Water Quality in the Little Sac River Basin near Springfield, Missouri, 1999–2001

By Brenda J. Smith

Abstract

The Little Sac River, north of Springfield, Missouri, flows through mainly agricultural and forest land. However, the quality of the river water is a concern because the river flows into Stockton Lake, which is a supplemental drinking water source for Springfield. Large bacterial densities and nutrient concentrations are primary concerns to the water quality of the river.

A 29-river mile reach of the Little Sac River is on the 1998 list of waters of Missouri designated under section 303(d) of the Federal Clean Water Act because of fecal coliform densities larger than the Missouri Department of Natural Resources standard (hereinafter referred to as Missouri standard) of 200 colonies per 100 milliliters for whole-body contact recreation. During an investigation of the water quality in the Little Sac River by the U.S. Geological Survey, in cooperation with the Watershed Committee of the Ozarks, fecal coliform bacteria densities exceeded the Missouri standard (the standard applies from April 1 through October 31) in one sample from a site near Walnut Grove. At other sites on the Little Sac River, the Missouri standard was exceeded in two samples and equalled in one sample upstream from the Northwest Wastewater Treatment Plant (NW WTP) and in one sample immediately downstream from the NW WTP.

Effluent from the NW WTP flows into the Little Sac River. Annually from April 1 through October 31, the effluent is disinfected to meet the Missouri standard for whole-body contact recreation. Fecal coliform bacteria densities in samples collected during this period generally were less than 100 colonies per 100 milliliters. For the rest of the year when the effluent was not disinfected, the bacteria densities in samples ranged from 50 (sample collected on November 1, 2000) to 10,100 colonies per 100 milliliters (both counts were non-ideal).

When the effluent was disinfected and the fecal coliform bacteria density was small, samples from sites upstream and downstream from the NW WTP had a bacteria density larger than the density in the effluent. Other sources of bacteria are likely to be present in the study area in addition to the NW WTP. These potential sources include effluent from domestic septic systems and animal wastes.

Nutrient concentrations in the Little Sac River immediately downstream from the NW WTP were affected by effluent from the NW WTP and possibly other sources. At two sites upstream from the NW WTP, median nitrite plus nitrate concentrations were 1.1 and 1.4 milligrams per liter. The median nitrite plus nitrate concentration for the effluent from the NW WTP was 6.4 milligrams per liter, and the median concentration decreased downstream in the Little Sac River to 2.2, 1.2, and 0.56 milligrams per liter.

The effects of the effluent from the NW WTP on the water quality of the Little Sac River downstream from the NW WTP were reflected in an increase in discharge (effluent from the NW WTP can be as much as 50 percent of the flow at the site about 1.5 river miles downstream from the NW WTP), an increase in specific conductance values, an increase in several inorganic constituent concentrations, including calcium, magnesium,

and sulfate, and a large increase in sodium and chloride concentrations. The effluent from the NW WTP seemed to have no effect on the pH value, temperature, and dissolved oxygen concentrations in the Little Sac River.

Results of repetitive element polymerase chain reaction (rep-PCR) pattern analysis indicated that most *Escherichia coli* (*E. coli*) bacteria in water samples probably were from nonhuman sources, such as horses and cattle. The rep-PCR pattern analysis indicated that horses were an important source of *E. coli* downstream from the NW WTP, which was consistent with horses pastured adjacent to the sampling site.

Fecal coliform bacteria loads increased upstream from the NW WTP from the most upstream site to the site immediately upstream from the NW WTP. Loads in the effluent from the NW WTP and also those in the Little Sac River downstream from the NW WTP were dependent on the treatment of the effluent. When the effluent was not disinfected, the loads in the effluent increased from those upstream. Downstream in the Little Sac River, the loads decreased, but then increased at the most downstream site. The increase may be a result of increased loads from tributaries and other sources not sampled during the study. When the effluent was disinfected, fecal coliform bacteria loads were less than loads in samples from the Little Sac River downstream from the NW WTP.

INTRODUCTION

The Little Sac River (fig. 1), in southwestern Missouri, is within the Springfield Plateau of the Ozark Plateaus physiographic province (Fenneman, 1938). From its headwaters in Greene County, the Little Sac River flows about 45 mi (miles) through mostly rural agricultural areas until it reaches Stockton Lake—a source of drinking water for the city of Springfield. The annual mean discharge for the Little Sac River upstream from Stockton Lake was 266 ft^3/s (cubic feet per second) for the 1999 calendar year and 75.4 ft^3/s for the 2000 water year (October 1999 through September 2000; Hauck and Nagel, 2001). The Little Sac River has five designated beneficial uses—livestock and wildlife watering, protection of warm-water aquatic life, cool-water fishery, whole-body contact recreation, and boating and canoeing (Missouri Department of Natural Resources, 1996).

Springfield is one of the fastest growing areas in the State. From 1990 to 2000, the population increased from 140,494 to 151,580, a 7.9 percent increase (U.S. Bureau of the Census, 1990, 2000). With this population increase comes a corresponding increase for drinking water supply. In the past, Springfield has used the surface-water sources of Fulbright Spring, Fellows and McDaniel Lakes, and the James River (in the southern part of Springfield) and ground water from municipal wells for its drinking water supply. In 1996, Springfield began receiving part of its public water supply from Stockton Lake and decreased its dependence on ground water. The water from Stockton Lake is used to supplement drinking water supplies from Fellows Lake. The intake for the Stockton Lake supply is located on an arm of the lake that receives inflow from the Little Sac River.

Densities of fecal coliform bacteria greater than the Missouri standard for whole-body contact recreation (Missouri Department of Natural Resources, 1996) of 200 col/100 mL (colonies per 100 milliliters) have been detected at a monitoring site on the Little Sac River north of Springfield (Little Sac River near Walnut Grove; site 6, fig. 1). The exceedences of fecal coliform bacteria at this site have resulted in a 29-river mi reach of the Little Sac River being included on the 1998 list of waters designated under section 303(d) of the Federal Clean Water Act by the Missouri Department of Natural Resources (MDNR; Missouri Department of Natural Resources, 1998). The presence of fecal coliform bacteria indicates contamination by fecal wastes of humans or other warm-blooded animals, or both. Potential sources of fecal contamination to this reach of the Little Sac River include the city of Springfield's Northwest Wastewater Treatment Plant (NW WTP), livestock within the basin, and domestic septic systems.

Nutrient concentrations are another water-quality factor in the Little Sac River and ultimately in Stockton Lake. Effluent from the NW WTP may be contributing to nutrient concentrations. Other possible nutrient sources are fertilizers and the presence of hobby and livestock farms with large numbers of livestock, including cattle, horses, swine, and sheep.

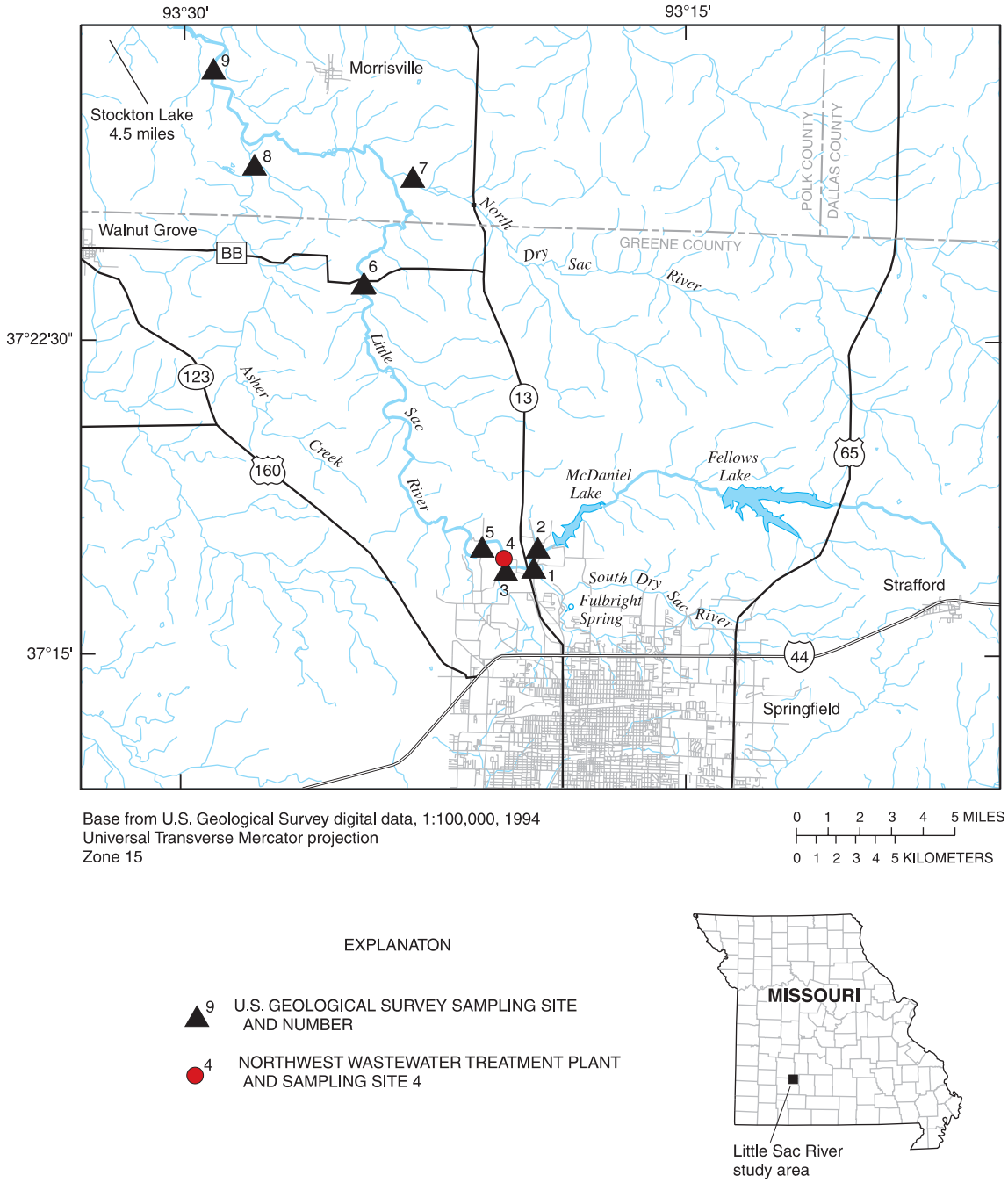

Figure 1. Location of U.S. Geological Survey sampling sites in the Little Sac River Basin near Springfield, Missouri.

Because of the water-quality concerns and the need to better understand the water quality in the Little Sac River, the U.S. Geological Survey (USGS), in cooperation with the Watershed Committee of the Ozarks, began a study of the water quality in the Little Sac River. The emphasis of the study was on the distribution and possible sources of fecal coliform bacteria and nutrients.

Purpose and Scope

This report describes water quality in the Little Sac River Basin, with an emphasis on distribution and possible sources of fecal coliform bacteria and nutrients. Data also are presented on the distribution of inorganic constituents, selected trace elements, and organic compounds commonly associated with municipal and domestic wastewater. The possible origin of fecal coliform bacteria in water samples was investigated using molecular genetic techniques being researched at the University of Missouri at Columbia College of Veterinary Medicine.

More than 100 samples were collected from a network of 9 sites during an 18-month study from November 1999 through April 2001. Water samples were submitted for various analyses including indicator bacterial densities, total nutrients, and optical brighteners. Selected samples were analyzed for inorganic constituents, selected trace elements, and a suite of 46 organic compounds.

Previous Investigations

The USGS has collected water samples from the Little Sac River near Walnut Grove (site 6; fig. 1) monthly in water years 1984 to 1986 (October 1983 through September 1986), 1988 to 1990 (October 1987 through September 1990), 1994 to 1996 (October 1993 through September 1996), and from October 1998 through April 2001. This sampling was done as part of the Ambient Water-Quality Monitoring Network in cooperation with the MDNR. The fecal coliform bacteria density was more than the Missouri standard of 200 col/100 mL in 21 of 88 samples and equalled the standard in 2 samples. In 16 of the samples, the fecal coliform density ranged from 230 to 11,000 col/100 mL. Most of the larger densities throughout the sampling period occurred when the streamflow was more than 110 ft^3/s, indicating large bacteria densities associated with high flow.

Nutrient concentrations also were determined for the samples collected by the USGS. The median total nitrite plus nitrate concentration as nitrogen (hereinafter referred to as nitrite plus nitrate) in 68 samples was 1.75 mg/L (milligrams per liter), and concentrations ranged from less than 0.10 to 4.5 mg/L. The median total phosphorus concentration in 87 samples was 0.18 mg/L; concentrations ranged from less than 0.02 to 2.2 mg/L. Total orthophosphorus as phosphorus (hereinafter referred to as orthophosphorus) concentrations in 18 samples collected from November 1993 through October 1999 ranged from less than 0.01 to 0.80 mg/L. The median concentration in these samples was 0.06 mg/L.

The MDNR, Water Pollution Control Program (WPCP) collected water samples from the Little Sac River upstream and downstream from the NW WTP. Samples were collected in July and August 1999 and in May, August, and September 2000. Upstream from the NW WTP, fecal coliform bacteria density was 370 col/100 mL in the May 2000 sample; the remaining four samples had densities less than 200 col/100 mL. A sample collected at the effluent from the NW WTP in September 2000 had a density of 700 col/100 mL. Other samples collected at the effluent from the NW WTP had densities less than 200 col/100 mL. The fecal coliform bacteria densities at the Little Sac River near Walnut Grove were 200 col/100 mL or larger 12.5 river mi downstream from the NW WTP in all samples collected during 2000. The samples collected during 1999 had densities less than 200 col/100 mL on the Little Sac River near Morrisville, 23 river mi downstream from the NW WTP. Two samples collected during 2000 had fecal coliform densities greater than 200 col/100 mL (410 and 430 col/100 mL). The other three samples had densities less than 200 col/100 mL (Scott Goodin, Missouri Department of Natural Resources, Water Pollution Control Program, written commun., 2001).

The Springfield Department of Public Works has collected water samples upstream and downstream from the NW WTP for analysis of ammonia and dissolved oxygen concentrations. From November 1999 through May 2001, concentrations of ammonia as nitrogen (hereinafter referred to as ammonia) in samples collected upstream from the NW WTP ranged from less than 0.1 to 1.1 mg/L. The dissolved oxygen concentrations ranged from 5.6 to 16.3 mg/L. Downstream from the NW WTP, ammonia concentrations

ranged from less than 0.1 to 1.52 mg/L. The dissolved oxygen concentrations ranged from 5.6 to 15.4 mg/L (Jim Burks, City of Springfield, Department of Public Works, written commun., 2001).

Springfield City Utilities has collected water samples from the Little Sac River at Farm Road 141 (old State Highway 13) and at the Hamilton Bridge west of Morrisville (near site 9; fig. 1). Samples were collected at the site on Farm Road 141 during 1995. Nitrite plus nitrate concentrations ranged from 0.208 to 0.777 mg/L. Orthophosphorus concentrations ranged from 0.021 to 0.034 mg/L. The nitrite plus nitrate concentrations in water samples collected from the Little Sac River near Morrisville from April 1991 through May 2001 ranged from less than the detection limit of 0.001 to 7.96 mg/L. Except for the concentration of 7.96 mg/L, the concentrations for all remaining samples were less than 1.80 mg/L. The orthophosphorus concentrations ranged from less than 0.001 to 0.390 mg/L (John Witherspoon, City Utilities of Springfield, written commun., 2001).

Three landfills are in the study area (fig. 2). Two of the landfills are in the upper part of the Little Sac River Basin, both upstream from the NW WTP. The Fulbright Landfill, on the south bank of the South Dry Sac River upstream from State Highway 13, accepted municipal and industrial wastes from 1962 to 1969; the Sac River Landfill, which accepted municipal and industrial wastes from 1968 to 1974, is in a bend of the Little Sac River upstream from the NW WTP. The two landfills, considered to be one site, are on the U.S. Environmental Protection Agency's (USEPA) National Priority List (Superfund). During 2000, the USEPA determined that further active remediation was not nec-

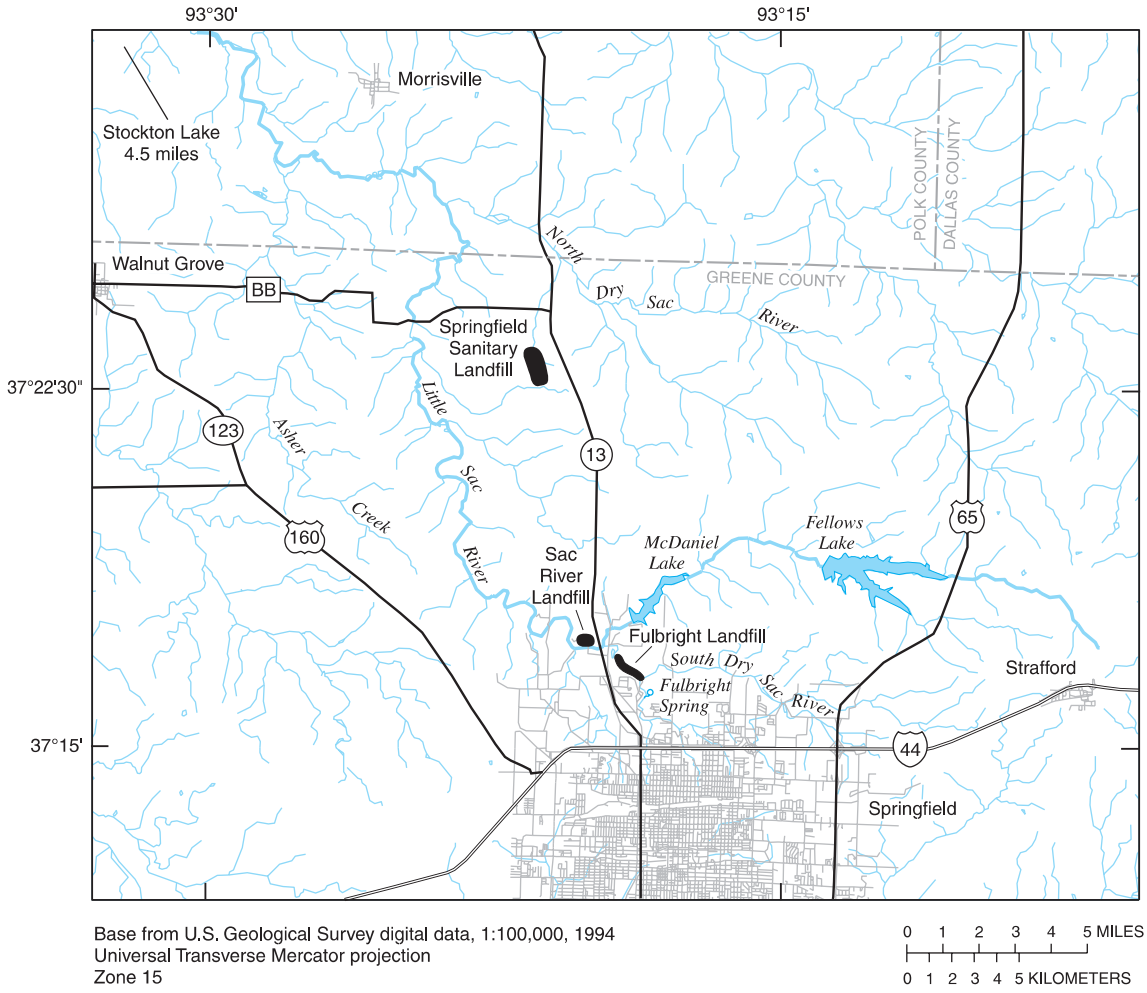

Figure 2. Location of landfills in the study area.

essary and that human health and the environment remain protected (Debbie Krieg, U.S. Environmental Protection Agency, written commun., 2001). Surface-water, ground-water, and leachate monitoring will continue to be conducted upgradient and downgradient from the two landfills. Arsenic, beryllium, cadmium, chromium, lead, mercury, nickel, zinc, and selected volatile organic compounds are the contaminants of concern. Concentrations were less than the detection limit for volatile organic compounds and near background concentrations for trace elements. Water samples from the Superfund site were not analyzed for bacteria and nutrients. The Springfield Sanitary Landfill, on a 1,000-acre site, is upstream from the junction of State Highway 13 and County Highway BB (fig. 2). With vegetation growing on bare soil and the construction of a stormwater retention basin to alleviate total dissolved solids concentrations, the landfill generally is in compliance with Missouri standards (Steve Short, Missouri Department of Natural Resources, oral commun., 2001).

Acknowledgments

Special acknowledgment is given to Charles Parrot of the NW WTP for his assistance in sampling efforts. John Ford and Scott Goodin of the MDNR, WPCP, and Steve Short, MDNR, John Witherspoon, City Utilities of Springfield, Jim Burks, Springfield Department of Public Works, and the National Weather Service at the Springfield-Branson Regional Airport graciously provided data collected by their respective organizations. The author thanks Andy Carson and Brian Shear, University of Missouri at Columbia, for analysis of repetitive element polymerase chain reaction (rep-PCR) patterns.

DESCRIPTION OF THE STUDY AREA

The Little Sac River Basin consists of about 390 mi^2 (square miles) in parts of Greene and Polk Counties. The study area included the Little Sac River downstream from McDaniel Lake to its junction with Stockton Lake and encompasses the reach of the Little Sac River that is on the 303(d) list of impaired waters by the MDNR. Major tributaries to the Little Sac River include the South Dry Sac River, North Dry Sac River, and Asher Creek.

Climate

The Little Sac River Basin is characterized by a temperate climate with warm, humid summers and cool, wet winters. The National Oceanic and Atmospheric Administration (NOAA) operates a climatological station at the Springfield-Branson Regional Airport, which is in the northwestern part of the city of Springfield. The average temperature range as measured at the airport is 67 to 90 °F (degrees Fahrenheit) during the summer and 20 to 42 °F during the winter (National Oceanographic and Atmospheric Administration, 1999, 2000, 2001).

The average annual precipitation is 43 in. (inches) of rainfall and 17 in. of snowfall in Springfield. The average annual precipitation during the sampling period from November 1999 through April 2001 was 58.1 in. Rainfall recorded at the Springfield-Branson Regional Airport during that time was 54.3 in. The departure from average monthly precipitation is shown in figure 3. Larger than average quantities of precipitation fell in 4 months of the sampling period, and during that time more than 12 in. of precipitation was recorded above average quantities. Most samples were collected in months that had less than average precipitation.

Land Use

Land use in the Little Sac River Basin predominantly is agricultural and forest with some urban areas. Cropland and pastureland comprise 63.5 percent of the basin; beef (cow-calf) and dairy operations are included in pastureland. Deciduous forest covers 27 percent of the basin with mixed forest covering 1.9 percent. Reservoirs, including Stockton, Fellows, and McDaniel Lakes comprise 3.8 percent of the basin. Residential areas comprise 1.5 percent and are restricted to northern Springfield along with several communities, including Morrisville, Walnut Grove, and Strafford. Other land uses are less than 2 percent (Richard Bradford, U.S. Geological Survey, written commun., 1999) and include landfills (fig. 2) and the NW WTP.

Agricultural data, available for Greene County, but not specifically for the Little Sac River Basin (U.S. Bureau of the Census, 1997), indicate that the number of farms and livestock are decreasing. In 1992, the number of farms in Greene County was 2,103 and had decreased to 1,997 by 1997. The number of cattle and

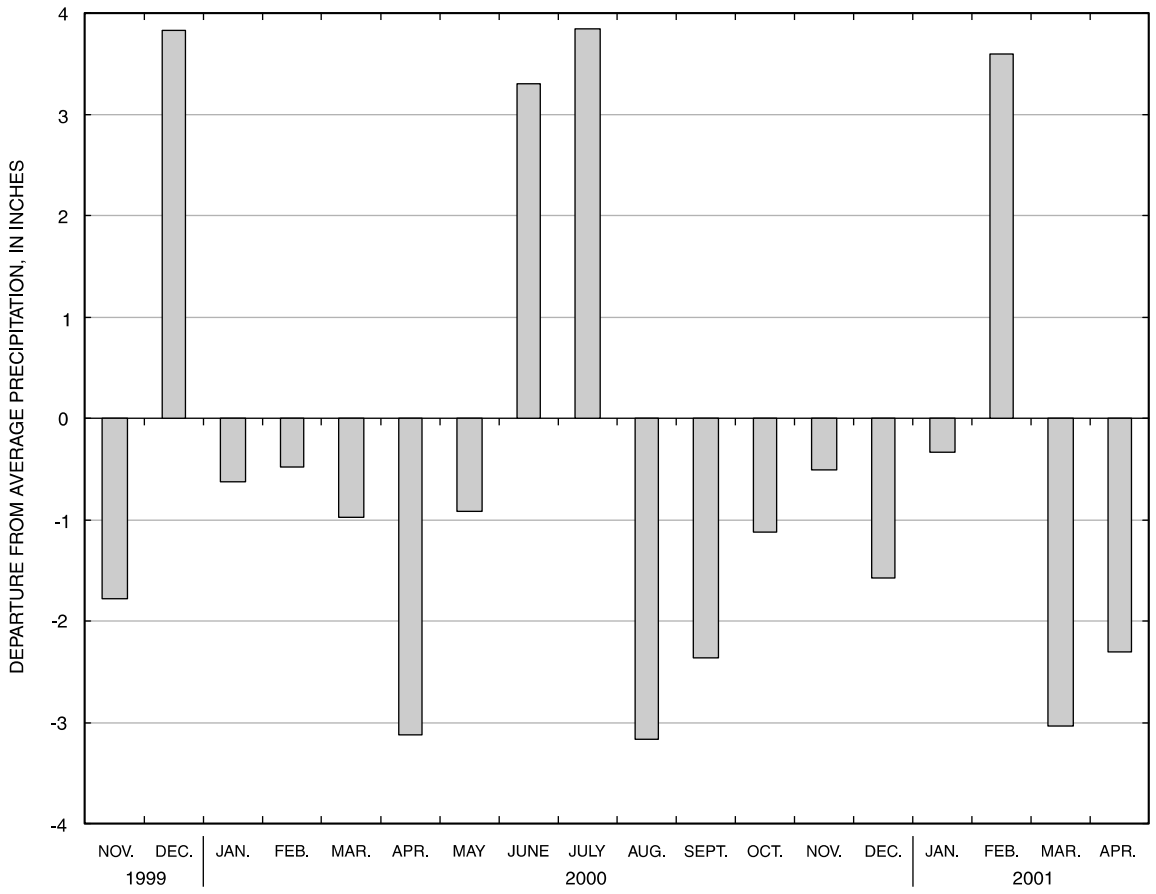

Figure 3. Departure from average precipitation at the Springfield-Branson Regional Airport, 1999–2001 (data from the National Oceanic and Atmospheric Administration, 1999–2001).

calves was 87,806 during 1992 and 79,119 during 1997. The number of swine during 1992 was 5,287 and during 1997 was 4,545.

Topography and Geology

Land-surface elevations in the study area range from about 950 ft (feet) at the downstream site on the Little Sac River in the northwestern part of the study area to about 1,100 ft in the southeast. Flow in the Little Sac River begins in the upland area northeast of the city of Springfield and continues northwest through the study area.

The slightly rolling hills of the Springfield Plateau contain numerous karst features, including sinkholes, springs, and caves. Numerous sinkholes are present in Greene County, especially north and northwest of Springfield near the southern part of the study area (Waite and Thomson, 1993). The presence of karst features makes ground water in the underlying rocks susceptible to contamination from surficial sources.

Mississippian-age cherty limestones form the bedrock in most of the study area (Imes, 1989). However, dolostones of Ordovician age crop out in the general vicinity of the North Dry Sac River (Imes, 1989; Missouri Division of Geology and Land Survey, 1979).

METHODS OF STUDY

To determine the 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 densities and inorganic and organic constituents.

Monitoring Network and Sample Collection

Following a reconnaissance of the study area in early November 1999, a network of nine sampling sites was established (fig. 1). Sampling site selection was based on accessibility to the stream and adequate site characteristics for collecting water-quality samples and measuring discharge at low and high flows. Water-quality samples were collected at 4 sites 14 times during the study (November 1999 through April 2001) and 6 times at the remaining 5 sites. Several sites were established upstream and downstream from the NW WTP to provide data on the effects of the effluent from the NW WTP on the water quality of the Little Sac River, in addition to providing data on the distribution of bacteria, nutrients, and other constituents.

Site 1 on the South Dry Sac River is upstream from the NW WTP. Although this site is on a tributary to the Little Sac River, in this report, it is considered as a site on the Little Sac River because at the start of the sampling period, in November 1999, no flow was observed in the Little Sac River upstream from the NW WTP (table 1, at the back of this report). When water was flowing in the Little Sac River upstream from the NW WTP, the flow at site 1 generally was larger than that in the Little Sac River. Sample collection at site 1 was downstream from the bridge on Farm Road 141. During the sampling period, no livestock were present in the adjacent pastureland, and access to the river was fenced. Beneficial uses of the South Dry Sac River are livestock and wildlife watering and protection of warm-water aquatic life.

Another site upstream from the NW WTP was the Little Sac River at Farm Road 141 (site 2). This site is immediately downstream from McDaniel Lake. Access to the river was fenced at this site also.

Site 3, the Little Sac River at Ritter Spring, also is upstream from the NW WTP. Water samples were collected about 1,000 ft downstream from the junction of the Little Sac River and Ritter Spring. The Ritter Spring City Park is upstream from site 3, and the Sac River Landfill is adjacent to the site on the east. The median discharge at this site during the study was 11.2 ft^3/s (table 2, at the back of this report).

The effluent from the NW WTP was designated as site 4. The pipe transporting the effluent from the NW WTP to the Little Sac River is at the northwestern part of the NW WTP. It is one of two treatment facilities for the city of Springfield. The NW WTP processes mainly domestic sewage, except for some industrial and commercial waste. The effluent from the NW WTP contributes substantial flow to the Little Sac River downstream from the NW WTP. The median discharge of the NW WTP when water samples were collected was 6.6 ft^3/s.

Site 5, the Little Sac River at Farm Road 125, is about 1.5 river mi downstream from the NW WTP. The sampling site was about 250 ft upstream from a low-water bridge across the Little Sac River. A commercial horse stable is less than 0.5 mi from the river. Cattle also were observed near the horse stable. Access to the river was fenced.

The Little Sac River near Walnut Grove was site 6, about 12.5 river mi downstream from the NW WTP. As previously discussed, this site is part of the Ambient Water-Quality Monitoring Network (station number 06918600), and as such, a monthly water-quality sample was collected, in addition to the 14 samples collected for this investigation, for a total of 32 samples. Samples were collected about 5 ft upstream from a bridge over the Little Sac River east of Walnut Grove. Cattle were observed in pastureland adjacent to the river. Access to the river was fenced.

Site 7 was on the North Dry Sac River, a tributary to the Little Sac River, at a low-water crossing at a commercial sod farm. In November 1999 and October 2000, no flow was observed at the site. However, a pool of water was sampled each time. During the sampling period, the streambed was disturbed, probably by heavy equipment. Beneficial uses of the North Dry Sac River include livestock and wildlife watering and protection of warm-water aquatic life.

Another tributary to the Little Sac River, Asher Creek, was sampled at site 8. The sampling site, near the junction of the creek and the Little Sac River, was at a low-water crossing on a gravel road southwest of Morrisville. Water samples were collected upstream from the low-water crossing. Cattle were observed adjacent to the creek and had direct access to the water. No beneficial uses have been designated for Asher Creek.

The most downstream site (site 9) was the Little Sac River near Morrisville. A USGS continuously recording streamflow gaging station is at this site (station number 06918740). Samples were collected about 0.25 river mi downstream from Hamilton Bridge on State Highway 215, about 2 mi west of Morrisville. No livestock were observed near the river.

At all sites, measurements of discharge, specific conductance, pH, water temperature, and dissolved oxygen were made, and water-quality samples were collected and analyzed for indicator bacteria and total (unfiltered) nutrients. Indicator bacteria included fecal coliform, fecal streptococcus, and *Escherichia coli* (*E. coli*). Nutrient analyses included nitrite, nitrite plus nitrate, ammonia, phosphorus, and orthophosphorus. Additional water samples were collected at each site and analyzed for dissolved inorganic constituents, including calcium, magnesium, sodium, potassium, sulfate, chloride, fluoride, and the trace elements boron and strontium. Selected samples were analyzed for compounds associated with wastewater, including optical brighteners.

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

Samples for the determination of nitrogen species and orthophosphorus were placed in amber 125-mL (milliliter) polyethylene bottles and chilled to 4 °C (degrees Celsius). Samples for the determination of phosphorus were put into 125-mL clear polyethylene bottles and preserved to pH less than 2 with sulfuric acid before chilling to 4 °C. Samples for the determination of dissolved inorganic constituents were filtered through a 0.45 μm (micrometer) pore-size disposable capsule filter using a peristaltic pump as the pressure source. Samples for inorganic constituents and trace elements were placed in acid washed 250-mL polyethylene bottles and acidified to pH less than 2 with nitric acid. Samples for the determination of organic constituents were placed in baked 40-mL amber vials (optical brighteners) or 1-L amber glass bottles (other compounds associated with wastewater). Blanks for inorganic constituents and nutrients were prepared by the USGS laboratory in Denver, Colorado. Concentrations of inorganic constituents and nutrients in the blank samples were at or less than laboratory reporting limits.

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

Bacteria Methods

Bacteria samples were collected in sterilized 500-mL polyethylene bottles. The bottles were filled by plunging them neck downward beneath the water surface at the centroid of flow in the stream. After collection, the samples were placed on ice until processing and enumerated using the membrane filter technique according to methods described in Wilde and Radtke (1998). Daily blanks were prepared by filtering 50 or 100 mL of sterile buffer water through the appropriate filters and incubation with the samples. No fecal coliform, fecal streptococcus, or *E. coli* colonies were detected in any of the blank samples.

Microbial Source Tracking (MST), the comparison of deoxyribonucleic acid (DNA) “fingerprints” of bacteria isolates from a sample to isolate groups from known sources, was used in this study to help identify the primary sources of *E. coli* in water samples. The use of MST has been shown to be useful in discriminating between human and nonhuman sources of *E. coli* in water samples from a Florida estuary (Parveen and others, 1999) and between various animal sources (Schlottmann and others, 2000). A form of MST called rep-PCR was used in this study. Different size DNA fragments are amplified from both an environmental sample and known-source feces to produce a banding pattern. The resulting banding patterns of the *E. coli* from the environmental water sample are compared to the banding patterns from *E. coli* in the known-source feces for similarity and possible identification.

Water samples collected from three sites (sites 1, 5, and 6) in March, June, and July 2001 and from four sites (sites 1, 4, 5, and 6) in April 2001 were submitted to the University of Missouri at Columbia College of Veterinary Medicine for rep-PCR pattern analysis. These samples were collected identically to the samples collected for indicator bacteria.

Water samples to determine the presence of the human pathogen *E. coli* O157:H7 were processed at the Department of Veterinary Pathobiology, University of Missouri, Columbia. Water samples were filtered with a 0.45-µm membrane filter and incubated at 37 °C for 12 to 16 hours on mEndo-LES agar. Between 20 and 30 shiny metallic colonies on the filters were transferred to a second set of mEndo-LES plates and to mFC plates using sterile toothpicks. The mEndo plates were incubated at 37 °C and the mFC plates were incubated at 44.5 °C for 24 hours. Only colonies that were positive for *E. coli* O157:H7 on the plates were further tested. Lastly, the positive colonies were transferred to McConkey Sorbitol plus MUG agar and incubated for 4 to 6 hours at 37 °C.

Data Analysis

Statistical tests on water samples were done using the computer software SYSTAT (SPSS Inc., 1998). Summary statistics for each site were computed using all data. Censored data included values reported as not detected at the laboratory reporting limit (less than values), estimated values (E values) for some measurements or chemical constituents detected at less than the reporting limit, and less than non-ideal plate counts (K values) for indicator bacteria. Because the less-than values and estimated values represented a small fraction of the data set, they were converted to numerical values by removing the remark code. Censored indicator bacteria densities, such as non-ideal plate counts, also were used in all statistical calculations by removing the remark code.

The distribution of selected physical property and chemical constituents were graphically displayed using truncated, side-by-side boxplots. The boxplots show selected percentiles of the data distribution including the 25th, 50th (median), and 75th. Points within 1.5 times the interquartile range are indicated by "whiskers" from the center of the box. Outlier values include those that are within 1.5 and 3 times the interquartile range and more than 3 times the interquartile range.

The nonparametric Kruskal-Wallis analysis of variance test (Helsel and Hirsch, 1992) was used to test for differences in the distributions of the data among groups. The distributions were considered significantly different from one another if the probability (p-value) is less than 5 percent (less than 0.05) that the observed difference occurs by chance. If a statistically significant difference was detected among the data groups, individual differences were evaluated by applying Tukey's multiple comparison test to the rank-transformed data (Helsel and Hirsch, 1992).

WATER QUALITY

Values of physical properties, bacteria densities, and concentrations of inorganic constituents, nutrients, selected trace elements, and organic compounds associated with wastewater were determined during the sampling period to provide additional data on the general water quality of the Little Sac River. Physical properties included discharge, specific conductance, pH, water temperature, and dissolved oxygen. Bacteria densities were determined for fecal coliform, fecal streptococcus, and *E. coli*. Inorganic constituents determined were calcium, magnesium, sodium, sulfate, and chloride. Samples were analyzed for total nitrite, nitrite plus nitrate, ammonia, phosphorus, and orthophosphorus. Boron and strontium were the trace elements analyzed. Concentrations of organic compounds associated with wastewater also were determined.

Physical Properties

The Little Sac River generally is a gaining stream. However, the uncertainty in the discharge measurement (estimated at 5 to 10 percent) and the relatively small quantity of flow makes it difficult to state this conclusion with certainty, especially in the upper reaches of the Little Sac River and during low-flow conditions. Discharge for sampling sites is shown in figure 4 for selected sample collection times in November 1999 and March and July 2000, including a low flow and two larger flows. High flow was measured at only a few sites during the study. During the time of sample collection shown in figure 4, all reaches of the Little Sac River gained flow, except for the reach from site 5 to 6 in March 2000; the discharge in this reach remained stable.

Discharge increased from site 1 to site 3, and on certain dates, inflow from site 2 substantially increased the discharge in the Little Sac River. For the rest of the sampling period, flow was small or nonexistent at site 2. Effluent from the NW WTP generally increased the discharge in the Little Sac River; the effluent was more than 50 percent of the discharge measured at site 5 immediately downstream from the NW WTP during 8

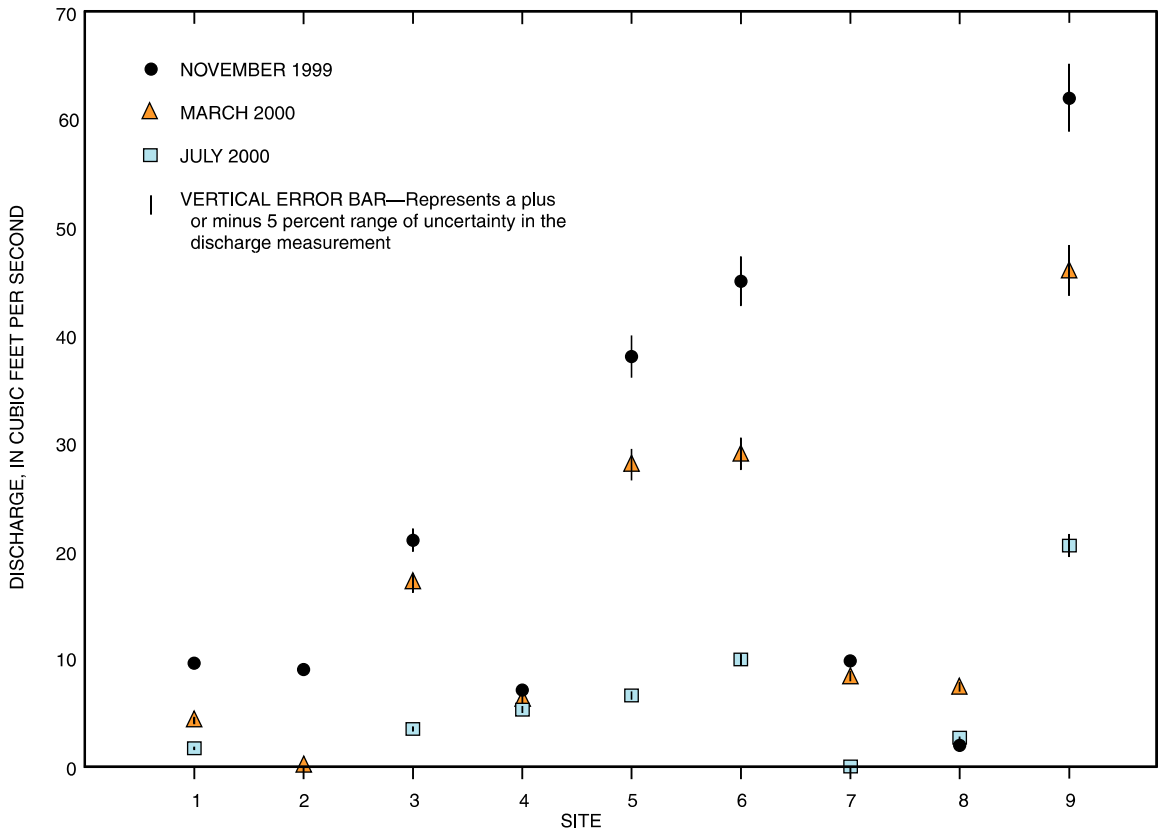

Figure 4. Variation in stream discharge in the Little Sac River, 1999–2000.

of the 14 sampling times. However, during periods of low flow in November 1999 and October 2000, the discharge at site 5 was less than the discharge from site 3, including the addition of effluent from the NW WTP (site 4). The discharge generally increased from site 5 to site 6. However, the discharge at site 5 was slightly larger or similar to that at site 6 for some measurements, indicating little if any inflow from small tributaries or ground-water contribution in this reach. The increase in discharge in the Little Sac River at site 9 from site 6 reflects contribution from tributary inflow from the largest tributaries in the lower part of the study area at sites 7 and 8, but also reflects water gained from ground water through the streambed.

Specific conductance values provide an indication of the dissolved solids concentration of the water. Median specific conductance values in the study area ranged from 365 µS/cm (microsiemens per centimeter at 25 degrees Celsius) (site 2) to 1,170 µS/cm (site 4; table 2, fig. 5). Median specific conductance values for tributaries to the Little Sac River generally were less than the values in the Little Sac River. In the Little Sac River, the median specific conductance was 505 µS/cm at site 1 and 500 µS/cm at site 3. Inflow from the NW WTP increased the median specific conductance value in the Little Sac River, with the largest increases immediately downstream from the NW WTP. Specific conductance values at site 9, the most downstream site, reflected continued dilution by increasing discharge. The median specific conductance value at site 5 was 745 µS/cm, and the value decreased from 636 µS/cm at site 6 to 518 µS/cm at site 9.

The pH values for all sites in the study area usually were between 7.5 and 8.2 (table 1). This range is consistent with surface water that is not affected by the oxidation of certain species, including sulfur and iron (Hem, 1992). Median pH values for all sites ranged from 7.7 to 8.1. The median pH value for most sites on the Little Sac River was 8.0 and slightly larger. The median pH value for the effluent from the NW WTP was 7.7.

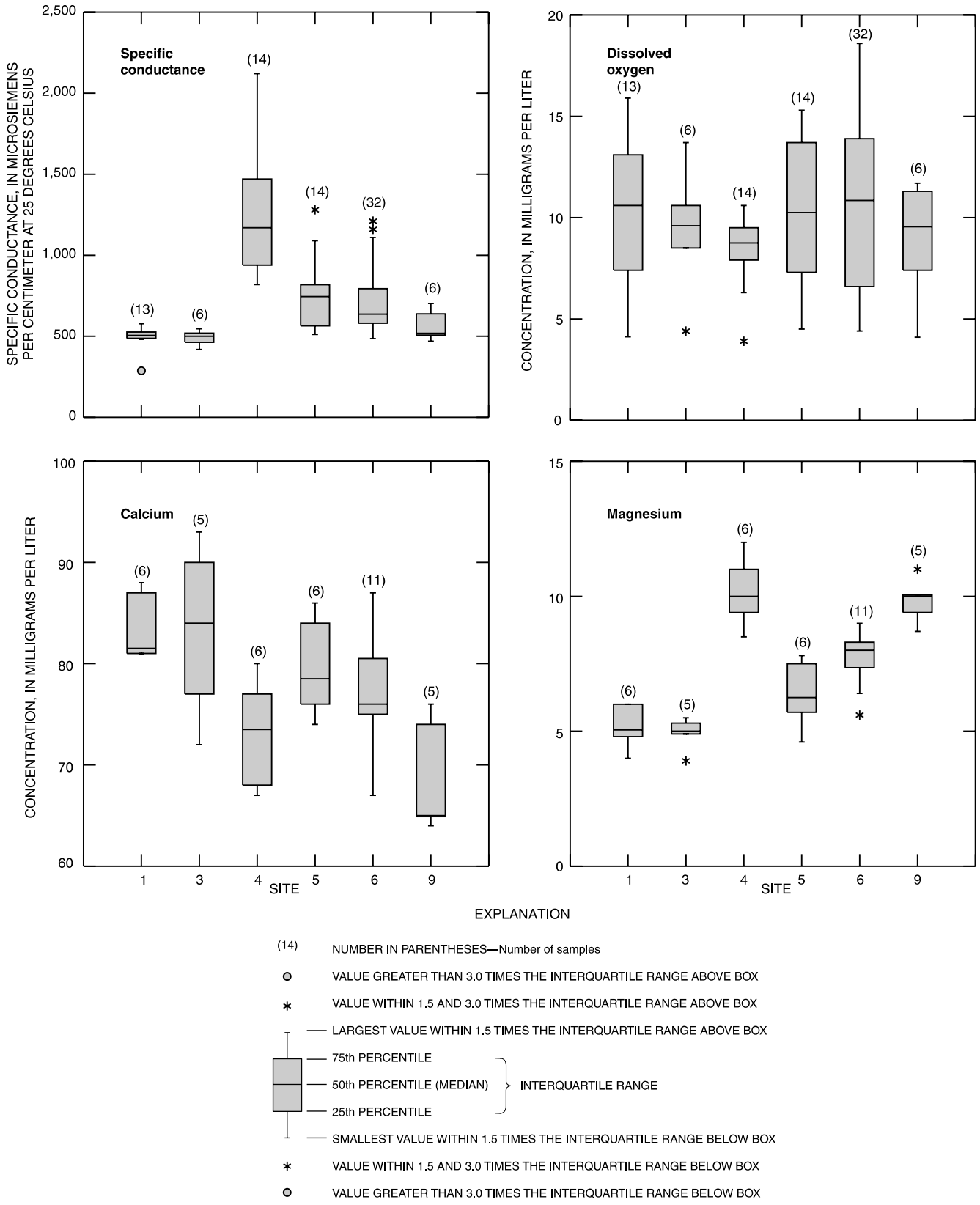

Figure 5. Distribution of selected physical properties and major constituents in the Little Sac River, 1999–2001.

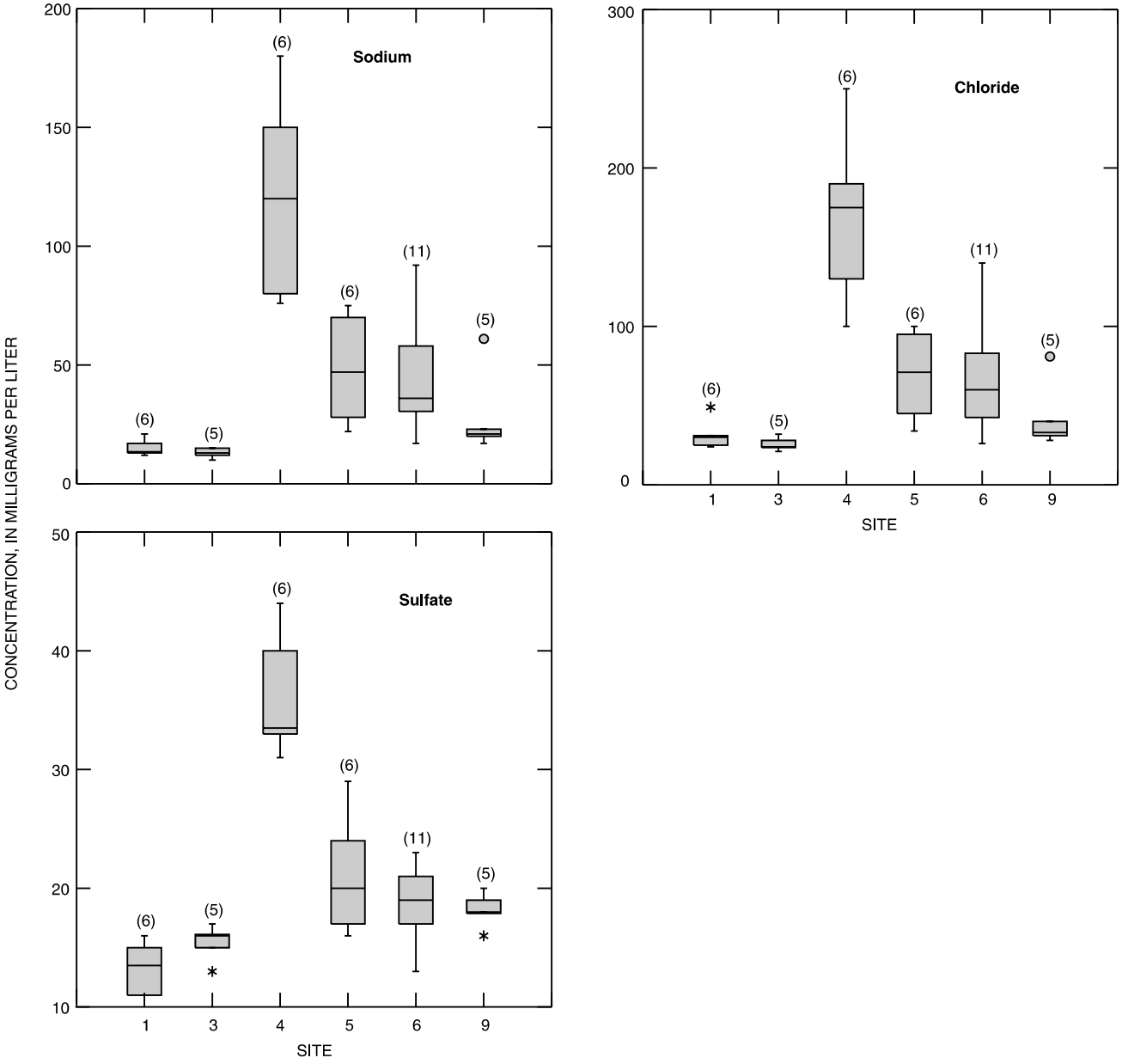

Figure 5. Distribution of selected physical properties and major constituents in the Little Sac River, 1999–2001—Continued.

Water temperature data (table 1) were divided into two categories—summer (March through August) and winter (September through February). The temperatures for all sites along the Little Sac River were not significantly different during the summer months. The temperature of the effluent from the NW WTP does not affect the temperature of the water in the Little Sac River downstream from the NW WTP. In the winter months, the water temperature of the effluent from the NW WTP is higher than the temperature at the other sites in the Little Sac River. However, the temperature of the effluent does not increase the water temperature at sites downstream from the NW WTP.

Median dissolved oxygen concentrations ranged from 5.0 at site 7 to 10.8 mg/L at site 6. The lowest dissolved concentration at site 7 occurred when there was no flow and pooled water was sampled. The median dissolved oxygen concentration in the Little Sac River ranged from 9.6 to 10.8 mg/L. The concentration in the outflow from the NW WTP was 8.8 mg/L, which was smaller than that of sites upstream and downstream from the NW WTP.

Bacteria

The fecal indicator bacteria measured in this study originate in the intestinal tracts of warm-blooded animals and include the fecal coliform and fecal streptococcus groups and *E. coli*. The fecal coliform test is not strictly specific to fecal coliform bacteria that originate in warm-blooded animals, but may include soil bacteria. *E. coli* can live for only short periods outside the intestinal tracts of warm-blooded animals and its presence in a water sample is evidence of fecal contamination from warm-blooded animals. *E. coli* in water also is an indicator of the possible presence of human pathogens (Eaton and others, 1995).

Fecal coliform densities in water samples from the Little Sac River near Walnut Grove (site 6) exceeded 200 col/100 mL in 2 of 32 samples (6 percent). The densities in these samples were 600 col/100 mL on November 22, 1999, and 240 col/100 mL on May 24, 2000. The Missouri standard for whole-body contact recreation applies only from April 1 through October 31 for each year during nonrunoff conditions, and it applies only to the Little Sac River, not any tributaries to the river (Missouri Department of Natural Resources, 1996). Therefore, the Missouri standard was exceeded in 1 of 16 samples (6 percent). Fecal coliform densities at site 6 ranged from 2 to 600 col/100 mL. The median fecal coliform bacteria density for the study period was 65 col/100 mL.

At other sites on the Little Sac River, fecal coliform densities exceeded 200 col/100 mL in 3 of 14 samples at site 1, in 2 of 6 samples at site 3, in 5 of 14 samples at site 5, and in 2 of 6 samples at site 9 (1 stormwater runoff sample). However, for the time that the Missouri standard applied to fecal coliform densities, the standard was exceeded once at sites 1 and 5 and equalled once at site 3. Fecal coliform densities in samples from tributaries to the Little Sac River exceeded 200 col/100 mL in one of five samples at site 2, in one of six samples at site 7, and in three of six samples at site 8.

Fecal coliform densities ranged from 8 to 692 col/100 mL, and the median density was 92 col/100 mL in the Little Sac River at site 1. The density at site 3 ranged from 4 to 800 col/100 mL with a median density of 159 col/100 mL. At site 4, the effluent from the NW WTP that flows into the Little Sac River, fecal coliform densities ranged from 1 to 10,100 col/100 mL. The median density was 462 col/100 mL. At site 5 downstream from the NW WTP, the density ranged from 60 to 430 col/100 mL. The median density was 142 col/100 mL. The fecal coliform density at site 9 ranged from 33 to 4,400 col/100 mL; the median density was 106 col/100 mL.

One of the factors determining the fecal coliform density in the Little Sac River is the effluent from the NW WTP that is discharged into the Little Sac River at site 4 (fig. 6). To meet the Missouri standard for whole-body contact recreation, the NW WTP disinfects the effluent that is discharged into the Little Sac River from April 1 to October 31. The fecal coliform densities at site 4 when disinfection was ongoing ranged from less than 10 to 100 col/100 mL. The median density was 30 col/100 mL.

For the period of no disinfection from November through March, the fecal coliform densities of the effluent from the NW WTP (site 4) ranged from 50 to more than 10,000 col/100 mL with one-half of the densities exceeding 4,560 col/100 mL. The fecal coliform density of 50 col/100 mL on November 1, 2000, the first day after disinfection ended for the year, likely reflected the residual effects of disinfection.

During the time that the NW WTP was disinfecting the effluent to the Little Sac River, the fecal coliform densities upstream and downstream from the NW WTP were larger than the density from the NW

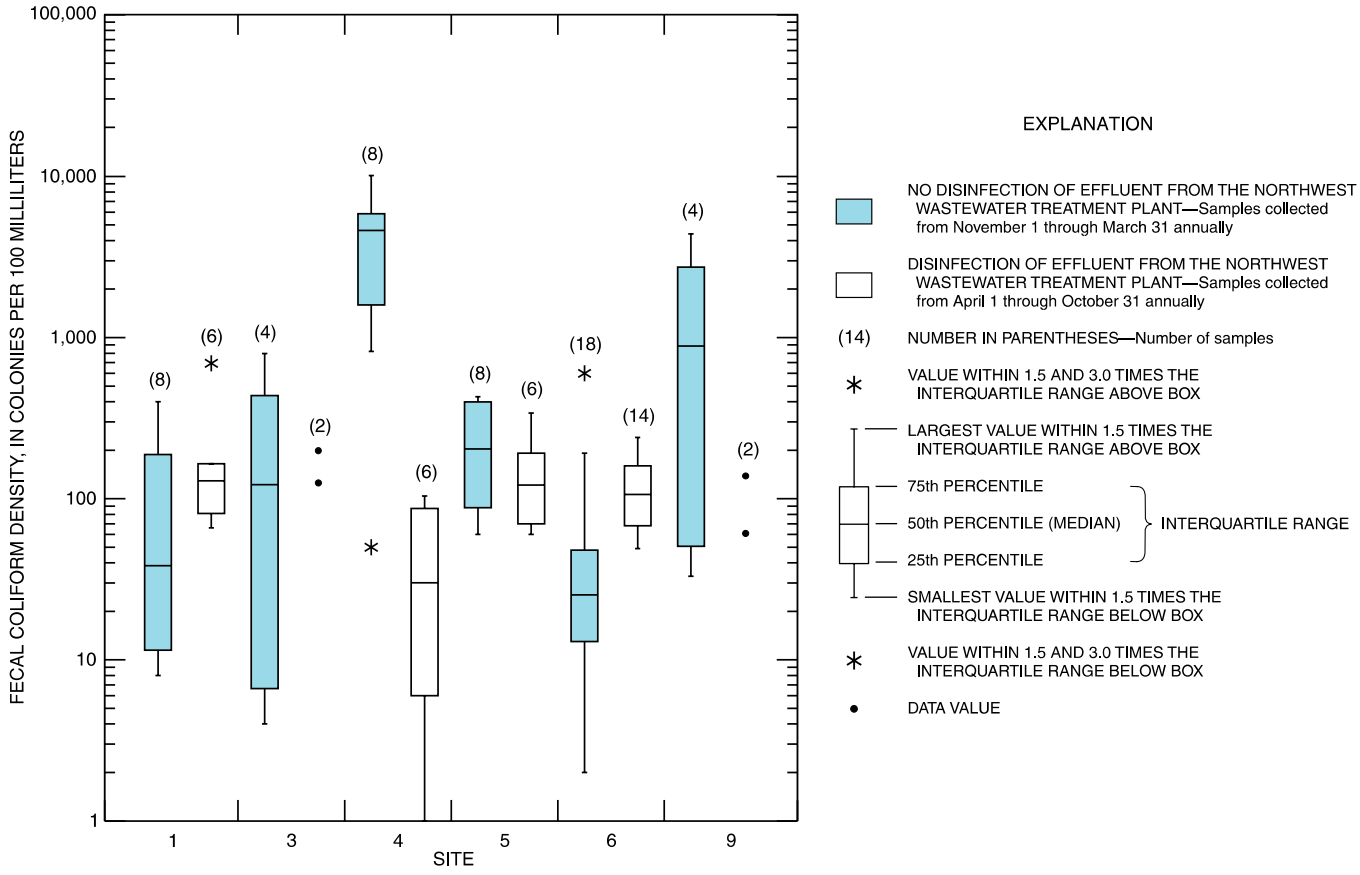

Figure 6. Fecal coliform bacteria densities in the Little Sac River, 1999–2001.

WTP. The fecal coliform densities at site 1 ranged from 66 to 692 col/100 mL. At site 2, the densities were 62 and 370 col/100 mL and were 120 and 200 col/100 mL at site 3. Downstream from the NW WTP, the fecal coliform densities ranged from 60 to 340 col/100 mL at site 5 and from 49 to 240 col/100 mL at site 6. The densities were 60 and 140 col/100 mL at site 9. The median densities ranged from 132 col/100 mL at site 1 to 159 col/100 mL at site 3 to 134 col/100 mL immediately downstream from the NW WTP (site 5) to 106 col/100 mL at site 6 to 98 col/100 mL at site 9.

When the NW WTP did not disinfect the effluent into the Little Sac River, the fecal coliform density ranged from 11 to 400 col/100 mL at site 1. At site 2, the density ranged from less than 5 to 18 col/100 mL. The density at site 3 ranged from 4 to 800 col/100 mL and at site 5 from 60 to 430 col/100 mL. The fecal coliform density for this period ranged from 2 to 600 col/100 mL at site 6 and from 78 to 4,400 col/100 mL at site 9. Immediately downstream from the NW WTP at site 5, the fecal coliform density was larger (median density of 222 col/100 mL) than that of the upstream site 1 (median of 38 col/100 mL). The density decreased substantially farther downstream at site 6 (median of 26 col/100 mL). The median fecal coliform density of 899 col/100 mL at site 9 was affected by large densities in November 1999 and during high flow in February 2001.

During the period when the NW WTP is not disinfecting the effluent, the discharged effluent contains large densities of fecal coliform. When the NW WTP is disinfecting the effluent, the effluent contains small densities of the bacteria. However, at site 5 the fecal coliform density was similar for samples when the NW WTP was disinfecting the effluent and when it was not. Therefore, the effluent from the NW WTP does not

seem to affect the fecal coliform density at this site. The fecal bacteria densities were similar at sites 1, 3, and 5 for both samples collected when the effluent was disinfected at the NW WTP and when the effluent was not disinfected. Based on these data, the fecal coliform bacteria density at these sites was not affected by the effluent from the NW WTP. The density farther downstream at site 6 is less than the density upstream from the NW WTP at site 1. When the NW WTP was disinfecting the effluent, bacteria densities were larger at sites upstream and downstream from the NW WTP than the density in the effluent from the NW WTP. Bacteria die-off and increased flow in the Little Sac River probably contribute to the decreased densities downstream from the NW WTP. Effect of the effluent from the NW WTP on fecal coliform density is surpassed by local sources of bacteria.

At site 6, the fecal coliform density in samples collected during the time of no effluent disinfection was significantly larger ($p<0.05$) than the density in samples collected when the effluent was disinfected, generally during the summer and early fall. This may be a result of increased bacteria growth from warmer air and water temperatures, generally smaller streamflow, and increased grazing by livestock adjacent to the river.

No Missouri standard exists for fecal streptococci or *E. coli* bacteria. However, the fecal streptococci density in most samples was less than the fecal coliform density, although the fecal streptococci density was larger in several samples upstream from the NW WTP. The *E. coli* density in samples generally was larger than that for fecal streptococci.

The fecal coliform instantaneous load is a measure of the number of fecal coliform bacteria present in the volume of water that passed a specific location per unit time. A small stream with large bacteria densities can contribute small loads, whereas a large stream with small bacteria densities can contribute large loads.

During the time that the effluent from the NW WTP was not disinfected, fecal coliform bacteria loads increased in the Little Sac River from site 1 to site 3 and further increased in the effluent from the NW WTP (fig. 7). Downstream from the NW WTP, the loads decreased from site 5 to 6 then increased at site 9. The increase at site 9 likely was the result of the large bacteria loads at sites 7 and 8, both tributaries to the Little Sac River, and other unidentified sources from streams that were not sampled in this study.

From April 1 through October 31 when the effluent from the NW WTP was disinfected, the fecal coliform bacteria densities generally increased from site 1 to site 3 (fig. 7). The load for the effluent from the NW WTP was less than the load at site 3. Loads at sites 5, 6, and 9 on the Little Sac River were larger than the load from the effluent from the NW WTP. Based on available data, the load for the effluent from the NW WTP was less than the load at site 3. The loads from tributary inflow at sites 2, 7, and 8 did not contribute substantially to the increased bacteria loads downstream from the NW WTP. However, some of the fecal coliform instantaneous loads from the tributaries were determined using only one or two samples.

Isolates of *E. coli* were obtained from water samples collected at site 1 (four samples), site 4 (one sample), site 5 (four samples), and site 6 (four samples) and were submitted for rep-PCR pattern analysis. This technique has been used in efforts to identify the source of fecal coliform and *E. coli* contamination in water bodies with varying results (Parveen and others, 1999; Schlottmann and others, 2000). Whereas most efforts to determine the source of bacteria in streams rely on indirect measures of inorganic and organic constituents, analysis of rep-PCR patterns has the promise of directly linking the bacteria to their source using DNA ribopatterns. The technique relies on the assumption that ribopatterns of *E. coli* from various animal species will be unique. However, little is known about the temporal and geographic variability of ribopatterns within a single animal group or the potential sharing of ribotypes between various animals. The rep-PCR pattern analysis involves using 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 data base—not the rigorous hypothesis test that is commonly done with water-quality data. For this study, three host classes were chosen for inclusion in the analysis—cow, horse, and human sewage. The choice was based on the presence of cows and horses in the basin and the effluent from the NW WTP. Pattern matching was done using discriminate analysis; a pattern was considered to be a match if the probability of a match was 0.80 or larger. As additional patterns are added to the data base of "known" patterns, the degree of similarity between unknown and known patterns constantly changes. Because of the large degree of uncertainty in the methods, results of the method were experimental

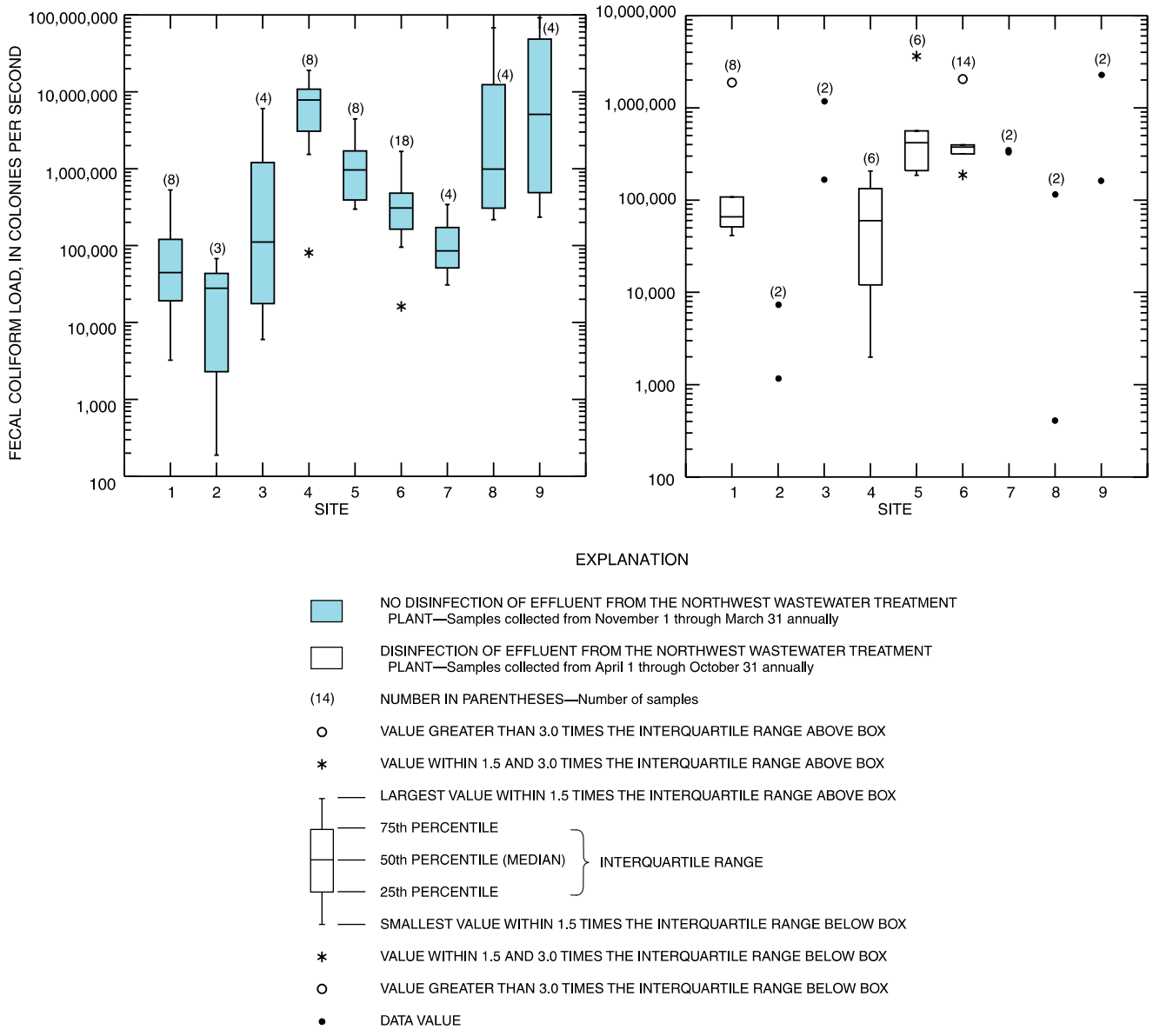

Figure 7. Instantaneous fecal coliform bacteria loads in the study area, 1999–2001.

for the purpose of this study, and interpretations of these data were made in conjunction with other data and information.

Water samples collected in March, April, June, and July 2001 were submitted for rep-PCR pattern analysis. Samples from sites 1, 5, and 6 were collected in March, June, and July. Samples collected in April were from the aforementioned sites with an additional sample from site 4. However, no readable patterns were obtained from site 4, probably because of interference from chlorine that was used to disinfect the effluent from the NW WTP.

Patterns were obtained from 102 isolates from 9 water samples (table 3). Results indicated that 62 of the 102 isolates matched the nonhuman group and 4 isolates matched the human group with a probability of 80 percent or larger. The rest of the isolate patterns were not matched to either the human or the nonhuman group.

In samples collected in March 2001, before disinfection of the effluent from the NW WTP, all matches were nonhuman. Of the 35 isolates, 30 were classified as horse. The lack of human matches at site 5 is inconsistent with the fact that the effluent from the NW WTP

Table 3. Number of *Escherichia coli* isolates in water samples assigned to various sources by rep-PCR pattern analysis

[rep-PCR, repetitive element polymerase chain reaction; sample collected at site 4 on April 24, 2001, produced no readable patterns; no suitable bacteria were cultured for ribotyping in samples collected on June 14, 2001]

Site (fig. 1)	Date	Number of isolates	Percent identified	Possible source using three-class analysis (probability of 0.80 or larger)			
				Horse	Cattle	Human	Unknown
1	03-22-01	15	93	14	0	0	1
	04-24-01	16	62	4	4	2	6
	07-05-01	10	50	4	1	0	5
5	03-22-01	10	100	10	0	0	0
	04-24-01	15	73	4	6	1	4
	07-05-01	7	0	0	0	0	7
6	03-22-01	10	70	6	1	0	3
	04-24-01	8	50	1	2	1	4
	07-05-01	11	45	2	3	0	6

is about 50 percent of the flow at the site. However, site 5 is adjacent to a horse stable and horses were observed within about 0.5 mi of the site during the time of most sample collections. Cows were observed within a few hundred feet of site 6 (one cow match of seven nonhuman matches).

In April 2001 after disinfection had begun at the NW WTP, samples upstream and downstream from the NW WTP had 4 of 39 isolates (10 percent) that matched the human group with a probability of 0.80 or larger. The one human group match for site 5 is consistent with the large quantity of effluent from the NW WTP that comprises the flow at the site, and a match at site 6 indicates that possibly a small component of the effluent is still present at this site or that other sources exist, such as leaking septic systems. However, most of the matches were nonhuman (21 of 39 isolates, or 54 percent). In samples at all sites, the number of matches for cow equalled or exceeded the number of matches for horse.

No suitable bacteria were cultured for rep-PCR pattern analysis in the samples collected in June 2001. Samples collected in July 2001 had 10 matches from 28 isolates (36 percent). All matches were nonhuman.

All water samples submitted for rep-PCR pattern analysis and one additional water sample collected from sites 1, 5, and 6 in July 2000 were tested for the human pathogen *E. coli* O157:H7. Cattle are thought to be the predominant source for this organism (Sargeant and others, 1999) although a variety of animals, including wild deer, could be the source. No *E. coli* O157:H7 were detected in any of the water samples.

The small number of human patterns is consistent with the relatively small human population in the study area. However, this number of human patterns is not consistent with the fact that effluent from the NW WTP is a large part of the flow in the Little Sac River at certain sampling sites. No sample was available for rep-PCR pattern analysis for the effluent from the NW WTP. The presence of effluent from the NW WTP may explain the detection of the human isolate at site 5. Site 6 was in the vicinity of several single-family residences with septic systems. Effluent from these systems may explain the detection of the human isolate at this site. The detection of human isolates at site 1 also probably is the result of effluent from septic systems. The presence of horse and cattle isolates is consistent with the presence of these animals near sites 1, 5, and 6.

The rep-PCR pattern results generally were consistent with land-use patterns, such as the identification of horse patterns near a horse stable at site 5. However, a concern of the concept of the rep-PCR pattern analysis is the variation in number of unknown isolates matching animal sources as the number of potential sources is changed. For example, in samples from sites 1, 5, and 6 collected March 2001, 30 of 35 isolates were

assigned to the horse group for a three-class analysis, with a match having a probability of 0.80 or larger. When the class was expanded to four by the addition of swine, the matches for sites 1 and 5 remained the same. For site 6, two additional isolates were reassigned to horse making the total of eight in this group. The one cattle isolate was removed. Based on these results, the rest of the samples were analyzed using only the three classes of horse, cattle, and human.

Because the techniques of the rep-PCR pattern analysis still are in the experimental stages, a large degree of uncertainty exists in the results. Additional samples are needed to confirm the results of this study. Technology for the rep-PCR pattern analysis, including the software available to analyze the results, has improved. Because regional differences exist in organisms (Hartel and others, 2002), samples of known sources need to be collected from the Little Sac River Basin.

Inorganic Constituents

Calcium concentrations in the study area were largest in samples collected upstream in the Little Sac River and tended to decrease downstream. The median calcium concentrations were 82 (site 1) and 84 mg/L (site 3) upstream from the NW WTP, 74 mg/L in the effluent from the NW WTP, 78 mg/L at site 5, 76 mg/L at site 6, and 65 mg/L downstream at site 9.

Median magnesium concentrations were largest at site 7 (18 mg/L), almost twice those detected at the NW WTP and at sites 8 and 9. Samples from site 7, in the lower part of the study area, had concentrations that ranged from 17 to 21 mg/L during the study. Rocks directly underlying the North Dry Sac River (site 7) are mostly dolostone (calcium-magnesium carbonate) instead of the limestone rocks (calcium carbonate) that occur throughout most of the study area (Missouri Division of Geology and Land Survey, 1979). The magnesium concentrations at site 7 possibly reflect magnesium-rich ground water that discharges to the river.

Median sodium and chloride concentrations, which can be indicators of municipal and industrial wastes and urban runoff, in the Little Sac River seem to be affected by effluent from the NW WTP (site 4; fig. 5). The median sodium concentration in water samples from the NW WTP was 120 mg/L, and the median chloride concentration was 175 mg/L. Effects of the increased sodium concentrations were still detected at the most downstream site (site 9), which had a median concentration of 21 mg/L. Immediately downstream from the NW WTP, the median sodium concentrations were 47 (site 5) and 36 mg/L (site 6). Median sodium concentrations of tributary inflow were less than 10 mg/L. Chloride concentrations were similarly small. Downstream from the NW WTP at site 5 the median chloride concentration was 71 mg/L, 60 mg/L at site 6, and decreased to 33 mg/L at site 9. Tributary inflow had a median chloride concentration of less than or equal to 15 mg/L.

A common source of sodium and chloride may be from the application of road salt during winter months. Samples collected during December through March, when road salt would be applied, generally had chloride concentrations larger than concentrations in samples collected during the other months of the year at site 1 and in the effluent from the NW WTP (site 4; table 1).

Sulfate concentrations, similar to other inorganic constituent concentrations, were largest in the effluent from the NW WTP (median concentration of 34 mg/L) and decreased downstream to 20 mg/L at site 5, 19 mg/L at site 6, and 18 mg/L at site 9. The median concentration of 23 mg/L at site 7 was the largest for all tributary inflows.

Nutrients

Nutrients, including nitrogen and phosphorus species, can have detrimental effects on desired uses of water and may serve as indicators of possible contamination. Possible sources of nutrients in the study area include horse, cattle, and human wastes, fertilizers, and the NW WTP. The USEPA has established a maximum contaminant level of 10 mg/L for nitrate (as nitrogen) in public drinking water supplies (U.S. Environmental Protection Agency, 1988) because high concentrations of nitrate can cause blue-baby syndrome in infants. Although beneficial uses for the Little Sac River do not include drinking water supply, the river flows into Stockton Lake, which is used as a drinking water source for Springfield. The nutrients analyzed in this study include total nitrite, nitrite plus nitrate, ammonia, phosphorus, and orthophosphorus.

Nitrite, considered unstable in water exposed to the atmosphere, is used as an indicator of contamination from sewage disposal or organic waste (Hem, 1992). Nitrite concentrations were less than 0.06 mg/L at all sites in the study area except for the effluent from

the NW WTP. The concentration at the effluent ranged from less than 0.01 to 0.31 mg/L. Nitrite concentrations from tributaries to the Little Sac River generally were less than 0.01 mg/L.

Nitrite plus nitrate concentration (fig. 8) ranged from 0.62 to 2.3 mg/L at site 1 (median of 1.1 mg/L) and from 0.87 to 1.7 mg/L at site 3 (median of 1.4 mg/L). Concentrations in the effluent of the NW WTP ranged from 0.13 to 8.1 mg/L. The median concentration at site 4 was 6.4 mg/L. The nitrite plus nitrate concentrations ranged from 1.3 to 5.0 mg/L (median of 2.2 mg/L) at site 5 decreased at site 6 to a median of 1.2 mg/L (ranged from 0.09 to 2.2 mg/L), and further decreased to a median of 0.56 mg/L at site 9. The concentrations at site 9 ranged from 0.02 to 1.2 mg/L. The nitrite plus nitrate concentration from tributaries generally was less than 1.0 mg/L. Runoff upstream from site 8 during the February 2001 sample collection increased the concentration in Asher Creek to 1.7 mg/L and in the Little Sac River at site 9 to 1.2 mg/L (table 1).

The nitrite plus nitrate concentrations were significantly different ($p<0.05$) from samples at sites 4 and 5 than for the rest of the sites on the Little Sac River. The concentrations at these two sites indicate effects from the effluent from the NW WTP. Concentrations at site 6 were similar to those from sites upstream from the NW WTP, and concentrations at site 9 were similar and gradually decreasing.

Total concentrations of ammonia were similar at site 1 upstream from the NW WTP and in the effluent from the NW WTP (fig. 6). At site 1, the concentration ranged from less than 0.01 to 0.28 mg/L, and at site 4 it ranged from 0.03 to 0.61 mg/L. The median ammonia concentrations were 0.12 and 0.11 mg/L at sites 1 and 4. The ammonia concentration in the Little Sac River decreased downstream from the NW WTP at sites 5, 6, and 9.

At site 1 upstream from the NW WTP, potential sources of ammonia were present that are unrelated to the NW WTP. These sources could include fertilizer use or livestock that are present in this part of the study area. The concentrations from the rest of the sites on the Little Sac River were less than those at sites 1 and 4.

Phosphorus is a component of sewage, is always present in animal waste, is used as fertilizer, and is present in certain insecticides. Although the use of phosphate (an oxidized state of phosphorus) has been limited in household detergents, domestic and industrial sewage effluent probably remains a large source of phosphorus in surface water (Hem, 1992). The largest concentrations of phosphorus detected during the study generally were in samples from the effluent at the NW WTP (fig. 6). Phosphorus concentrations ranged from 0.08 to 5.0 mg/L. The median phosphorus concentration at site 4 was 0.20 mg/L. Median phosphorus concentrations at sites 5 and 6 decreased from those detected in the effluent of the NW WTP and further decreased at site 9. The median phosphorus concentration at site 9 was 0.08 mg/L. Phosphorus concentrations were similar at sites 1 and 3 upstream from the NW WTP. Median phosphorus concentrations detected upstream from the NW WTP for sites 1 and 3 were 0.02 mg/L.

Orthophosphorus concentrations in the Little Sac River were statistically similar for sites downstream from the NW WTP, including the effluent from the NW WTP. These concentrations likely indicate effects from the effluent at the NW WTP, with a slight decrease in othophosphorus concentrations in the lower part of the basin.

Selected Trace Elements

Boron may be present in industrial wastes and sewage because it has numerous industrial uses and is used in cleaning products in the form of tetraborate (borax). Boron concentrations at site 1 [range of 10 to 34 μg/L (micrograms per liter), median of 18 μg/L] and site 3 (range of 10 to 33 μg/L, median of 23 μg/L) were similar. Boron concentrations in samples of the effluent from the NW WTP ranged from 160 to 220 μg/L, and the median concentration was 195 μg/L, which contributed to the increase in boron concentrations in the Little Sac River downstream from the NW WTP. The boron concentration at site 5 ranged from 30 to 110 μg/L (median of 55 μg/L) and at site 6 from 30 to 88 μg/L (median of 45 μg/L). The median concentration at site 9 was 31 μg/L. Median boron concentrations from tributaries to the Little Sac River were less than 10 μg/L.

The median strontium concentrations were similar in the Little Sac River between site 1 (80 μg/L), site 3 (81 μg/L), and site 4 (86 μg/L). The median concentrations downstream from the NW WTP were 79 μg/L at site 5 and 62 μg/L at site 9. The strontium concentrations in samples from tributaries to the Little Sac River were less than 60 μg/L.

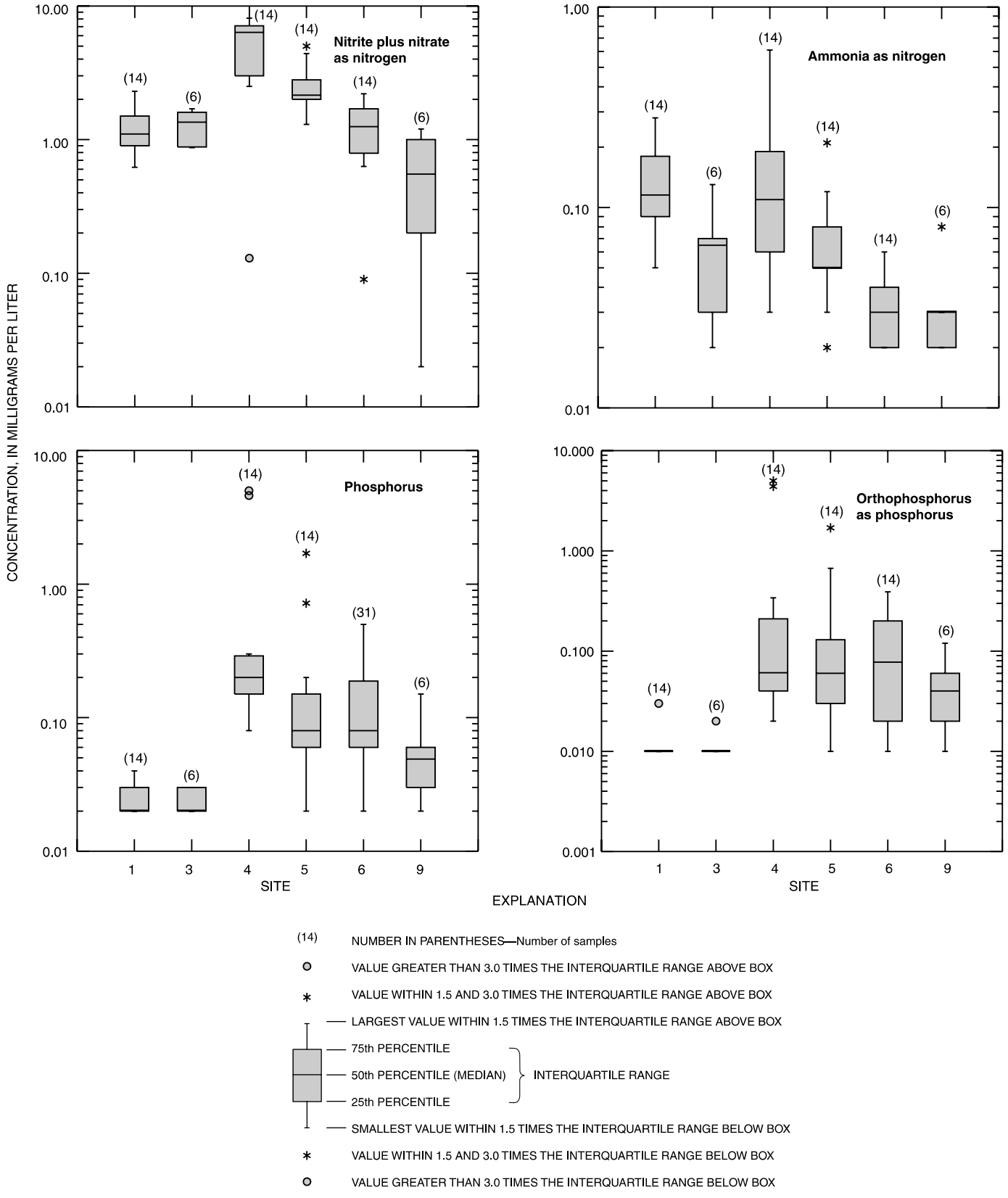

Figure 8. Distribution of selected nutrients in the Little Sac River, 1999–2001.

Organic Compounds

Water samples from several sites contained trace quantities of organic compounds commonly associated with municipal or domestic sewage effluent (table 4, at the back of this report). However, several of the compounds detected have sources other than sewage effluent. Polynuclear aromatic hydrocarbons (PAHs) can be associated with asphalt roads, concrete water proofing on foundations, and asphalt roof shingles. Plasticizers are extremely common in an industrialized society because they are in many products and, therefore, are commonly reported laboratory contaminants. For these reasons, PAH data were not used in interpretations in this report. Cholesterol and 3-beta-coprostanol are indicators of fecal contamination and can be associated with other waste sources besides municipal or domestic sewage effluent, such as animals. The wood preservative para-cresol may be present in treated lumber used in structures. Unless para-cresol is detected in combination with other compounds more specific to human wastes, its detection is not indicative of municipal or domestic sewage effluent.

Of the organic compounds detected in the water samples from the Little Sac River Basin, the most reliable indicators of municipal or domestic sewage effluent are caffeine, the disinfectants phenol and triclosan, nonylphenol (detergent metabolite), nonylphenol monoethoxylate (NPEO1) and nonylphenol diethoxylate (NPEO2) (associated with nonionic detergent use), the fire retardant tri(2-chloroethyl) phosphate, and 1,4-dichlorobenzene (lavatory fumigant). These eight compounds are referred to as human wastewater compounds and their detection may indicate effects from municipal or domestic sewage effluent. The number of detections of human wastewater compounds was normalized by dividing the number of detections at a particular site by the number of samples collected from that site to give a human wastewater "score" (fig. 9).

Site 4, the effluent from the NW WTP, had the largest human wastewater score of 6.5 with 39 detections in 6 samples, indicating the largest effect from human wastes. Only 6 of the 46 compounds analyzed for were not detected—stigmastanol, cis-chlordane, codeine, dieldrin, methyl parathion, and diethyl phthalate. Ten compounds were detected only once.

The second largest wastewater score (4.3 with 26 detections in 6 samples) was at site 5 immediately downstream from the NW WTP. The scores decreased with increasing distance downstream in the Little Sac River, reaching a low of 1.5 at site 9. The decrease in the human wastewater score indicates dilutions of municipal wastewater effluent downstream in the river.

Upstream from the NW WTP, the human wastewater scores in the Little Sac River were 1.8 at site 1 (11 detections in 6 samples) and 0.75 at site 3 (3 detections in 4 samples). Samples from these two sites contained caffeine, phenol, and cholesterol, which may indicate effects from septic tanks.

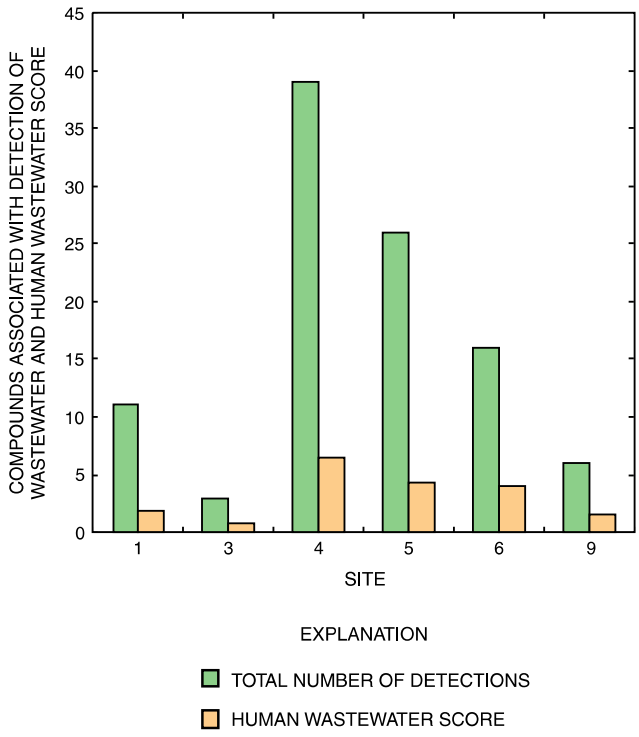

Figure 9. Number of detections of compounds associated with wastewater and human wastewater score in the Little Sac River, 1999–2001.

The human wastewater scores from samples of tributaries flowing into the Little Sac River ranged from 0.5 at site 7 to 1.5 at site 2. The scores from sites 7 and 8 in the lower, sparsely populated part of the basin indicate little effect from municipal or domestic sewage effluent even though cholesterol, an indicator of fecal contamination, was detected in a sample from site 8. This detection also is consistent with large bacteria densities and pastured animals observed near the sampling site. The detection of caffeine, phenol, and triclosan at site 2 may indicate effects from septic tanks in this part of the study area.

The compound N,N-diethyltoluamide or DEET is an insecticide and repellent. This compound was present in samples from all sites on the Little Sac River and its tributaries (detected in 29 of 43 samples) and was detected in samples collected throughout the entire sampling period. The presence of this compound possibly can be attributed to widespread use of the compound, continual usage throughout the year, little ability to biodegrade in water (National Library of Medicine, 2002), adsorption to suspended solids and sediment (National Library of Medicine, 2002), or analytical error.

Optical brighteners are added to laundry detergents as whiteners for cotton and other plant-derived textiles and enter septic systems in gray water. Detection of optical brighteners in a water sample is an indicator of effects from septic systems. Optical brighteners were detected in water samples from site 1 and site 4 collected in November 1999, July 2000, and April 2001. The intensity of the optical brighteners detected was larger at site 4 than the intensity at site 1.

Detections of human wastewater compounds at site 1 are consistent with the detection of human patterns by ribopattern analysis and with the possibility of effluent leaking from septic systems. The large human wastewater score at site 4 is reasonable because this site is the effluent from the NW WTP. The decreases in the number of detections of wastewater compounds and in the human wastewater score downstream from the NW WTP are consistent with dilution of the effluent as discharge increases in the Little Sac River. The detection of a human pattern in the ribotype analysis at sites 5 and 6 is consistent with the presence of wastewater compounds.

SUMMARY AND CONCLUSIONS

The Little Sac River, north of Springfield, Missouri, flows through mainly agricultural and forest land. Most of the study area is sparsely populated, even though the northern part of Springfield is drained by the Little Sac River, so runoff from an urban area contributes flow to the river. Farms in the area have livestock, mainly horses and cattle, some with direct access to the river. The Little Sac River flows into Stockton Lake, which is a supplemental drinking water source for Springfield.

The 29-river mi (mile) reach of the lower part of the Little Sac River is on the 1998 list of waters of Missouri designated under section 303(d) of the Federal Clean Water Act. Fecal coliform bacteria densities exceeded the Missouri standard of 200 col/100 mL (colonies per 100 milliliters) for whole-body contact recreation in water samples from the Little Sac River near Walnut Grove (site 6).

The U.S. Geological Survey (USGS), in cooperation with the Watershed Committee of the Ozarks, conducted a study from November 1999 through April 2001 to determine the water quality of the Little Sac River, upstream from the Northwest Wastewater Treatment Plant (NW WTP) to the junction of the Little Sac River with Stockton Lake, with an emphasis on fecal coliform bacteria density and nutrient concentrations. Fecal coliform bacteria densities exceeded 200 col/100 mL in 2 of 32 samples and exceeded the Missouri standard for whole-body contact recreation (standard only applies from April 1 through October 31) in 1 of 32 samples from site 6. Fecal coliform bacteria densities at other sites along the Little Sac River both upstream and downstream from the NW WTP exceeded the Missouri standard in one sample from sites 1, 2, and 5, and the standard was equalled once at site 3. Sites 1, 2, and 3 were upstream from the NW WTP, and site 5 was downstream.

To meet the standard for whole-body contact recreation for the Little Sac River, the NW WTP disinfects the effluent it discharges into the river from April 1 through October 31 annually. When the outflow from the NW WTP was not disinfected, fecal coliform bacteria densities at the NW WTP ranged from 50 (sample collected on November 1, 2000) to 10,100 col/100 mL. Densities during the time of disinfection (April 1 through October 31) ranged from 10 to 100 col/100 mL.

When the outflow is not disinfected from November to March, large fecal coliform densities in the effluent are input into the Little Sac River. However, the density at site 5 is similar to those at sites 1 and 3. This probably is the result of bacteria die-off and dilution by increased discharge.

From April 1 through October 31, when the NW WTP is disinfecting the outflow to the Little Sac River, fecal coliform densities in the outflow were less than 100 col/100 mL (median density of 30 col/100 mL). Fecal coliform densities generally were larger upstream and downstream in the Little Sac River than coliform densities from the NW WTP. Other sources of bacteria likely are present in the study area, in addition to the NW WTP. These potential sources include effluent from domestic septic systems and animal wastes.

Fecal coliform bacteria loads increased upstream from the NW WTP from the most upstream site to the site immediately upstream from the NW WTP. Loads in the effluent from the NW WTP and also those in the Little Sac River downstream from the NW WTP were dependent on the treatment of the effluent. When the effluent was not disinfected, the loads in the effluent increased from those upstream. Downstream in the Little Sac River, the loads decreased, but then increased at the most downstream site. The increase may be a result of increased loads from tributaries and other sources not sampled during the study. When the effluent was disinfected, fecal coliform bacteria loads were less than loads in samples from the Little Sac River downstream from the NW WTP.

Results of repetitive element polymerase chain reaction (rep-PCR) pattern analysis indicate that 62 of 102 isolates of *Escherichia coli* (*E. coli*) extracted from 9 water samples probably were from nonhuman sources. The detection of human patterns at sites 5 and 6 is consistent with effluent from the NW WTP being part of the discharge in the Little Sac River. Human patterns detected at site 1, with no obvious wastewater source, may be indicative of leakage from domestic septic systems. The predominance of horse patterns detected in the study area is consistent with the presence of horses near the sampling sites.

Nutrient concentrations in the effluent from the NW WTP affect the water quality of the Little Sac River downstream from the NW WTP. The median nitrite plus nitrate concentration was 6.5 mg/L (milligrams per liter) at the NW WTP and decreased to 2.2 mg/L at site 5 and 1.2 mg/L at site 6, which was the approximate median concentration upstream from the NW WTP (1.3 mg/L at site 1 and 1.4 mg/L at site 3). Other sources of bacteria likely are present in the study area, in addition to the NW WTP. These potential sources include effluent from domestic septic systems, fertilizer use, and runoff from areas containing livestock.

Organic compounds associated with wastewater were detected throughout the study area, although the frequency of detections was small at sites 7, 8, and 9. The effluent from the NW WTP had 39 detections in 6 samples. Downstream at site 5, there were 26 detections in 6 samples and at site 6, 16 detections in 6 samples. The effluent from the NW WTP contributes organic compounds to the Little Sac River. However, organic compounds associated with wastewater effluent were detected upstream from the NW WTP and their presence indicates likely contamination from other sources, which could include seepage from septic tanks.

The effects of the effluent from the NW WTP on the water quality of the Little Sac River downstream from the inflow are reflected in an increase in discharge (inflow from the NW WTP can be as much as 50 percent of the flow at site 5), an increase in specific conductance values, an increase in several inorganic constituent concentrations, including calcium, magnesium, and sulfate, and a large increase in sodium and chloride concentrations. The effluent from the NW WTP seems to have no effect on the pH value, temperature, and dissolved oxygen concentrations.

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