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Assessment of Possible Sources of Microbiological Contamination and Water-Quality Characteristics of the Jacks Fork, Ozark National Scenic Riverways, Missouri—Phase II

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Abstract

In 1998, an 8-mile reach of the Jacks Fork was included on Missouri's list of impaired waters as required by Section 303(d) of the Federal Clean Water Act. The identified pollutant on the Jacks Fork was fecal coliform bacteria. Potential sources of fecal contamination to the Jacks Fork include a wastewater treatment plant; campground pit-toilet or septic-system effluent; a large commercial, cross-country horseback trail riding facility; canoeists, boaters, and tubers; and cows.

The U.S. Geological Survey, in cooperation with the National Park Service, conducted a study to better understand the extent and sources of microbiological contamination within the Jacks Fork from Alley Spring to the mouth, which includes the 8-mile 303(d) reach. Identification of the sources would provide the National Park Service and the State of Missouri with the information needed to craft a solution of abatement, regulation, prevention, and mitigation with the end result being the removal of the Jacks Fork from the 303(d) list. Fifteen sites were sampled from November 1999 through December 2000. An additional site was sampled one time. Samples were collected mostly during base-flow conditions during a variety of nonrecreational and recreational season river uses. Samples were analyzed for selected fecal indicator bacteria, physical properties, nutrients, and wastewater organic compounds.

During the sampling period, the whole-body-contact recreation standard for fecal coliform (200 colonies per 100 milliliters of sample) was exceeded at three sites on August 10, 2000, and also at one site on May 11, June 7, and October 3, 2000. Fecal coliform densities and instantaneous loads generally increased from background concentrations at the Eminence site, peaked about 2 river miles downstream, and then decreased until the most downstream site sampled. Generally, the largest densities and loads at sites downstream from Eminence not related to wet-weather flow were observed during a trail ride held August 6 to12, 2000.

A 24-hour sample collection effort was conducted the weekend of July 15 and 16, 2000, to investigate the effect that large numbers of swimmers, canoeists, and tubers had on fecal coliform densities in the Jacks Fork. Five or six samples were collected at six sites between Saturday morning and the following Sunday afternoon. No fecal coliform density at any of the sites sampled exceeded the whole-body-contact recreation standard.

Because bacteria survive longer in streambed sediments than in water, a source of bacteria in the water column could be from resuspension of accumulated bacteria from streambed sediments. Water and streambed-sediment samples were collected at three sites on August 3, 2000, 1 week before a trail ride and again at three sites on

August 8, 2000, during a trail ride. Results indicate that fecal coliform bacteria densities increased substantially in the streambed sediment and the water column during the trail ride.

Sixty-five *Escherichia coli* isolates obtained from water samples collected at 9 sites and 23 *Escherichia coli* isolates obtained from streambed-sediment samples collected at 5 sites were submitted for ribotyping analysis. Samples were collected in 2000 during a variety of nonrecreational and recreational season river uses, including trail rides, canoeing, tubing, and swimming. Of the 65 isolates from water samples, 40 percent were identified as originating from sewage, 29 percent from horse, 11 percent from cow, and 20 percent from an unknown source. Of the 23 isolates from streambed-sediment samples, 39 percent were identified as originating from sewage, 35 percent from horse, 13 percent from cow, and 13 percent from unknown sources.

Analysis of physical property (dissolved oxygen, pH, specific conductance, and temperature) and nutrient (dissolved nitrite plus nitrate and total phosphorus) data indicated that overall few statistically significant differences occurred among the main stem sites of the Jacks Fork. A significant increase in total phosphorus concentrations did occur at site 75 immediately downstream from the Eminence Wastewater Treatment Plant, but the effect diminished quickly downstream. Unlike fecal coliform bacteria, most variations in physical property values or nutrient concentrations were related to seasonal changes, time of day the sample was collected, or hydrologic conditions and not to certain recreational activities.

Trace quantities of wastewater organic compounds were detected in all waters sampled for these constituents. Two of the compounds were detected in associated laboratory blanks, and other detected compounds have sources other than sewage effluent. The best indicators of municipal or domestic sewage effluent were the non-ionic detergent metabolites (nonylphenol monoethoxylate, octylphenol monoethoxylate, and para-nonylphenol), phenol, and caffeine; but possible sources of these compounds, which were detected in one or more of the samples, could be the numerous campers, swimmers, and canoeists that were present when the samples were collected.

INTRODUCTION

The Ozark National Scenic Riverways (ONSR), the Nation's first federally protected riverway, was created by an Act of Congress on August 24, 1964, for "the purposes of conserving and interpreting unique scenic and other natural values and objects of historic interest, including preservation of parts of the Current River and the Jacks Fork in Missouri as free-flowing streams, preservation of springs and caves, management of wildlife, and provisions for use and enjoyment of the outdoor recreation resources thereof by the people of the United States" (Public Law 88-492). The primary natural resources protected by the ONSR are 134 river mi (miles) of the Current River and Jacks Fork (fig. 1). About 1.5 million people visit the ONSR annually for recreational purposes, including canoeing, johnboating, swimming, fishing, tubing, camping, hiking, caving, horseback riding, and hunting.

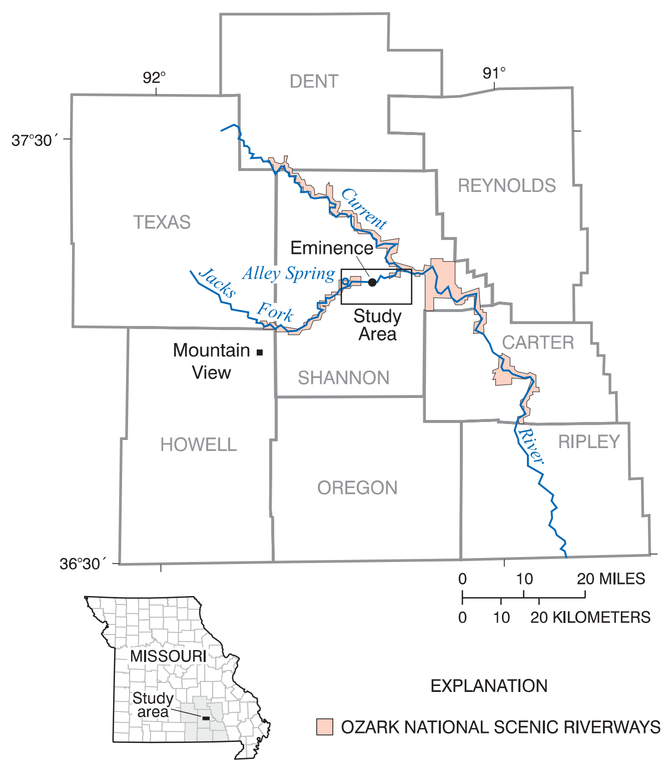

Figure 1. Location of study area.

The Jacks Fork is the largest tributary of the Current River, and similar to the Current River, has been classified as an Outstanding National Resource Water (Missouri Department of Natural Resources, 2000). Outstanding National Resource Waters have national recreational and ecological significance and receive special protection against any degradation in quality. The Jacks Fork has been designated for the following five beneficial uses by the State of Missouri: livestock and wildlife watering, aquatic life protection, cool-water fishery, whole-body-contact recreation, and boating and canoeing (Missouri Department of Natural Resources, 2000).

The intense recreational use of the Jacks Fork has caused concerns regarding the effects that this use might be having on the river. A river use management plan prepared by the National Park Service (NPS) (Sullivan and others, 1989) states that the increasing popularity of the recreational area has created concerns associated with greater competition for the use of a finite resource base. Also, because of inappropriate or intensive use, resource damage has increased in some areas. Concerns include crowding and increased conflicts between river users, the need to improve and provide more sanitation facilities, the proliferation of litter, congestion at river accesses and campgrounds, and balancing the need to protect water quality with the recreational needs of the public.

Section 303(d) of the Federal Clean Water Act requires that each State identify those stream segments with documented pollution problems for which existing pollution controls are not adequate to implement the State water-quality standards. For these impaired stream segments, States are required to establish total maximum daily loads (TMDL) of the identified pollutant. A TMDL specifies the maximum amount of the identified pollutant allowed to be present in a water body, allocates allowable pollutant loads among sources, and provides the basis for attaining or maintaining water-quality standards within the affected water body.

In 1998, an 8 river mi reach of the Jacks Fork was included on Missouri’s list of impaired waters as required by Section 303(d) of the Federal Clean Water Act. The identified pollutant on the Jacks Fork was fecal coliform bacteria, whose presence in large numbers indicates contamination by fecal wastes of humans and other warm-blooded animals. The State fecal coliform standard for safe whole-body-contact recreation is a maximum of 200 col/100 mL (colonies per 100 milliliters) of sample (Missouri Department of Natural Resources, 2000). In the Jacks Fork Basin, the standard applies only to the main stem of the Jacks Fork during base-flow conditions during the recreational season from April 1 through October 31. Potential sources of fecal contamination to the Jacks Fork include the Eminence Wastewater Treatment Plant (WWTP); campground pit-toilet or septic-system effluent; a large commercial, cross-country horseback trail riding facility; canoeists, boaters, and tubers; and cows. Studies conducted by the U.S. Geological Survey (USGS) (Barks, 1978; Davis and Bell, 1998), Emrie (1986), NPS (National Park Service, written commun., 1997), and the Missouri Department of Natural Resources (MDNR) (1998) have indicated that intense recreational use is causing adverse affects on the water quality of the river, including fecal coliform bacteria densities that, on occasion, exceed the water-quality standard for whole-body-contact recreation.

Substantive regulatory efforts by the State to control and eliminate fecal coliform bacteria inputs to the Jacks Fork depend on identification of sources. The USGS, in cooperation with the NPS, is conducting a three-phase study to better understand the extent and sources of microbiological contamination within the Jacks Fork from Alley Spring to the mouth (fig. 1), which includes the 8 river mi 303(d) reach. The results of this study are expected to provide the NPS and other natural resource management agencies in Missouri with information needed to make effective resource-management decisions. Specific objectives of the three-phase study include determining the location and magnitude of microbiological contamination (Phase I); establishing a water-quality sampling network to further document and understand the sources of microbiological contamination (Phase II); and establishing sampling locations for routine long-term water-quality monitoring (Phase III).

A summary of the Phase I study is described in Davis and Richards (2001). The purpose of this report is to describe Phase II of the study. This report includes a description of the study area, including geology, climate, land use, and population; a description of the sampling network; a description of the sample collection and analysis methods; and an assessment of the microbiological contamination and water quality of the Jacks Fork using statistical and graphical methods.

DESCRIPTION OF STUDY AREA

The Jacks Fork is in the Ozark Plateaus physiographic province (Fenneman, 1938) in southeast Missouri, which is an area characterized by deep, narrow valleys and sharp ridges (fig. 2). The river flows through mature karst topography and gains most of its base flow from springs. Karst topography (springs, sinkholes, and losing streams) and structural features (folds, faults, and fractures) greatly affect water quantity and quality. From its source in Texas County, the Jacks Fork drains an area of about 422 mi^2 (square miles); the average discharge of the Jacks Fork at Eminence, Missouri, is 466 ft^3/s (cubic feet per second) (Hauck and Nagel, 2000). Alley Spring (fig. 3), Missouri's seventh largest spring, discharges an average of about 125 ft^3/s into the Jacks Fork (Vandike, 1995) about 6 river mi upstream from the town of Eminence (fig. 1).

Figure 2. Typical reach of the Jacks Fork immediately upstream from Alley Spring. Photograph courtesy of R.B. Jacobson, U.S. Geological Survey.

Josiah Bridge mapped and described the geology of much of the ONSR area (Barks, 1978). Geologic mapping of the Eminence 7-1/2 minute quadrangle was completed in 2000, and additional quadrangles are currently (2002) being mapped in the area (R.W. Harrison, U.S. Geological Survey, written commun., April 2001). The ONSR area is situated on the southwest slope of the St. Francois Mountains. Precambrian igneous knobs crop out east of Eminence near the center of the ONSR. The igneous rocks are mainly rhyolite, a dense, fine-grained to porphyritic rock that generally is red but may be gray or green. Streams and orifices of large springs are in the early Ordovician Gasconade Dolomite and late Cambrian Eminence and Potosi Dolomites. These cherty dolomites generally are several hundred feet thick, light gray to brown, and extremely soluble. Solution-enlarged openings throughout the formations store and transport large quantities of water. Soils produced from weathering of the dolomitic rock are deep red clays with numerous small chert fragments. The Roubidoux Formation, which is composed of interbedded sandstones and dolomites, overlies the Gasconade Dolomite and caps the divides between most of the streams. Alluvial material in the Jacks Fork is comprised of boulders, cobbles, gravel, and sand overlying bedrock.

The Jacks Fork area has a temperate climate with average annual precipitation of about 42 in/yr (inches per year). Average monthly precipitation generally is greatest in the spring [March through May; about 4 to 5 in/mo (inches per month)] and least in the late fall and winter (December through February; about 2 to 3 in/mo; Dugan and Peckenpaugh, 1985). Mean annual air temperature is 56 °F (degrees Fahrenheit); the mean air temperature during January is 32 °F and during July is 78 °F (Dugan and Peckenpaugh, 1985). Monthly precipitation during the sampling period at Eminence, Missouri (November 1999 through December 2000), generally was below average (fig. 4).

Land use in the Jacks Fork Basin primarily is forest (76 percent) and agricultural (23 percent). Second-growth, deciduous forest mixed with evergreen forest is predominant. About 92 percent of the agricultural land is used for pasture or hay production (Missouri Spatial Data Information Service, 2001). The permanent population in the Jacks Fork Basin is about 8,000; the largest towns are Eminence (548) and Mountain View (2,430) (University of Missouri, 2001). Estimates of monthly horseback trail riders, canoe (fig. 5), tube, and angler use for 1999 and 2000 were compiled by the NPS as part of the ONSR rangers' monthly visitor use reports (table 1). During the Phase II sampling period, six guided week-long trail rides were held on May 7 to

Figure 3. Mill at Alley Spring. Photograph courtesy of C.A. Pepmiller, U.S. Geological Survey.

13, June 4 to 10, August 6 to 12, October 1 to 7, November 5 to 11, and December 3 to 8, 2000. The number of trail riders (fig. 6) is based only on guided trail rides inside the ONSR and does not include unguided rides in the ONSR. This estimate is, therefore, substantially less than the actual number of trail riders that participate in any of the week-long trail rides. During the largest trail rides held in June, August, and October 2000, an estimated 3,000 total trail riders may have taken advantage of the recreational opportunities on the Jacks Fork (Dr. Kenneth Chilman, Southern Illinois University at Carbondale, written commun., 2001).

METHODS OF STUDY

To determine the microbiological contamination and water quality of the study area, a network of sampling sites was established. Water samples were collected from these sites and analyzed for a variety of indicator bacteria and chemical constituents.

Description of Sampling Network

The locations of microbiological contamination were determined in Phase I through three intensive synoptic surveys (Davis and Richards, 2001). A synoptic survey

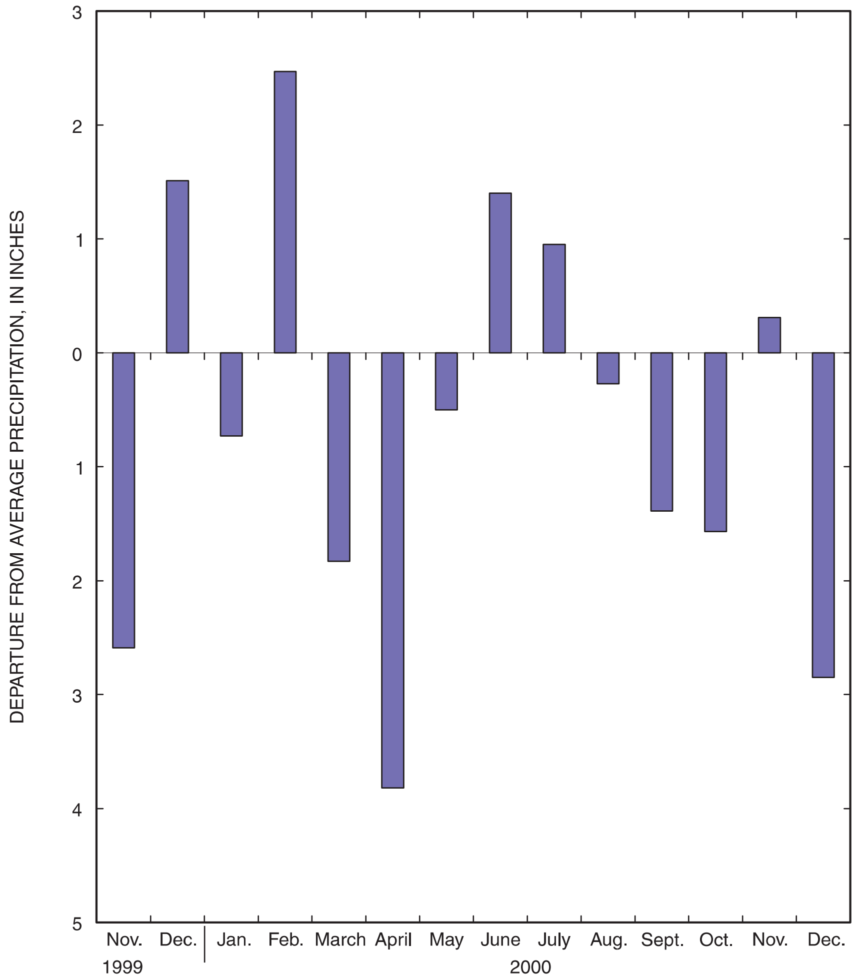

Figure 4. Departure from average monthly precipitation at Eminence, Missouri, November 1999 through December 2000. Data from National Oceanic and Atmospheric Administration (1999, 2000).

consists of the measurement of selected constituents at many sites during a brief period representative of a particular hydrologic or seasonal condition. A reconnaissance of the Jacks Fork from the Alley Spring Campground to the mouth was done to locate sampling sites, locate potential sources of microbiological contamination, and map hydrologic features for inclusion in a geographic information system (GIS) database. Each location or feature was identified by geographic coordinates as determined by a global positioning system (GPS). Based on information collected during the reconnaissance, 42 sampling sites, including the Eminence WWTP, were selected (Davis and Richards, 2001). These sites were located on tributaries, spring branches, and on the main stem of the Jacks Fork near potential sources of microbiological contamination. The first intensive synoptic survey was done May 10 to 12, 1999, during spring base-flow conditions at the beginning of the recreational season (defined by the State of Missouri as April 1 through October 31). The second and third intensive synoptic surveys were done during the recreational season on June 22 to 24 and August 10 to 12, 1999, during early-summer and late-summer base-flow conditions.

Figure 5. Canoeing on the Jacks Fork.

Table 1. Estimates of Jacks Fork visitor use for 1999 and 2000

Month	Horseback riders[a]	Canoes[b]	Tubes[b]	Anglers[c]
		1999[d]		
January	137	0	0	119
February	0	20	0	82
March	6	47	0	104
April	41	186	0	233
May	125	665	5	729
June	65	3,326	497	986
July	224	4,302	387	1,786
August	296	3,760	308	382
September	49	1,079	98	453
October	403	222	0	398
November	142	12	0	282
December	25	4	0	154
Total	**1,513**	**13,623**	**1,295**	**5,708**
		2000[d]		
January	31	8	0	209
February	17	20	0	115
March	12	50	0	150
April	213	225	0	457
May	387	828	5	1,176
June	787	3,513	114	1,454
July	383	4,561	566	760
August	283	4,075	363	1,892
September	254	1,665	91	558
October	642	150	0	428
November	126	29	0	286
December	16	20	0	43
Total	**3,151**	**15,144**	**1,139**	**7,528**

[a] The number of horseback riders is derived from both the cross-country trail riding facility and National Park Service rangers during their normal duties in the area. The cross-country trail riding facility does not report all riders participating in a week-long trail ride. Only guided trail rides inside the Ozark National Scenic Riverways are reported; unguided trail riders in the Ozark National Scenic Riverways are not counted by the cross-country trail riding facility.

[b] The number of canoes and tubes reflects the number of canoes and tubes reported as rented by concessionaires in the area.

[c] The number of anglers reflects the number of anglers observed by National Park Service rangers during their normal duties in the area.

[d] These numbers are totals for the entire Jacks Fork within the boundaries of the Ozark National Scenic Riverways derived from the National Park Service rangers' monthly visitor use reports.

Figure 6. Trail riders crossing the Jacks Fork.

Phase I results indicated that fecal coliform bacteria are a concern in the Jacks Fork (Davis and Richards, 2001). Fecal coliform bacteria densities and loads generally were larger in the 303(d) reach downstream from Eminence than upstream from Eminence during the June 22 to 24 and August 10 to 12, 1999, synoptic surveys. The State standard for whole-body-contact recreation was exceeded at two sites in the 303(d) reach during the June synoptic survey and at five sites in the 303(d) reach during the August synoptic survey.

To further understand the sources of microbiological contamination in the Jacks Fork, sampling was done during Phase II (November 1999 through December 2000) at 16 of the 42 sites from Phase I (fig. 7, table 2). An additional site not sampled during Phase I was sampled one time. Samples were collected mostly during base-flow conditions during a variety of nonrecreational and recreational season river uses, including canoeing, swimming, tubing, camping, and horseback riding. River conditions were above base flow when samples were collected during December 1999 and late February and early March 2000. Continuously recording streamflow gaging stations are located on the Jacks Fork immediately downstream from site 5 and at Eminence (site 60; fig. 7). The hydrologic conditions at the two gaged sites and river uses occurring during Phase II sampling are shown in figure 8 and listed in table 3. Although the recreational season is defined as occurring between April 1 and October 31, most recreational river use takes place between late May and early September.

Sample Collection and Analysis Methods

During Phase II, 15 sites were sampled approximately monthly from November 1999 through April 2000 and from September 2000 through December 2000 and twice per month from May 2000 through August 2000 (table 3). Samples were collected and analyzed by the USGS at each site for indicator bacteria [fecal coliform, *Escherichia coli* (*E. coli*), fecal streptococci, and enterococci] using the membrane filtration procedure described in Myers and Wilde (1997). Indicator bacteria samples were collected in a sterile 500-mL (milliliter) polypropylene bottle by facing the bottle into the current and dipping quickly into the stream at 3 to 5 equally spaced locations in the stream cross section. The samples were placed on ice and held a maximum of 6 hours until processing. Because densities of indicator bacteria can be quite variable, generally two sample volumes ranging from 10 to 100 mL were filtered from individual stream samples. Reagent blanks were run twice each day to check for contamination of equipment and reagents.

Samples for the enumeration of fecal indicator bacteria (fecal coliform and *E. coli*) in streambed sediments were collected at selected sites in August 2000 using a modification of the membrane filtration procedure described in Francy and Darner (1998). Streambed sediments, which consist primarily of gravel, sand, and silt, were collected by scooping the stream bottom with a sterile wide-mouth 250-mL polypropylene jar. The jars were kept covered until they touched the stream bottom to minimize contamination by the overlying water and then quickly closed after scooping the sediment. Streambed sediments were subsampled in three locations in the stream cross section. The samples were placed on ice and held no more than 24 hours before processing. The three subsamples were composited in a sterile 1-L (liter) polypropylene bottle and then split into two sterile 500-mL polypropylene jars. Phosphate buffer was added to one of the subsamples to isolate fecal coliform, and saline buffer was added to the other subsample to isolate *E. coli*. The samples were shaken vigorously for 5 minutes, the buffer-sediment mixture decanted into two 250-mL polypropylene bottles, and the sediment allowed to settle. Three sample volumes ranging from 1 to 10 mL were filtered from the individual streambed-sediment sample extracts for analysis of fecal coliform and *E. coli*.

A technique known as Microbial Source Tracking (MST) was used in this study to help identify the primary sources of *E. coli* in water and streambed-sediment samples. Ribotyping, which can be used for MST, has been shown to be useful in discriminating between human and nonhuman sources of *E. coli* in water samples (Hartel and others, 1999; Parveen and

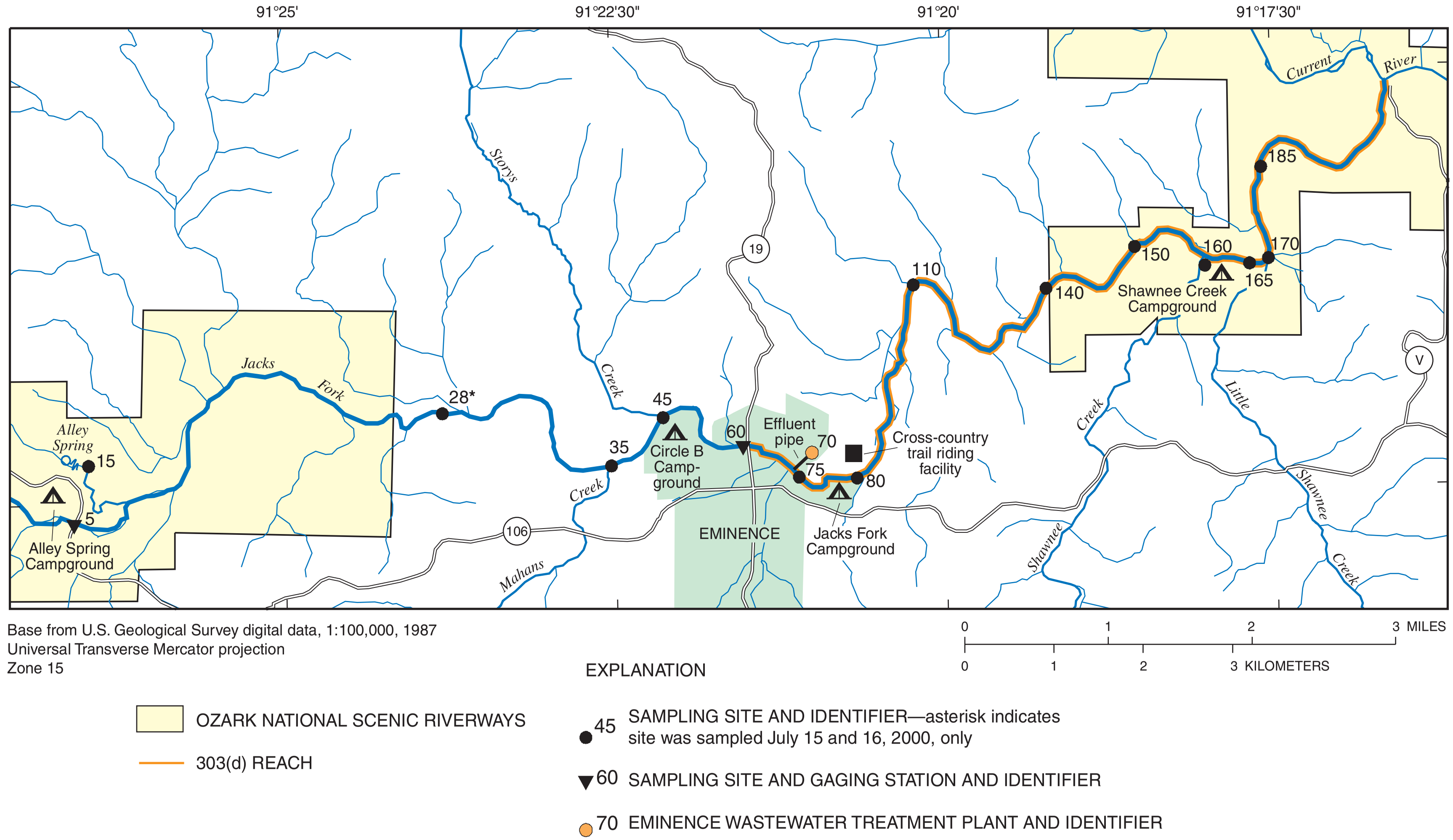

Figure 7. Location of Jacks Fork sites sampled in Phase II.

Table 2. Jacks Fork sampling sites, Phase II

[mi^2, square miles; NA, not applicable]

Site number (fig. 7)	Site name	Latitude	Longitude	Drainage area (mi^2)
5	Jacks Fork above Alley Spring	370857	912659	302
15	Alley Spring at Alley	370914	912629	a
28	Jacks Fork above Eminence[b]	370931	912341	340
35	Mahans Creek above Eminence	370911	912232	54.1
45	Storys Creek above Eminence	370928	912208	5.34
60	Jacks Fork at Eminence	370918	912131	404
70	Eminence Wastewater Treatment Plant	370915	912101	NA
75	Jacks Fork below Wastewater Treatment Plant below Eminence	370907	912108	405
80	Jacks Fork above 2nd unnamed hollow (south) below Eminence	370905	912040	406
110	Jacks Fork above Lick Log Hollow below Eminence	371014	912013	409
140	Jacks Fork above Bald Knob Hollow below Eminence	371012	911913	412
150	Jacks Fork above Powell Spring above Two Rivers	371026	911833	413
160	Shawnee Creek above Two Rivers	371019	911801	20.0
165	Jacks Fork above Little Shawnee Creek above Two Rivers	371020	911741	433
170	Little Shawnee Creek above Two Rivers	371021	911732	9.4
185	Jacks Fork below 3rd unnamed hollow (north) above Two Rivers	371054	911735	444

[a] Recharge area of Alley Spring is estimated to be greater than 125 square miles (Aley and Aley, 1987).
[b] This site was sampled only during the weekend of July 15 and 16, 2000.

others, 1999; Schlottmann and others, 2000; Carson and others, 2001). Ribotyping involves isolating pure cultures of *E. coli* from both an environmental sample and known-source feces. The deoxyribonucleic acid (DNA) is isolated from these pure bacterial strains, cut in fragments using a restriction enzyme, and the resulting fragments are separated by molecular weight using agarose-gel electrophoresis. Hybridization with a labeled DNA probe creates a chemiluminescent pattern of the fragments containing ribosomal ribonucleic acid (rRNA) genetic information. The resulting banding patterns from water-isolated *E. coli* are compared to the banding patterns of isolated *E. coli* from known-source feces for similarity and possible identification. Water samples for ribotyping analysis were collected from selected sites in April, June, July, and August 2000 using the same technique described previously for indicator bacteria and sent by overnight mail to the College of Veterinary Medicine at the University of Missouri in Columbia (UMC), Missouri. For this study, the unknowns were compared to the banding patterns from sewage, horses, and cows.

Samples for nutrient analyses (dissolved nitrite plus nitrate, ammonia, and orthophosphate and total ammonia plus organic nitrogen and phosphorus) also were collected at the 15 Phase II sites according to standard USGS sample collection and processing protocols described by Edwards and Glysson (1998) and Wilde and others (1999a, 1999b). All chemical analyses were done by the USGS National Water Quality Laboratory in Lakewood, Colorado, according to procedures described in Fishman and Friedman (1989) or Fishman (1993) (table 4). Selected sites (sites 5, 15, 60, 80, and 110; fig. 7; table 2) also were sampled for wastewater organic compounds (table 5) on July 16 and August 10, 2000 (Zaugg and others, 2002). Onsite measurements of dissolved oxygen (DO), pH, specific conductance, and temperature were made at each site according to procedures described by Wilde and Radtke (1998).

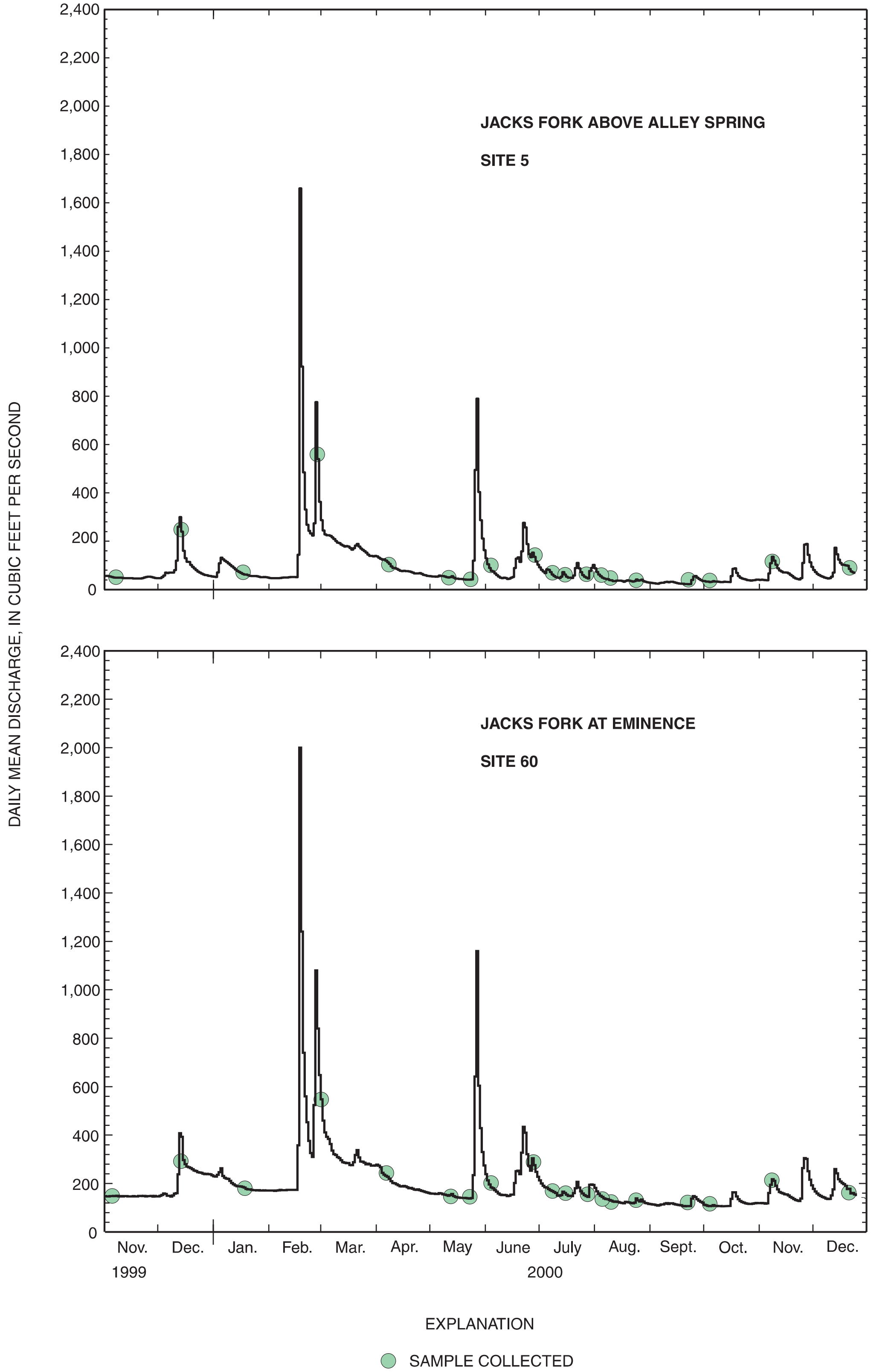

Figure 8. Hydrologic condition during Jacks Fork sampling at two gaged sites, November 1999 through December 2000.

Table 3. Sample collection dates, hydrologic conditions, and predominant river uses during sampling

Date of sample collection	Hydrologic condition	River use
November 8–10, 1999	Base flow	Nonrecreational season (trail ride October 31–November 6)
December 14–16, 1999	Falling stage	Nonrecreational season
January 18–20, 2000	Base flow	Nonrecreational season
February 29–March 2, 2000	Falling stage	Nonrecreational season
April 4–6, 2000	Base flow	Early recreational season
May 10–12, 2000	Base flow	Trail ride, canoeing
May 23–25, 2000	Base flow[a]	Canoeing, swimming, tubing, and camping
June 6–8, 2000	Base flow	Trail ride, canoeing, swimming, tubing, and camping
June 28–30, 2000	Base flow	Canoeing, swimming, tubing, and camping
July 10–12, 2000	Base flow	Canoeing, swimming, tubing, and camping
July 15–16, 2000	Base flow	Canoeing, swimming, tubing, and camping
July 26–28, 2000	Base flow	Canoeing, swimming, tubing, and camping
August 2, 2000	Base flow	Canoeing, swimming, tubing, and camping
August 8–11, 2000	Base flow	Trail ride, canoeing, swimming, tubing, and camping
August 21–22, 2000	Base flow	Canoeing, swimming, tubing, and camping
September 19–20, 2000	Base flow	Late recreational season
October 3–4, 2000	Base flow	Trail ride and late recreational season
November 8–9, 2000	Base flow	Trail ride and nonrecreational season
December 12 and 20, 2000	Base flow	Nonrecreational season

[a] Base-flow conditions existed on May 23 and 24. Rainfall on the evening of May 24 and the early morning of May 25 resulted in runoff. The whole-body-contact recreation standard does not apply during wet-weather flow.

Stream discharge was measured at all tributary sites and at most of the other Jacks Fork main stem sites during sample collection.

Twenty-two quality-assurance samples were collected at the 15 sites. The quality-assurance samples included 8 field equipment blanks (FEB) collected to monitor for contamination and carry-over between environmental samples and 14 replicate environmental samples collected to monitor analytical precision. Nutrients were analyzed in the FEB and replicate samples. Most constituent concentrations were less than the method reporting level (MRL) in the 8 FEB with the following exceptions: total ammonia plus organic nitrogen detected in 4 FEB [MRL 0.10 mg/L (milligram per liter), and detected concentrations ranged from an estimated concentration of 0.06 to 0.18 mg/L]; and dissolved orthophosphate detected in 1 FEB (MRL 0.01 mg/L, detected concentration of 0.012 mg/L). Nutrient concentrations in the replicate environmental samples were comparable and within laboratory analytical error.

Data Analysis Methods

The indicator bacteria, physical property, and nutrient data were evaluated to determine factors affecting their occurrence in the Jacks Fork and tributaries. Descriptive statistics were used to show the central tendency and variation in the data. The minimum and maximum and the values at the 25th, 50th (median), and 75th percentiles were calculated. The water-quality data collected during Phase II are available in Hauck and Nagel (2001).

Table 4. Analytical methods and method reporting levels

[mg/L, milligram per liter; N, nitrogen; ASF, automated-segmented flow; P, phosphorus; EPA, U.S. Environmental Protection Agency]

Analyte	Analytical method[a]	Method number	Method reporting level (mg/L)
Nitrite plus nitrate, dissolved as N	Colorimetry, ASF	I-2545-90	0.05
Ammonia, dissolved as N	Colorimetry, ASF	I-2522-90	.02
Ammonia plus organic nitrogen, total as N	Colorimetry, ASF, microkjeldahl digestion	I-4515-91	.10
Phosphorus, total as P	Colorimetry, ASF, microkjeldahl digestion	EPA 365.1	.004
Orthophosphate, dissolved as P	Colorimetry, ASF	I-2601-90	.01

[a]Fishman, 1993 and Fishman and Friedman, 1989.

The distribution of selected indicator bacteria, physical property, and nutrient constituent data was graphically displayed using side-by-side boxplots (fig. 9; Helsel and Hirsch, 1992, p. 24–26). The plots show the center of the data (median—the center line of the box), the variation (interquartile range—the box height), the skewness (quartile skew—the relative size of the box halves), and the presence or absence of unusual values ("outside" or "far-out" values). The boxplot consists of a center line (the median) splitting a rectangle defined by the interquartile range (25th to 75th percentiles) and whiskers that extend to the last observation within one step beyond either end of the box ("adjacent values"). A step equals 1.5 times the height of the box. Observations between one and two steps from the box in either direction are plotted with an asterisk ("outside values"), and observations farther than two steps beyond the box are plotted with a circle ("far-out values"). If the median equals the 25th percentile, no center line will be present. If the median equals the 25th and 75th percentiles, the box is represented by a line. Boxplots constructed for sites with censored data (data reported as less than some threshold) were modified by making the lower limit of the box equal to the MRL.

The nonparametric Kruskal-Wallis analysis-of-variance test (Helsel and Hirsch, 1992, p. 163) was used to test for differences in the distributions of the data from Alley Spring and the nine Jacks Fork main stem sampling sites. The distributions were considered significantly different from one another if the probability (p-value) was less than 5 percent (less than 0.05) that the observed difference occurred by chance. If a statistically significant difference was detected between the sites, individual differences were evaluated by applying Tukey's multiple comparison test to the rank-transformed data (Helsel and Hirsch, 1992, p. 196).

ASSESSMENT OF MICROBIOLOGICAL CONTAMINATION

To determine the probable sources of fecal bacteria, multiple lines of evidence are necessary, including microbiological, physical, chemical, and hydrological data and ancillary information on land, water, and recreational use of the resource. The indicator bacteria analyzed for this study include fecal coliform, *E. coli*, fecal streptococci, and enterococci. Summary statistics of the indicator-bacteria data for each of the 15 sites are listed in table 6. A complete analysis of the fecal coliform data is presented in the following sections. Results for *E. coli*, fecal streptococci, and enterococci are similar to those for fecal coliform and will not be discussed.

Fecal Indicator Bacteria Densities and Loads

Fecal indicator bacteria are measures of the sanitary quality of water. The density of these bacteria is one indicator of whether water is safe for whole-body-contact recreation and consumption and/or free from disease-causing organisms. Indicator bacteria are not typically disease causing, but two indicator organisms, *E. coli* and enterococci, have been strongly correlated

Table 5. Wastewater organic compounds analyzed in selected water samples

[All compounds analyzed by gas chromatography/mass spectrometry using method O-1433-01 (Zaugg and others, 2002); µg/L, micrograms per liter]

Compound	Method reporting level (µg/L)	Compound	Method reporting level (µg/L)
Non-ionic detergent metabolites		**Stimulants and metabolites**	
NPEO1 (nonylphenol monoethoxylate)	1.00	Caffeine	0.08
NPEO2 (nonylphenol diethoxylate)	1.10	Codeine (analgesic)	.20
OPEO1 (octylphenol monoethoxylate)	.10	Cotinine (nicotine metabolite)	.04
OPEO2 (octylphenol diethoxylate)	.20		
para-Nonylphenol (total)	.50	**Plasticizers and polymer precursors**	
		bis(2-Ethylhexyl) adipate	2.00
Disinfectants		Bisphenol A (polymer manufacture)	.09
Phenol	.25	bis(2-Ethylhexyl) phthalate	2.50
Triclosan (antimicrobial)	.05	Diethylphthalate	.25
		Ethanol, 2-butoxy-, phosphate	.20
Fecal indicator and hormones		Phthalic anhydride (plastic manufacture)	.29
3-beta-Coprostanol (carnivores)	.60	Triphenyl phosphate	.10
Cholesterol	1.50		
17-beta-Estradiol (estrogen metabolite)	.50	**Polynuclear Aromatic Hydrocarbons (PAHs)**	
Stigmastanol (plant sterol)	2.00	Anthracene	.06
		Benzo[a]pyrene	.05
Fire retardants		Fluoranthene	.03
Tri(2-chloroethyl) phosphate	.04	Naphthalene	.02
Tri(dichloroisopropyl) phosphate	.10	Phenanthrene	.05
		Pyrene	.03
Fragrance			
Acetophenone	.22	**Food and other preservatives**	
		5-Methyl-1H-benzotriazole (industrial use)	.10
Fumigant		2,6-Di-tert-butylphenol	.08
1,4-Dichlorobenzene	.03	2,6-Di-tert-para-benzoquinone	.50
		Butylated hydroxyanisole (BHA)	.12
Pesticides		Butylated hydroxytoluene (BHT)	.08
Carbaryl	.06	para-Cresol (wood preservative)	.06
Chlorpyrifos	.02		
cis-Chlordane	.04	**Solvent**	
Dieldrin	.08	Tetrachloroethylene	.03
Diazinon	.03		
Lindane	.05		
Methyl parathion	.06		
N,N-diethyltoluamide (DEET)	.04		

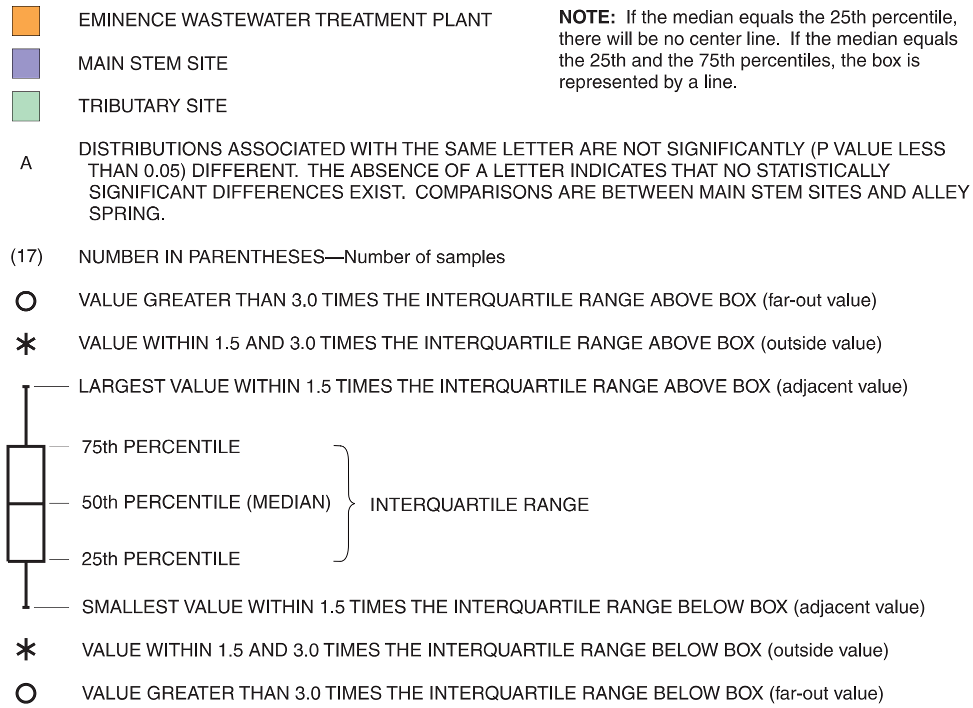

Figure 9. Boxplot example.

to the presence of water-borne pathogens (U.S. Environmental Protection Agency, 1986). Common sources of fecal indicator bacteria include municipal wastewater-treatment effluents that have not been disinfected; septic tanks; animal wastes from feedlots, barnyards, and pastures; and stormwater. The fecal indicator bacteria species used in the study are bacteria of the fecal coliform and fecal streptococci groups, *E. coli*, and enterococci; all of which commonly are found in the intestinal tracts of warm-blooded animals. *E. coli* and enterococci are strictly inhabitants of the gastrointestinal tracts of warm-blooded animals, and their presence in water is direct evidence of fecal contamination from warm-blooded animals and the possible presence of pathogens (Dufour, 1977).

Because fecal bacteria normally inhabit the gastrointestinal tracts of warm-blooded animals, they die off within days of exposure to the relatively cold environment of streams. Fecal bacteria have been shown to survive from a few hours to a few days in streams and a few weeks to a few months in streambed sediments (Roszak and Colwell, 1987; Doyle and others, 1992; Pommequy and others, 1992; Myers and others, 1998). Bacterial decay results from cell starvation, predation by stream protozoans, and destruction by the ultraviolet component of sunlight and other physicochemical processes such as chlorination (U.S. Environmental Protection Agency, 1985; Roszak and Colwell, 1987).

During the sampling period, the whole-body-contact recreation standard (200 col/100mL)—applicable from April 1 through October 31 during base-flow conditions on the main stem of the Jacks Fork—was exceeded at one or more sites on four sampling occasions. The standard was exceeded at sites 80, 110, and 140 on August 10, 2000 (210, 340, and 210 col/100 mL of sample) and also at site 110 on May 11 (340 col/100 mL), June 7 (240 col/100 mL), and October 3, 2000 (780 col/100 mL) (fig. 10; site 140 is not included on the figure because fewer samples were collected at site 140 than at the other sites shown on the graph). Each of these exceedences occurred in samples collected during trail rides (table 3). Values above the standard also were measured in samples collected on May 25, 2000, at sites 165 and 185 (450 and 490 col/100 mL), but these probably occurred as a result of runoff during wet-weather flow when the standard does not apply (fig. 8).

The distribution of fecal coliform bacteria densities at nine main stem sites, five tributary sites, and the Eminence WWTP is shown in figure 11. The only statistically significant difference (p value less than 0.05)

Table 6. Statistical summary of indicator bacteria, physical property, and nutrient data for samples collected on the Jacks Fork, tributaries, and the Eminence Wastewater Treatment Plant, November 1999 through December 2000

[col/100 mL, colonies per 100 milliliters; *E. coli*, *Escherichia coli*; ft^3/s, cubic feet per second; mg/L milligrams per liter; µS/cm, microsiemens per centimeter at 25 degrees Celsius; °C, degrees Celsius; N, nitrogen; P, phosphorus; <, less than; --, no data available]

	Fecal coliform (col/100 mL)	*E. coli* (col/100 mL)	Fecal streptococci (col/100 mL)	Enterococci (col/100 mL)	Discharge (ft^3/s)	Dissolved oxygen (mg/L)	pH	Specific conductance (µS/cm)	Water temperature (°C)	Nitrite plus nitrate, dissolved (mg/L as N)	Ammonia, dissolved (mg/L as N)	Ammonia plus organic N, total (mg/L as N)	Phosphorus, total (mg/L as P)	Ortho-phosphate, dissolved (mg/L as P)
Jacks Fork above Alley Spring (site 5, fig. 7)														
Number of samples	18	18	18	14	18	18	18	18	18	18	18	18	18	18
Minimum	<1	<1	<1	3	25	5.8	7.2	246	1.0	<.05	<.02	<.10	<.004	<.01
25th percentile	2	1	14	8	42	8.5	7.9	311	11.7	.09	<.02	.10	<.004	<.01
Median	8	3	40	18	57	9.9	8.1	332	21.9	.11	<.02	.10	<.004	<.01
75th percentile	23	12	67	32	117	10.7	8.2	337	25.5	.22	<.02	.10	<.004	<.01
Maximum	64	55	180	236	359	15.6	8.4	346	29.5	.68	<.02	.14	.005	<.01
Alley Spring at Alley (site 15, fig. 7)														
Number of samples	18	18	18	14	18	18	18	18	18	18	18	18	18	18
Minimum	2	1	4	6	66	7.2	7.5	244	13.6	.53	<.02	<.10	.006	<.01
25th percentile	6	2	27	27	74	9.6	7.7	296	14.0	.56	<.02	<.10	.009	<.01
Median	10	8	41	38	80	10.2	7.7	310	14.6	.58	<.02	<.10	.009	<.01
75th percentile	18	17	50	52	87	10.7	7.8	316	14.9	.63	<.02	.10	.010	<.01
Maximum	92	56	84	124	173	11.3	7.9	322	15.3	.77	<.02	.10	.012	.01
Mahans Creek above Eminence (site 35, fig. 7)														
Number of samples	17	17	17	13	17	17	17	17	17	12	12	12	12	12
Minimum	16	2	24	50	2.8	7.9	7.9	319	3.2	.16	<.02	<.10	<.004	<.01
25th percentile	32	20	94	98	5.4	9.0	8.1	388	11.4	.19	<.02	.10	<.004	<.01
Median	96	48	130	160	8.5	9.5	8.1	411	19.6	.20	<.02	.10	.004	<.01
75th percentile	180	110	220	220	12	10.0	8.1	435	21.9	.26	<.02	.10	.005	<.01
Maximum	440	280	430	1,270	58	14.2	8.3	458	25.9	.32	<.02	.14	.006	<.01
Storys Creek above Eminence (site 45, fig. 7)														
Number of samples	10	10	10	6	10	10	10	10	10	5	5	5	5	5
Minimum	27	16	30	24	.30	6.9	8.0	367	5.7	.15	<.02	.10	<.004	<.01
25th percentile	50	31	60	410	.52	8.0	8.2	427	10.2	--	--	--	--	--
Median	85	64	295	555	.69	9.2	8.3	452	17.8	.19	<.02	.12	.004	<.01
75th percentile	540	320	630	670	1.2	10.2	8.4	466	23.6	--	--	--	--	--
Maximum	1,500	680	1,200	1,200	4.6	13.4	8.5	511	28.4	.28	<.02	.19	.017	<.01
Jacks Fork at Eminence (site 60, fig. 7)														
Number of samples	21	21	21	16	21	21	20	21	21	18	18	18	18	18
Minimum	2	<1	4	<1	110	7.9	7.8	263	4.2	.21	<.02	<.10	<.004	<.01
25th percentile	5	3	15	14	136	9.2	8.0	320	13.0	.28	<.02	<.10	<.004	<.01
Median	25	13	40	27	154	9.9	8.1	327	17.9	.30	<.02	.10	<.004	<.01
75th percentile	35	21	92	62	224	11.2	8.3	331	20.2	.38	<.02	.10	.004	<.01
Maximum	120	86	230	170	542	15.1	8.5	338	24.7	.66	<.02	.13	.010	<.01

Table 6. Statistical summary of indicator bacteria, physical property, and nutrient data for samples collected on the Jacks Fork, tributaries, and the Eminence Wastewater Treatment Plant November 1999 through December 2000–Continued

	Fecal coliform (col/100 mL)	*E. coli* (col/100 mL)	Fecal streptococci (col/100 mL)	Enterococci (col/100 mL)	Discharge (ft^3/s)	Dissolved oxygen (mg/L)	pH	Specific conductance (µS/cm)	Water temperature (°C)	Nitrite plus nitrate, dissolved (mg/L as N)	Ammonia, dissolved (mg/L as N)	Ammonia plus organic N, total (mg/L as N)	Phosphorus, total (mg/L as P)	Ortho-phosphate, dissolved (mg/L as P)
Eminence Wastewater Treatment Plant (site 70, fig. 7)														
Number of samples	13	13	13	9	--	6	6	12	12	9	9	9	9	9
Minimum	<1	<1	<1	<1	--	1.1	7.7	509	9.8	.07	<.02	.43	.87	.77
25th percentile	<1	<1	<1	<1	--	2.2	7.7	608	11.6	7.04	<.02	.75	1.5	1.2
Median	<1	<1	<1	<1	--	3.6	7.7	752	21.2	16.2	<.02	.89	3.0	2.4
75th percentile	<1	<1	2	5	--	5.3	7.8	805	24.0	19.8	.36	1.8	3.0	2.7
Maximum	5	10	21	11	--	5.6	7.8	1040	27.0	24.8	8.2	10	4.5	4.1
Jacks Fork below Wastewater Treatment Plant below Eminence (site 75, fig. 7)														
Number of samples	14	14	14	10	--	14	14	14	14	14	14	14	14	14
Minimum	2	1	5	5	--	8.6	7.9	265	8.9	.29	<.02	<.10	<.004	<.01
25th percentile	11	6	14	16	--	9.2	8.0	317	13.4	.36	<.02	.10	.005	<.01
Median	22	18	44	30	--	10.2	8.1	328	18.0	.38	<.02	.10	.009	<.01
75th percentile	48	24	72	45	--	11.0	8.2	331	21.3	.42	<.02	.10	.016	.01
Maximum	200	180	300	120	--	12.4	8.4	338	25.3	.67	.02	.20	.020	.02
Jacks Fork above 2nd unnamed hollow below Eminence (site 80, fig. 7)														
Number of samples	17	17	17	13	17	17	17	17	17	17	17	17	17	17
Minimum	4	<1	3	5	109	8.9	8.0	265	6.7	.22	<.02	<.10	<.004	<.01
25th percentile	10	3	14	13	128	9.3	8.1	323	13.1	.30	<.02	<.10	<.004	<.01
Median	17	7	34	18	155	10.2	8.2	330	18.1	.34	<.02	.10	.005	<.01
75th percentile	48	41	70	69	228	11.5	8.3	333	19.6	.40	<.02	.10	.007	<.01
Maximum	210	220	700	190	547	14.0	8.5	338	24.2	.66	<.02	.19	.009	<.01
Jacks Fork above Lick Log Hollow below Eminence (site 110, fig. 7)														
Number of samples	19	19	19	15	17	19	19	19	19	18	18	17	17	18
Minimum	<1	<1	4	1	105	7.4	7.8	267	3.3	.22	<.02	<.10	<.004	<.01
25th percentile	18	6	21	10	133	9.4	8.2	321	14.0	.30	<.02	.10	<.004	<.01
Median	50	29	48	60	151	10.4	8.3	331	19.2	.31	<.02	.10	.005	<.01
75th percentile	130	82	160	180	227	11.2	8.4	338	21.5	.36	<.02	.10	.007	<.01
Maximum	780	210	710	1,080	524	14.9	8.5	340	24.4	.64	<.02	.38	.013	.02
Jacks Fork above Bald Knob Hollow below Eminence (site 140, fig. 7)														
Number of samples	12	12	12	10	--	11	12	12	11	12	12	12	12	12
Minimum	1	<1	4	2	--	7.6	8.1	301	9.0	.16	<.02	<.10	<.004	<.01
25th percentile	12	4	16	8	--	9.6	8.1	322	17.9	.27	<.02	.10	<.004	<.01
Median	23	11	36	30	--	10.7	8.3	331	21.8	.28	<.02	.10	.004	<.01
75th percentile	33	16	88	62	--	11.1	8.4	338	22.5	.33	<.02	.11	.006	<.01
Maximum	210	48	210	130	--	12.6	8.5	344	25.7	.40	.02	.18	.009	.01

Table 6. Statistical summary of indicator bacteria, physical property, and nutrient data for samples collected on the Jacks Fork, tributaries, and the Eminence Wastewater Treatment Plant November 1999 through December 2000–Continued

	Fecal coliform (col/100 mL)	*E. coli* (col/100 mL)	Fecal streptococci (col/100 mL)	Enterococci (col/100 mL)	Discharge (ft^3/s)	Dissolved oxygen (mg/L)	pH	Specific conductance (μS/cm)	Water temperature (°C)	Nitrite plus nitrate, dissolved (mg/L as N)	Ammonia, dissolved (mg/L as N)	Ammonia plus organic N, total (mg/L as N)	Phosphorus, total (mg/L as P)	Ortho-phosphate, dissolved (mg/L as P)
Jacks Fork above Powell Spring above Two Rivers (site 150, fig. 7)														
Number of samples	17	17	17	13	16	17	17	17	17	17	17	17	17	17
Minimum	1	<1	6	3	114	6.7	7.4	273	2.6	.21	<.02	<.10	<.004	<.01
25th percentile	15	2	13	8	135	9.1	8.1	324	12.9	.26	<.02	.10	<.004	<.01
Median	33	15	28	14	160	10.7	8.3	333	20.2	.28	<.02	.10	.004	<.01
75th percentile	49	23	46	55	218	11.2	8.4	340	22.5	.35	<.02	.10	.006	<.01
Maximum	100	66	120	220	489	14.5	8.5	346	26.1	.67	<.02	.19	.008	<.01
Shawnee Creek above Two Rivers (site 160, fig. 7)														
Number of samples	17	17	17	13	17	17	17	17	17	12	12	12	12	12
Minimum	<1	2	7	8	2.3	6.0	7.9	338	4.1	.10	<.02	<.10	<.004	<.01
25th percentile	18	13	70	120	3.9	8.7	8.1	458	12.3	.19	<.02	<.10	.004	<.01
Median	72	40	160	170	5.7	9.3	8.1	476	19.6	.23	<.02	.10	.006	<.01
75th percentile	120	82	220	490	11	11.5	8.2	501	20.1	.35	<.02	.10	.009	<.01
Maximum	600	420	1,500	1,000	24	14.5	8.4	526	26.2	.59	<.02	.36	.014	.01
Jacks Fork above Little Shawnee Creek above Two Rivers (site 165, fig. 7)														
Number of samples	17	17	17	13	--	17	17	17	17	17	17	17	17	17
Minimum	3	<1	5	5	--	6.8	7.9	284	5.5	.21	<.02	<.10	<.004	<.01
25th percentile	10	7	12	18	--	9.5	8.2	329	12.8	.25	<.02	<.10	<.004	<.01
Median	50	18	50	61	--	10.4	8.2	335	19.4	.30	<.02	.10	.005	<.01
75th percentile	69	34	84	120	--	11.4	8.3	340	20.7	.37	<.02	.10	.006	<.01
Maximum	450	570	4,100	3,100	--	14.2	8.4	347	27.0	.65	<.02	.20	.013	<.01
Little Shawnee Creek above Two Rivers (site 170, fig. 7)														
Number of samples	8	8	8	5	8	8	8	8	8	4	4	4	4	4
Minimum	12	2	23	25	.56	5.3	7.8	414	6.5	.17	<.02	<.10	<.004	<.01
25th percentile	13	12	30	86	.68	8.0	8.0	449	8.6	.25	--	--	--	--
Median	18	16	100	97	2.6	10.2	8.2	482	15.2	.32	<.02	.10	<.004	<.01
75th percentile	44	24	150	140	3.6	11.3	8.2	496	19.4	.38	--	--	--	--
Maximum	66	74	660	210	6.4	11.8	8.4	503	20.1	.43	<.02	.10	.008	<.01
Jacks Fork below 3rd unnamed hollow above Two Rivers (site 185, fig. 7)														
Number of samples	17	17	17	13	15	17	17	17	17	17	17	17	17	17
Minimum	1	<1	2	7	113	7.6	7.9	280	4.8	.20	<.02	<.10	<.004	<.01
25th percentile	13	5	18	20	156	9.1	8.2	332	12.8	.26	<.02	.10	<.004	<.01
Median	30	6	36	44	177	10.5	8.2	340	18.9	.30	<.02	.10	.004	<.01
75th percentile	36	11	49	69	233	11.2	8.3	349	21.4	.35	<.02	.10	.005	<.01
Maximum	490	640	3,900	1,900	470	13.8	8.6	356	26.4	.63	<.02	.23	.014	.01

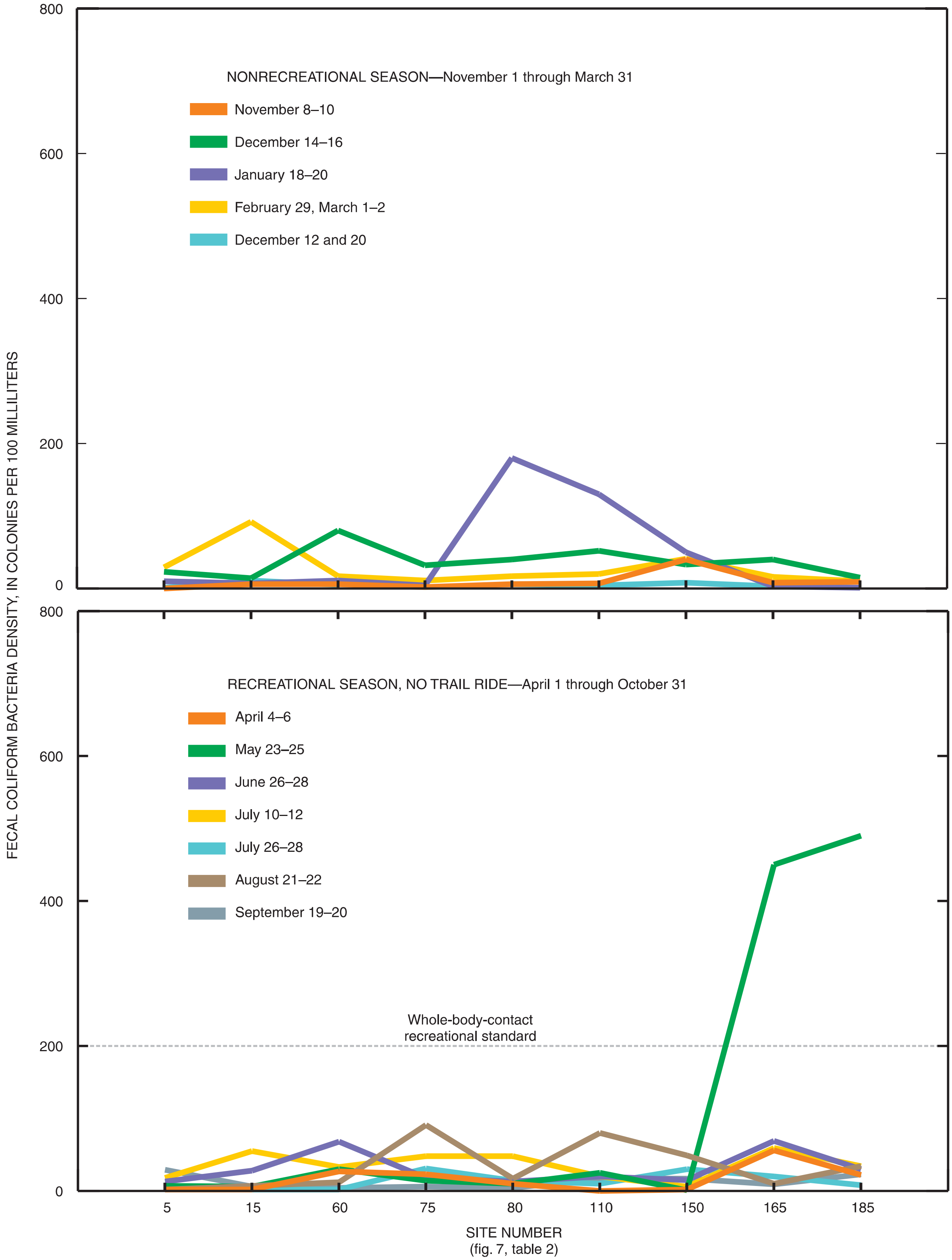

Figure 10. Fecal coliform bacteria density at selected Jacks Fork main stem sites and Alley Spring, November 1999 through December 2000.

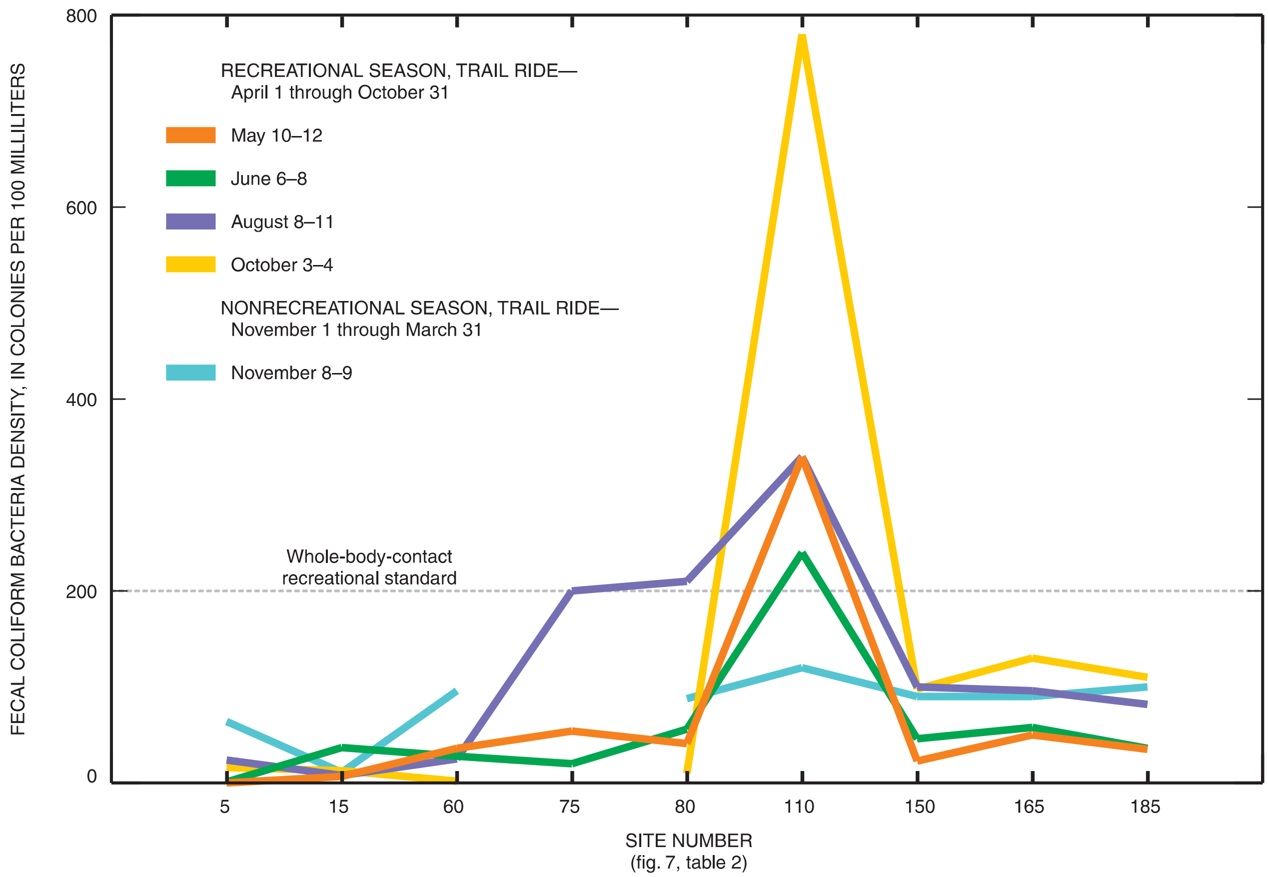

Figure 10. Fecal coliform bacteria density at selected Jacks Fork main stem sites and Alley Spring, November 1999 through December 2000—Continued.

between the distributions of the nine main stem and Alley Spring sites was at site 110, which overall tended to have larger fecal coliform bacteria densities than the other sites. Similar to Phase I of the study, four of the five tributaries [not including Alley Spring (site 15)] generally had larger fecal coliform densities than the main stem sites, but the tributaries again had little effect on the fecal coliform bacteria densities in the Jacks Fork because of the relatively small discharge of the tributaries relative to the discharge of the Jacks Fork (Davis and Richards, 2001). The Eminence WWTP also was not a contributor to the fecal coliform bacteria densities in the Jacks Fork main stem. Out of 13 samples collected from the WWTP, 11 had less than 1 colony of fecal coliform bacteria per 100 mL; the largest density was 5 col/100 mL.

The fecal coliform instantaneous load (the product of bacteria density times stream discharge) is a measure of the number of fecal coliform bacteria present in the volume of water that passes by a specific location in 1 second. Fecal coliform densities and loads generally were smallest at sites 5 and 15 (figs. 10, 12), which are upstream from the 303(d) reach (fig. 7) and are considered to have background concentrations. The fecal coliform densities and instantaneous loads generally increased from background concentrations at site 60 at Eminence, peaked at site 110, and then continued to decrease to site 185, which is the downstream-most site sampled during Phase II of the study. Large densities and loads at sites 165 and 185 on May 25, 2000, are associated with runoff. Generally, the largest densities and loads at sites 75 to 150 not related to wet-weather flow (figs. 11, 12) were measured August 8 to 10, 2000, during a trail ride, when the river discharge was at a minimum, water temperatures were at their maximum, and river use (trail riding, canoeing, tubing, and swimming) was at a maximum. The most affected site was site 110, with large densities and loads that exceeded the whole-body-contact standard occurring during trail rides that were held May 7 to 13, June 4 to 10, August 6 to 12, and October 1 to 7, 2000. During the May and October trail rides, few canoes and no swimmers or tubers were observed using the river. Although trail riders are observed along and in the river from Alley

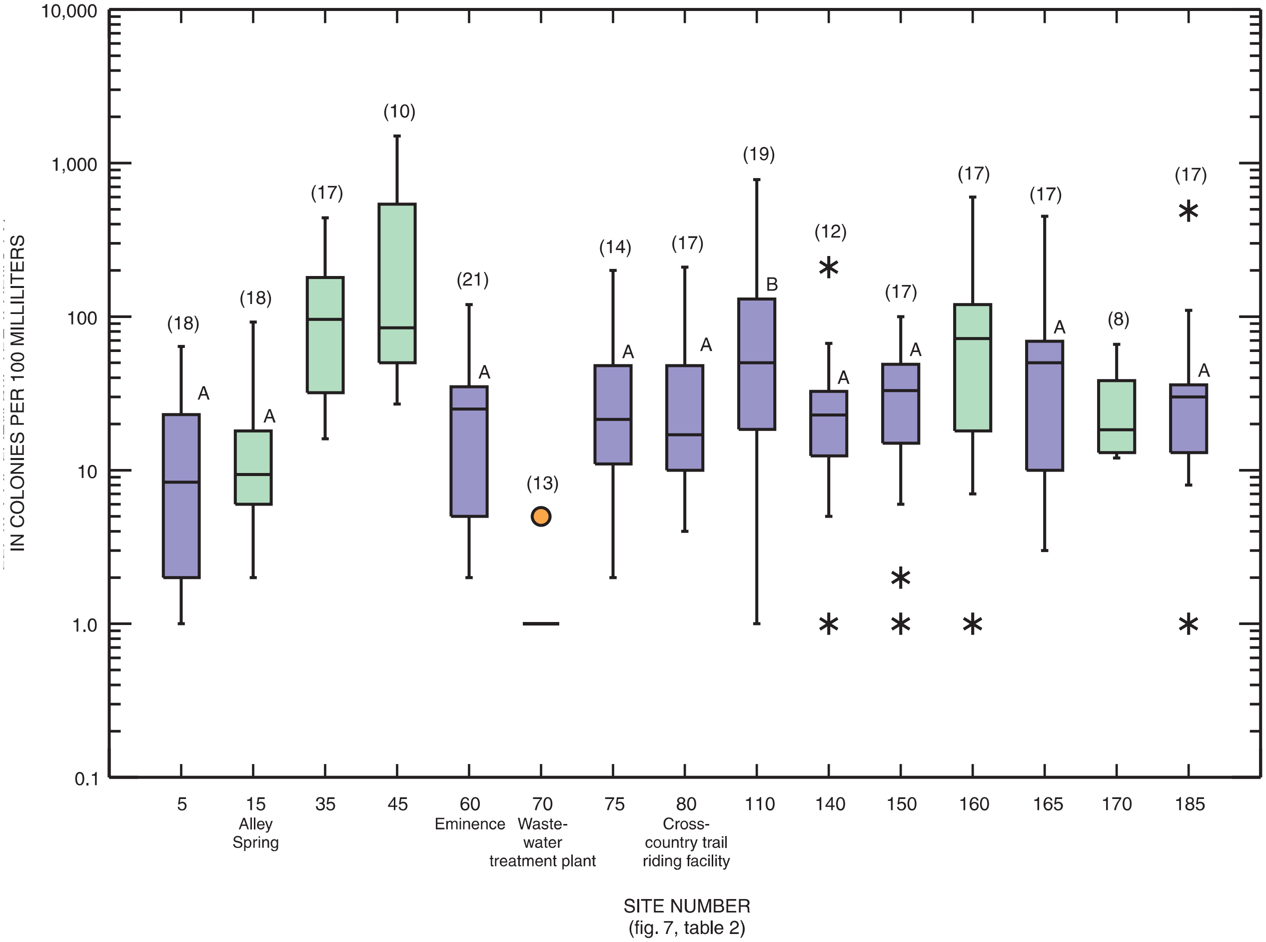

Figure 11. Statistical distribution of fecal coliform bacteria density at main stem and tributary sites and the Eminence Wastewater Treatment Plant on the Jacks Fork, November 1999 through December 2000.

Spring downstream to site 185, the largest concentration of trail riders occurred between sites 75 and 150, with site 110 at an intensely used trail-ride crossing outside of the ONSR boundary (fig. 7).

Swimmers, canoeists, and tubers were observed during most sample collection times in June, July, and August 2000, including during trail rides. All of the sample collection was done Monday through Friday, whereas the largest volume of swimmers, canoeists, and tubers use the Jacks Fork on the weekend. To investigate the potential effects of large numbers of swimmers, canoeists, and tubers on the fecal coliform bacteria densities, a 24-hour sample collection was conducted the weekend of July 15 and 16, 2000. Five or six samples were collected at sites 5, 15, 28 (a previously unsampled site located about 4 river mi upstream from Eminence), 60, 110, and 150 (fig. 7) between Saturday morning until the following Sunday afternoon. The samples were analyzed for fecal coliform and *E. coli* bacteria and physical properties, including discharge, DO, pH, specific conductance, and water temperature. In addition, samples were sent to the College of Veterinary Medicine at the UMC for ribotyping analysis. No fecal coliform density at any of the sites sampled during the 24-hour period exceeded the whole-body-contact recreation standard (fig. 13). The largest measured density of 110 col/100 mL was in a sample collected at site 110 at 9:00 a.m. on Sunday. Generally, fecal coliform densities were largest in samples collected at site 110; densities ranged from 10 to 110 col/100 mL, with a median density of 56 col/100 mL in six samples. Densities ranged from 6 to 52 col/100 mL with a median of 18 col/100 mL at the other five sites. The reason for the elevated densities at site 110 may be because of the herd of cows that resides in the pasture adjoining the river, although the cows were not observed crossing the river during sampling. With the exception of site 28, which was not sampled between 12:55 a.m. and 2:25 a.m. or 10:45 a.m. and 11:55 a.m., and site 110, fecal coliform densities generally were

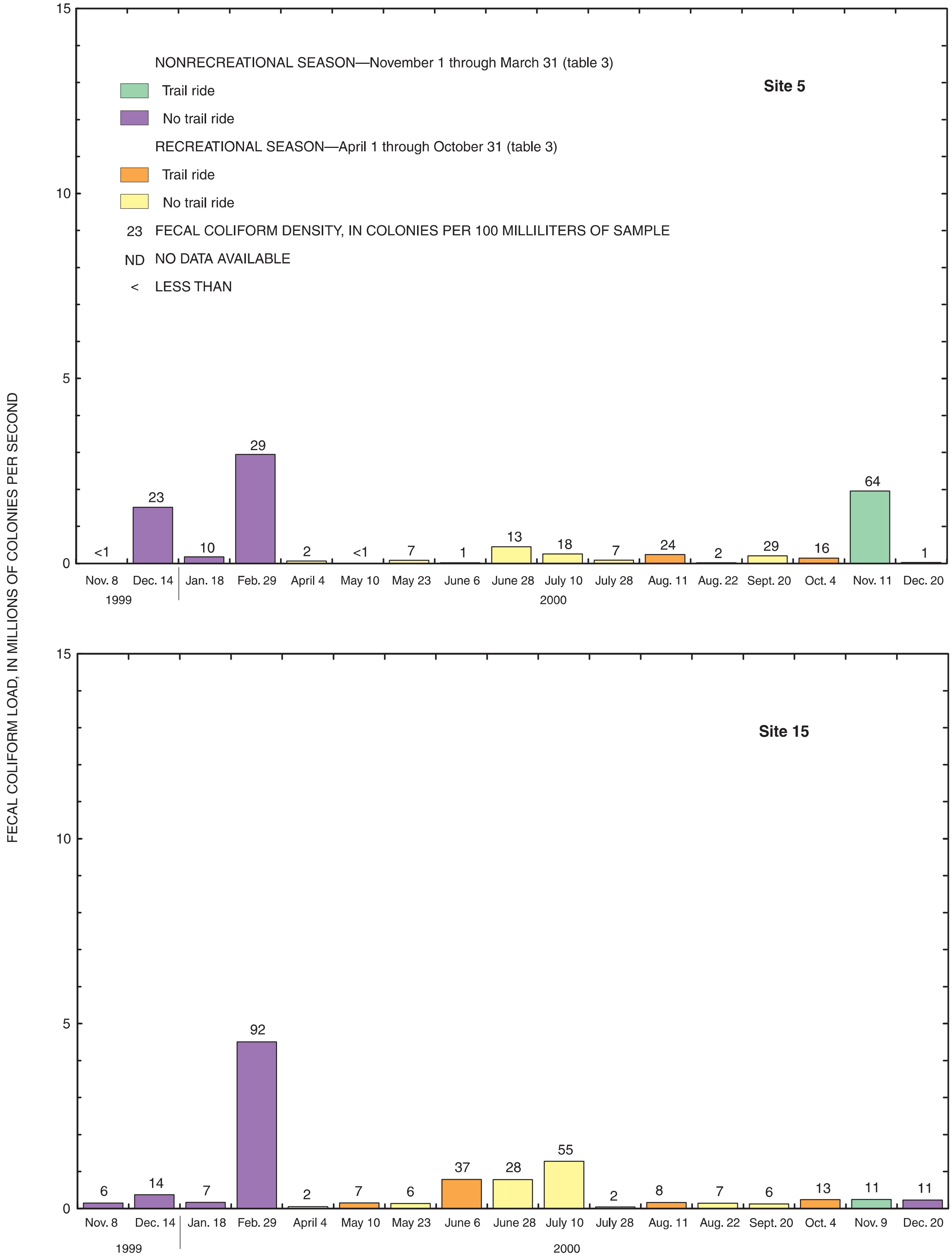

Figure 12. Fecal coliform bacteria density and instantaneous load for Jacks Fork main stem sites and Alley Spring, November 1999 through December 2000 (fig. 7, table 2).

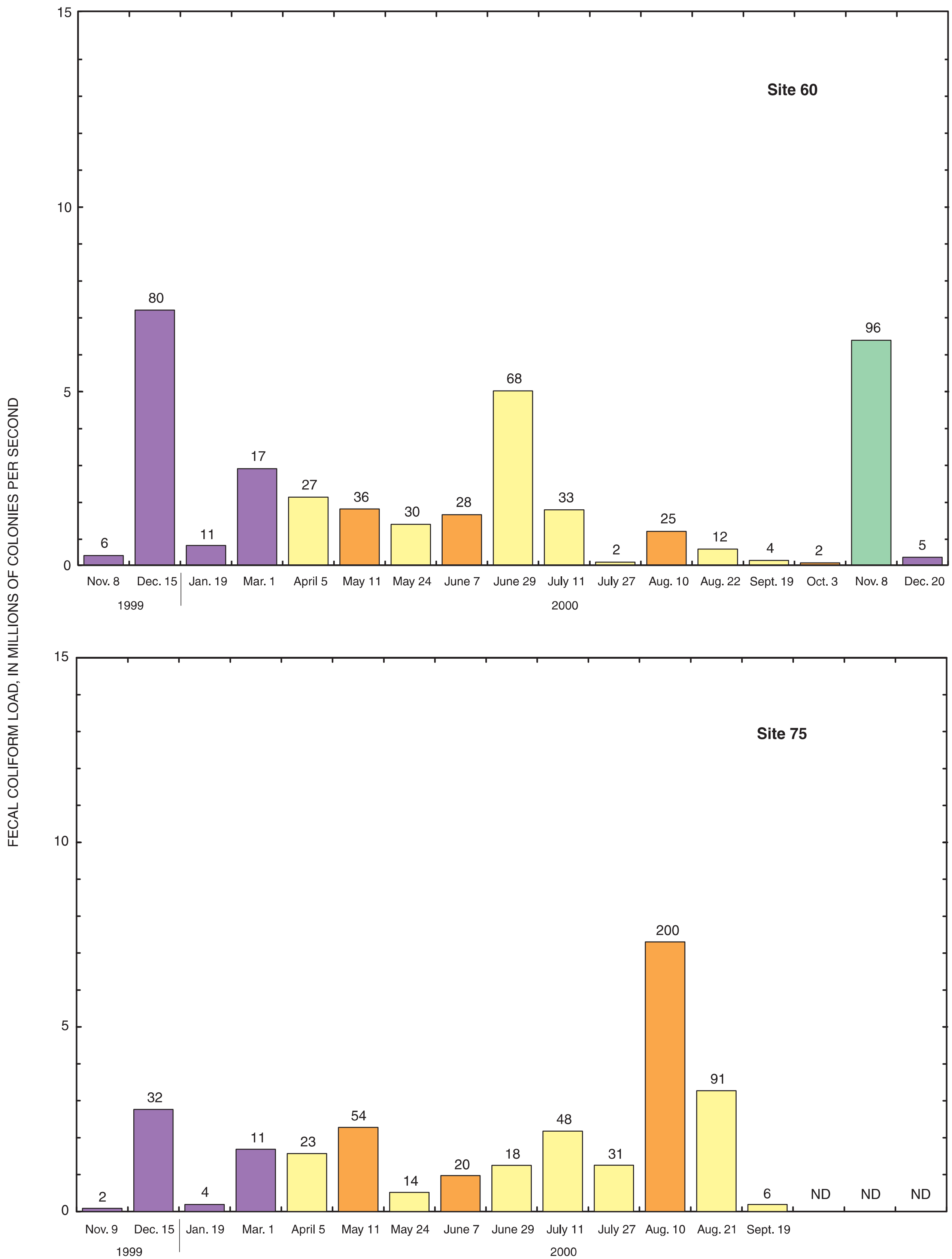

Figure 12. Fecal coliform bacteria density and instantaneous load for Jacks Fork main stem sites and Alley Spring, November 1999 through December 2000 (fig. 7, table 2)—Continued.

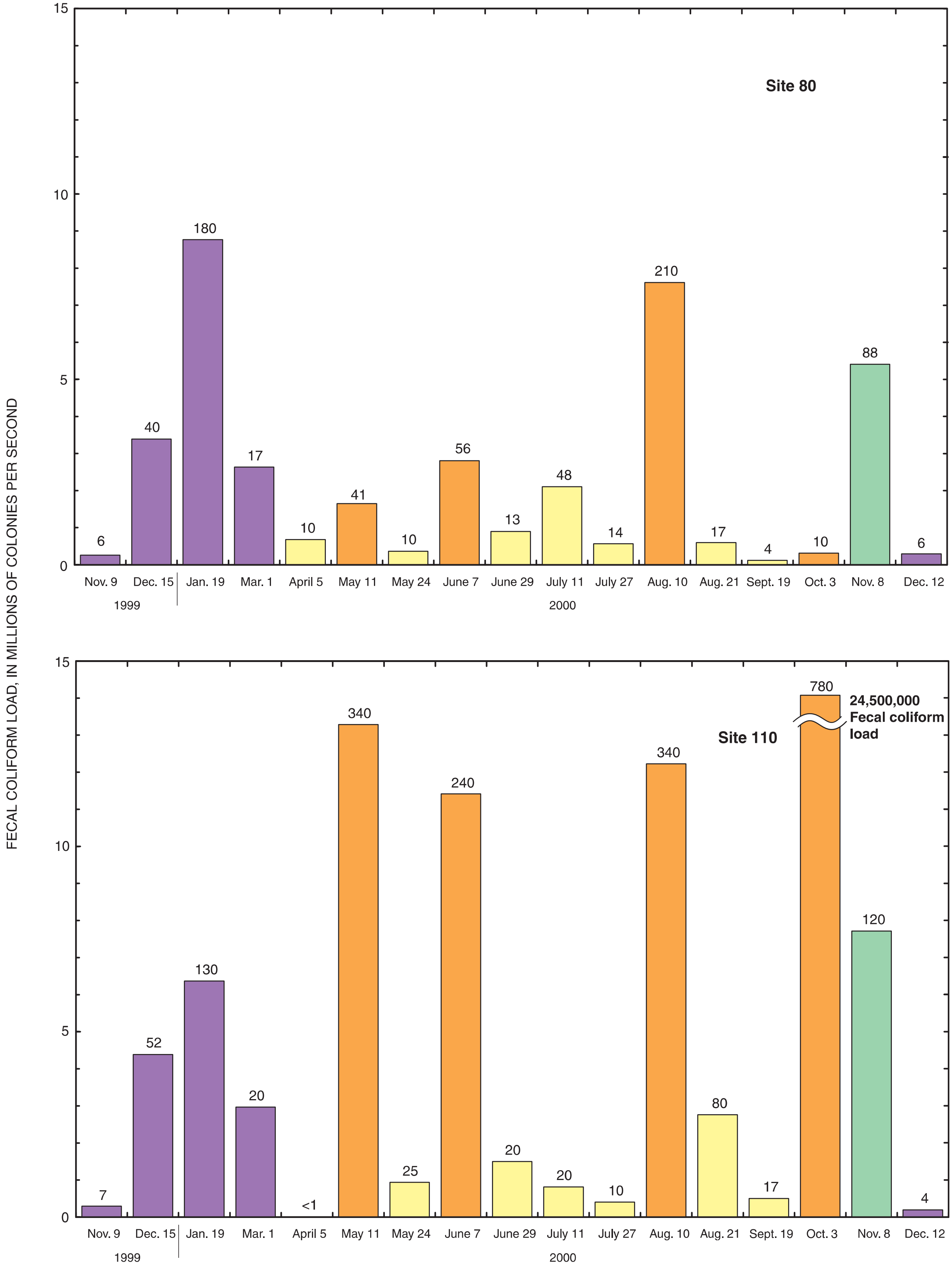

Figure 12. Fecal coliform bacteria density and instantaneous load for Jacks Fork main stem sites and Alley Spring, November 1999 through December 2000 (fig. 7, table 2)—Continued.

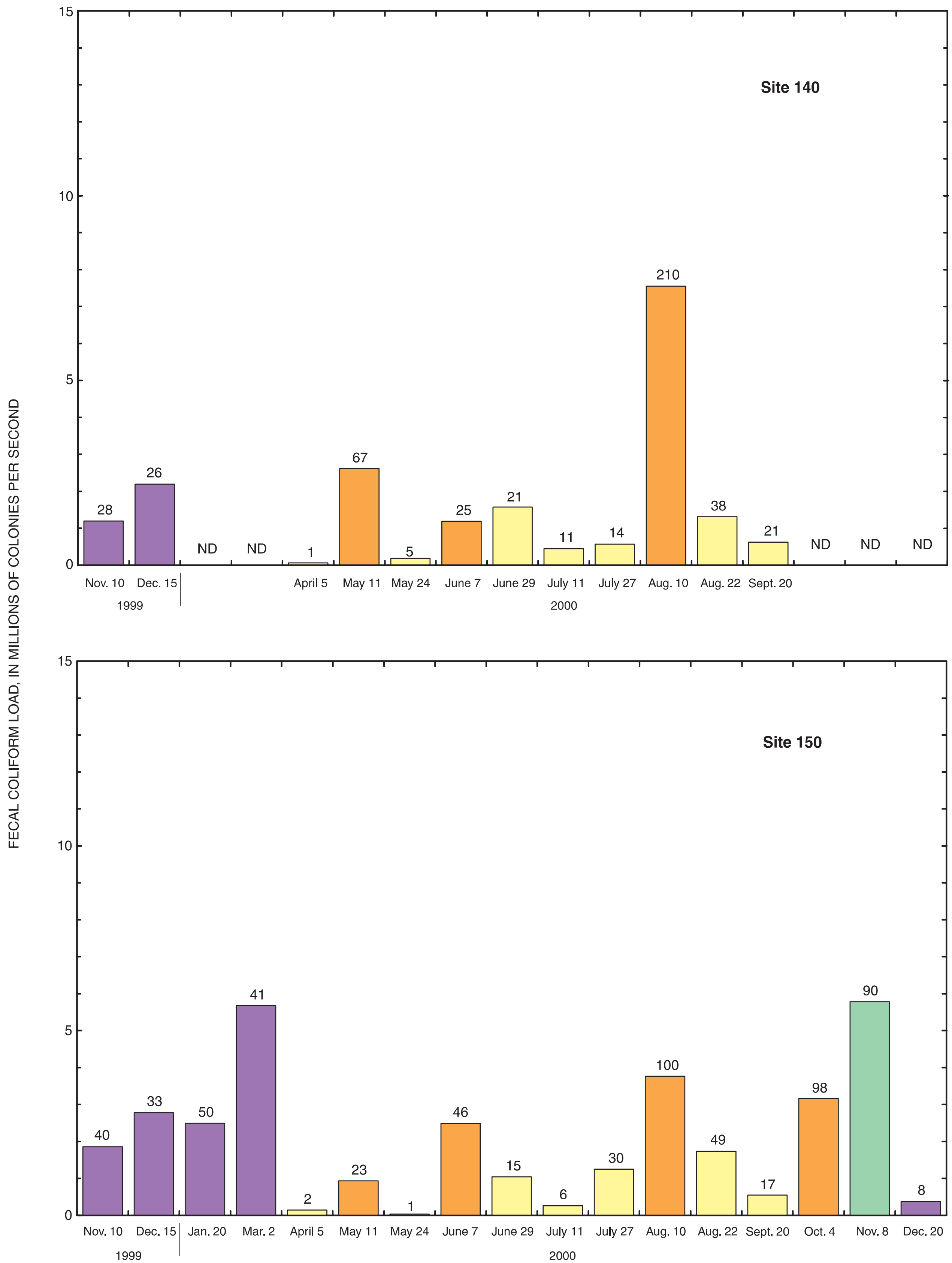

Figure 12. Fecal coliform bacteria density and instantaneous load for Jacks Fork main stem sites and Alley Spring, November 1999 through December 2000 (fig. 7, table 2)—Continued.

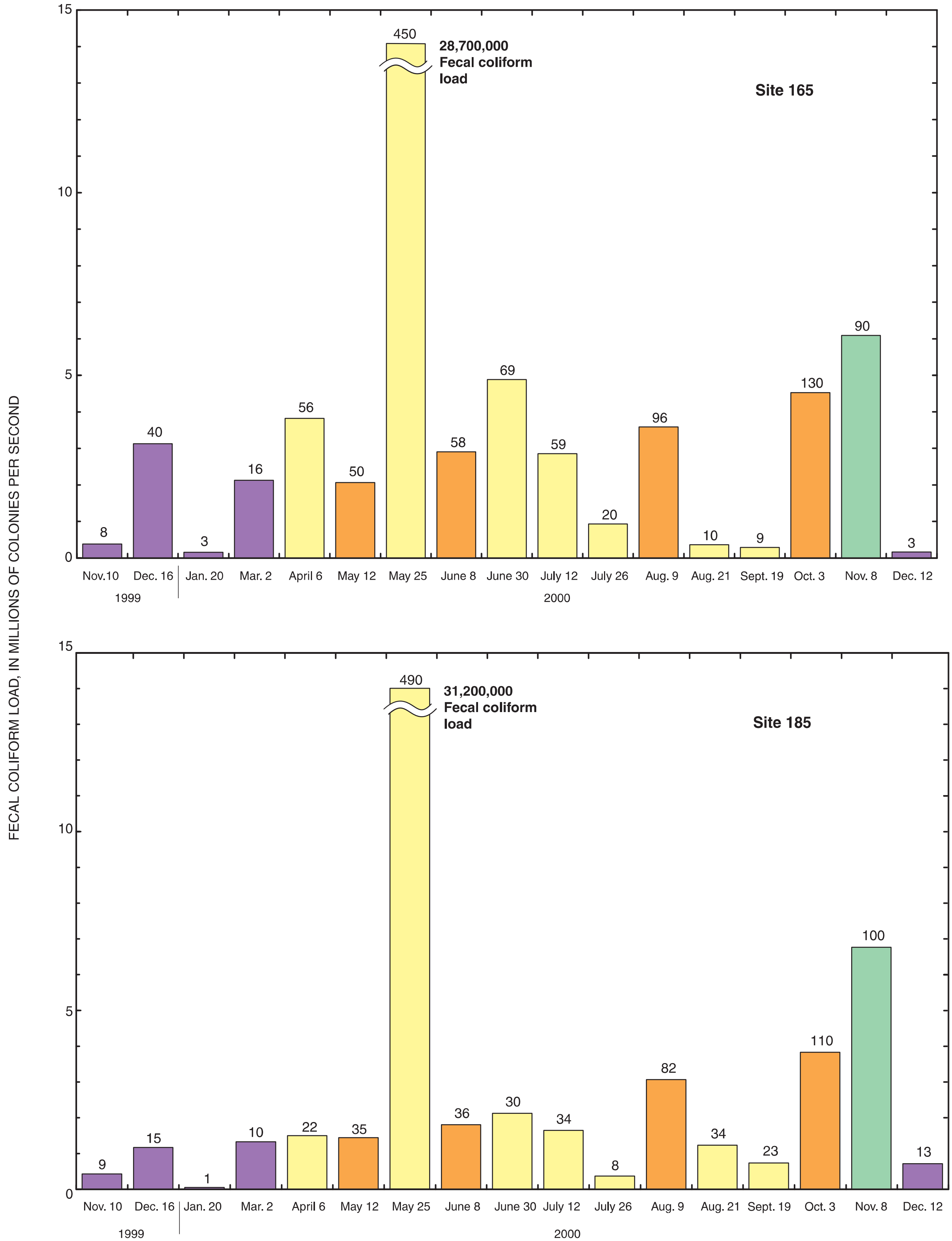

Figure 12. Fecal coliform bacteria density and instantaneous load for Jacks Fork main stem sites and Alley Spring, November 1999 through December 2000 (fig. 7, table 2)—Continued.

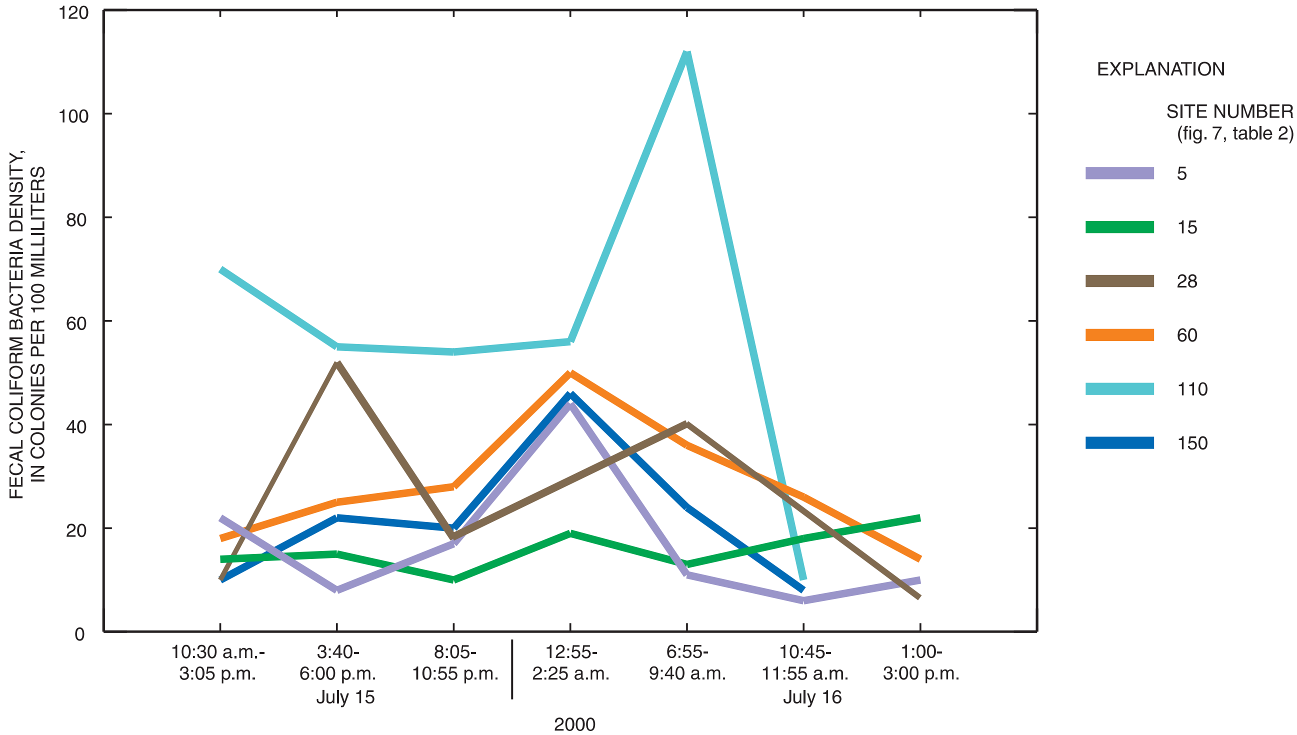

Figure 13. Fecal coliform bacteria density at selected Jacks Fork main stem sites and Alley Spring, July 15 and 16, 2000.

largest in the samples collected just after midnight at sites 5, 60, and 150. The large number of swimmers, canoeists, and tubers using the Jacks Fork on the weekend of July 15 to 16, 2000, did not appear to affect the fecal coliform densities.

Because bacteria survive longer in streambed sediments than in water, a source of bacteria in the water column could be from resuspension of accumulated bacteria from streambed sediments (Marino and Gannon, 1991). The fecal bacteria stored in the streambed sediments may be returned to the water column by physical disturbance of the streambed sediments by processes such as dredging (Grimes, 1980), wind and wave action (Lehman and Fogel, 1976), and by swimmers or boaters (Bromel and others, 1978). In the case of the Jacks Fork, the mechanical action could be caused by the movement of horses through the water or by swimmers, canoeists, and tubers. Water and streambed-sediment samples were collected at three sites (5, 60, and 110) on August 2, 2000, 1 week before a trail ride. Additional water and streambed-sediment samples were collected at three sites (110, 150, and 160) on August 8, 2000, during a trail ride. These samples were analyzed for fecal coliform and *E. coli* bacteria and physical properties, including discharge, DO, pH, specific conductance, and water temperature. In addition, samples were sent to the College of Veterinary Medicine at the UMC for ribotyping analysis. The results are listed in table 7. Although only site 110 was sampled before and during the trail ride, the results indicate that the fecal-bacteria densities increased at this site in both the streambed sediment and water column during the trail ride. Additional water-column and streambed-sediment samples need to be collected to determine if this increase routinely occurs.

Analysis of the fecal coliform bacteria data indicates that during the Phase II sampling period, bacteria densities not related to wet-weather flow were largest during trail rides. During the June 4 to 10 and August 6 to 12 trail rides, numerous people were camping near the river or swimming, canoeing, or tubing in the river, which may have contributed to the elevated bacteria densities. However, few people were camping or swimming, canoeing, or tubing during the May, October, and November trail rides, and bacteria densities were still elevated. These elevated densities may be related to four factors: (1) physical disturbance of streambed sediments causing resuspension of accumulated bacteria, (2) defecation directly into the river, (3) fecal material carried into the river on the feet of animals, and (4) leakage of sewage effluent from an unknown source into the river.

Table 7. Fecal coliform and *Escherichia coli* densities in water and streambed-sediment samples collected at selected sites, August 2000, in colonies per 100 millilitiers

Site number (fig. 7)	Fecal coliform		*Escherichia coli*	
	Water	Sediment	Water	Sediment
		August 2, 2000		
5	10	88	2	87
60	5	190	1	160
110	11	570	6	340
		August 8, 2000		
110	310	5,100	148	3,000
150	124	480	23	206
160	1,460	920	1,520	680

Microbial Source Tracking

Isolates of *E. coli* were obtained from water samples collected at sites 5 (1 sample), 35 (1 sample), 45 (2 samples), 60 (5 samples), 80 (2 samples), 110 (5 samples), 150 (3 samples), 160 (1 sample), and 165 (1 sample) and from streambed-sediment samples collected at sites 5 (1 sample), 60 (1 sample), 110 (2 samples), 150 (1 sample), and 160 (1 sample) and were submitted for ribotyping analysis (table 8). Whereas most efforts to determine the source of bacteria in streams rely on indirect measures of inorganic and organic constituents, ribotyping has the promise of directly linking the bacteria to their source using DNA ribopatterns. The technique relies on the assumption that ribotypes of *E. coli* from various animal species will be unique. However, little is known about the temporal and geographic variability of ribotypes within a single animal group or the potential sharing of ribotypes between various animals. Hartel and others (2002) determined that ribotypes for cows in Idaho and Georgia differed significantly. Sargeant and others (1999) showed that wild deer foraging in fields where dairy cows were pastured became colonized with the identical strain of *E. coli* carried by the cows.

Ribotyping analysis involves the use of multivariate statistical methods to compare patterns in large data sets. The method compares the degree of similarity of the patterns from unknown samples to known patterns in a database. For this study, the unknowns were compared to known patterns obtained from sewage, horses, and cows (table 8). Patterns from sewage differ from patterns obtained directly from human fecal material. Sources of sewage in the study area could include the Eminence WWTP or campground pit-toilet or septic-system effluent. The three choices were based on the presence of all of these sources in the study area. Matching of patterns was made using discriminate analysis, and a pattern was considered to be a match if the probability of a match was 70 percent or higher. If no pattern was matched, the isolate was considered to come from unknown sources, which could include domesticated animals such as pigs, dogs, cats, or chickens, or wild animals such as deer, raccoons, geese, and ducks. As additional patterns are added to the database of “known” patterns, the degree of similarity between unknown and known patterns constantly changes. Because of the large degree of uncertainty in the method, results of the method should be treated as experimental for the purpose of this study, and interpretations of these data were made in conjunction with other data and information.

The samples for ribotyping analysis were collected in 2000 on April 6 at the beginning of the recreational season; June 8 during the June 4 to 10 trail ride; July 16 during intense swimming, canoeing, and tubing use; August 2, 1 week before a trail ride; and August 8 during the August 6 to 12 trail ride. Ribopatterns were obtained from 65 *E. coli* isolates from 13 water samples and from 23 *E. coli* isolates from 4 streambed-sediment samples (table 8). No isolates were obtained from water and/or streambed-sediment samples collected at site 110 on June 8; sites 60, 110, and 150 on July 16; sites 5, 60, and 110 on August 2; and at sites 60 and 160 on August 8 because of a failed *E. coli* biochemical confirmation test, no *E. coli* colonies were isolated for ribotyping, or the DNA fragments from the *E. coli* isolates smeared during gel electrophoresis.

The isolates were compared to several hundred isolates obtained from animal sources at the UMC School of Veterinary Medicine farms, selected locations in southwest Missouri, and the Eminence area. Results indicated that of the 65 *E. coli* isolates obtained from the water samples, 26 (40 percent) were identified as originating from sewage, 19 (29 percent) from horse, 7 (11 percent) from cow, and 13 (20 percent) from unknown sources. Of the 23 isolates from the streambed-sediment samples, 9 (39 percent) were identified as originating from sewage, 8 (35 percent) from horse, 3 (13 percent) from cow, and 3 (13 percent) from

Table 8. Number of *Escherichia coli* isolates in water or streambed-sediment samples assigned to various sources by ribotyping

[col/100 mL, colonies per 100 milliliters of sample; *E. coli*, *Escherichia coli*; W, water; <, less than; --, no data available; S, streambed sediment]

Site (fig. 7)	Date	Sample matrix	Fecal coliform (col/100 mL)[a]	*E. coli* (col/100 mL)[a]	Total number of isolates[b]	Possible animal source using three knowns (probability of 0.70 or larger)			
						Sewage	Horse	Cow	Unknown
45	04/06/00	W	27	20	2	0	0	1	1
60	04/06/00	W	27	15	1	0	0	1	0
110	04/06/00	W	<1	<1	3	0	1	1	1
45	06/08/00	W	380	310	6	0	5	0	1
60	06/08/00	W	28	27	10	7	2	0	1
80	06/08/00	W	56	43	10	5	1	1	3
110	06/08/00	W	240	80	[c]0	--	--	--	--
150	06/08/00	W	46	3	2	2	0	0	0
60	07/16/00	W	14	<2	[d]0	--	--	--	--
110	07/16/00	W	10	12	[d]0	--	--	--	--
150	07/16/00	W	8	2	[d]0	--	--	--	--
5	08/02/00	W	10	2	[e]0	--	--	--	--
	08/02/00	S	88	87	6	2	3	0	1
60	08/02/00	W	5	1	[d]0	--	--	--	--
	08/02/00	S	190	160	[e]0	--	--	--	--
110	08/02/00	W	11	6	[d]0	--	--	--	--
	08/02/00	S	570	340	3	2	1	0	0
35	08/08/00	W	180	48	6	1	3	1	1
60	08/08/00	W	25	13	[c]0	--	--	--	--
80	08/08/00	W	210	180	4	4	0	0	0
110	08/08/00	W	310	148	5	4	1	0	0
	08/08/00	S	5,100	3,000	9	5	3	1	0
150	08/08/00	W	124	23	8	1	5	1	1
	08/08/00	S	480	206	5	0	1	2	2
160	08/08/00	W	1,460	1,520	7	2	0	1	4
	08/08/00	S	920	680	[d]0	--	--	--	--
165	08/08/00	W	96	20	1	0	1	0	0

[a] Samples collected and processed by the U.S. Geological Survey.
[b] Samples collected by the U.S. Geological Survey and processed by the College of Veterinary Medicine at the University of Missouri in Columbia, Missouri.
[c] Ribotyping analysis failed for technical reasons (smeared pattern).
[d] No *Escherichia coli* colonies isolated for ribotyping.
[e] Failed biochemical confirmation test.

unknown sources. The overall results indicate that the predominant sources of *E. coli* bacteria were sewage and horses.

At the beginning of the recreational season, no matches were classified as human. Of the six isolates obtained from three samples collected on April 6, three were classified as cow, one as horse, and two unknown (fig. 14). The small number of isolates is consistent with the small fecal coliform and *E. coli* densities observed in these samples at these sites and can be considered representative of base-flow conditions during the early part of the recreational season (table 8, fig. 8).

Twenty-eight isolates were obtained from samples collected June 8 during a trail ride. Fourteen isolates were assigned to sewage, 8 to horse, 1 to cow, and 5 to unknown sources (fig. 14). Although some trail riders do ride along the Jacks Fork upstream from site 75, most trail-ride activity occurs between sites 75 and 165. On June 8, no isolates were obtained at site 110, and only two were obtained at site 150. Site 45 is located on Storys Creek, a small (5.34 mi^2), predominantly agricultural basin. Ribotyping results for site 45 indicate that five of the six isolates were assigned to horse. Cows have been observed grazing in pastures located immediately upstream from the site.

In the samples collected August 2, 1 week before a trail ride, isolates were obtained only from streambed-sediment samples collected at sites 5 and 110. Of the nine isolates, four were assigned to sewage, four to horse, and one to an unknown source (fig. 14). The small number of isolates is consistent with the small fecal coliform and *E. coli* densities in these samples at these sites and can be considered representative of base-flow conditions during the recreational season (table 8, fig. 8).

The most complete set of results was obtained from the samples collected August 8 during a trail ride. Of the 31 isolates obtained from 7 water samples, 12 were classified as originating from sewage, 10 from horse, 3 from cow, and 6 from unknown sources. Of the 14 isolates obtained from streambed-sediment samples, 5 were classified as originating from sewage, 4 from horse, 3 from cow, and 2 from unknown sources (fig. 14). At the sites most affected by trail-ride activity (sites 80, 110, 150, and 160), water sample isolates identified as originating from sewage accounted for 46 percent of the total number of isolates, and isolates identified as originating from horse accounted for 25 percent of the total. Of the 14 isolates obtained from streambed-sediment samples collected at sites 110 and 150, 36 percent were identified as originating from sewage, and 29 percent were identified as originating from horse.

As discussed previously, ribotyping results should be treated as experimental for the purpose of this study because of the large degree of uncertainty in the method. However, based on the results of comparing unknown patterns to patterns for sewage, horses, and cows, sewage and horses appear to be possible sources of *E. coli* bacteria in the Jacks Fork. Additional MST using ribotyping and other techniques needs to be done to further pinpoint *E. coli* sources. Since 2000, when these samples were analyzed, MST technology, including the software available to analyze the results, has improved. Obtaining additional samples of known sources from the Eminence area also would assist in MST efforts.

WATER-QUALITY CHARACTERISTICS

The measurement of physical properties and nutrient concentrations in surface water can provide additional information on the general condition of the stream and the effects of hydrologic events and recreational uses on stream water quality. The physical characteristics and chemical composition of the stream can indicate whether the stream environment can support diverse aquatic life and is desirable for recreational use.

Physical Properties

The physical properties measured at each site included DO, pH, specific conductance, and temperature. A statistical summary of the physical-property data is given in table 6.

Dissolved Oxygen

The distribution of DO concentrations on the Jacks Fork and tributaries and Eminence WWTP is shown in figure 15. Median DO concentrations at the nine main stem sites and Alley Spring ranged from 9.9 to 10.7 mg/L (table 6) with no statistically significant differences in the distributions. No DO concentration on the Jacks Fork main stem was less than the minimum of 5 mg/L required by the State of Missouri to sustain a cool-water fishery (Missouri Department of Natural Resources, 2000). Although the DO concentra-

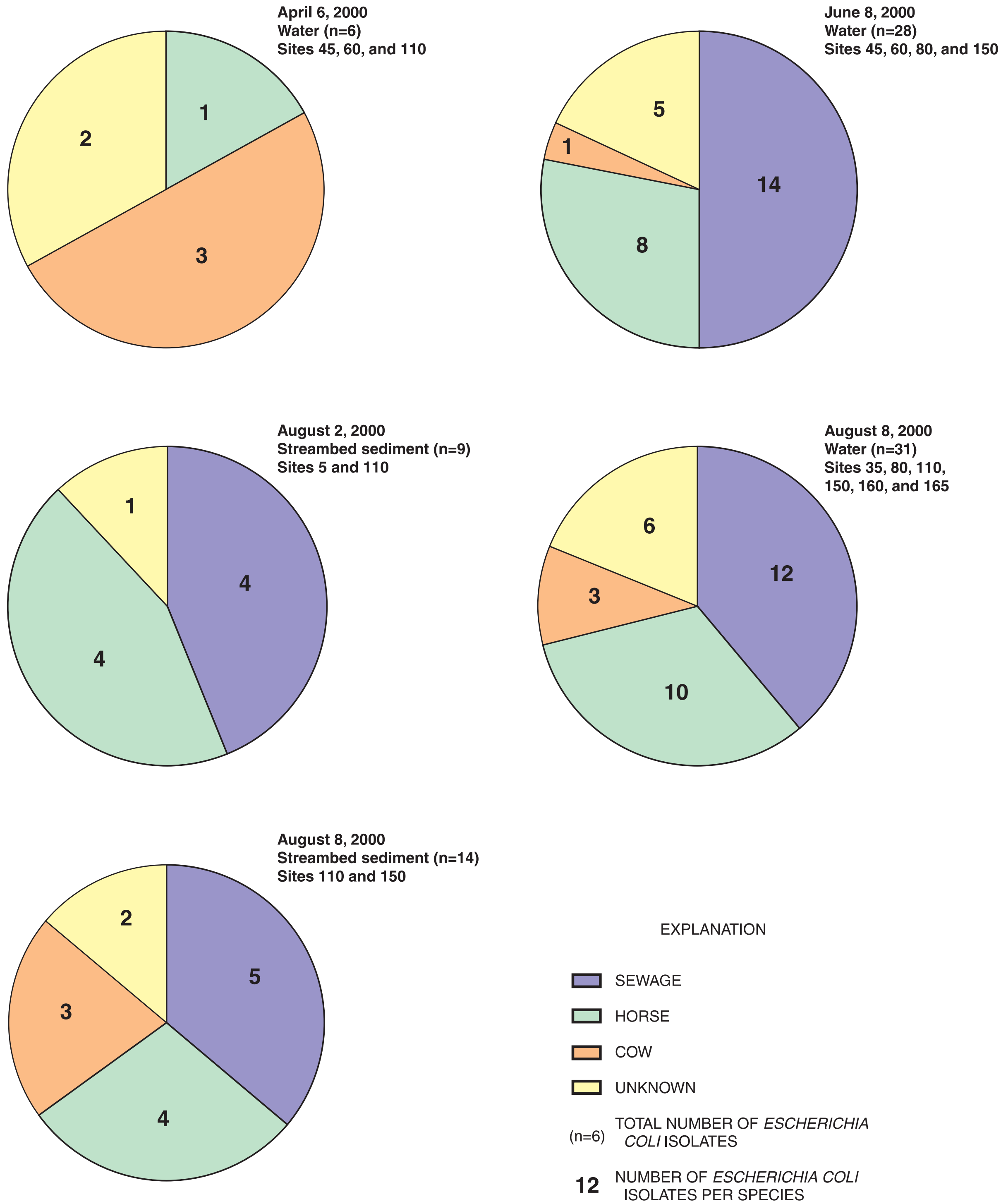

Figure 14. Percentage of *Escherichia coli* isolates in water or streambed-sediment samples assigned to various sources by ribotyping (fig. 7, table 2).

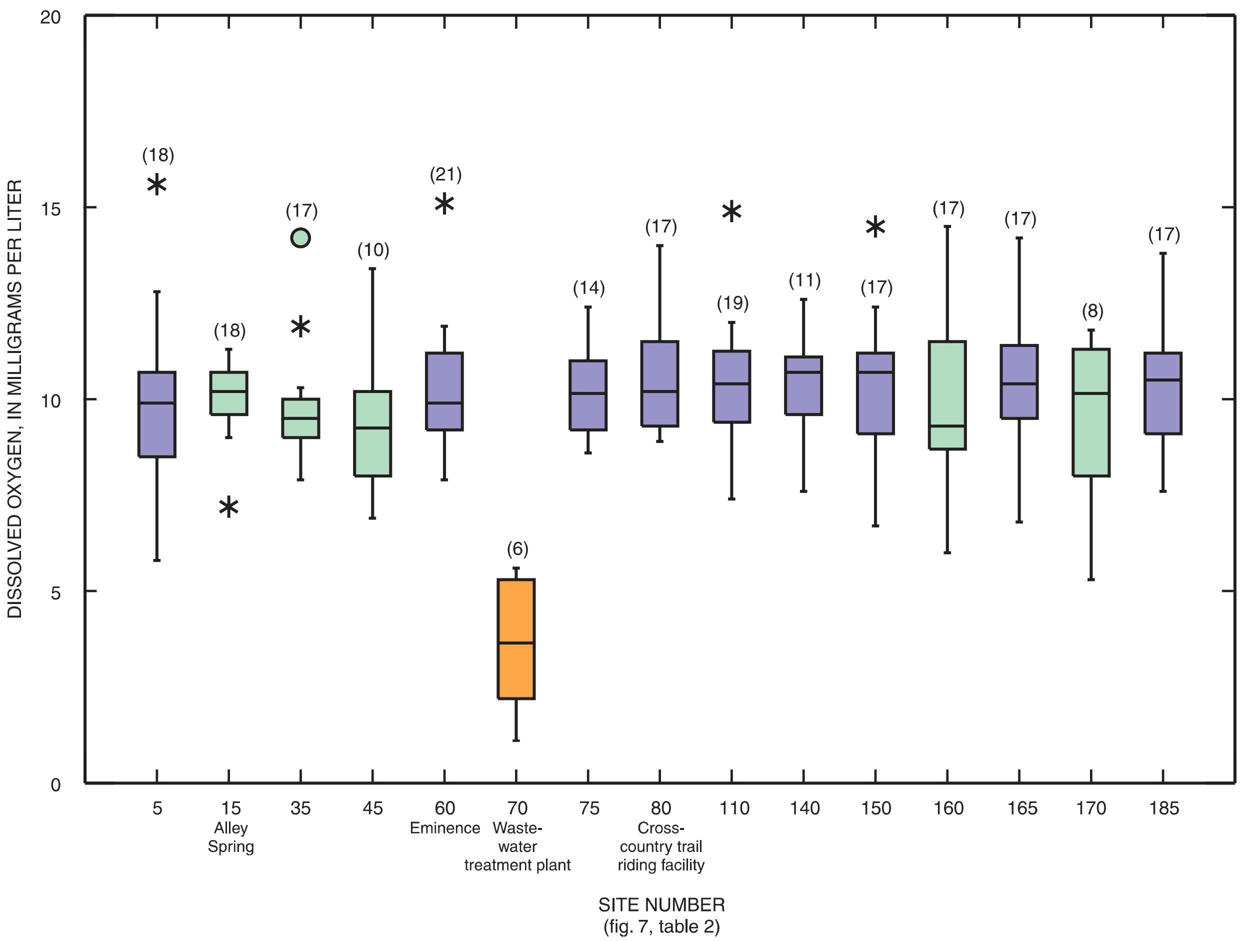

Figure 15. Statistical distribution of dissolved-oxygen concentrations at main stem and tributary sites and the Eminence Wastewater Treatment Plant on the Jacks Fork, November 1999 through December 2000.

tion was extremely small (minimum of 1.1 mg/L) in the effluent from the Eminence WWTP, no DO effects were observed at site 75 immediately downstream from the WWTP, probably because of the small volume of the effluent. Variations in DO concentrations appear to be seasonal, with the largest DO concentrations tending to occur in the cooler months, or related to the time of day the DO concentration was determined.

pH

The median pH for Jacks Fork main stem sites ranged from 8.1 to 8.3 (fig. 16, table 6) with no statistically significant differences between the sites. The median pH for samples collected at Alley Spring was 7.7, which was significantly different (p-value less than 0.05) than the values of the main stem sites. This is not unexpected because carbon dioxide in ground water normally occurs at a much higher partial pressure than in the earth’s atmosphere causing the pH in ground water to be lower than the concentration in streams. When ground water is exposed to the atmosphere, carbon dioxide will escape and the pH will increase (Freeze and Cherry, 1979, p. 140). The median pH of the samples collected at the Eminence WWTP was 7.7, and although the median pH increased steadily at sites downstream from the WWTP (median of 8.1, 8.2, and 8.3 at sites 75, 80, and 110), this increase probably is not directly related to the effluent. The median pH at site 60 located immediately upstream from site 70 also was 8.1. Variations in pH primarily appear to be related to hydrologic events or related to the time of day the pH measurement was taken because the smallest pH values tended to occur in samples collected at higher flows or in the morning.

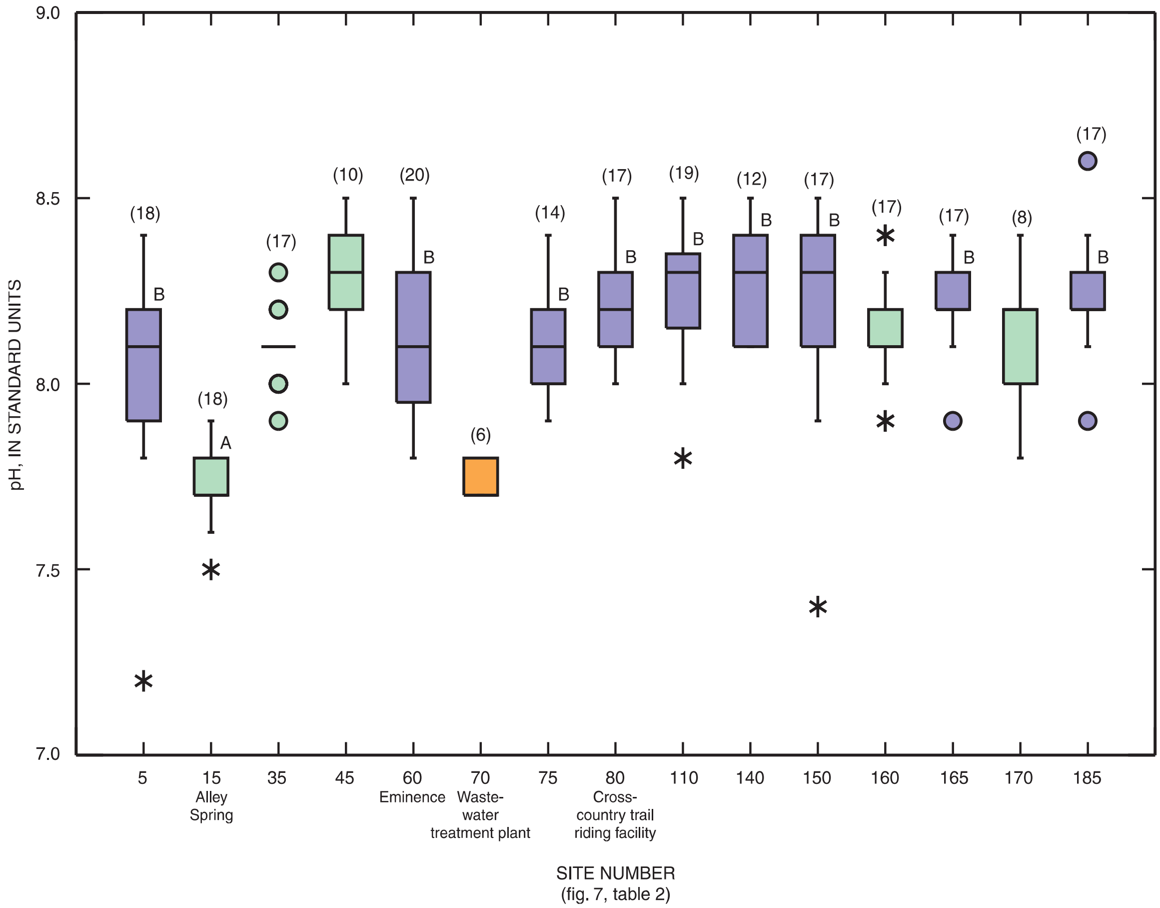

Figure 16. Statistical distribution of pH values at main stem and tributary sites and the Eminence Wastewater Treatment Plant on the Jacks Fork, November 1999 through December 2000.

Specific Conductance

Median specific conductance values ranged from 327 to 340 µS/cm [microsiemens per centimeter at 25 °C (degrees Celsius)] (fig. 17, table 6) at the Jacks Fork main stem sites with no statistically significant differences between the sites. The median specific conductance value at Alley Spring (site 15; 310 µS/cm) was significantly smaller (p-value less than 0.05) than the values of the main stem sites, but did not appear to have a dilution effect on the Jacks Fork. Likewise, the median specific conductance at the Eminence WWTP (site 70; 752 µS/cm) does not cause a significant increase in the specific conductance in the Jacks Fork immediately downstream from the WWTP at site 75. Variations in specific conductance values (figs. 8 and 18, table 3) appear to be related primarily to hydrologic events, with the smallest specific conductance values occurring at the largest flows.

Water Temperature

Median water temperature for the Jacks Fork main stem sites ranged from 17.9 to 21.9 °C (table 6) with no significant differences between the sites. The median water temperature for Alley Spring (site 15) was 14.6 °C (typical of spring water), which was significantly smaller (p-value less than 0.05) than the values of the main stem sites. Variation in water temperature throughout the sampling period was because of seasonal changes, with the lowest temperatures occurring in the winter and the highest temperatures occurring in the summer (fig. 19). The water temperature of Alley Spring did not vary with season.

Nutrients

The fixation of atmospheric nitrogen by plants and animals, atmospheric deposition of nitrogen, the dissolution of phosphorus-bearing rocks or minerals in

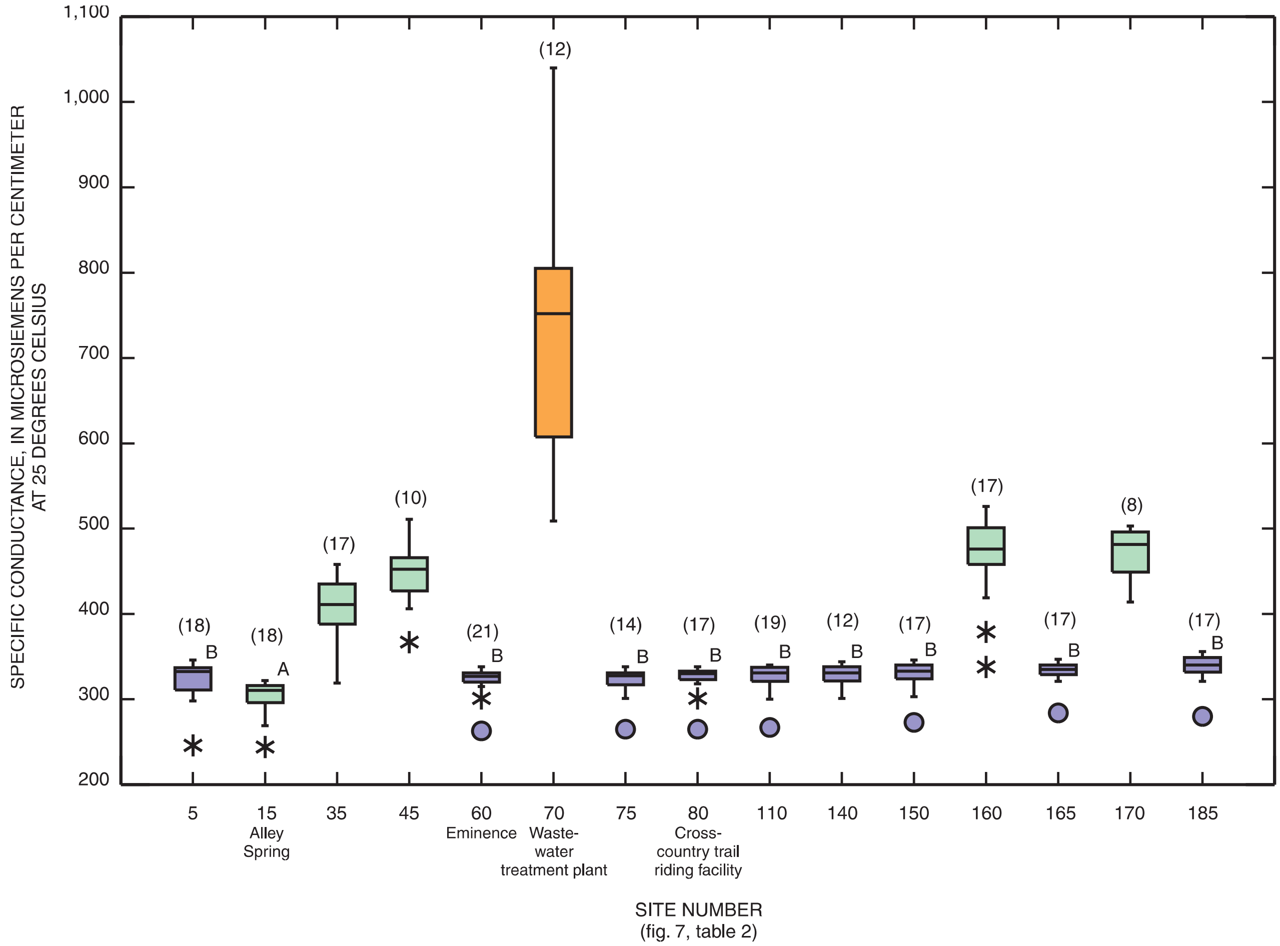

Figure 17. Statistical distribution of specific conductance values at main stem and tributary sites and the Eminence Wastewater Treatment Plant on the Jacks Fork, November 1999 through December 2000.

the soils, and organic matter, including soil organic matter and decaying plants and animals, are natural sources for nitrogen and phosphorus in streams. Anthropogenic sources include sewage discharges, fertilizers, animal waste, and septic tanks (Hem, 1985). Background concentrations of nitrogen and phosphorus in streams generally are small because the dissolved forms of the two elements are assimilated rapidly by plants and bacteria. Excessive concentrations of nutrients can have detrimental effects on desired uses of water and may indicate possible contamination. Anthropogenic sources of nutrients in the study area are the Eminence WWTP, septic systems, cows, and horses.

The nutrients analyzed included dissolved species of nitrite plus nitrate, ammonia, and orthophosphate and total species of ammonia plus organic nitrogen and phosphorus. Summary statistics of the nutrient data for the Jacks Fork are listed in table 6. The dissolved nitrite plus nitrate and total phosphorus data will be discussed in detail in the following sections. Differences in dissolved ammonia and orthophosphate and total ammonia plus organic nitrogen were not statistically significant between sites.

Dissolved Nitrite Plus Nitrate

On the main stem of the Jacks Fork, dissolved nitrite plus nitrate concentrations were statistically smaller (p-value less than 0.05) at the most-upstream site above Alley Spring (site 5; median 0.11 mg/L) and increased below Alley Spring at Eminence (site 60; median 0.30 mg/L) (fig. 20, table 6). This increase also was observed during Phase I data collection (Hauck and Nagel, 2000) and is caused by high dissolved nitrite plus nitrate concentrations in Alley Spring (site 15; median 0.58 mg/L), whose input at lower main stem flows will double the discharge of the Jacks Fork. A small, but not statistically significant, increase in dissolved nitrite plus nitrate concentrations occurred at

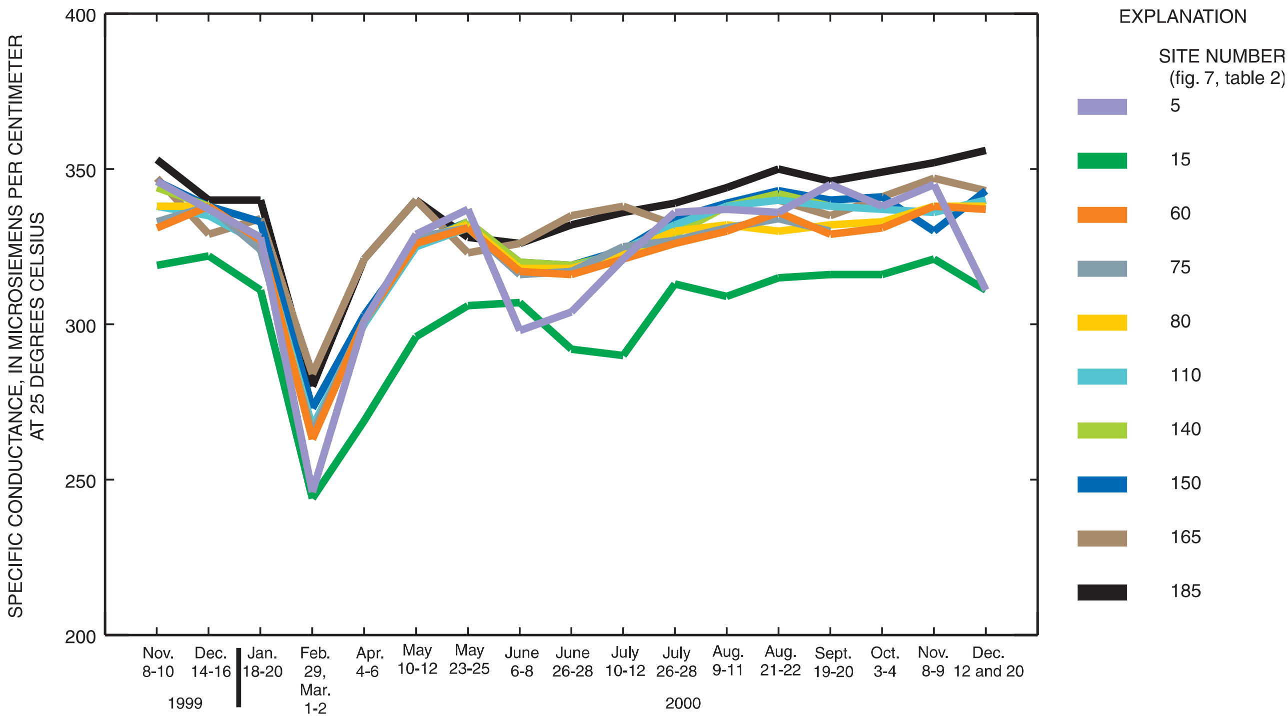

Figure 18. Specific conductance values at Jacks Fork main stem sites and Alley Spring, November 1999 through December 2000.

site 75 (median 0.38 mg/L) immediately downstream from the WWTP (site 70; median 16.2 mg/L). The effect diminishes quickly downstream from the WWTP at sites 80 (median 0.34 mg/L), 110 (median 0.31 mg/L), 140 (median 0.28 mg/L), and 150 (median 0.28 mg/L). The median concentration of dissolved nitrite plus nitrate was 0.30 mg/L at sites 165 and 185. The minimal effects of the Eminence WWTP also were observed in Phase I (Hauck and Nagel, 2000). Unlike fecal coliform bacteria, dissolved nitrite plus nitrate concentrations appear to be related mostly to hydrologic conditions and do not increase during recreational activities (fig. 21).

Total Phosphorus

Similar results were observed with total phosphorus as with dissolved nitrite plus nitrate. On the main stem of the Jacks Fork, total phosphorus concentrations again tended to be smallest at the most upstream site above Alley Spring (site 5; median less than 0.004 mg/L), but did not increase significantly downstream from Alley Spring (site 15; median 0.009 mg/L) at site 60 (median less than 0.004 mg/L) (fig. 22, table 6). Similar results also were reported during Phase I data collection (Hauck and Nagel, 2000). A statistically significant increase in total phosphorus concentrations occurred at site 75 (median 0.009 mg/L) immediately downstream from the WWTP (site 70; median 3.0 mg/L). Similar to dissolved nitrite plus nitrate concentrations, the effect diminishes quickly downstream from the WWTP at sites 80 (median 0.005 mg/L), 110 (median 0.005 mg/L), 140 (median 0.004 mg/L), and 150 (median 0.004 mg/L). Median concentrations of total phosphorus were 0.005 and 0.004 mg/L at sites 165 and 185. The effects of the Eminence WWTP also were observed in Phase I (Hauck and Nagel, 2000).

Wastewater Organic Compounds

Trace quantities of organic compounds commonly associated with municipal or domestic sewage effluent were detected in samples collected at sites 5, 15, 60, 80, and 110 (tables 5, 9). Most of the detected concentrations were estimated because they were less than the MRL. Octylphenol monoethoxylate (OPEO1), nonylphenol monoethoxylate (NPEO1), and bispenol A were detected in the associated laboratory blanks. In the cases of OPEO1 and bisphenol A, the laboratory-blank concentrations were larger than the concentration

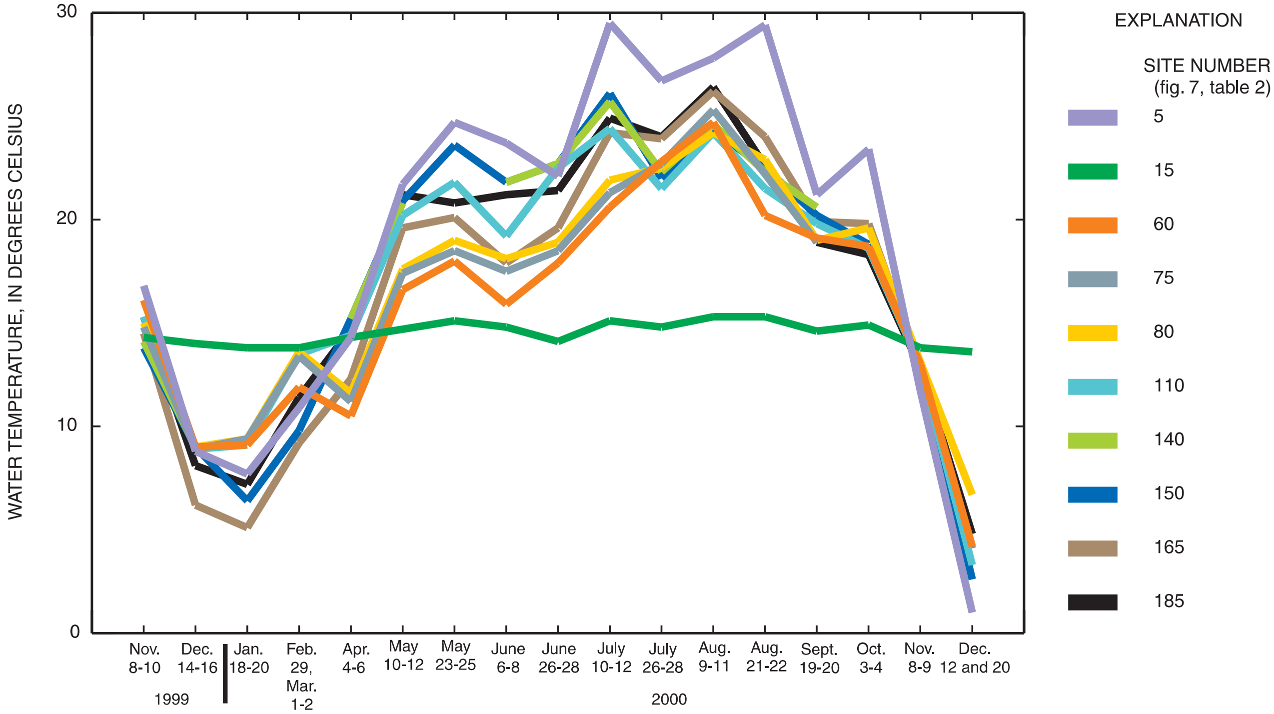

Figure 19. Water temperature at Jacks Fork main stem sites and Alley Spring,

detected in the environmental sample, thereby making data interpretation impossible. Some of the compounds detected have sources other than sewage effluent. Polynuclear aromatic hydrocarbons (PAHs) can be associated with asphalt roads, water proofing of concrete foundations, asphalt roof shingles, and fuel combustion. Plasticizers are extremely common in an industrialized society because they are in many products and, therefore, are commonly reported laboratory contaminants. N,N-diethyltoluamide, or DEET, is a common ingredient in insect repellents, and its presence in the Jacks Fork would not be unexpected.

Of the organic compounds detected in the water samples from the Jacks Fork and Alley Spring, the best indicators of municipal or domestic sewage effluent are the non-ionic detergent metabolites (NPEO1, OPEO1, and para-nonylphenol), the disinfectant phenol, and caffeine. However, possible sources of these compounds could be the numerous campers, swimmers, and canoeists that were present in the area on July 16 and August 10, 2000, when the wastewater organic compound samples were collected. Associated fecal coliform bacteria densities were relatively small in the samples collected on July 16 and August 10, or both, at sites 5 (10 col/100 mL), 15 (22 col/100 mL), 60 (14 and 25 col/100 mL), and 110 (10 col/100 mL), indicating a source other than sewage. The fecal coliform density at site 80 was 210 col/100 mL, but the only compounds detected were caffeine [estimated 0.08 µg/L (microgram per liter)] and N,N-diethyltoluamide (estimated 0.038 µg/L). At the time of sample collection, numerous swimmers and tubers were in the water, which may account for the presence of these compounds.

SUMMARY

In 1998, an 8-mile reach of the Jacks Fork was included on Missouri's list of impaired waters as required by Section 303(d) of the Federal Clean Water Act. The identified pollutant on the Jacks Fork was fecal coliform bacteria, whose presence in large numbers indicates contamination by fecal wastes of humans and other warm-blooded animals. The standard for safe whole-body-contact recreation is 200 col/100 mL (colonies per 100 milliliters) of sample. Potential sources of fecal contamination to the Jacks Fork include a wastewater treatment plant; campground pit-toilet or septic-system effluent; a large commercial, cross-country horseback trail riding facility; canoeists, boaters, and tubers; and cows. The U.S. Geological Survey, in cooperation with the National Park Service, conducted

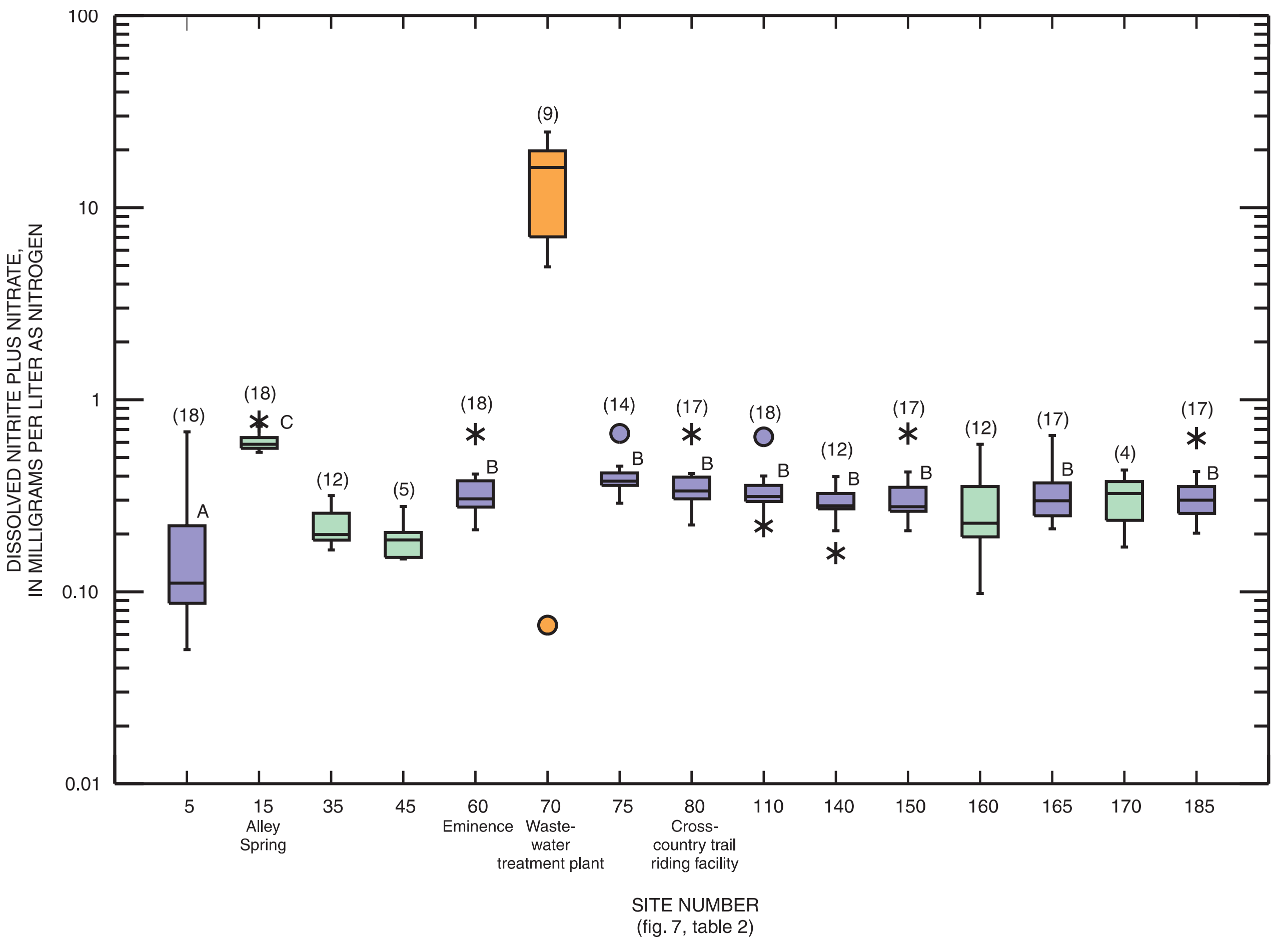

Figure 20. Statistical distribution of dissolved nitrite plus nitrate concentrations at main stem and tributary sites and the Eminence Wastewater Treatment Plant on the Jacks Fork, November 1999 through December 2000.

a study to better understand the extent and sources of microbiological contamination within the Jacks Fork from Alley Spring to the mouth, which includes the 8-mile 303(d) reach. Identification of the sources would provide the National Park Service and the State of Missouri with the information needed to craft a solution of abatement, regulation, prevention, and mitigation with the end result being the removal of the Jacks Fork from the 303(d) list.

During Phase II of the study, 15 sites were sampled approximately monthly from November 1999 through April 2000 and from September 2000 through December 2000 and twice per month from May 2000 through August 2000. An additional site was sampled one time. Samples were collected mostly during base-flow conditions during a variety of nonrecreational and recreational season river uses, including canoeing, swimming, tubing, and horseback riding. Samples were analyzed for fecal indicator bacteria [including fecal coliform, *Escherichia coli* (*E. coli*), fecal streptococci, and enterococci], physical properties, nutrients, and wastewater organic compounds. Additional samples were collected and sent to the College of Veterinary Medicine at the University of Missouri in Columbia, Missouri, for ribotyping analysis.

During the sampling period, the whole-body-contact recreation standard was exceeded from upstream to downstream at sites 80, 110, and 140 on August 10, 2000 (210, 340, and 210 col/100 mL), and also at site 110 on May 11, June 7, and October 3, 2000, (340, 240, and 780 col/100 mL). Each of these exceedences occurred in samples collected during trail rides.

The only statistically significant difference between the overall fecal coliform bacteria density distributions of the Jacks Fork main stem sites was at site 110, which tended to have larger fecal coliform bacteria densities than the other main stem sites. Four of the five tributaries (not including Alley Spring) sampled generally had larger fecal coliform densities than the

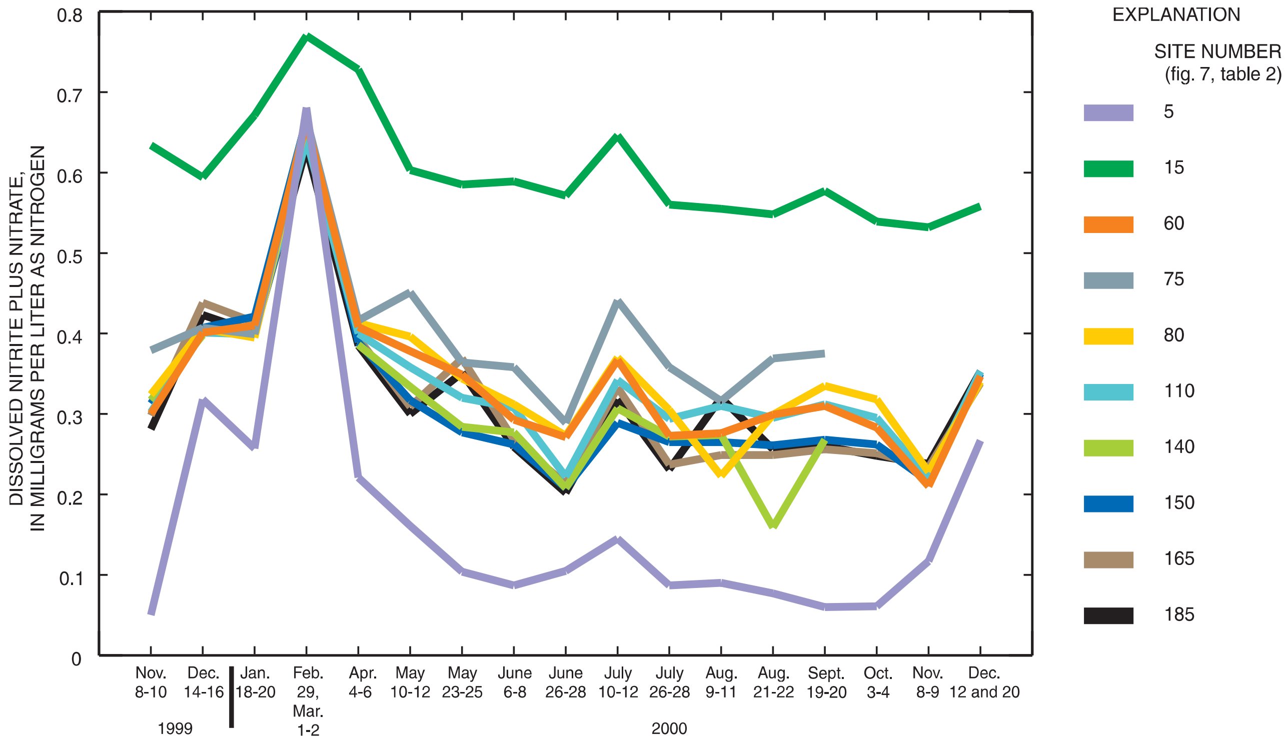

Figure 21. Dissolved nitrite plus nitrate concentrations at Jacks Fork main stem sites and Alley Spring, November 1999 through December 2000.

main stem sites, but the tributaries had little effect on the fecal coliform bacteria densities in the Jacks Fork because of the relatively small discharge of the tributaries relative to the discharge of the Jacks Fork. The Eminence Wastewater Treatment Plant also was not a contributor to the fecal coliform bacteria densities in the Jacks Fork.

The fecal coliform densities and instantaneous loads generally increased from background concentrations at site 60 at Eminence, peaked at site 110, and then continued to decrease to site 185, which is the downstream-most site sampled. The largest concentration of trail riders occurred between sites 75 and 150, and site 110 is located at an intensely used trail-ride crossing outside of the Ozark National Scenic Riverways boundary. Generally, the largest densities and loads at sites 75 to 150 not related to wet-weather flow were observed during a trail ride held August 6 to 12, 2000.

To investigate the effects that large numbers of swimmers, canoeists, and tubers would have on the fecal coliform densities, a 24-hour sample collection was conducted the weekend of July 15 to 16, 2000. Between five or six samples were collected at sites 5, 15, 28, 60, 110, and 150 between Saturday morning until the following Sunday afternoon. No fecal coliform density at any of the sites sampled during the 24-hour period exceeded the whole-body-contact recreation standard. The largest observed density of 110 col/100 mL was in a sample collected at site 110 at 9:00 a.m. on Sunday. The large number of swimmers, canoeists, and tubers using the Jacks Fork did not appear to affect the fecal coliform densities.

Because bacteria survive longer in streambed sediments than in water, a source of bacteria in the water column could be from resuspension of accumulated bacteria from streambed sediments. Water and streambed-sediment samples were collected at three sites (5, 60, and 110) on August 3, 2000, one week before a trail ride. Additional water and streambed-sediment samples were collected at three sites (110, 150, and 160) during a trail ride. Results indicate that fecal-bacteria densities increased substantially in the streambed sediment and water column during the trail ride.

Analysis of the fecal coliform bacteria data indicates that during the Phase II sampling period bacteria densities not related to wet-weather flow were largest during trail rides. During the June 4 to 10 and August 6 to 12 trail rides, numerous people were camping near the river or swimming, canoeing, or tubing in the river, which may have contributed to the elevated bacteria densities. However, few people were camping or swim-

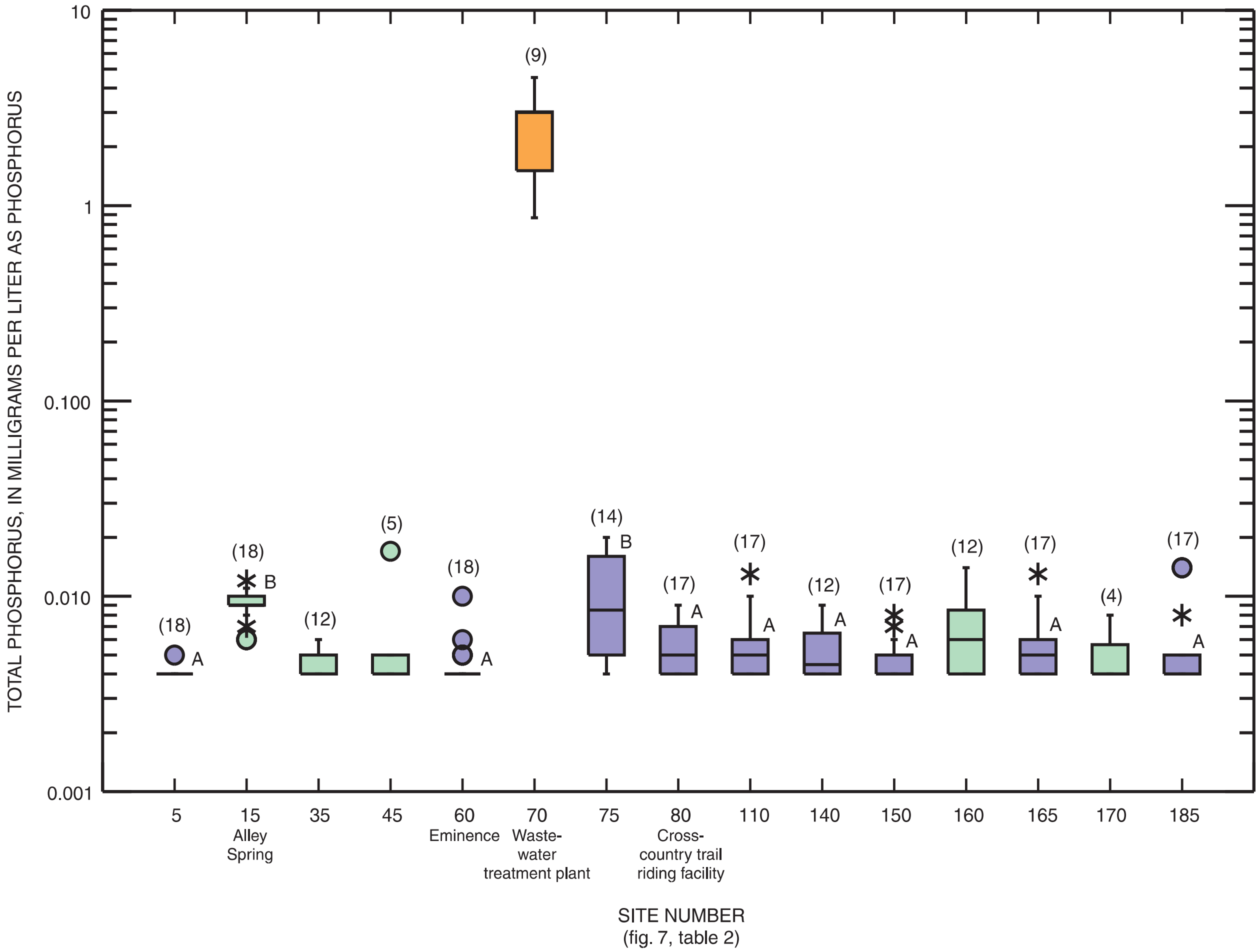

Figure 22. Statistical distribution of total phosphorus concentrations at main stem and tributary sites and the Eminence Wastewater Treatment Plant on the Jacks Fork, November 1999 through December 2000.

ming, canoeing, or tubing during the May, October, and November trail rides, and bacteria densities were still elevated. The reasons for these elevated densities may be related to four factors: (1) physical disturbance of streambed sediments causing resuspension of accumulated bacteria, (2) defecation directly into the river, (3) fecal material carried into the river on the feet of animals, and (4) leakage of sewage effluent from an unknown source into the river.

Sixty-five *E. coli* isolates obtained from water samples collected at 9 sites (5, 35, 45, 60, 80, 110, 150, 160, and 165) and 23 *E. coli* isolates obtained from streambed-sediment samples collected at 5 sites (5, 60, 110, 150, and 160) were submitted for ribotyping analysis. Samples were collected in 2000 during a variety of nonrecreational and recreational season river uses, including trail rides, canoeing, tubing, and swimming. Ribotypes were compared to a library of ribotypes from three known sources: sewage, horses, and cows. Of the 65 isolates from water samples, 40 percent were identified as originating from sewage, 29 percent from horse, 11 percent from cow, and 20 percent from unknown sources. Of the 23 isolates from streambed-sediment samples, 39 percent were identified as originating from sewage, 35 percent from horse, 13 percent from cow, and 13 percent from unknown sources. Ribotyping results should be treated as experimental for the purpose of this study because of the large degree of uncertainty in the method. However, based on the results of comparing unknown patterns to patterns for sewage, horses, and cows, sewage and horses do appear to be possible sources of *E. coli* in the Jacks Fork.

An analysis of the physical property data, including dissolved oxygen, pH, specific conductance, and temperature, for the main stem of the Jacks Fork and Alley Spring indicated that no statistically significant differences occurred among the main stem sites for any of the physical properties. The median pH, specific

Table 9. Selected wastewater organic compound concentrations and fecal coliform densities in water samples collected on July 16 and August 10, 2000

[All concentrations in micrograms per liter; bold numbers indicate that compound was detected; NPEO1, nonylphenol monoethoxylate; OPEO1, octylphenol monoethoxylate; col/100 mL, colonies per 100 milliliters; <, less than; E, estimated; b, detected in laboratory blank]

Date	NPEO1	OPEO1	para-Nonylphenol	Phenol	N,N-diethyltoluamide	Caffeine	Bisphenol A	Ethanol, 2-butoxy-, phosphate	Naphthalene	Fecal coliform (col/100 mL)
Jacks Fork above Alley Spring (site 5, fig. 7)										
07/16/00	<1.00	<0.10	<0.50	**E0.44**	<0.040	<0.08	<0.09	<0.20	<0.02	10
Alley Spring at Alley (site 15, fig. 7)										
07/16/00	<1.00	<.10	<.50	**E.45**	<.040	<.08	<.09	<.20	<.02	22
Jacks Fork at Eminence (site 60, fig. 7)										
07/16/00	<1.00	<.10	**E.95**	**E.34**	**E.018**	<.08	<.09	<.20	<.02	14
08/10/00	<1.00	<.10	<.50	**E.20**	**E.034**	<.08	**.45 b**	**.21**	**.02**	25
Jacks Fork above 2nd unnamed hollow below Eminence (site 80, fig. 7)										
08/10/00	<1.00	<.10	<.50	<.25	**E.038**	**E.08**	**.30 b**	<.20	<.02	210
Jacks Fork above Lick Log Hollow below Eminence (site 110, fig. 7)										
07/16/00	**E1.26 b**	**E.06 b**	**E.95**	**E1.03**	**.044**	<.08	<.09	<.20	<.02	10

conductance, and temperature for samples collected at Alley Spring were significantly smaller than the values for the main stem sites. In samples collected from the Eminence Wastewater Treatment Plant, the median dissolved oxygen concentration and pH were smaller than the medians for the main stem sites, and the median specific conductance was larger than the medians for the main stem sites. However, no effects from the Eminence Wastewater Treatment Plant were observed immediately downstream at site 75, probably because of the small volume of the effluent. Unlike fecal coliform bacteria, most variation in dissolved oxygen, pH, specific conductance, and temperature values was related to seasonal changes, time of day the samples were collected, or hydrologic conditions and not to recreational activities.

On the main stem of the Jacks Fork, dissolved nitrite plus nitrate concentrations were statistically smaller at the most upstream site above Alley Spring [site 5; median 0.11 mg/L (milligrams per liter)] and increased below Alley Spring at Eminence (site 60; median 0.30 mg/L). A small, but not statistically significant, increase in dissolved nitrite plus nitrate concentrations occurred at site 75 (median 0.38 mg/L) immediately downstream from the Eminence Wastewater Treatment Plant (site 70; median 16.2 mg/L). The effect diminished quickly downstream from the plant. Unlike fecal coliform bacteria, dissolved nitrite plus nitrate concentrations were related mostly to hydrologic conditions and did not increase during recreational activities. Similar results were observed with total phosphorus. However, the increase in total phosphorus concentrations at site 75 immediately downstream from the Eminence Wastewater Treatment Plant was statistically significant, but the effect diminished quickly downstream.

Trace quantities of organic compounds commonly associated with municipal or domestic sewage effluent were detected in samples collected at sites 5, 15, 60, 80, and 110. Octylphenol monoethoxylate and bisphenol A were detected in associated laboratory blanks at concentrations larger than the concentration detected in the environmental sample, thereby making interpretation of the data impossible. Other detected compounds, such as polynuclear aromatic hydrocarbons, have sources other than sewage effluent. The best indicators of municipal or domestic sewage effluent were the non-ionic detergent metabolites (nonylphenol monoethoxylate, octylphenol monoethoxylate, and para-nonylphenol), phenol, and caffeine. However, possible sources of these compounds, which were detected in one or more of the samples, could be the numerous campers, swimmers, and canoeists that were present when the samples were collected.

Although both Phase I and II sampling indicate that fecal coliform densities tend to increase to sometimes unacceptable levels during trail rides, the exact causes for this increase and the sources of the fecal coliform bacteria have not been positively identified. Phase III sampling has continued at 10 sites plus the Eminence Wastewater Treatment Plant since January 2001, with specific recreational activities (canoeing and trail rides) being the primary focus of the sampling efforts. Additional work is being done to examine the role that storage of fecal coliform in the streambed sediments and then subsequent resuspension may contribute to the overall concern and to try to positively identify the sources of the bacteria using ribotyping and other Microbial Source Tracking techniques.

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