2 to a maximum of 0.098 h^{-1} for fecal coliform at site 3. The decay rates for *E. coli* were similar, ranging from 0.075 h^{-1} at site 2 to 0.095 h^{-1} at site 3. The stream channel at both locations where the diffusion chambers were placed was mostly open with scattered trees on the bank. Climatic conditions were clear with scattered light clouds. A steep hillside west of site 3 resulted in diffusion chambers at this site receiving about 2 to 3 hrs less sunlight in the late afternoon than those at site 2. Because of the limited number of data points, no significant difference (alpha level of 0.05) was detected between the calculated decay rates at the two sites or between fecal coliform and *E. coli* densities; therefore, the decay rates were averaged to give an estimated fecal coliform and *E. coli* decay rate of 0.084 h^{-1}. This estimated decay rate indicates that about 8 percent of the fecal coliform and *E. coli* bacteria in Shoal Creek "die-off" each hour. This rate of 0.084 h^{-1} is larger than decay rates of fecal coliform and *E. coli* of 0.002 to 0.0407 h^{-1} measured during the summer in the Cuyahoga River by Myers and others (1998). A likely reason for the larger decay rates measured in this study is the larger degree of sunlight penetration and warmer water in Shoal Creek as compared to that of the Cuyahoga River. Sunlight penetration is reduced by water turbidity. Myers and others (1998) measured decay rates during periods of runoff where suspended-sediment concentrations ranged from 38 to 848 mg/L. In contrast, suspended-sediment concentrations in Shoal Creek at base-flow conditions are less than 20 mg/L. In addition, the diffusion chambers in the Cuyahoga River were placed 18 in. below the surface, more than twice the depth the chambers at sites 2 and 3 were placed. The shallower depth of the chambers in Shoal Creek would allow additional sunlight, and possible UV radiation, to reach the chambers. Bacteria decay in surface water is inversely proportional to water temperature (Craig and others, 2002). The median water temperature of Shoal Creek (26.8 °C) during sample collection was larger than the median summertime water temperature (21.4 to 25.2 °C) during the Cuyahoga River study (Myers and others, 1998).

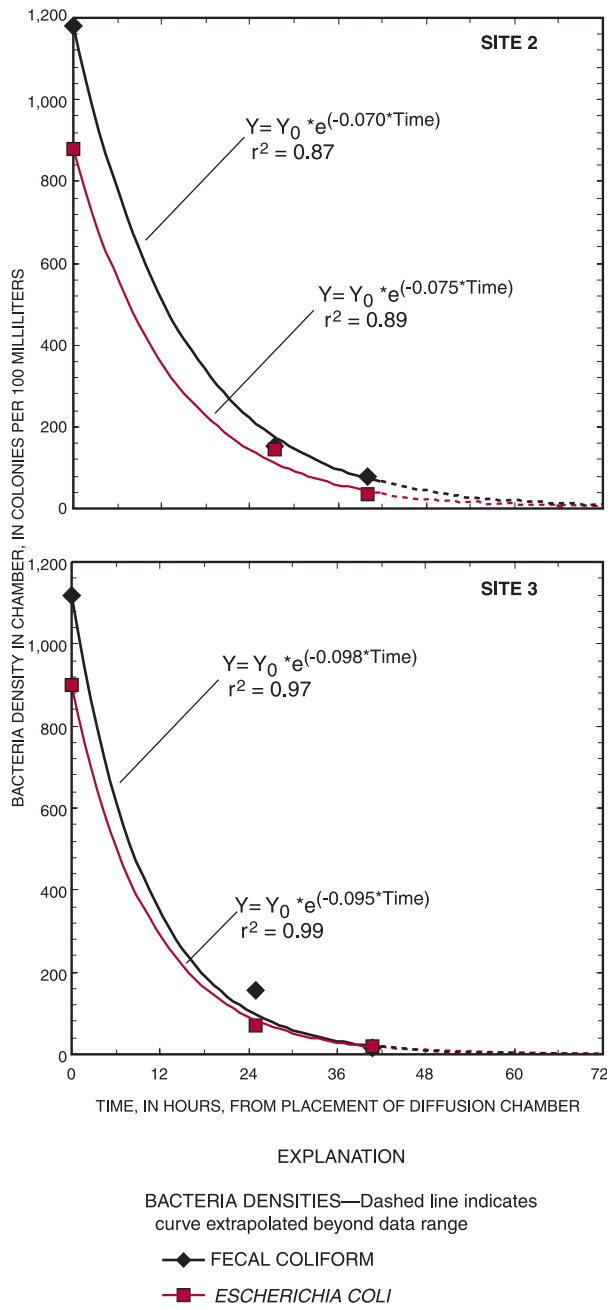


Figure 9. Measured fecal coliform and *Escherichia coli* densities in diffusion chambers at sites 2 and 3 on Shoal Creek, July 23 to 26, 2001.

Table 1. Densities of fecal coliform and *Escherichia coli* bacteria in diffusion chambers placed in Shoal Creek at sites 2 and 3, July 23 to 25, 2001

[*E. coli, Escherichia coli*; col/100 mL, colonies per 100 milliliters; k, non-ideal count]

Sample chamber number	Date	Time	Water temperature (degrees Celsius)	Elapsed time (hours)	*E. coli* density (col/100 mL)	Fecal coliform density (col/100 mL)
Site 2-initial	07/23/01	1730	28.5	0	880	1,200
tube1	07/24/01	2000	26.8	26.50	120	145
tube2	07/24/01	2000	26.8	26.50	170	160
tube6	07/25/01	1030	25.0	41.00	36 k	80 k
Site 3-initial	07/23/01	1700	26.8	0	900	1,100
tube1	07/24/01	1800	27.5	25.00	80 k	180
tube2	07/24/01	1800	27.5	25.00	56 k	150
tube3	07/24/01	1800	27.5	25.00	72 k	135
tube4	07/25/01	1045	23.9	41.75	25 k	5 k
tube5	07/25/01	1045	23.9	41.75	25 k	25 k
tube6	07/25/01	1045	23.9	41.75	12 k	16 k

Travel Times of Bacteria in Shoal Creek

The transport and travel times of fecal bacteria at low base-flow conditions between sites 2 and 3 on Shoal Creek were investigated by following the pulse of the fluorescent dye (rhodamine-WT) injected into the stream immediately upstream from site 2 on July 23, 2001. The underlying assumption is that the transport of fecal bacteria is similar to that of the dye. Less than 0.6 in. of precipitation was recorded by the NOAA weather station in Monett during the 7-day period preceding the dye injection, and the record from the streamflow-gaging station 0.2 mi downstream from the site indicated that Shoal Creek was at stable low base-flow conditions. Shoal Creek discharge generally increased downstream from the dye injection site. The discharge between sites 2 and 2a more than doubled, increasing from 7.30 to 14.8 ft^3/s (cubic feet per second) (table 2). Inflow from Pogue Creek (5.14 ft^3/s) accounted for most of the 7.50 ft^3/s increase in discharge between sites 2 and 2a. The remaining 2.36 ft^3/s (about 16 percent of the increase) was from springs and diffuse ground-water inflows. Discharge between sites 2a and 2b increased only slightly (14.8 to 15.0 ft^3/s) and was within 5 percent measurement error, indicating no detectable inflow between these sites. Discharge increased substantially between sites 2b (15.0 ft^3/s) and 2c (23.6 ft^3/s). Inflow from Joyce Creek accounted for only 1.55 ft^3/s of the increase in discharge between site 2b and 2c. Most of the increase in discharge between sites 2b and 2c was attributed to the presence of several large springs between these sites. The discharge between sites 2c and 3 increased slightly (23.6 to 24.6 ft^3/s); however, the increase (4 percent) was within the accepted discharge measurement error. A small spring (discharge estimated at 0.05 ft^3/s on August 27, 2002) about 0.25 mi upstream from site 3 accounted for part of the small increase in discharge between sites 2c and 3.

A total of 595 g of rhodamine-WT dye was injected at 1828 on July 23, 2001, into a riffle immediately upstream from a pool upstream from site 2 (figs. 4, 5). About 50 percent of this dye was recovered downstream at sites 2b, 2c, and 3 (table 2). Dye concentrations at site 3 peaked at about 2100 hrs the following day, indicating that the total time of travel from the injection to site 3 (distance of 5.7 mi) was about 26 hrs. As the dye peak moved downstream, dilution and dispersion resulted in a decrease of the peak dye concentration and broadening of the peak at each successive downstream site (fig. 10). The peak dye concentrations decreased more than 30-fold between sites 2 and 2a from 2,070 to 69.8 µg/L, reflecting substantial dilution by the large increase in discharge (which more than

Table 2. Summary of travel times and dye quantity recovered from selected sites during the July 2001 dye-trace study

[--, no data available]

		Site number (fig. 2)				
	Injection[a]	2	2a	2b	2c	3
Date of dye peak arrival	07/23/01	07/23/01	07/24/01	07/24/01	07/24/01	07/24/01
Time of dye peak arrival	1828	1843	0205	0745	1505	2048
Maximum dye concentration (micrograms per liter)	--	2,070	69.8	39.4	14.4	14.1
Discharge (cubic feet per second)	7.30	7.30	14.8	15.0	23.6	24.6
Travel time of dye peak from injection site (hours)	--	.25	7.62	13.28	20.62	26.33
Travel time of dye peak between sites (hours)	--	.25	7.37	5.67	7.33	5.72
Distance from injection point (miles)	--	.08	1.6	2.8	4.1	5.7
Average travel rate of dye peak from injection point (mile per hour)	--	.32	.21	.21	.20	.22
Rate of dye peak travel between sites (mile per hour)	--	.32	.21	.21	.18	.28
Estimated mass of dye recovered (grams)	595	504	412	333	278	327
Fraction of dye recovered	--	.85	.69	.56	.47	.55

[a] Injection site was riffle at the upstream end of a pool about 100 feet upstream from State Highway W bridge. Water samples were collected at the pool outlet about 300 feet downstream from State Highway W bridge.

doubled) and dispersion along this reach. Despite a nearly uniform discharge, dye concentrations decreased by more than 40 percent between sites 2a and 2b, indicating substantial dispersion along this reach as evidenced by the slight broadening of the dye peak (fig. 10). The substantial dispersion of the dye peak probably was the result of a number of long pools that were present between sites 2a and 2b. Absorption of dye to streambed material and organic matter within the channel also contributed to the dye decrease. Dye concentrations decreased by about 64 percent and the peak broadened between sites 2b and 2c, whereas the discharge increased by more than 50 percent. Unlike the upstream reaches, the peak dye concentrations and shape of the dye peak at sites 2c and 3 were similar (fig. 10), indicating the channel characteristics between these sites are different from those sites upstream. The reach between sites 2c and 3 is shallow and narrow with few pools as compared to the reach upstream from site 2c. The general lack of pools and eddies also was expressed in the slightly larger rate of dye movement between sites 2c and 3 [0.28 mi/hr (mile per hour)] compared to between sites 2a and 2c (0.21 to 0.18 mi/hr, table 2).

Assuming that the transport characteristics of the dye are similar to fecal bacteria, results of the dye study indicate that transport of fecal bacteria during low base-flow conditions between sites 2 and 3 occurred in about 26 hrs. As bacteria are transported along this reach of Shoal Creek, dilution and dispersion will cause substantial decreases in bacterial densities between sites 2 and 2c. Dilution and dispersion will not cause a measurable decrease in bacteria densities between sites 2c and 3 at low base-flow conditions.

Distribution of Fecal Bacteria and Nutrients

During the July 2001 dye-trace study, densities of fecal coliform bacteria in samples from Shoal, Pogue, and Joyce Creeks generally exceeded the MDNR standard of 200 col/100 mL for whole-body contact recreation. All of the samples collected from Shoal Creek at sites 2, 2a, 2b, and 3 and Pogue Creek at site 11 had fecal coliform densities larger than the MDNR standard. Seven of eight samples collected from Shoal Creek at site 2c and five of seven samples collected from Joyce Creek at site 12 also had fecal

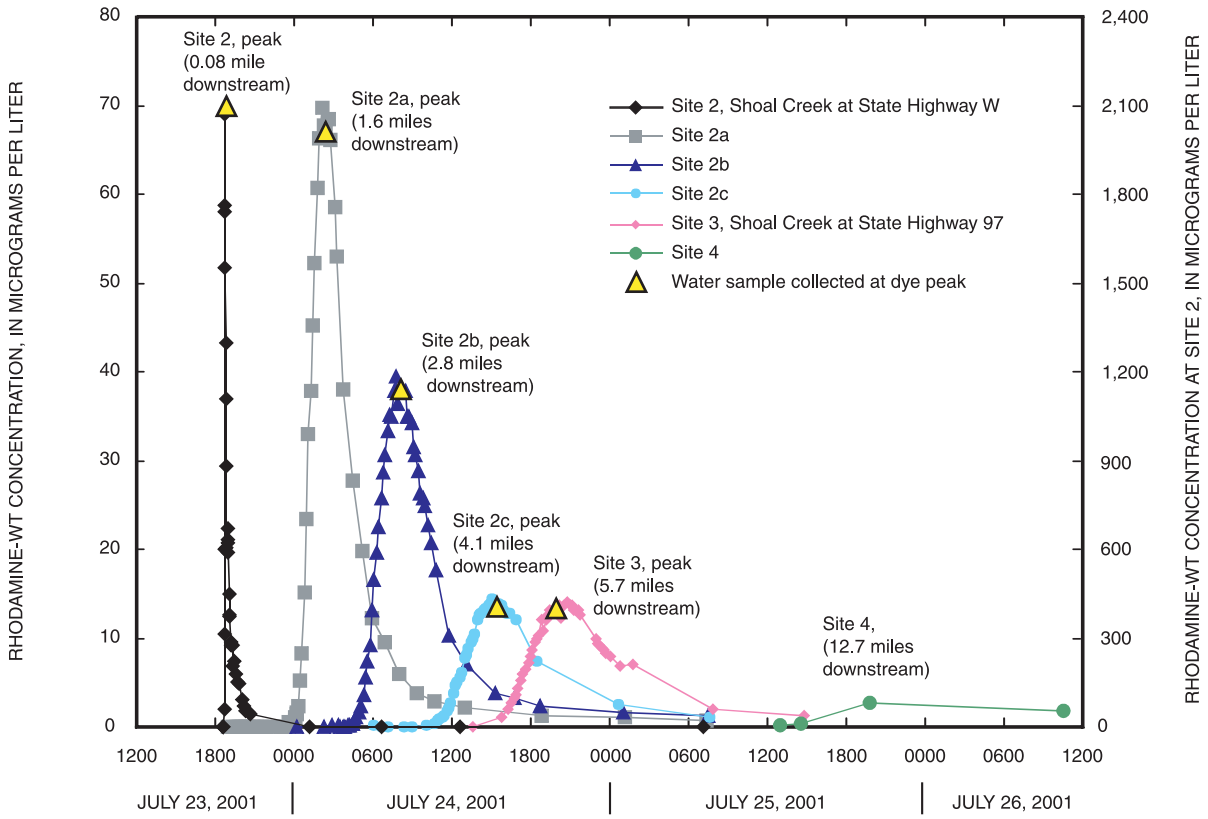


Figure 10. Concentrations of rhodamine-WT dye in water samples from the main stem of Shoal Creek, July 23 to 26, 2001.

coliform densities larger than the MDNR standard. None of the three samples collected from Shoal Creek at site 4 contained fecal coliform densities larger than the MDNR standard. Samples from site 2a contained the largest densities of fecal coliform (average of 2,600 col/100 mL) and *E. coli* (average of 2,200 col/100 mL) detected in the main stem of Shoal Creek (fig. 11). Average fecal coliform and *E. coli* densities decreased to less than or equal to 1,400 col/100 mL downstream at site 2b and further decreased to less than 500 col/100 mL at site 2c, but increased significantly at site 3. The average densities of fecal coliform (2,100 col/100 mL) and *E. coli* (1,400 col/100 mL) measured at site 3 during the July 2001 dye-trace study were comparable to densities previously reported for this site by Schumacher (2001). At an average decay rate of 0.084 hr^{-1}, fecal bacteria densities in a parcel of water at site 2c would be expected to decrease about 40 percent during the 5.7 hr it takes to travel to site 3. The significant increase in fecal bacteria densities between sites 2c and 3 and lack of measurable inflow between these sites indicates a substantial source of fecal bacteria along this stream reach.

Fecal bacteria densities in Pogue Creek (site 11, fig. 11) were the largest detected during the July 2001 dye-trace study. The average fecal coliform and *E. coli* densities in Pogue Creek (3,000 and 2,500 col/100 mL) measured during the July 2001 dye-trace study were about twice the average densities for this site reported by Schumacher (2001). Samples from sites 11a and 11c had the largest fecal coliform (9,900 and 4,300 col/100 mL) and *E. coli* densities (4,900 and 4,300 col/100 mL) detected in the 2001 dye-trace study (table 3), indicating substantial sources of fecal bacteria in the upper reaches of Pogue Creek. Although not measured, the discharge at both sites was estimated to be about one-half (site 11c) to three-fourths (site 11a) of that measured downstream at site 11, which supports the presence of substantial sources of fecal bacteria in the upper reaches of Pogue Creek. Densities of fecal bacte-

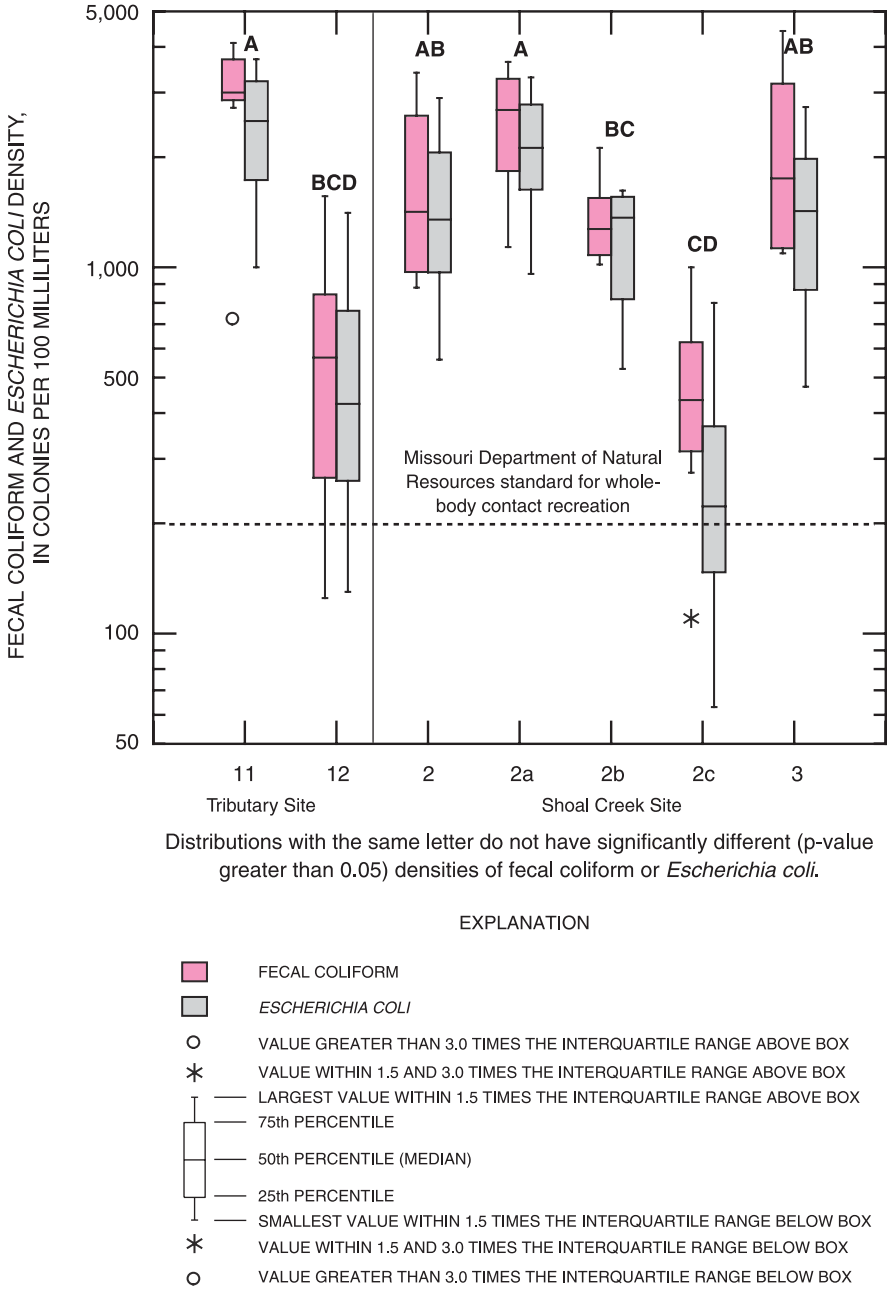


Figure 11. Fecal coliform and *Escherichia coli* densities in water samples from Shoal Creek and tributary sites during the July 2001 dye-trace study.

ria in samples from Pogue Creek were significantly larger (alpha level of 0.05) than densities in samples from Joyce Creek at site 12 and Shoal Creek at sites 2b and 2c (fig. 11).

Fecal bacteria densities varied substantially during the July 2001 dye-trace study and tended to exhibit some type of diurnal or sinusoidal variability. For example, densities of *E. coli* at site 2 tended to peak during the early morning hours and decrease in the early evening (fig. 12). A similar pattern in *E. coli* densities was observed at sites 2b and 2c. Densities of *E. coli* at sites 2a and 3 also exhibited some sort of sinusoidal variability, but were out of phase with the other main stem sites. *E. coli* densities tended to peak in the

Table 3. Discharge, physical properties, and densities of fecal coliform and *Escherichia coli* in water samples collected from Shoal Creek and selected tributary sites during the July 2001 dye-trace study

[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; col/100 mL, colonies per 100 milliliters; *E. coli*, *Escherichia coli*; --, no data; pk, sample collected at dye peak; k, non-ideal count]

Date	Time	Q	Temp	SC	DO	DO percent saturation	Fecal coliform (col/100 mL)	*E. coli* (col/100 mL)
Site 2, Shoal Creek at State Highway W								
07/23/01	1658	7.3	28.5	258	5.96	76.6	--	--
07/23/01 pk	1850	--	28.4	318	6.92	89.4	1,000	560
07/23/01 pk	1906	--	--	--	--	--	880	920
07/24/01	0110	--	25.9	303	5.06	62.3	1,300k	1,900k
07/24/01	0640	--	24.7	296	5.47	66.0	3,400k	2,300k
07/24/01	1240	--	27.2	227	8.60	108.4	2,000k	1,300
07/24/01	1930	--	26.8	232	5.50	73.0	910k	1,000
07/25/01	0123	--	24.9	303	5.46	66.0	1,500k	1,400k
07/25/01	0705	--	24.0	232	5.15	61.4	3,400	2,900
Site 2a, Shoal Creek at County Road 2110								
07/23/01	1630	14.8	28.7	331	5.29	75.1	--	--
07/23/01	1857	--	--	--	--	--	3,600k	3,300
07/24/01	0025	--	--	--	--	--	3,100k	960k
07/24/01 pk	0225	--	25.1	329	4.74	57.5	2,400k	2,300k
07/24/01	0655	--	24.2	321	5.14	61.5	3,000k	1,700k
07/24/01	1255	--	26.4	322	7.94	98.5	1,500k	1,900k
07/24/01	1850	--	26.7	314	6.30	79.5	2,300	3,100
07/25/01	0110	--	24.5	325	5.27	63.3	3,500	2,500
07/25/01	0720	--	23.5	328	4.74	55.6	1,100k	1,600k
Site 2b, Shoal Creek at County Road 2100								
07/23/01	1550	15	28.5	332	5.78	74.5	--	--
07/23/01	1948	--	--	--	--	--	1,100k	1,500
07/24/01	0010	--	27.0	325	4.07	51.8	1,800k	1,600k
07/24/01 pk	0805	--	--	--	--	--	1,300k	1,300
07/24/01	0840	--	24.9	331	4.96	60.0	2,100k	1,500
07/24/01	1305	--	27.3	325	7.49	93.9	1,100	530k
07/24/01	1840	--	27.3	325	6.27	79.2	1,000k	580
07/25/01	0100	--	25.7	325	4.73	58.0	1,300k	1,200k
07/25/01	0730	--	24.2	330	4.92	58.4	1,300k	1,600k
Site 2c, Shoal Creek at County Road 2090								
07/23/01	1520	23.6	26.5	325	6.28	77.8	--	--
07/23/01	1955	--	--	--	--	--	110	63
07/24/01	0035	--	24.8	326	5.89	70.6	460	275
07/24/01	0635	--	23.9	326	6.05	71.5	780	480k
07/24/01	1318	--	25.4	319	7.78	94.5	1,000	180
07/24/01 pk	1520	--	25.9	313	7.66	95.1	280	130
07/24/01	1830	--	25.6	316	7.55	92.6	360	170k
07/25/01	0040	--	24.1	321	6.54	78.0	500	280
07/25/01	0738	--	23.2	325	5.77	67.8	410	800k
Site 3, Shoal Creek at State Highway 97								
07/23/01	1430	24.6	26.8	--	--	--	--	--
07/23/01	2010	--	--	--	--	--	1,100k	1,500
07/23/01	2340	--	25.9	320	6.10	80.0	1,100k	1,400k
07/24/01	0655	--	24.2	329	5.84	69.6	1,100k	580
07/24/01	1335	--	27.2	315	9.06	112.8	3,600k	470k
07/24/01	1950	--	--	--	--	--	2,100k	1,800k
07/24/01 pk	1956	--	--	--	--	--	4,400k	2,700k
07/25/01	0047	--	24.5	322	6.12	73.6	1,400k	1,300
07/25/01	0755	--	23.5	328	5.73	67.5	2,800	2,200k
07/25/01	1450	--	--	--	--	--	790	360

Table 3. Discharge, physical properties, and densities of fecal coliform and *Escherichia coli* in water samples collected from Shoal Creek and selected tributary sites during the July 2001 dye-trace study—Continued

[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; col/100 mL, colonies per 100 milliliters; *E. coli*, *Escherichia coli*; --, no data; pk, sample collected at dye peak; k, non-ideal count]

Date	Time	Q	Temp	SC	DO	DO percent saturation	Fecal coliform (col/100 mL)	*E. coli* (col/100 mL)
Site 4, Shoal Creek								
07/25/01	1300	--	--	--	--	--	73k	20k
07/25/01	1430	--	--	--	--	--	91k	60k
07/26/01	0915	--	--	--	--	--	190	130
Site 11a, Pogue Creek at County Road								
07/24/01	1530	--	22.5	350	5.44	63.5	9,900	4,900
Site 11c, Pogue Creek at County Road								
07/24/01	1600	--	16.2	365	6.25	63.3	4,300	4,300
Site 11, Pogue Creek near mouth								
07/23/01	1925	5.14	--	--	--	--	--	--
07/23/01	2200	--	--	--	--	--	3,800	3,700
07/24/01	0115	--	22.2	358	5.20	58.6	3,000	3,300
07/24/01	0646	--	20.9	356	5.94	66.5	3,000	2,000
07/24/01	1246	--	22.6	356	6.84	79.0	4,100	1,000k
07/24/01	1620	--	--	--	--	--	3,600k	3,200k
07/25/01	0120	--	21.3	349	5.99	67.8	2,700k	2,500k
07/25/01	0713	--	20.2	324	5.77	63.8	700	1,500
Site 12, Joyce Creek near mouth								
07/23/01	1600	1.55	--	--	--	--	170	200
07/24/01	0030	--	26.7	306	4.45	56.4	130	350
07/24/01	0645	--	26.2	301	4.64	58.0	570k	420k
07/24/01	1325	--	27.6	297	5.61	74.8	830k	130
07/24/01	1823	--	27.6	294	5.67	74.7	1,600k	650
07/25/01	0033	--	26.3	299	4.30	51.8	430	890k
07/25/01	0745	--	25.5	302	4.66	57.0	860k	1,400k
Bacteria concentrations in streambed-sediment samples (colonies per gram wet weight)								
Site 2, Shoal Creek at State Highway W								
07/25/01	1045	--	--	--	--	--	28	30
Site 3, Shoal Creek at State Highway 97								
07/25/01	0930	--	--	--	--	--	8	8

early evening and decrease during the late morning. The patterns of *E. coli* densities at the various sites indicated that inputs of fecal bacteria to the stream or their decay, or both, have some sort of diurnal or sinusoidal variability. Unfortunately, the sampling period was not sufficiently long enough to determine if the variability in fecal bacteria densities had a repeatable frequency. The fluctuations in fecal bacteria densities were not a simple function of advection and dispersion because the patterns at successive downstream sites were not shifted in time by the travel time of the dye between the sites. For example, at the time of injection, *E. coli* densities at site 2 were less than 1,000 col/100 mL, but increased steadily during the next 12 hrs (fig. 12). As the dye peak passed site 2a about 8 hrs after injection, the *E. coli* densities at site 2a (2,300 col/100 mL) were larger than those at site 2 when the dye was injected and remained generally steady during the next 12 hrs. Pogue Creek accounts for a large part of the increase in discharge between sites 2 and 2a, but the *E. coli* densities in Pogue Creek near the time of dye injection (3,700 col/100 mL) were not large enough to generate the *E. coli* densities observed as the dye peak passed site 2a. This suggests additional inputs of *E. coli* to Shoal Creek between sites 2 and 2a independent of Pogue Creek.

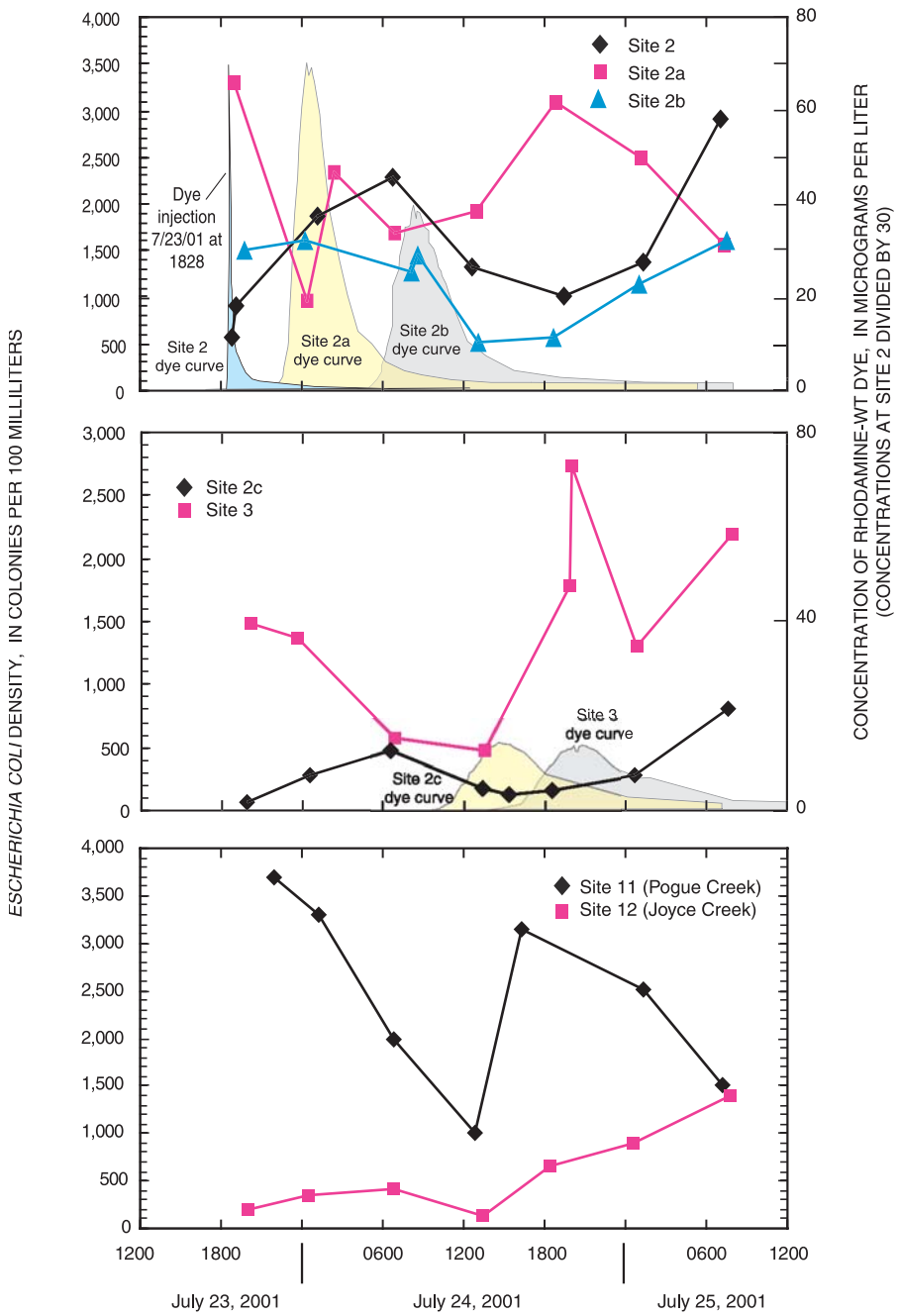
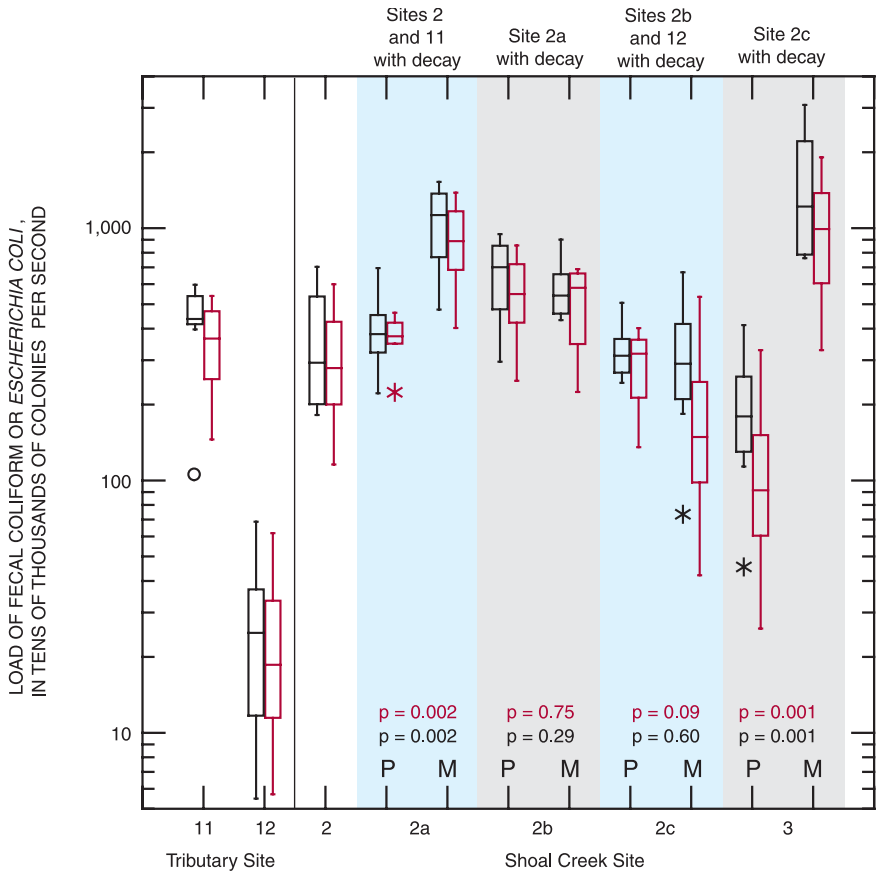


Figure 12. Variation of *Escherichia coli* densities and rhodamine-WT dye concentrations at main stem sites on Shoal Creek, July 23 to 25, 2001.

A comparison of the dye concentrations and *E. coli* densities at sites 2c and 3 indicated that the significant increase in fecal bacteria densities between these sites was caused by a large influx of bacteria along this stream reach. As the dye peak traveled between sites 2c and 3, the fecal bacteria densities in the parcel of water carrying the dye peak increased nearly ten-fold (fig. 12). Because the advection of the dye peak along this reach occurred during the daytime, a time when bacterial decay rates should be at a maximum because of warmer temperatures and sunlight exposure, the increase in the *E. coli* densities at site 3 was caused by an influx of bacteria along the 1.6-mi reach between sites 2c and 3.

A mass balance of fecal bacteria loads confirms significant sources of fecal bacteria to Shoal Creek between sites 2 and 2a and between sites 2c and 3 during the July 2001 dye-trace study. The mass balance included summing the measured bacteria loads from upstream and tributary sites and allowing for bacteria decay over the estimated transport time between the sites. For example, the fecal bacteria loads at site 2 and Pogue Creek (site 11) were summed and then allowed to decay (rate of 0.084 h^{-1}) for the 7.37 hrs it took the dye peak to travel between sites 2 and 2a (table 2). The results are predicted distributions of fecal coliform and *E. coli* bacteria loads at site 2a (fig. 13). Results of the Kruskal-Wallis test indicated that the predicted fecal coliform and *E. coli* loads at sites 2a and 3 were significantly smaller than the measured loads. At site 3, the predicted loads were nearly an order of magnitude smaller than the measured loads, indicating an extremely large input of fecal bacteria between sites 2c and 3 (fig. 13).

Analysis of streambed-sediment samples from sites 2 and 3 indicated that the accumulation of substantial quantities of fecal bacteria in the streambed



EXPLANATION

P PREDICTED BACTERIA LOAD

M MEASURED BACTERIA LOAD

Predicted loads at the downstream sites were calculated as the sum of the load of the upstream site plus tributaries and allowing for bacterial decay. Bacteria decay was calculated using the equation:

$$y=y_0{}^{*}e^{(0.84^{*}\ \text{time})},$$

where time is hours for the dye peak to travel from the upstream site to the downstream site. For example, the predicted loads at site 2a were calculated as the sum of the measured loads at upstream site 2 plus the measured loads at upstream tributary site 11 and allowing for bacterial decay according to the above equation using a dye peak travel time of 7.37 hours from site 2 to 2a (table 2).

Distributions in each shaded bar were compared using the Kruskal-Wallis non-parametric test. The probability values for each test are listed below the boxplots. Values in red represent p-values for *Escherichia coli* comparisons and values in black are for fecal coliform. Distributions with p-values larger than 0.05 were not considered statistically significant.

FECAL COLIFORM

ESCHERICHIA COLI

VALUE GREATER THAN 3.0 TIMES THE INTERQUARTILE RANGE ABOVE BOX

VALUE WITHIN 1.5 AND 3.0 TIMES THE INTERQUARTILE RANGE ABOVE BOX

LARGEST VALUE WITHIN 1.5 TIMES THE INTERQUARTILE RANGE ABOVE BOX

75th PERCENTILE

50th PERCENTILE (MEDIAN)

25th PERCENTILE

SMALLEST VALUE WITHIN 1.5 TIMES THE INTERQUARTILE RANGE BELOW BOX

VALUE WITHIN 1.5 AND 3.0 TIMES THE INTERQUARTILE RANGE BELOW BOX

VALUE GREATER THAN 3.0 TIMES THE INTERQUARTILE RANGE BELOW BOX

Figure 13. Results of statistical tests of predicted and measured loads of fecal coliform and *Escherichia coli* at selected sites during the July 2001 dye-trace study.

sediment in Shoal Creek probably does not occur. Fecal coliform and *E. coli* bacteria concentrations in streambed sediment from sites 2 and 3 were less than or equal to 30 col/g (colonies per gram) wet weight (table 3). The relatively small fecal bacteria concentrations in streambed sediment from Shoal Creek compared to water samples was not surprising because of the coarse-grained (sand to cobble) streambed sediment. Myers and others (1998) reported that smaller fecal bacteria densities were present in sandy sediment as compared to silty sediment in the Cuyahoga River. The small concentration of fecal bacteria in streambed sediment from Shoal Creek indicates that resuspension of streambed sediment during runoff probably is not a major source of fecal bacteria to the water column. However, the streambed-sediment samples collected from Shoal Creek were not from areas where livestock had access to the stream and may not represent fecal bacteria concentrations in streambed sediment in areas of intense livestock activity.

Concentrations of NO_{23t} in water samples collected during the July 2001 dye-trace study from Shoal Creek increased from 2.3 mg/L at site 2 to between 2.9 and 3.2 mg/L at sites 2a, 2b, 2c, and 3 (table 4). Samples from Pogue Creek contained the largest NO_{23t} concentrations detected (4.5 to 4.8 mg/L). A comparison of instantaneous NO_{23t} loads during the July 2001 dye-trace study indicated that inflow from Pogue Creek accounted for nearly all of the increase in NO_{23t} concentrations between sites 2 and 2a. Schumacher (2001) postulated that the large NO_{23t} concentrations in Pogue Creek might be the result of liquid waste from a poultry processing plant. Liquid wastes from this plant, which has a discharge in excess of 1 Mgal/d, are applied to the land surface about 1-mi upstream from site 11c (fig. 2). All the flow in Pogue Creek at site 11c was attributable to two springs (Dilbeck Spring and an unnamed spring) between the processing plant and site 11c, and upstream from these springs Pogue Creek is usually dry. According to data from Schumacher (2001) and Mugel (2002), Dilbeck Spring contained NO_{23t} concentrations larger than other springs in the upper Shoal Creek Basin.

Concentrations of NO_{23t} in samples collected during August 2002 at all sites were similar or slightly smaller than those detected in samples during the July 2001 dye-trace study. The smaller concentrations during the August 2002 sampling probably were caused by dilution, because the discharges measured during August 2002 were larger than those measured during 2001 (table 4). Total phosphorus (P_t) concentrations at all sites were less than 0.1 mg/L, with a tendency for concentrations in August 2002 to be larger than those in July 2001. Overall, NO_{23t} and P_t concentrations in water samples collected during this study were similar to values reported by Schumacher (2001).

SOURCES OF FECAL BACTERIA IN SHOAL CREEK

Attempts were made to identify the potential sources of fecal bacteria along the study reach using detailed observations and sampling during a seepage study of a select reach of Shoal Creek during August 2002. Additional efforts to identify the sources of fecal bacteria included the analysis of wastewater organic compounds and pharmaceutical compounds in water samples along the study reach and DNA-fingerprinting of *E. coli* isolated from selected water samples. Shoal Creek is an important source of water for livestock, and livestock generally are not fenced out of the stream. Schumacher (2001) observed that the livestock have free access to most of Shoal Creek and its tributaries upstream from site 3 and may be an important source of fecal bacteria to Shoal Creek and its tributaries upstream from site 3.

Detailed Seepage Study

In response to the suspected large source of fecal bacteria identified between sites 2c and 3 during the dye-trace study, a detailed seepage study was conducted along this 1.6-mi reach during low base-flow conditions on August 27, 2002. Results of this detailed seepage study indicated that the large increase in fecal bacteria densities in Shoal Creek between site 2c and site 3 (fig. 14) probably was caused by pastured cattle between these sampling sites. Water-quality samples collected during the detailed seepage study indicated a substantial increase in fecal bacteria densities downstream from areas where cattle had been fed along the streambank, or in areas where cattle were wading in the stream and congregating under trees along the streambank. Fecal coliform and *E. coli* densities at site 2c were small (100 and 40 col/100 mL), but increased to 1,100 and 580 col/100 mL at site 2c-L downstream from an area of intense cattle activity (fig. 14). Inflow from a spring branch (site 2c-J) where more than 100 cattle were wading or grazing nearby also contained

Table 4. Physical properties, nutrient concentrations, and densities of fecal coliform and *Escherichia coli* bacteria in surface- and ground-water samples collected during July 2001 and August 2002

[Q, discharge in cubic feet per second; Temp, temperature in degrees Celsius; SC, specific conductance in microsiemens per centimeter at 25 degrees Celsius; pH, pH, in standard units; NH_{3t}, total ammonia; mg/L, milligrams per liter; NO_{23t}, total nitrite plus nitrate as nitrogen; NO_{2t}, total nitrite as nitrogen; P_t, total phosphorus; PO_{4t}, total orthophosphorus; FC, fecal coliform; col/100 mL, colonies per 100 milliliters; *E. coli*, *Escherichia coli*; --, no data; <, less than; e, estimated]

Site (fig. 2)	Site type	Date	Time	Q	Temp	SC	pH	NH_{3t} (mg/L)	NO_{23t} (mg/L)	NO_{2t} (mg/L)	P_t (mg/L)	PO_{4t} (mg/L)	FC (col/100 mL)	*E. coli* (col/100 mL)
July 2001 dye-trace study														
2	Stream	07/23/01	1850	7.3	--	318	--	0.06	2.3	0.03	0.04	0.04	1,000	560
2a	Stream	07/24/01	0250	14.8	24.5	329	--	.06	3.2	.03	.04	.05	2,400	2,300
2b	Stream	07/24/01	0800	15.0	24.9	331	--	.05	3.1	.03	.04	.05	1,300	1,300
2c	Stream	07/24/01	1520	23.6	--	313	--	.03	3.1	.01	.03	.04	280	120
3	Stream	07/24/01	1950	24.6	--	322	--	.06	2.9	.02	.04	.04	2,100	1,800
11a	Stream	07/24/01	1550	--	--	350	--	.10	4.5	.03	.08	.07	9,900	4,900
11c	Stream	07/24/01	1600	--	--	352	--	.05	4.6	.01	.03	.03	4,300	4,300
11	Stream	07/24/01	1620	5.1	--	352	--	.11	4.8	.04	.07	.07	3,600	3,100
August 2002 detailed seepage study														
2	Stream	08/27/02	1400	12	22.7	320	8.1	<0.01	2.6	<0.01	0.03	0.03	780	140
2c	Stream	08/27/02	1552	28	22.6	317	--	.01	3.0	<.01	.03	.04	100	40
2c-E	Stream	08/27/02	1645	--	--	--	--	--	--	--	--	--	330	60
2c-G	Stream	08/27/02	1744	--	--	--	--	--	--	--	--	--	200	73
2c-J	Spring	08/27/02	1745	.05 e	30.9	325	--	--	--	--	--	--	5,600	4,000
2c-L	Stream	08/27/02	1822	--	--	319	--	--	--	--	--	--	1,100	580
3	Stream	08/27/02	1040	29.5	20.8	337	8.1	.02	3.0	.01	.03	.03	200	660
11	Stream	08/28/02	0830	5.9	17.9	342	7.4	.01	3.6	<.01	.04	.02	700	680
Dilbeck Spring	Spring	08/27/02	1635	2.0	14.6	384	7.1	<.01	3.9	<.01	.02	.02	<20	<20
12	Stream	08/27/02	1210	2.4	21.7	315	8.2	.01	3.1	<.01	.04	.02	440	500

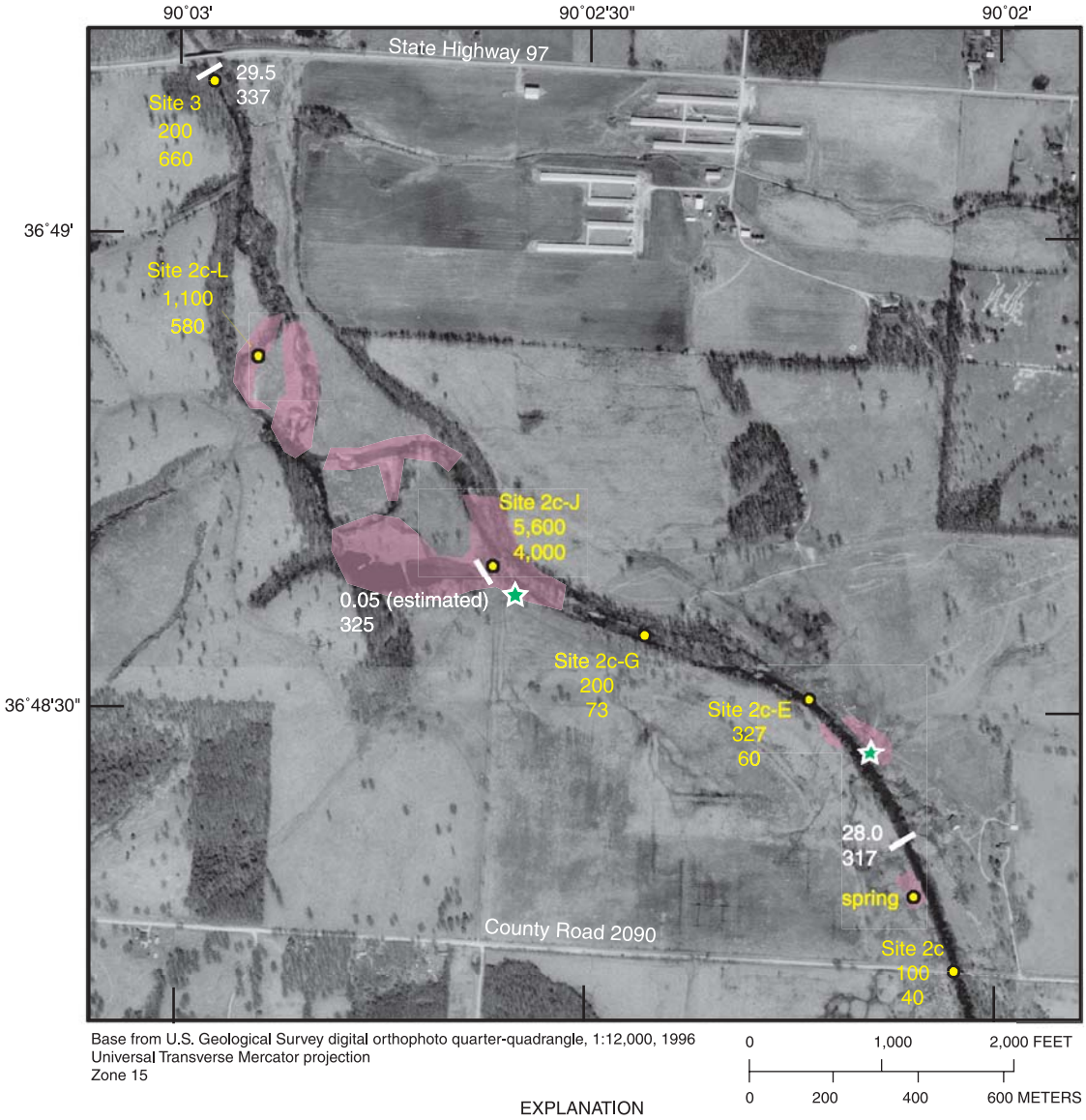


Figure 14. Discharge and fecal bacteria densities measured during the detailed seepage study between sites 2c and 3, August 27, 2002.

large densities of fecal coliform (5,600 col/100 mL) and *E. coli* (4,000 col/100 mL). Fecal coliform densities downstream in the sample from site 3 (200 col/100 mL) were twice as large as those at the upstream site (site 2c), but densities of *E. coli* at site 3 (660 col/100 mL) were more than 10 times larger than those at site 2c (fig. 14). Results of the detailed seepage study are consistent with results obtained during the dye-trace study and indicate that at low base-flow conditions during the time of these studies, a substantial input of fecal bacteria into Shoal Creek occurred between sites 2c and 3 likely from cattle pastured along this reach.

Fecal bacteria densities in samples collected during the August 2002 detailed seepage study tended to be smaller than those in samples collected during the July 2001 dye-trace study (table 4). The large fecal bacteria densities in the July 2001 samples might be related to the higher temperatures and the land application of poultry litter that was occurring at the time of the dye-trace study. The average maximum temperature for the 3-day period ending July 26, 2001, was 93 °F compared to 87 °F for the 3-day period ending August 27, 2002. Because the study results indicated that cattle are an important source of the fecal bacteria measured in Shoal Creek, the cattle may be expected to congregate for longer periods in and along the stream during hotter weather. According to the NRCS, during the July 2001 dye-trace study, several hundred truckloads of poultry litter were spread on fields along Shoal Creek between sites 2 and 3. Poultry litter was observed along roadsides and on low-water bridges at sites 2a and 2b during the July 2001 dye-trace study. In contrast, widespread application of poultry litter was not known to be occurring at the time of the detailed seepage study.

Distribution of Wastewater Organic Compounds and Pharmaceutical Compounds

To provide additional information on the possible sources of fecal bacteria in Shoal Creek, water samples collected during the July 2001 and August 2002 sampling trips were analyzed for a suite of organic compounds commonly associated with wastewater and a suite of pharmaceutical compounds. During the July 2001 dye-trace study, only water samples from the main stem sites (sites 2, 2a, 2b, 2c, and 3) were analyzed for these compounds, whereas during the August 2002 detailed seepage study, only samples from the upstream and downstream sites (sites 2 and 3) and major tributaries (Pogue and Joyce Creeks) were analyzed. A sample was collected from Dilbeck Spring during August 2002 because a large expansion of the poultry processing plant wastewater irrigation system upstream from the spring was underway and the spring supplies a large part of the flow of Pogue Creek.

A total of seven wastewater compounds and four pharmaceutical compounds were detected in one or more water samples collected during this study (table 5). The most frequently detected compounds were the stimulant caffeine (eight detections), the fecal indicator cholesterol, and the human drug acetaminophen (three detections each). Except for an estimated concentration of cholesterol of 1.2 µg/L, concentrations of all compounds were below the method reporting limit and estimated at less than 1 µg/L. Analytical error and the potential of sample contamination are substantial at these small concentrations and care should be used in interpreting the results listed in table 5. Trends in the type of compounds and concentrations detected along the study reach of Shoal Creek were not clearly evident. The largest number of compounds were detected in water samples collected during July 2001 from sites 2a and 2c (five detections). Samples collected during July 2001 from sites 2 and 3 had only four or less detections, and the sample from site 2b had one detection. The number of compounds detected had no relation to the densities of fecal bacteria because site 2a had among the largest fecal densities whereas site 2c had among the smallest fecal bacteria densities (fig. 12). The detection of acetaminophen at sites 2, 3, and 11, and the antimicrobial agent triclosan at sites 2b and 3 indicated that some human wastewater is entering Shoal Creek. Triclosan was detected in a previous study from site 3 by Schumacher (2001). The detection of thiabendazole, a common anthelmintic used to treat a variety of worm infections in cattle, at sites 2 and 2a, may indicate cattle waste was entering Shoal Creek. Although the presence of thiabendazole may indicate cattle waste, the absence of its detection does not necessarily indicate the absence of cattle waste. For example, the August 2002 sample from site 3 contained no thiabendazole, but did contain acetaminophen despite evidence from fecal bacteria samples collected during the detailed seepage study along this reach that indicated a large influx of fecal bacteria from cattle between sites 2c and 3 (fig. 14). The sample from Dilbeck Spring contained phenol (estimated concentration

Table 5. Concentrations of wastewater organic compounds and selected pharmaceutical compounds in surface- and ground-water samples collected during July 2001 and August 2002

[All concentrations in micrograms per liter; <, less than; e, estimated concentration below the reporting limit; ND, not detected and method detection level not established]

Compound	Shoal Creek at State Highway W (site 2)		Pogue Creek near mouth (site 11)	Shoal Creek (site 2a)	Shoal Creek (site 2b)	Joyce Creek near mouth (site 12)	Shoal Creek (site 2c)	Shoal Creek at State Highway 97 (site 3)		Dilbeck Spring
	07/23/01 1850	08/27/02 1400	08/28/02 0830	07/24/01 0225	07/24/01 0800	08/27/02 1210	07/24/01 1520	07/24/01 1950	08/27/02 1040	08/27/02 1635
Wastewater organic compound analysis										
3-beta-Coprostanol	<2	<2	<2	0.24 e	<2	<2	0.57 e	<2	<2	<2
beta-Sitosterol	<2	<2	<2	.60 e	<2	<2	<2	.93 e	<2	<2
Caffeine	.13 e	<.5	<.5	.13 e	<.5	<.5	.33 e	<.5	<.5	<.5
Cholesterol	<2	<2	<2	.58 e	<2	<2	.81 e	1.2 e	<2	<2
para-Cresol	<1	<1	.25 e	<1	<1	<1	<1	<1	<1	<1
Phenol[a]	<.5	<.5	<.5	<.5	<.5	<.5	<.5	<.5	<.5	.68 e
Triclosan	<1	<1	<1	<1	.21 e	<1	<1	.12 e	<1	<1
Pharmaceutical compound analysis										
Acetaminophen	ND	0.004 e	0.003 e	ND	ND	ND	ND	ND	0.0035 e	ND
Caffeine	ND	.015	ND	ND	ND	0.015	0.003	0.0043	.036	0.019
1,7-Dimethylxanthine	ND	ND	ND	ND	ND	ND	.012	ND	ND	ND
Thiabendazole	0.0003	ND	ND	0.0004	ND	ND	ND	ND	ND	ND

[a] Phenol was detected at an estimated concentration of 0.26 microgram per liter in the blank sample submitted in July 2001 and at an estimated concentration of 0.22 microgram per liter in the August 2002 blank sample.

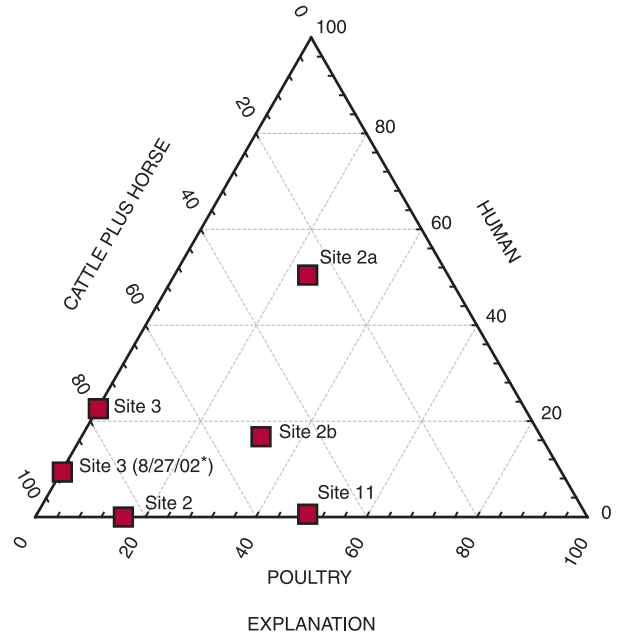
of 0.68 µg/L), which is a common disinfectant used in domestic and industrial applications. Phenol is used as a disinfectant in the poultry industry in the area and also was detected in samples from Dilbeck Spring and Shoal Creek at site 3 by Schumacher (2001). However small concentrations of phenol (0.22 and 0.26 µg/L) were detected in the blank samples submitted with these samples.

The variety of wastewater and pharmaceutical compounds detected, and their relatively low concentrations, provides indirect evidence of a variety of human or industrial and animal waste sources to streams in the study area. Interpretation of these data is complicated by the small number of samples collected and analyzed because of their relatively high cost compared to other analyses, such as indicator bacteria. In addition, the potential for substantial laboratory error and sample contamination is greatly increased at the small concentrations detected. For example, during the July 2001 dye-trace study the sample from site 2b contained one detection of these compounds, despite the detection of five compounds upstream at site 2a and downstream at site 2c. Dilution and dispersion unlikely would decrease concentrations detected at site 2a to less than detection at site 2b because the discharge at sites 2a and 2b was essentially the same and the peak dye concentration at site 2b was more than 50 percent of that at site 2a. The detection of only one compound in the sample from site 2b was unusual and suggests that a higher sampling frequency was needed to confirm the detected concentrations; care should be exercised when interpreting the detection of trace concentrations of these compounds.

Genetic Identification of Fecal Bacteria

A total of 48 *E. coli* isolates from 5 sites (2, 2a, 2b, 3, and 11) sampled during the July 2001 dye-trace study were evaluated using the rep-PCR method at the University of Missouri-Columbia Department of Veterinary Pathobiology. DNA-fragmentation patterns from these isolates were compared to the University library of known host patterns using a discriminate analyses technique. Results of the analysis yielded the presumptive source of each individual *E. coli* isolate. An initial comparison (two-class problem) was done to determine if the isolate patterns from the water samples were human or nonhuman. Twenty-five of the 48 patterns (52 percent) matched non-human patterns and 6 patterns (13 percent) matched human patterns. Seventeen of the patterns (35 percent) were not matched. Using discriminate analysis, matching of a pattern from an unknown isolate was defined to occur when the pattern was matched to known patterns of a particular source group in the database at a probability of greater than or equal to 70 percent.

Results of a second comparison indicated that *E. coli* in the water samples collected during the July 2001 dye-trace study were from a variety of sources with cattle, horses, and humans being the most common. The second five-class comparison (cattle, chicken, horse, human, and turkey) was done only on those patterns presumptively identified as either human (n=6) or nonhuman (n=25) in the first comparison. About 80 percent of the *E. coli* patterns isolated from sites 2 and 3 were matched to cattle and horses (fig. 15) with 44 percent of the patterns from these sites being matched to cattle. This match was consistent with observations of large numbers of cattle grazing in fields upstream from sites 2 and 3 and the detection of thiabendazole in water samples from sites 2 and 2a. Horses are scattered



Only isolates that were initially assigned to either a human or a non-human source during an initial two-class screening by discriminate analysis at a probability (p-value) of 0.70 or greater were used.

*Sample collected by the the Food and Agricultural Policy Research Institute (FAPRI) on August 27, 2002. Remaining samples were collected in July 2001.

Figure 15. Percentage of *Escherichia coli* isolates in water samples collected from Shoal and Pogue Creeks matched to a major source group.

throughout the basin but not in as large of a density as cattle. Large quantities of poultry litter were being applied to fields along the study reach and poultry litter was observed along roads and low-water crossings; however, the small percentage of isolates matched to poultry indicated that measurable amounts of this litter had not entered the stream at the time of the dye-trace study. Results of DNA-fingerprinting from a sample collected at site 3 by the FAPRI on August 27, 2002, indicated that 7 of 13 isolates (54 percent) were matched to cattle, and only 1 isolate was matched to humans (Claire Baffaut, written commun., 2002). Results of DNA-fingerprinting of the FAPRI sample support the results of the detailed seepage study, which identified a large influx of fecal coliform and *E. coli* to Shoal Creek from an area of intense cattle grazing and feeding upstream from site 3.

Humans appeared to be an important source of *E. coli* at site 2a, where about 50 percent of the patterns were matched to human sources. The presence of human patterns at site 2a was consistent with the detection of several wastewater organic compounds, including caffeine, in the July 2001 water sample from this site. About 20 percent of the patterns from site 3 were matched to humans, which was consistent with the detection of triclosan in the July 2001 water sample from this site. However, humans accounted for less than 10 percent of the patterns in the August 27, 2002, water sample collected by the FAPRI at site 3. Pogue Creek at site 11 was the only site where an appreciable number of the isolates (about 50 percent) were matched to poultry. The detection of poultry patterns at site 11 was consistent with a large density of commercial turkey operations upstream from this site and previous DNA-fingerprinting, which indicated a predominance of turkey patterns in some water samples from Pogue Creek (Schumacher, 2001). In addition, Schumacher (2001) identified tylosin, an antibiotic used to treat respiratory infections in poultry, in a sample from Pogue Creek.

A summary of the DNA-fingerprinting indicated that cattle are an important source (about 50 percent) of *E. coli* at the MDNR sampling site (site 3) on Shoal Creek. The identification of cattle patterns at site 3 was consistent with the results of the August 2002 seepage study, which indicated large influxes of fecal bacteria from an area of intense cattle activity. However, because of the small number of samples and *E. coli* isolates examined in this study, absolute identification of the source of all water-borne *E. coli* in the study reach cannot be made. A small number of patterns from known wildlife in the region, such as turkeys, deer, and raccoon, have been examined, and these animals also may contribute to *E. coli* detected in Shoal Creek. In addition, most of the cattle isolates in the reference library are from outside the study area and this may cause some cattle isolates in Shoal Creek to be unidentified and classified as unknown origin. A detailed discussion of some of the limitations of DNA-fingerprinting of *E. coli* isolates from the Shoal Creek Basin is given in Schumacher (2001).

OCCURRENCE AND SURVIVAL OF FECAL BACTERIA IN LAND-APPLIED POULTRY LITTER

Fecal bacteria were detected in all of the poultry litter samples examined during the study described in this report with concentrations varying nearly three orders of magnitude. Concentrations of fecal coliform ranged from 73 col/g in the broiler litter compost applied at test plot P-3 to 47,000 col/g in the turkey litter applied at test plot P-2 (table 6). Concentrations of *E. coli* generally were similar and ranged from 570 col/g in broiler litter compost to 45,000 col/g in turkey litter. The concentrations of fecal coliform in poultry litter samples measured in this study were comparable to concentrations reported by Hartel and others (2000). Hartel and others (2000) measured fecal coliform bacteria in 10 of 20 samples of fresh poultry litter with concentrations ranging from $10^{3.06}$ to $10^{6.66}$ col/g. The small fecal bacteria density in the broiler litter compost as compared to the turkey litter probably was caused by the poor survival of fecal bacteria at the temperatures that can exist in compost piles. Compost used at test plot P-3 was removed from a covered stacking shed with a front-end loader immediately before application to the test plot. Temperatures measured by the NRCS inside the compost pile immediately before application ranged from 120 to 125 °F. The pile had previously reached 150 °F and was in the process of cooling down (Kari Rhoades, Natural Resources Conservation Service, written commun., 2001). In a comparison of fecal coliform survival in fresh and stacked broiler litter, Hartel and others (2000) concluded that the rapid decrease in fecal coliform concentrations from greater than 10^7 col/g to less than 10 col/g within 8 days in stacked broiler litter was the result of spontaneous heating within the piles and that bacteria survival was

Table 6. Description of litter application test plots and initial fecal coliform and *Escherichia coli* densities in poultry litter

[--, no data; *E. coli*, *Escherichia coli*]

Description	Unit	Test plot			
		P-1	P-2	P-3	P-4
Litter type	--	Turkey litter	Turkey litter	Broiler litter compost	Broiler litter
Start date	--	05/23/01	05/07/02	08/10/01	04/19/02
End date	--	07/03/01	07/02/02	08/31/01	07/03/02
Plot size	Feet	302 x 75	302 x 75	180 x 30	200 x 80
Plot area	Square feet	22,650	22,650	5,400	16,000
	Acres	.5	.5	.1	.4
Litter condition	--	Dry	Dry	Damp-wet	Damp
Application rate					
Mat 1	Tons per acre	2.1	4.2	7.7	4.2
Mat 2	Tons per acre	3.5	3.4	16.5	3.4
Mat 3	Tons per acre	1.7	--	--	--
Average rate	Tons per acre	2.4	3.8	12.1	3.8
Vegetation	--	Fescue/hay	Fescue/hay	Mixed grasses	Fescue/hay
Initial fecal coliform density, in litter	Colonies per gram (dry weight)	17,000	47,000	73	45,000
Initial *E. coli* density, in litter	Colonies per gram (dry weight)	17,000	45,000	570	44,000

inversely related to temperature. Jeffrey and others (2001) also noted that increased temperature was an important factor in the poor survival of fecal bacteria in stacked broiler litter. The high temperatures in test plot P-3 broiler litter compost pile were sufficient to kill fecal bacteria, and the small concentrations of fecal coliform and *E. coli* that were measured at the time of application may be the result of small pockets of bacteria surviving in cooler parts of the pile or contamination of the pile by rodent feces or the manure spreader used to apply the litter compost to the test plot.

An important finding of the test plots was that fecal bacteria in poultry litter can survive in fields for several weeks after the application of the litter to the land surface. Densities of fecal coliform and *E. coli* in slurry-water samples from the test plots where litter from turkey (test plots P-1 and P-2) or broiler barns (test plot P-4) was applied were significantly larger after the application of litter than before litter application (tables 7, 8). Excluding the broiler litter compost, median densities of fecal coliform and *E. coli* at each test plot increased from less than 60 col/100 mL before litter application to at least 40,000 col/100 mL immediately after litter application (tables 7, 8). The largest increases were at test plot P-4 (broiler litter) where median fecal coliform and *E. coli* densities immediately after application were 420,000 and 290,000 col/100 mL. Densities of fecal coliform and *E. coli* at the test plots decreased in a generally exponential manner with increasing time after application (fig. 16). Estimated decay rates ranged from 0.085 to 0.185 d^{-1} (per day) for fecal coliform to between 0.100 and 0.250 d^{-1} for *E. coli* (fig. 16). These values were comparable to the range of die-off rates (0.11 to 0.37 d^{-1} for fecal coliform to 0.17 to 0.94 d^{-1} for *E. coli*) measured by Teague and Vendrell (1995) in laboratory studies using soil amended with broiler litter from Arkansas. Median fecal coliform and *E. coli* densities remained significantly larger than the initial densities for 4 to 8 weeks after application. The larger fecal bacteria densities measured after litter application indicated that bacteria in the litter applied to the land surface were surviving despite the apparent harsh environmental

Table 7. Densities of fecal coliform bacteria in slurry-water samples from test plots of land-applied poultry litter

[<, less than; --, no data]

Date	Time	Elapsed time since application (days)	Test plot subsample location							Control 1	Control 2	Summary			Probability value[a]
			s1	s2	s3	s4	s5	s6	s7			Average	Median	Standard deviation	
Turkey litter (Test plot P-1)															
05/23/01	1130	0	<10	<10	<10	30	<10	69	80	--	--	31	10	31	--
05/23/01	1430	.13	26,000	12,000	85,000	20,000	42,000	58,000	57,000	--	--	43,000	42,000	26,000	0.000
05/24/01	1200	1.02	48,000	5,200	98,000	32,000	12,000	220,000	64,000	--	--	68,000	48,000	74,000	.000
05/29/01	1100	5.98	136,000	3,400	10,000	12,000	1,700	90,000	42,000	--	--	42,000	12,000	52,000	.000
06/05/01	1200	13.02	5,200	220,000	4,500	2,600	4,600	4,700	4,900	--	--	35,000	4,700	81,000	.000
06/12/01	1130	20.00	10	50	160	<20	150	1,400	400	--	--	362	160	530	.232
06/19/01	1130	27.00	940	130	560	4,300	200	910	420	--	--	1,100	560	1,500	.000
06/26/01	1200	34.02	10	240	10	460	60	10	440	--	--	180	60	200	.314
07/03/01	1130	41.00	44	12	10	40	24	76	40	--	--	35	40	23	1.000
Turkey litter (Test plot P-2)															
05/07/02	1030	0	26	5	10	10	20	10	10	10	--	13	10	7	--
05/07/02	1200	.06	74,000	110,000	170,000	100,000	500,000	290,000	92,000	<1,000	--	191,000	110,000	155,000	0.000
05/14/02	1020	6.99	380,000	280,000	110,000	23,000	7,300	140,000	72,000	1,000	--	140,000	110,000	138,000	.000
05/29/02	1030	22.00	15,000	27,000	86,000	200,000	16,000	7,000	10,000	11,000	--	52,000	16,000	71,000	.000
06/13/02	1730	37.29	<330	2,700	1,300	3,300	1,300	11,300	8,000	13,000	--	4,000	2,700	4,100	.008
07/02/02	1340	56.13	60	1,900	600	100	1,600	200	1,300	1,800	--	820	600	770	.739
Broiler litter compost (Test plot P-3)															
08/10/01	1445	0	19,000	95	15	24	100	10	900	180	7,900	2,900	95	7,100	--
08/10/01	1545	.04	2,200	1,900	100	200	600	300	890	--	--	880	600	840	0.914
08/11/01	1400	.97	130	210	1,900	100	9,100	180	210	130	2,100	1,700	210	3,300	1.000
08/15/01	1030	4.82	20	3,000	2,500	500	20	1,700	8,300	20	9,100	2,300	1,700	2,900	.996
08/31/01	1310	20.93	4,900	5,300	6,000	6,000	8,700	6,000	--	4,700	--	6,200	6,000	1,300	.984
Broiler litter (Test plot P-4)															
04/19/02	1300	0	31,000	65	5	2,600	29	5	5	710	--	4,800	29	12,000	--
04/19/02	1500	.08	245,000	930,000	250,000	540,000	100,000	1,000,000	420,000	1,200	--	500,000	420,000	350,000	0.000
04/20/02	1130	.94	220,000	240,000	840,000	110,000	850,000	190,000	190,000	730	--	400,000	220,000	320,000	.000
04/26/02	1000	6.88	<1,000	21,000	370,000	36,000	67,000	110,000	370,000	<1,000	--	140,000	67,000	160,000	.000
05/06/02	1730	17.19	3,000	57,000	42,000	160,000	2,000	110,000	37,000	<1,000	--	59,000	42,000	58,000	.002
05/22/02	1100	32.92	14,000	2,000	2,000	14,000	220,000	15,000	140,000	30,000	--	58,000	14,000	87,000	.041
06/05/02	1215	46.97	30,000	19,000	6,100	30,000	19,000	2,500	2,100	27	--	16,000	19,000	12,000	.846
07/03/01	1000	74.03	30,000	19,000	6,100	30,000	19,000	2,500	2,100	34	--	16,000	19,000	12,000	.234

[a] Probability value from analysis of variance on ranked data and Tukeys multiple comparison test comparing average rank to initial conditions before application of poultry litter. Probability values less than 0.05 indicate a significant difference from the initial concentration.

Table 8. Densities of *Escherichia coli* bacteria in slurry-water samples from test plots of land-applied poultry waste

[nd, not detected because of overgrowth on plate; --, no data; <, less than; > greater than]

Date	Time	Elapsed time since application (days)	Test plot subsample location							Control 1	Control 2	Summary			Probability value[a]
			s1	s2	s3	s4	s5	s6	s7			Average	Median	Standard deviation	
Turkey litter (Test plot P-1)															
05/23/01	1130	0	nd	nd	nd	30	nd	nd	20	--	--	25	25	7	--
05/23/01	1430	.13	27,000	25,000	110,000	2,700	47,000	53,000	44,000	--	--	44,000	44,000	34,000	0.000
05/24/01	1200	1.02	400,000	9,000	85,000	21,000	8,900	300,000	65,000	--	--	130,000	65,000	160,000	.000
05/29/01	1100	5.98	500,000	3,000	2,200	27,000	2,400	68,000	134,000	--	--	100,000	27,000	180,000	.000
06/05/01	1200	13.02	2,000	410,000	3,500	2,400	6,500	8,000	3,800	--	--	62,000	3,800	150,000	.000
06/12/01	1130	20.00	40	200	20	--	60	1,000	360	--	--	280	130	380	.539
06/19/01	1130	27.00	456	130	200	10,000	--	2,600	700	--	--	2,300	580	3,900	.001
06/26/01	1200	34.02	nd	60	nd	460	40	400	420	--	--	280	400	210	.864
07/03/01	1130	41.00	nd	64	nd	nd	nd	25	nd	--	--	45	45	28	.999
Turkey litter (Test plot P-2)															
05/07/02	1030	0	200	<5	<10	<10	<10	<10	<10	<10	--	36	10	72	--
05/07/02	1200	.06	99,000	60,000	150,000	77,000	330,000	200,000	79,000	1,000	--	140,000	99,000	96,000	0.000
05/14/02	1020	6.99	340,000	200,000	80,000	18,000	14,000	250,000	96,000	1,000	--	140,000	96,000	120,000	.000
05/29/02	1030	22.00	16,000	24,000	92,000	100,000	14,000	3,600	10,000	44,000	--	37,000	16,000	41,000	.000
06/13/02	1730	37.29	<330	330	670	3,000	330	9,300	1,800	6,000	--	2,300	670	3,300	.000
07/02/02	1340	56.13	nd	nd	nd	20	6,000	20	20	1,600	--	1,500	20	3,000	.037
Broiler litter compost (Test plot P-3)															
08/10/01	1445	0	19,000	5	100	5	19	130	500	300	1,000	2,800	100	7,100	--
08/10/01	1545	.04	100	5,100	100	300	100	100	100	--	--	840	100	1,900	0.767
08/11/01	1400	.97	20	700	20	20	100	900	20	14	700	250	20	380	.831
08/15/01	1030	4.82	20	700	20	20	100	900	20	20	5,100	250	20	380	.602
08/31/01	1310	20.93	14	14	14	14	14	6,000	6,000	19,000	24,000	1,700	14	2,900	.008
Broiler litter (Test plot P-4)															
04/19/02	1300	0	25,000	68	55	1,400	25	5	5	600	--	3,800	55	9,400	--
04/19/02	1500	.08	150,000	590,000	290,000	200,000	140,000	460,000	490,000	2,500	--	330,000	290,000	180,000	0.000
04/20/02	1130	.94	82,000	220,000	880,000	200,000	710,000	194,000	54,000	1,100	--	330,000	200,000	320,000	.000
04/26/02	1000	6.88	1,000	21,000	370,000	36,000	67,000	110,000	370,000	1,000	--	139,000	67,000	160,000	.002
05/06/02	1730	17.19	3,000	57,000	42,000	160,000	2,000	110,000	37,000	1,000	--	59,000	42,000	58,000	.037
05/22/02	1100	32.92	1,500	3,500	10,000	51,000	500	200,000	40,000	15,000	--	44,000	10,000	72,000	.222
06/05/02	1215	46.97	670	2,700	3,000	8,000	4,000	4,700	<330	<330	--	3,300	3,000	2,600	.069
07/03/01	1000	74.03	10,000	460	>30,000	10,000	28,000	16,000	1,000	30,000	--	14,000	10,000	11,800	.556

[a] Probability value from analysis of variance on ranked data and Tukeys multiple comparison test comparing average rank to initial conditions before application of poultry litter. Probability values less than 0.05 indicate a significant difference from the initial concentration.

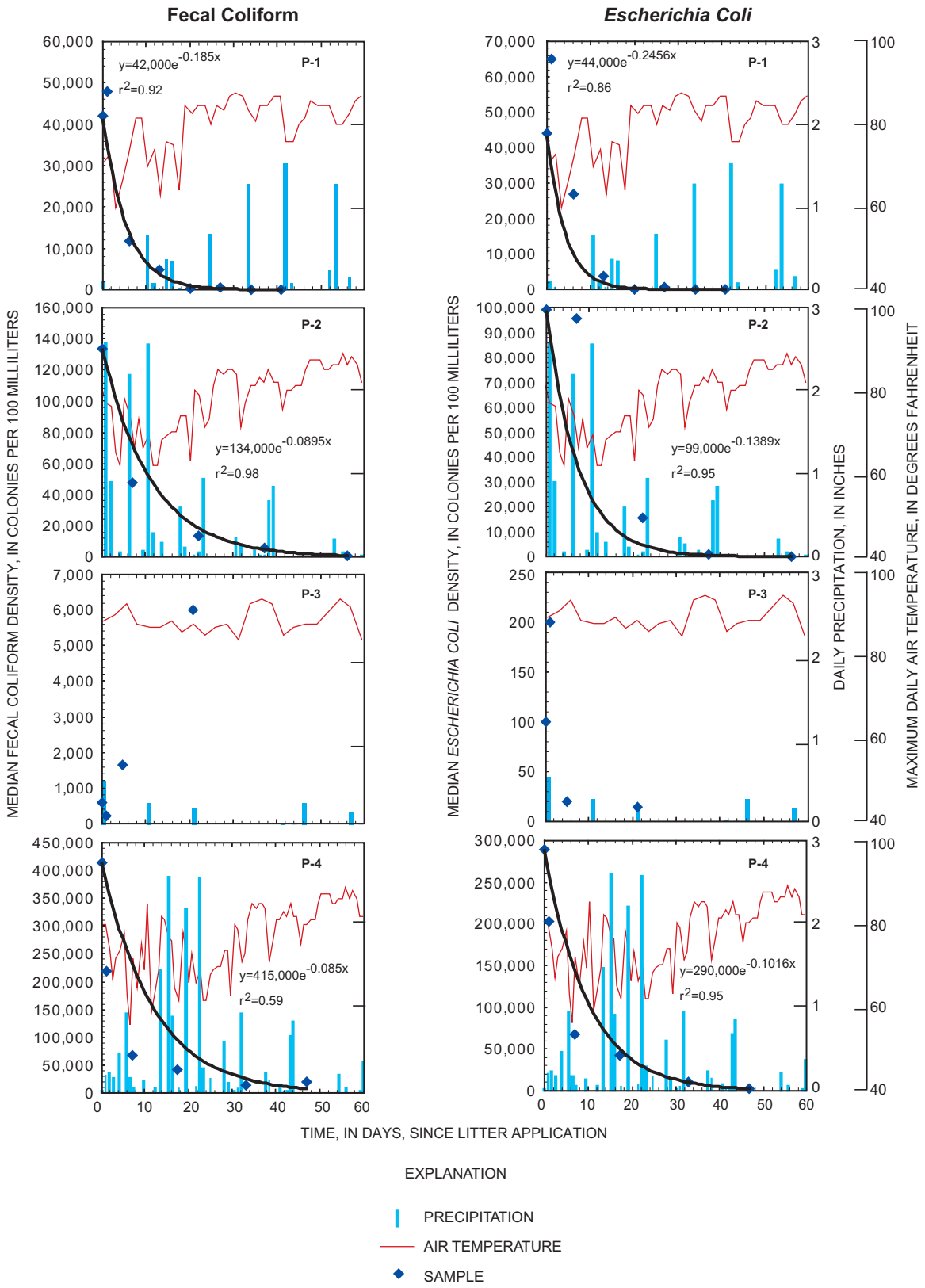


Figure 16. Median densities of fecal coliform and *Escherichia coli* bacteria, total daily precipitation, and mean daily air temperature at the poultry litter field plots.

conditions at the land surface. Bacteria from test plot P-1 had the shortest survival times (about 4 weeks) and those from test plot P-2 had the largest survival times (6 to 8 weeks). Fecal bacteria densities at the broiler litter compost test plot (P-3) did not decrease significantly after the application of the compost and generally tended to increase with increasing time (fig. 16) because of exhaust dust from a nearby poultry barn.

While generally replicating actual conditions on the fields, the uncontrolled conditions of the test plots created variability in the data. For example, the erratic fecal bacteria densities in the control and test plot samples from broiler litter compost test plot (P-3) probably were related to the poor location chosen for the test plot and the near absence of fecal bacteria in the compost. Because of a lack of access to nearby fields, test plot P-3 was located about 60 ft north of a broiler house. Although the house was empty when the test began, litter from the house was being removed from the house during the first 2 days that test plot P-3 was monitored. About 3 weeks after monitoring began, a flock of broilers was moved into the house, and fresh dust and feathers were observed on the test plot near the exhaust fans on the north side of the house. Sub-sample locations s6, s7, and control 1 and control 2 were located in the vicinity of the exhaust fans, and *E. coli* densities at these locations increased during the test (table 8). Fecal bacteria densities in control samples at test plot P-4 also were variable. Sometime after monitoring of test plot P-4 began, several cows entered the field where test plot P-4 was located. Fresh cow manure was observed in the vicinity of the control site during the May 22, 2002, sampling visit, and fecal bacteria densities in control samples collected that day were larger than expected (tables 7, 8).

The presence of significant densities of fecal bacteria at the test plots following the land application of poultry litter (test plots P-1, P-2, and P-4) was consistent with the published literature. Edwards and Daniel (1994) detected densities in fecal coliform as large as about $10^{6.4}$ col/100 mL in runoff from plots where poultry litter had been applied. Coyne and Blevins (1995) also reported increased fecal coliform densities in runoff from plots amended with poultry litter. Increased total coliform densities [from 0 to 33,000 col/mL (colonies per milliliter)] were observed in runoff from field plots treated with poultry litter (Giddens and Barnett, 1980). Unlike the study described in this report none of the published studies, however, attempted to monitor the survival of the fecal bacteria on the fields for an extended time.

The apparent survival of significant numbers of fecal bacteria on fields where poultry litter has been applied indicated that runoff from fields where this litter is applied is a potential source of fecal bacteria to vicinity streams for many weeks following application. The finding is consistent with the results of previous DNA-fingerprinting reported by Schumacher (2001), which identified poultry patterns in *E. coli* isolates from water samples collected from Shoal Creek and its tributaries, even during the summer months when litter application generally was decreased. The potential for land application of poultry litter to affect fecal bacteria densities in Shoal Creek and its tributaries is compounded by the fact that litter is removed from most poultry barns and spread to fields during the spring, a time when the potential for runoff from spring thunderstorms probably is the greatest.

SUMMARY AND CONCLUSIONS

Results of the study described in this report indicate that densities of fecal coliform bacteria along a 5.7-mi (mile) reach of Shoal Creek between State Highway 97 (site 3) and State Highway W (site 2) exceeded the Missouri Department of Natural Resources (MDNR) standard of 200 col/100 mL (colonies per 100 milliliter). Fecal coliform densities above the MDNR standard also were detected in two tributaries, Pogue Creek and Joyce Creek, along this reach of Shoal Creek. A combination of techniques was used in this study to determine the possible sources of the increased fecal coliform densities along this reach of Shoal Creek, including a determination of the survival or "die-off" rates of fecal coliform and *Escherichia coli* (*E. coli*) in the stream, a dye-trace study to determine travel times and dispersion characteristics along this stream reach, a detailed seepage study and water-quality sampling to locate specific sources of fecal bacteria to the stream, and a field study to monitor the quantity and survival of fecal bacteria in land applied poultry litter. A network of five sites on this reach of Shoal Creek (sites 2, 2a, 2b, 2c, and 3) was monitored during this study, in addition to a downstream site (site 4), and two tributaries—Pogue Creek and Joyce Creek.

Measurements of fecal bacteria survival in Shoal Creek during July 2001 indicated that about 8 percent of fecal coliform and *E. coli* bacteria decay each hour with an average first-order decay constant of 0.084 h^{-1}

(per hour). Results of a dye-trace study indicate that at low base-flow conditions, the travel time in Shoal Creek between State Highway W (site 2) and the MDNR sampling site (site 3) about 5.7-mi downstream at State Highway 97 is about 26 hours. Substantial dispersion and dilution of the dye peak occurred between the injection site (site 2) and site 2c (about 4.1 mi downstream) where there are several inflows, long pools, and channel meanders; however, minimal dispersion and dilution of the dye peak occurred between site 2c and the MDNR sampling site (site 3) about 1.6-mi downstream.

Measurements of fecal bacteria densities for 36 hours during the July 2001 dye-trace study indicated that samples from site 2a contained the largest average densities of fecal coliform (2,600 col/100 mL) and *E. coli* (2,200 col/100 mL) detected in the main stem of Shoal Creek. Average densities of fecal coliform and *E. coli* decreased to less than or equal to 1,400 col/100 mL downstream at site 2b, and further decreased to less than 500 col/100 mL at site 2c, but increased significantly at site 3. At site 3 the average fecal coliform and *E. coli* densities were 2,100 and 1,400 col/100 mL. The largest bacteria densities were detected in two samples from the upper reaches of Pogue Creek, where densities of fecal coliform and *E. coli* exceeded 4,000 col/100 mL. Densities of fecal bacteria at sampling sites along the main stem of Shoal Creek exhibited sinusoidal variability with time, with bacterial densities at sites 2, 2b, and 2c generally being in phase with each other, but out of phase with bacteria densities at sites 2a and 3. The variability in the bacteria densities with time could not be attributed to simple advection and dispersion because the patterns at successive downstream sites were not predictable based solely on travel time between the sites. Large concentrations of fecal bacteria were not detected in streambed-sediment samples from Shoal Creek, and re-suspension of these sediments during runoff probably is not a large source of fecal bacteria to the water column.

Results of a mass balance of bacteria densities indicated that substantial amounts of fecal bacteria enter Shoal Creek between sites 2 and 2a and between sites 2c and the MDNR sampling site (site 3). Results of a detailed seepage study upstream from site 3 indicated that cattle likely are a large source of the increased fecal bacteria densities at site 3. Substantial increases in fecal bacteria densities were observed downstream from areas where cattle were fed along the stream bank or where cattle were wading in the stream and congregating under trees along the stream bank. Densities of fecal coliform and *E. coli* increased from 100 and 40 col/100 mL at site 2c to 1,100 and 580 col/100 mL downstream from an area of intense cattle activity immediately upstream from the MDNR sampling site (site 3).

Analysis of selected water samples for a suite of wastewater organic compounds and pharmaceutical compounds detected small (less than 1.5 micrograms per liter) concentrations of seven wastewater compounds and four pharmaceutical compounds in one or more samples. The most frequently detected compounds were caffeine (eight detections), cholesterol, and acetaminophen (three detections each). The antimicrobial agent triclosan was detected at sites 2b and 3, and the common cattle anthelmintic thiabendazole was detected at sites 2 and 2a. The detection of organic compounds associated with human wastewater and thiabendazole indicates that some human and animal wastes probably are entering Shoal Creek.

Results of deoxyribonucleic acid (DNA) fingerprinting of *E. coli* isolates extracted from water samples collected from Shoal Creek and its tributaries during the July 2001 dye-trace study indicated that cattle, horses, and humans were the predominate sources of fecal bacteria in the stream. About 50 percent of the *E. coli* isolates from site 2a were matched to a human source, which was consistent with the detection of several wastewater organic compounds, including caffeine, at this site. About 50 percent of the *E. coli* isolates from Pogue Creek were matched to poultry, which was consistent with a large density of poultry barns in its basin. DNA-fingerprinting indicated that cattle are an important source of *E. coli* (about 50 percent) at the MDNR site (site 3), which is consistent with substantial increases of fecal bacteria densities immediately downstream from areas where cattle congregated in and along the stream bank immediately upstream from this site.

Results of field test plots indicated that substantial numbers of fecal bacteria present in poultry litter can survive in fields for up to 8 weeks after the application of the litter to the land surface. Experiments were done using broiler litter, turkey litter, and composted broiler litter. Median densities of fecal coliform and *E. coli* in water samples from fields increased from less than 60 col/100 mL before the application of turkey and broiler litter to as large as 420,000 and 290,000 col/100 mL after the application of litter. Bacteria densities in the test plots generally decreased in an exponential

manner over time, with decay rates ranging from 0.085 to 0.185 d^{-1} (per day) for fecal coliform to between 0.100 and 0.250 d^{-1} for *E. coli*. However, fecal bacteria densities at the broiler litter compost test plot did not vary significantly after the application. The apparent survival of substantial numbers of fecal bacteria on fields where poultry litter has been applied indicated that runoff from fields where this litter is applied is a potential source of fecal bacteria to vicinity streams for many weeks following application.

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