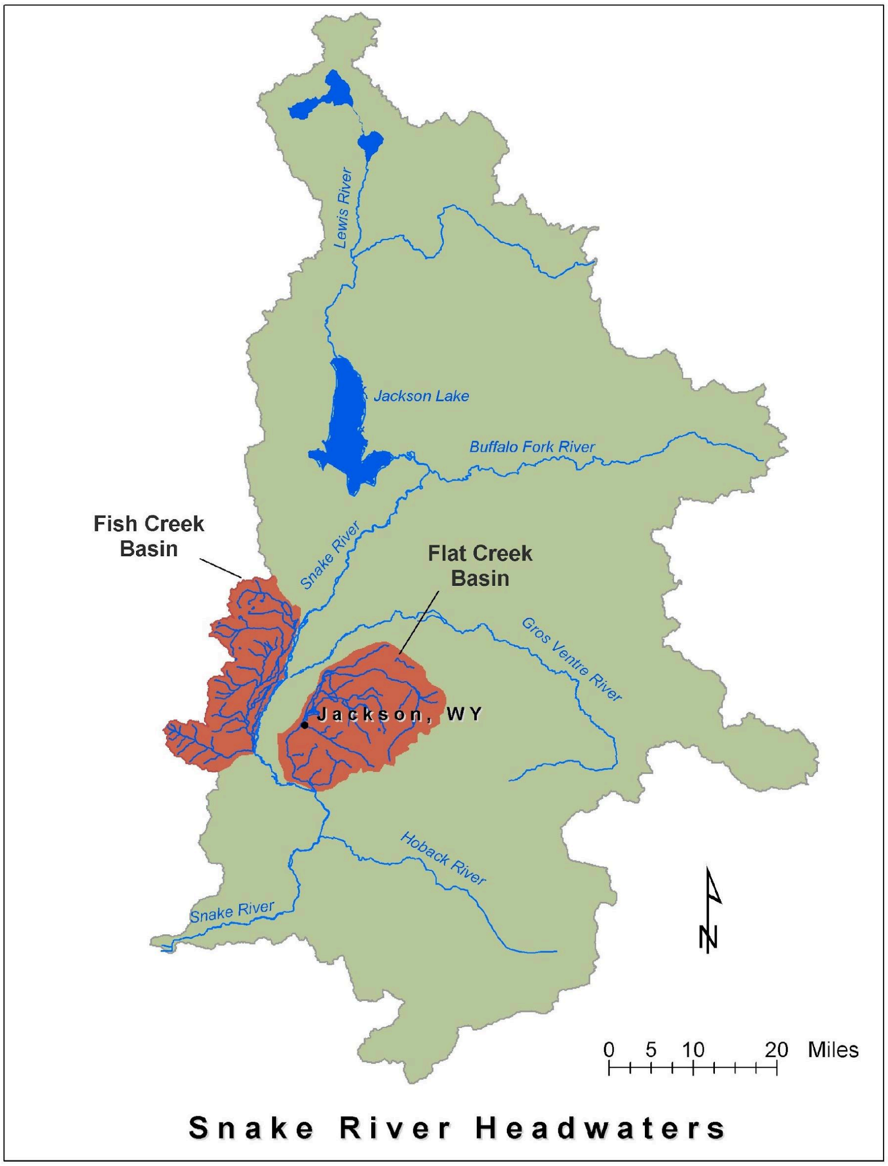
Microbial Source Tracking for *Escherichia coli* in Two Upper Snake River Basins

Fish Creek and Flat Creek Basins, Teton County, Wyoming, March-November 2003



Final Report, May 2005

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Teton Conservation District
Est. 1946

Teton Conservation District is a sub-entity of the State of Wyoming, Department of Agriculture and is supervised by a five member publicly elected board. Teton Conservation District's boundaries include Teton County, Wyoming and Yellowstone National Park in Wyoming. Funding for the District comes from a locally elected property mill levy.

The **mission** of the Teton Conservation District is to promote conservation and management of natural resources -- air, land, water, vegetation, and wildlife -- through watershed-based research, education, conservation practices, cooperative projects, and on-the-ground actions to ensure the health, safety and general welfare of the people and resources of this area.

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Teton Conservation District would like to thank those individuals and organizations who participated and cooperated in this study.

Abbreviations used in this reports:

<	less than
>	greater than
BTNF	Bridger-Teton National Forest
CC	Cache Creek
col/100mL	colonies per 100 milliliters
E. coli	*Escherichia coli* fecal-indicator bacteria
EPA	U.S. Environmental Protection Agency
FC	Fish Creek
FL	Flat Creek
GC	Game Creek
IEH	Institute for Environmental Health
MST	Microbial Source Tracking
RPD	Relative percent difference
USGS	U.S. Geological Survey
WDEQ	Wyoming Department of Environmental Quality

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ABSTRACT

In 2002, Fish Creek and Flat Creek basins were chosen as study units for microbial source tracking for *Escherichia coli* fecal-indicator bacteria. Public concern about fecal contamination in surface waters and stream committee guidance led TCD to seek public input and eventually develop a plan to identify the sources of fecal bacteria in the two selected basins. Monitoring efforts began in March of 2003 and continued into November of 2003. This final report summarizes the microbial source tracking efforts in the Fish Creek and Flat Creek basin and will be used to provide information and planning tools to local organizations, government decision makers and private individuals.

Twice monthly, from March through November 2003, samples were collected at ten sites. Seventeen sampling events over the nine month period resulted in 162 water samples. The lowest concentration of *E. coli* of >1 colonies/100mL was measured at all sites. The highest concentration of 162 *E. coli* samples was 1060 colonies/100mL for the Game Creek site in the Flat Creek basin (GC4).

Mean concentrations of *E. coli* indicator bacteria at individual sites in the Fish Creek basin varied from 12 to 66 col/100mL. Median concentrations were 3 to 23 col/100 mL. Concentrations ranged from 0 to 320 *E. coli* col/100mL. One sample, site TV1 October 7, 2003, exceeded WDEQ proposed standard for moderate use recreational contact of 298 col/100mL. Mean concentrations of *E. coli* indicator bacteria at individual sites in the Flat Creek basin varied from 17 to 142 col/100mL. Median concentrations were 1 to 20 col/100 mL. Concentrations ranged from 0 to 1060 *E. coli* col/100mL. Four samples in Game and Cache Creek exceeded WDEQ proposed standard for moderate use recreational contact of 298 col/100mL.

Microbial Source Tracking (MST) results identified 686 *E. coli* isolate matches to specific warm-blooded animal sources. For both basins, 178 isolate matches for avian was the highest and 26 percent of the total. Coyote had the fewest isolate matches of 5 and less than 1 percent. Five percent, or 38 isolates, were not matched to any source and were considered unknown. The MST data indicate that both human and non-human sources contribute *E. coli* to surface water in the Fish and Flat creek basins. A higher total percentage of the known isolates are from wildlife sources than from humans, domestic pets, and livestock. Isolates matching human sources totaled 40, about 6 percent of total matches. The highest numbers of domestic sources were dog, about 40 percent of domestic matches.

Wyoming DEQ single-sample standard for moderate full body contact criteria (298 organisms per 100 mL) was used for comparisons in this report. Five of the 162 water samples taken during the study period exceeded the moderate full body contact criteria. Two of the five samples contained domestic source matches for domestic dog. No other domestic source matches were associated with water quality standard exceedances.

INTRODUCTION

In 1996, Teton Conservation District (TCD) initiated a watershed assessment process for Fish Creek and Flat Creek within Teton County, Wyoming (fig. 1). The objectives of the watershed assessment are to: 1) develop an understanding of baseline water quality; 2) identify potential sources contributing to the degradation of water quality; and 3) provide technical support to decision makers and private landowners to improve water quality and prevent further degradation. In order to achieve these objectives biological, physical and chemical assessment parameters were selected by two stream committees, primarily consisting of landowners and interested organizations. Over 20 study units were selected that represent the diverse hydrologic and land cover features of these basins.

In 2002, Fish Creek and Flat Creek basins were chosen as study units for microbial source tracking for *Escherichia coli* fecal-indicator bacteria. Public concern about fecal contamination in surface waters and stream committee guidance led TCD to seek public input and eventually develop a plan to identify the sources of fecal bacteria in the two selected basins. Monitoring efforts began in March of 2003 and continued into November of 2003. This final report summarizes the microbial source tracking efforts in the Fish Creek and Flat Creek basin and will be used to provide information and planning tools to local organizations, government decision makers and private individuals.

Background

In 1914, the U.S. Public Health Service adopted the coliform group of bacteria as an indicator of fecal contamination of drinking water. Coliform bacteria are nonpathenogenic bacteria that occur in the feces of warm-blooded animals. Their presence in a water sample can indicate that harmful pathogenic bacteria may also be present. Coliform bacteria do not necessarily cause illness themselves, but they are found in association with pathogens that cause such waterborne diseases as gastroenteritis and bacillary dysentery, typhoid fever, and cholera (Clark and Gamper, 2003). Coliforms are found in numbers corresponding to the degree of pollution, are relatively easy to detect, and overall hardier than pathogenic bacteria (Maier, et al, 2000). For these reasons coliforms are important in that researchers may look for the presence of these “indicator organisms” in an environmental sample to assess water quality prior to or in place of culturing other organisms.

Fecal-indicator bacteria, include total coliform, fecal coliform, and *Escherichia coli (E. coli*), fecal streptococci, and enterococci, have been adopted around the world as official standards for drinking water, recreational bathing water, and wastewater discharges. Fecal-indicator bacteria can come from both point and non-point source bacteria. Point sources of bacterial contamination include wastewater treatment plants. Non-point sources are diffuse in nature and include: 1) urban/residential – failed septic systems, pet waste, landfill leakage; 2) agricultural – animal waste, application of manure on fields, crop irrigation from

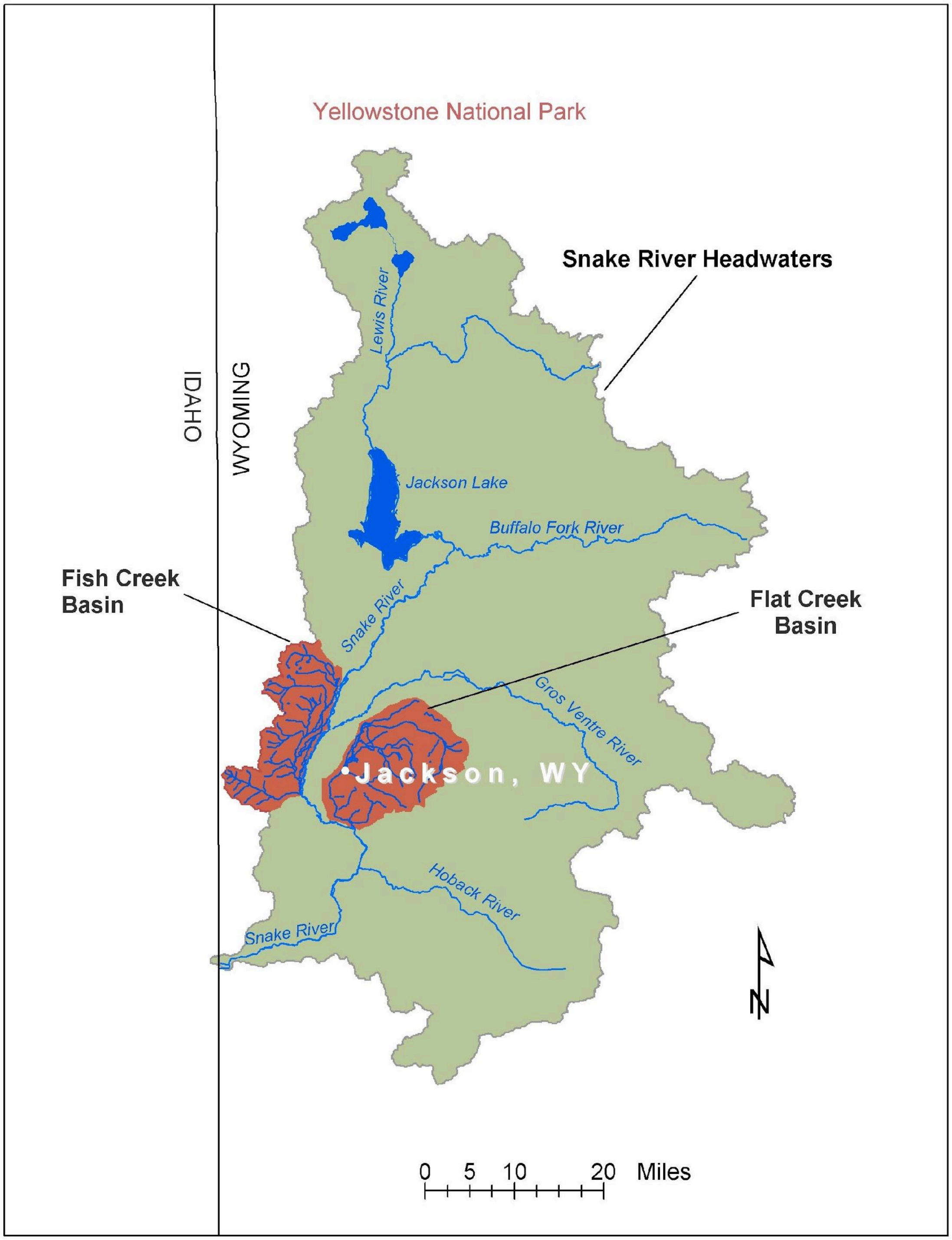


Figure 1. Location of Fish Creek and Flat Creek basins in the Snake Headwaters basin.

contaminated storage ponds;
3) recreational – direct discharge of sewage or waste; and 4) wildlife waste (Clark and Gamper, 2003).

In 1986, the U.S. Environmental Protection Agency (EPA) finalized their recommendation to states for ambient bacteriological water quality criteria. The EPA's recommendation was based on an estimate of bacterial indicator counts and gastrointestinal illness rates. *E. coli* fecal-indicator bacteria is currently recommended by the EPA because one of the fecal-coliform group, Klebsiella, has non-fecal bacteria sources, including pulp and paper mill effluents, cotton mill wastewater and sugar beet wastes (EPA, 1986). At the time of this report, an *E. coli* surface water quality standard is proposed by Wyoming Department of Environmental Quality (WDEQ). The proposed standard separates surface water into two categories: 1. primary contact recreation and 2. secondary contact recreation (WDEQ, 2005). The standard proposes a primary contact recreation season (May 1 –October 1) during which the concentrations of *E. coli* bacteria shall not exceed a geometric mean of 126 colonies per 100 milliliters based on a minimum of not less than 5 samples obtained during separate 24 hour periods for any 30-day period. Single sample concentrations for moderate full body contact shall not exceed 298 col/100 mL(colonies per 100 milliliters). All surface waters within the Flat and Fish Creek basins are designated for primary contact recreation.

The presence of fecal indicator bacteria, primarily fecal coliform and *E. coli*, has been reported in both Fish and Flat Creek basins. In Fish Creek, *E. coli,* concentrations were greater than 400 col/100mL at some sites and exceeding 3,000 col/mL at one site in June and July of 2000. In the Flat Creek basin, *E. coli* concentrations exceeded 300 col/mL at some sites and exceeded 1,000 col/mL in one tributary. Fecal indicator bacteria concentrations exceeded WDEQ proposed surface water standards sporadically and did not show any site specific or time of year specific trends. On a few occasions during the summer of 1996 and 1997, *E. coli* concentrations exceeded proposed WDEQ standards in Cascade Creek and Garnet Creek in Grand Teton National Park (Farag et al. 2001). Isolation of the *E. coli* colonies from Cascade Creek matched ribosome patterns of avian, deer, canine, elk, rodent and human fecal coliforms.

Fish Creek and Flat Creek basin landowners, along with the Bridger-Teton National Forest(BTNF), Jackson Ranger District, determined there was a need to distinguish between the sources of *E. coli* in surface water. A wide variety of human pathogens may be found in the excrement of humans as well as from domestic animals contributing to human health concerns(Laws, 2000). For this reason, both sanitary sewage and land runoff may contaminate water supplies and recreational waters with human pathogens. In both Fish and Flat Creek basins, domestic well water sources are typically not treated with any disinfecting chemicals or filtering apparatus. Additional concerns about the impacts of septic tanks and treated wastewater affecting groundwater and surface water quality have prompted interest in identifying *E. coli* sources. The abundance of wildlife intertwined with rural development and agriculture has posed additional questions as to the percentages of wildlife contributing to *E. coli* concentrations in surface water.

Project Scope and Goals

The intent of this report is to characterize and identify sources of fecal contamination in Fish and Flat Creek basins in Teton County, Wyoming. The main goals of the project are the following:

- Provide resource managers and landowners in the Fish and Flat Creek basins with information regarding the sources of fecal contamination in surface waters,
- Educate community members living and recreating in the Fish and Flat Creek basins regarding the results of the project, and
- Disseminate the project results throughout the state of Wyoming to assist other regions in their decision making processes and watershed planning efforts.

BASIN DESCRIPTIONS

Flat Creek Basin

The eastern mountainous portions of the Flat Creek basin are composed of porous carbonate rocks lying on top of impervious granitic rock (Love et al., 1992). The portion of Flat Creek basin in the valley consists of alluvial deposits and unconsolidated fluvioglacial outwash. Flat Creek basin is composed of federal, state, local and privately owned land (fig. 2). Flat Creek basin is approximately 100,844 acres. The headwaters for Flat Creek and its main tributaries are in the Gros Ventre and Hoback Ranges to the south and east of the Town of Jackson. The corporate limits of the Town of Jackson are completely contained within the Flat Creek basin and cover an area of 2450 acres. The BTNF makes up the largest portion of basin which covers approximately 66,000 acres. Cache Creek and Game Creek basins, major tributaries on the BTNF, are popular recreational areas for hikers, mountain bikers, skiers, snowmobilers and horseback riders. The National Fish and Wildlife Service's National Elk Refuge is the second largest landowner in the Flat Creek basin covering 24,000 acres. Approximately 7,000 to 10,000 elk winter on this refuge. The remaining area in the basin is primarily mixed residential and ranching land.

The flow regime of Flat Creek is typical of a spring-fed stream, but is heavily influenced by water diverted from the Gros Ventre River. According to Galbraith et al. in the *Flat Creek Riparian Survey, 1997*, 50 to 90% of Flat Creek flows originate from ground water originating in the porous carbonate rocks to the east. Other than during the month of June, there is generally no connectivity between the upper Flat Creek canyon on the BTNF and the lower valley meadows on the National Elk Refuge. Mean monthly flows for the U.S. Geological Survey (USGS) gage located on the southern boundary of the town of Jackson (#13018350, Flat Creek below Cache Creek near Jackson, WY 1989-present) range from 56.7 cubic feet per second (cfs) in September up to 131 cfs in June. The maximum peak flow of record, 277 cfs, occurred on July 12, 1995 while the lowest daily mean, 14 cfs, occurred on September 22, 2001. Within the Flat Creek basin below the National Elk Refuge, BRCI (1996) has identified 50 adjudicated water rights served by 14 diversions totaling 52 cfs

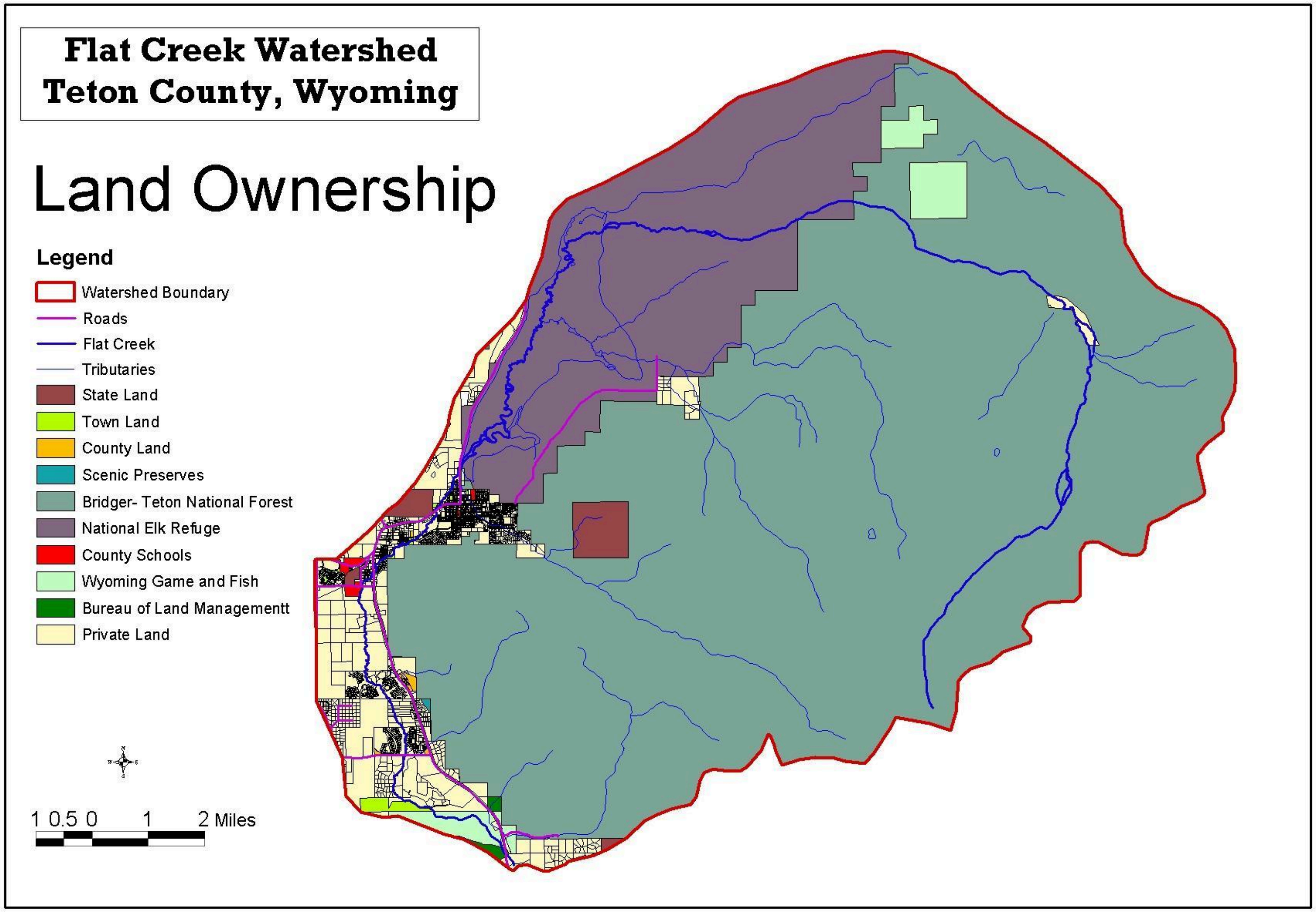


Figure 2. Land ownership in Flat Creek basin, Teton County, Wyoming, 2003.

which can also influence the streams hydrology. The largest of these diversions is the Leek diversion which is above the USGS gage. The town of Jackson contributes increased surface water runoff from impervious surfaces through fifteen storm drain pipes. During periodic winter snowmelt and rainfall events, large quantities of water containing road sand and other non-point source pollution create and artificial increase in the Flat Creek hydrograph. The Town of Jackson maintains a WDEQ discharge permit for its wastewater facility approximately one mile from Flat Creek's confluence with the Snake River.

Fish Creek Basin

Fish Creek basin consists of alluvial deposits and unconsolidated fluvioglacial outwash (Love and Reed, 1975). Floodplain deposits of sand, silt and clay create lenses within the aquifer, some having significant lateral extent (Adams, 1997). Fish Creek basin is composed of federal, state, local and privately owned land (fig. 3). Fish Creek basin is approximately 76,219 acres. The headwaters for Fish Creek and its main tributaries are in the Teton Range to the northwest and Snake River Range to the southwest of Wilson, Wyoming. Grand Teton National Park composes 24,362 acres in the northern portion of the basin. The Bridger-Teton National Forest (BTNF) makes up largest portion of basin which covers approximately 34,841acres. The Jackson Hole Mountain Resort, a 2,500 acres full service ski mountain, lies within the headwaters of Fish Creek on the BTNF. The remaining area in the basin is primarily mixed residential and ranching land.

The flow regime of Fish Creek is typical of a spring-fed stream, but is heavily influenced by water diverted from the Snake River and its largest tributary, Lake Creek. Mean monthly flows for the USGS gage located 2000 ft upstream of State Highway 22 (#13016450 Fish Creek at Wilson, WY, 1994-present) range from 38.6 cubic feet per second (cfs) in February up to 583 cfs in June. The maximum peak flow of record, 1080 cfs, occurred on June 16, 1996 while the lowest daily mean, 29 cfs, occurred on January 10, 2001. During the months of April to September, Lake Creek carries water diverted from the Snake River and has an average flow of 140 cfs with a peak flow of 310 cfs (Nelson Engineering, 1992). The Granite Creek Supplemental diversion increases the groundwater table in the area of Lake Creek and increases the return flow to Fish Creek. Diverted water in the Fish Creek basin totals more than 300 cfs in the summer months (Nelson Engineering, 1992).

The groundwater in the Fish Creek basin is primarily influenced by the Snake River to the east and flood irrigation recharge (Nelson Engineering, 1992). Two groundwater discharges are permitted by the WDEQ in the Fish Creek basin. Tertiary treated wastewater from Teton Village and the Aspens Sewer Districts is injected to the groundwater within the basin. In addition, over 2,000 residential septic tanks exist in the Fish Creek basin. High transmissivity in the aquifer and groundwater levels at or near the surface make soils within the Fish Creek basin not suitable for septic tank effluent often requiring raised leachfields. In the fall of 2004, a sewer collection system was completed for the Wilson area (fig. 4). The sewage collection system and advanced wastewater treatment proposed

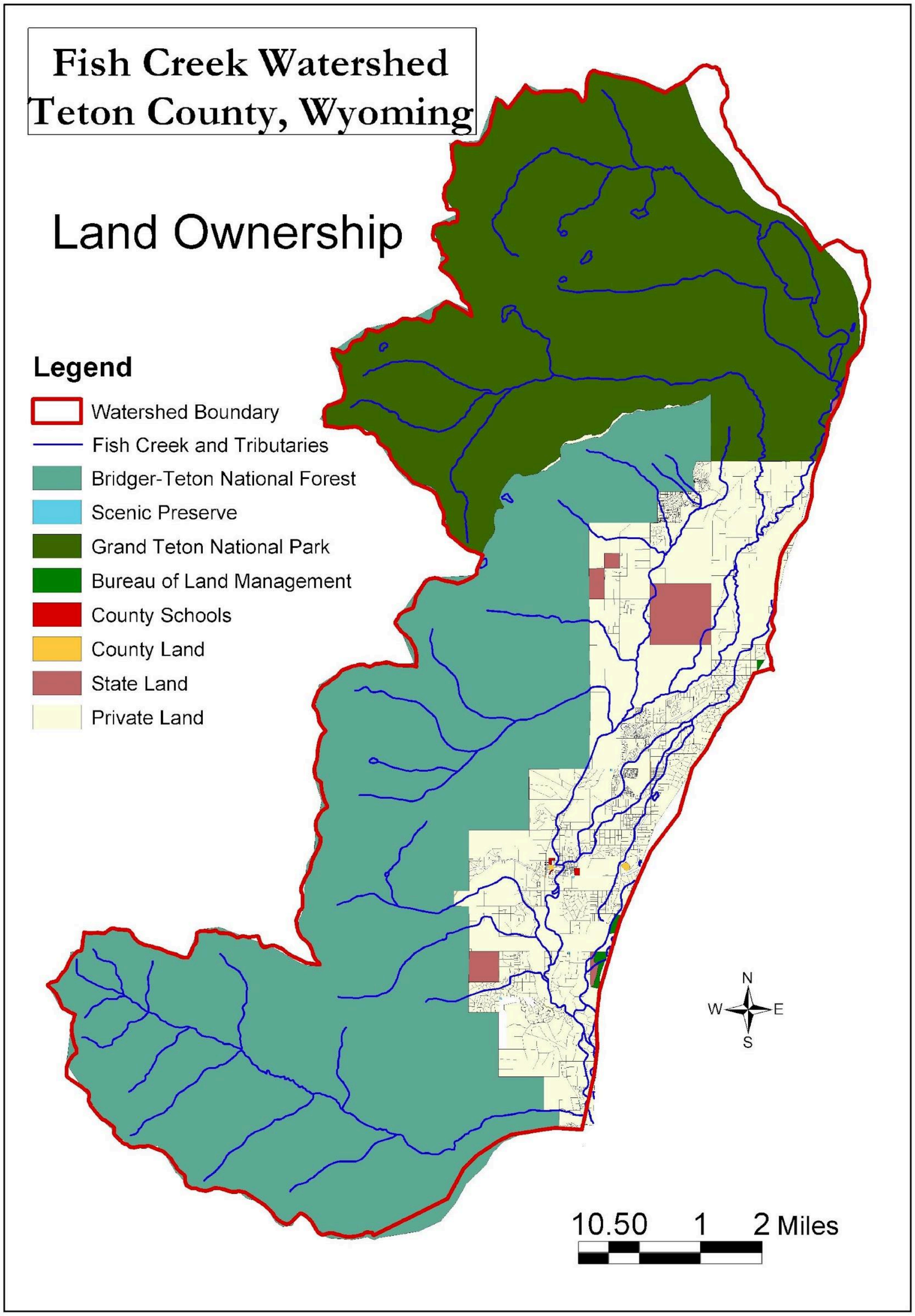


Figure 3. Land ownership in Fish Creek basin, Teton County, Wyoming, 2003.

would eliminate over 284 existing septic systems, 66% of which are more than 20 years old (Nelson Engineering, 2000).

SAMPLING SITES AND METHODS

Sampling sites were determined based upon previous fecal-indicator bacteria data, site access, land use and ownership, and input from public meetings and stream committees. Ten sites were sampled on multiple creeks in the Fish and Flat Creek basins (fig. 4 and 5). The upstream site on Fish Creek is below Teton Village (site TV1) and the downstream most site (FC7) is approximately a quarter mile from its confluence with the Snake River. An additional site was added (site FC1) in June of 2003 at the request of the landowner. The upstream site (FLRB) on Flat Creek is at the northern Town of Jackson boundary where the creek leaves the National Elk Refuge. The downstream site (FL5) on Flat Creek is located on the Wyoming Game and Fish Department's South Park Elk Feed Grounds approximately one mile from its confluence with the Snake River. Two sites were sampled at the mouths of the Cache Creek diversion tube (site CC3) and Game Creek (site GC4).

Sample Collection

Water samples were collected according to Wyoming Department of Environmental Quality Protocol (WDEQ, 2001). Sterile conditions are required for collection, preservation and analysis of fecal-indicator bacteria analysis. To minimize analytical variability, all samples were collected and processed by the same equipment and same person. Water samples were collected using sterile 150 mL sample bags and cooled at 4 degrees Celsius. A duplicate sample was collected at one randomly selected site during each sampling event.

Laboratory Methods and Enumeration of *E. coli*

Water samples were processed through membrane filters in 1 mL, 10 mL and 100 mL aliquots in order to achieve an ideal enumeration. The enumeration method is described in WDEQ's Standard Operating Procedures, 2001. The Environmental Protection Agency approved Hach Company m-ColiBlue24® Broth was used as medium during incubation. The ideal range for *E. coli* is 20-80 colonies per plate. *E. coli* concentrations outside the ideal range are listed with and 'e' and filters with no colonies are given a <1 col/100 mL. Occasionally, a plate was unreadable or partially readable due to large sediment content in the sample. These samples were labeled 'nr' for no result. The plated samples were then immediately shipped to the Institute for Environmental Health (IEH) in Seattle, Washington for DNA ribotyping analysis.

Quality control samples, including blanks and duplicates, were collected during the study. One field blank and one field duplicate were collected for each sampling event. Sterile-distilled water was used for field blanks to determine if contamination occurred during sample collection and processing. Field blanks were processed first and field duplicates were processed along with the regular samples. The purpose of the duplicate sample is to estimate the inherent variability of the sampling procedure, technique, characteristic or fecal contamination in the source water.

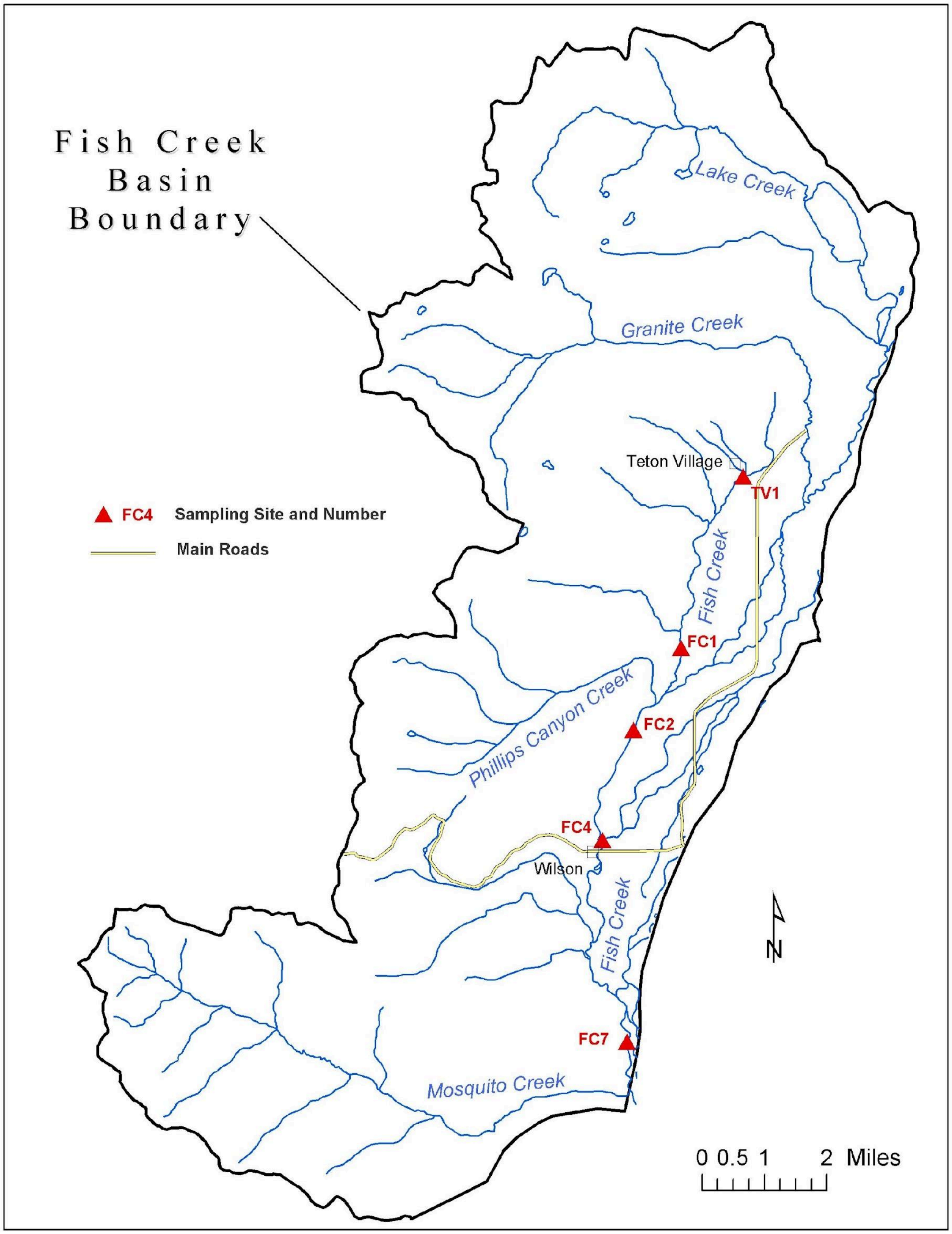


Figure 4. Location of sampling sites in Fish Creek basin, Teton County, Wyoming, 2003.

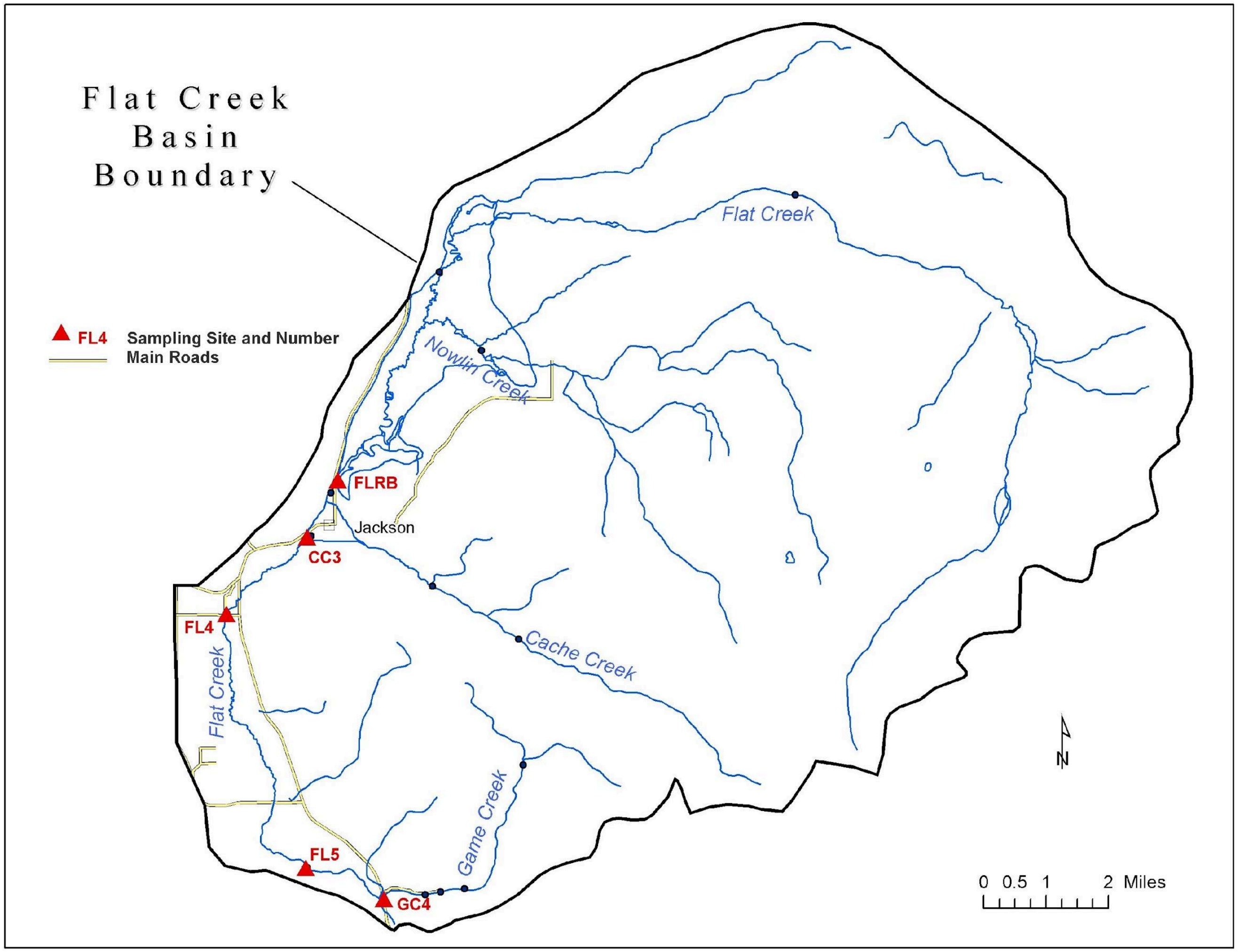


Figure 5. Location of sampling sites in Flat Creek basin, Teton County, Wyoming, 2003.

The equation used for determining the relative percent difference (RPD) between the water sample and duplicate sample is:

$$RPD = \left(\frac{duplicate - sample}{\left(\frac{duplicate + sample}{2} \right)} \right) \times 100$$

Duplicate samples were collected simultaneously with the regular sample.

Microbial Source Tracking

Microbial source tracking (MST) is based on the use of a genetic fingerprinting of *Escherichia coli* strains isolated from water and source samples by a method of ribotyping modified by Dr. Mansour Samadpour, termed EcoRI / PvuII variant. In a 2004 study published in Environmental Science and technology Journal, seven protocols to identify fecal contamination sources using *E. coli* were compared (Stoeckel, 2004). In accuracy tests, measuring the seven protocols abilities to correctly classify isolates, ribotyping with EcoRI / PvuII approached 100% when 6% of the isolates were classified. The other six protocols attempted to classify 100% of the isolates, but have much lower accuracies averaging 65%.

It is the DNA fingerprint of the *E. coli* that lives within the animal that is typed-not the DNA of the specific animal. Though fecal coliform bacteria found in many animal species are very similar genetically, there are differences among members of the same species that have adapted to live in different host species. It is thought that these distinctions in bacteria occur because the intestinal environments differ between animals, including humans. It is in identifying these genetic differences that it is possible to associate bacteria with sources (human, avian, dog, etc.).

The MST method has been developed on the basis of the principles of microbiology, epidemiology, molecular epidemiology, sanitary engineering, and hydrogeology. The ability to identify the sources of microbial pollution of fecal origin depends on whether or not there are host-specific lineages (Ecotypes) within a given species of bacteria (Maynard Smith, 1996), particularly among bacterial species like *E. coli.* The existence of Ecotypes would then allow the principles of epidemeology and molecular epidemeology to be applied in identifying the host species for bacterial strains that are isolated from environmental samples in the manner that outbreaks of infectious diseases are detected (Samadpour, 1995). According to Dr. Mansour Samadpour, Ph.D., with the University of Washington in Seattle, WA, there are several foundations which the MST method is based:

- First, in any given pollution scenario there are multiple contributing animal sources of microbial pollution, each of which has its own unique clones of bacteria that constitute their normal flora.
- Second, collections of isolates from an appropriate bacterial species can be compiled from the polluted sites and the suspected animal sources of pollution, which are identified through a sanitary survey of the region surrounding the polluted site.
- Third, using an appropriate molecular subtyping method,

all bacteria in the collection can be subtyped.

- Finally, the genetic fingerprints of the bacterial isolates from the polluted site can be compared to those of the bacteria from suspected animal sources.

The MST method is based upon two principles. The first principle is that bacterial population genetic structure is clonal. Bacteria divide by binary fission. The two daughter cells that result of this division are virtually identical in all aspects. Microbial Source Tracking makes use of the clonal population structure of bacteria to classify organisms based on their genetic fingerprints into groups of clonal descent. The second principle behind the MST methodology is the assumption that within a given species of bacteria, various members have adapted to living/environmental conditions in specific hosts. As a result, there is a high degree of host specificity among bacterial strains that are seen in the environment. A resident strain of bacteria is one that has adapted to a particular host (e.g., warm-blooded animal intestinal tract) and is capable of colonizing that environment and competing favorably with its indigenous flora. The *E. coli* strain that inhabits the intestines of one species is genetically different from the strain that might inhabit another species.

The Institute for Environmental Health has an extensive library of *E. coli* ribotypes (>120,000) from strains that were isolated from the feces of scores of different animals. The University of Washington's library contained strains from the local area, but the library was lacking local source samples from domestic dogs. Twenty domestic dog feces samples were collected in the Fish and Flat Creek watersheds and sent to the Institute for Environmental Health.

Samples were received by the IEH and *E. coli* colonies were randomly sampled from the plate cultures. Ten percent of the plated colonies (minimum of three and maximum of ten colonies) were sampled and the ribotype strain was determined. These E. coli strains were matched to sources in IEH library.

Data Analysis

Data in this report are summarized using tables, boxplots, charts and general statistics. For boxplots, the lower and upper edges of the box indicate the 25th and 75th percentiles, respectively. The line within the box is the median and the whiskers represent the 10th and 90th percentiles. Values outside the whiskers are shown as individual points. Circles are outlier values with more than 1.5 box lengths above the box and stars are extreme values with more than 3 box lengths above the box. Microbial source tracking data is summarized using pie charts to illustrate relative percentages and bar charts to show quantities of isolates to matching sources.

RESULTS AND DISCUSSION

Sampling times and duration were designed to best characterize representative hydrologic conditions for a complete year. Twice monthly, from March through November 2003, samples were collected at each site. Seventeen sampling events over the nine month period resulted in 162 water samples. The *E. coli* fecal-indicator bacteria concentrations are presented in table 1. The lowest concentration of *E. coli* of >1 colonies/100mL was measured at all sites. The highest concentration of 162 *E. coli* samples was 1060 col/100mL for the Game Creek site in the Flat Creek basin (GC4). Boxplots for both basins are shown in figures 6 and 7.

The MST results identified 686 *E. coli* isolates shown in table 2. The highest number of matches was avian with 178 isolates. The lowest number was coyote with 5 isolate matches.

Sampling Conditions

Sampling events for this source tracking study represented variability in weather and water runoff into streams in Fish and Flat Creek basins. Samples were collected during transient snowmelt events, spring mountain snowmelt, intense and light rainfall, and during cold and warm dry periods. Three stream gaging stations maintained by the United States Geological Survey provided streamflow and hydrograph data for the study (Figure 8.) Streamflows in the basins are affected by irrigation diversions and return flows which alter the natural hydrologic regime.

Stream water temperature may have influenced *E. coli* colony formation on the filtered plates using *Hach's m-ColiBlue24® Broth.* Stream water temperatures in Fish and Flat Creek basins are generally below 10° Celsius between the beginning of October and the middle of June. Very few, if any positive *E. coli* indicator bacteria colonies showed on samples collected during these months during 2003. Although water temperature was not collected with each sample, it appears there is a correlation between very low *E. coli* results and temperature. Despite USEPA and the manufacturer's approval of the product for stream water and no limitations on water temperature, a future comparative analysis of different fecal indicator bacteria broth manufacturer's could be initiated to support this assumption. The July-September time period when stream water temperatures generally exceed 10° Celsius provides valuable information in relation to human exposure to pathogens through recreational contact, which is highest during these months.

Quality Control Samples

No *E. coli* colonies were detected in the seventeen field blanks processed for each sampling event. Seventeen replicate samples were processed. The results for these samples are listed in table 1. The mean and median RPD for E. coli replicates were 54 and 22 percent respectively. Six of the RPD for replicates exceeded 100 percent. For example, a sample result of 0 *E. coli* colonies and a replicate result of 1 colony will result in an RPD of 200 percent.

	Site Map Number												
Sample Date	**TV1**	**FC1**	**FC2**	**FC4**	**FC7**	**FLRB**	**FL4**	**FL5**	**GC4**	**CC3**	**Duplicate**	**RPD**	**Blank**
					E. coli (colonies/100mL)								
03-10-03	10e		10e	21	1e	<1	4e	2e	5e	2e	12 GC4	82	0
03-24-03	58		6e	8e	7e	<1	1e	1e	8e	65	0 FL5	100	0
04-07-03	7e		1e	1e	2e	<1	<1	2e	3e	<1	2 FC7	0	0
04-15-03	16e		1e	2e	1e	60	6e	2e	<1	60	10 TV1	46	0
05-05-03	3e		<1	2e	<1	<1	1e	1e	1e	<10	0 CC3	0	0
05-22-03	17e		14e	87e	5e	<1	1e	10e	nr	1e	0 FLRB	0	0
06-09-03	14e		64	90e	30e	10e	<1	10e	40e	510	410 CC3	22	0
06-23-03	12e	38	27	28	21	4e	20	9e	<1	20	1 FLRB	120	0
07-25-03	282e	11	34	30	18	150	74	36	640	368	720 GC4	12	0
08-05-03	70e	30e	100e	110e	50e	250	50e	60e	80e	150e	240 GC4	100	0
08-18-03	100e	130e	100e	40e	20e	220	50e	40e	270	190e	220 GC4	20	0
09-04-03	130e	20e	290	20e	10e	120e	40e	100e	120e	180e	120 GC4	0	0
09-23-03	60e	50e	20e	20e	5e	170e	5e	20e	1060e	50e	60 FL5	100	0
10-07-03	320	<1	20e	<1	30e	<1	<1	<1	10e	<1	0 FL5	0	0
10-22-03	20e	<1	10e	<1	<1	30e	<1	<1	40e	10e	10 GC4	120	0
11-03-03	<1	20e	10e	<1	<1	<1	<1	<1	<1	<1	0 FC4	0	0
11-19-05	1e	<1	<1	<1	1e	1e	<1	<1	<1	9e	1FL4	200	0

e, estimated value (outside ideal range); nr, no result; Red, exceeds WDEQ proposed moderate full body contact surface water criteria (298 organisms per 100 mL)

Mean	66	30	42	27	12	60	15	17	142	95
Standard Error	23	12	17	9	3	21	6	7	74	36
Median	17	20	14	20	5	4	1	2	9	20
Standard Deviation	96	39	72	35	14	87	23	28	295	147
Range	320	130	290	110	50	250	74	100	1060	510
Minimum	0	0	0	0	0	0	0	0	0	0
Maximum	320	130	290	110	50	250	74	100	1060	510

Table 1. *E. coli* fecal-indicator bacteria results for Fish and Flat Creek basins, Teton County, Wyoming, March-November 2003.

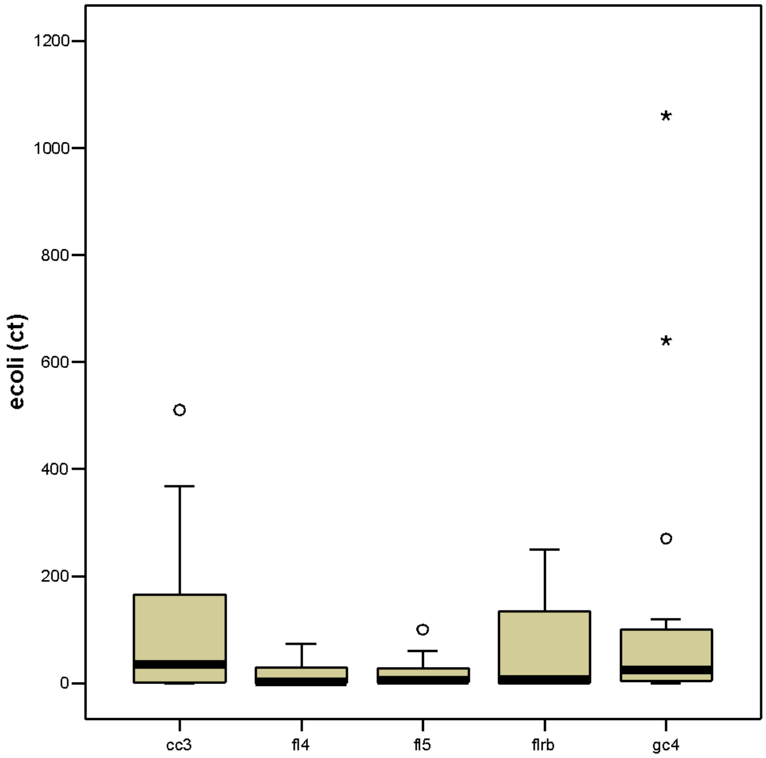
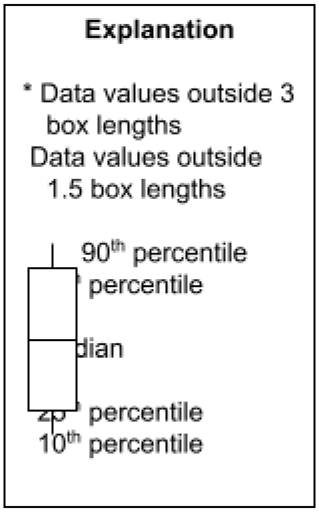


Figure 6. Boxplot showing *E. coli* results for Flat Creek basin.



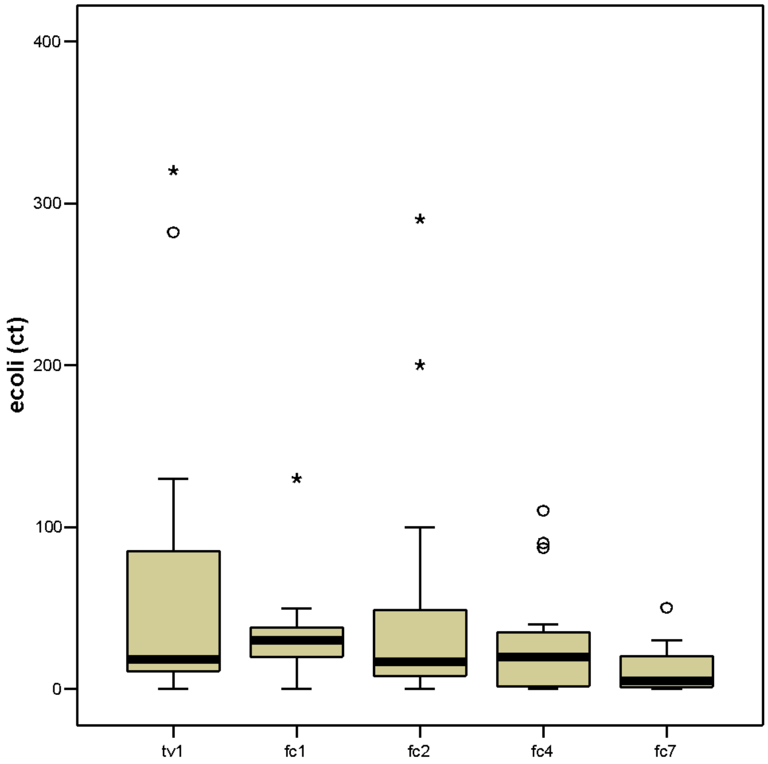


Figure 7. Boxplot showing *E. coli* results for Fish Creek basin.

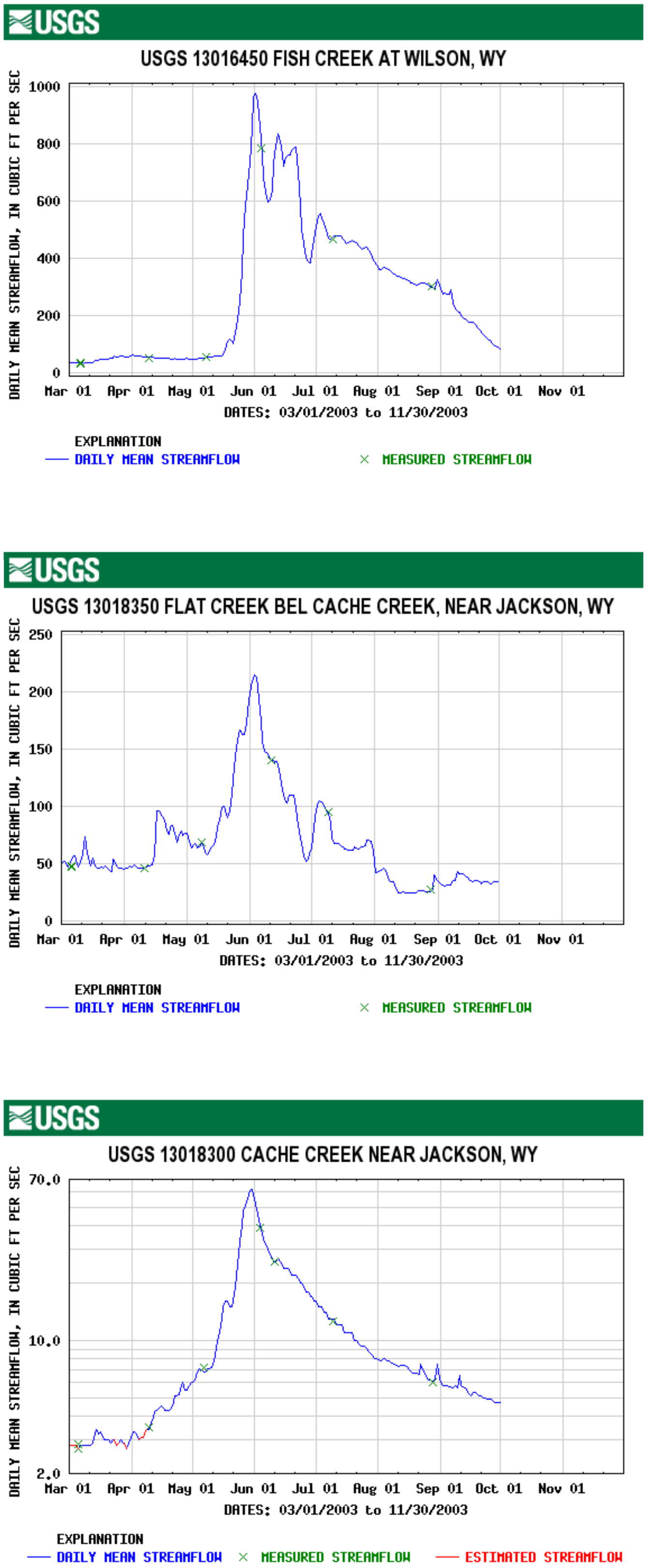


Figure 8. Annual hydrographs during the study for Fish Creek, Flat Creek and Cache Creek, 2003. (Note: October and November data not available at the time of this report)

E. coli Bacteria Results by Basin

Fish Creek Basin

Mean concentrations of *E. coli* indicator bacteria at individual sites in the Fish Creek basin varied from 12 to 66 col/100mL (table 1). Median concentrations were 3 to 23 col/100 mL. Concentrations ranged from 0 to 320 *E. coli* col/100mL. One sample, site TV1 October 7, 2003, exceeded the WDEQ proposed standard for moderate use recreational contact of 298 col/100mL.

Flat Creek Basin

Mean concentrations of *E. coli* indicator bacteria at individual sites in the Flat Creek basin varied from 17 to 142 col/100mL (table 1). Median concentrations were 1 to 20 col/100 mL. Concentrations ranged from 0 to 1060 col/100mL. Four samples in Game and Cache Creek exceeded the WDEQ proposed standard for moderate use recreational contact of 298 col/100mL.

Distribution & Source Matches of *E. coli* Indicator Bacteria

A total of 686 *E. coli* bacteria were matched to specific warm-blooded animal sources (table 2). For both basins, 178 isolate matches for avian (birds) was the highest and 25 percent of the total. Coyote had the fewest isolate matches of 5 and less than 1 percent. Six percent or 38 isolates were not matched to any source and were considered unknown. The MST data indicate that both human and non-human sources contribute to *E. coli* to the Fish and Flat creek basins, and a higher total percentage of the known isolates are from wildlife sources than from humans, sewage, domestic pets, and livestock. Isolates matching human and sewage sources totaled 40, about 6 percent. Isolate matches that were not identified as dog or coyote were labeled canine. The canine family includes dog, coyote, wolf, and fox. Canine sources contributed a total of 103 matches or 15 percent. Canine matches identified as dog contributed 47 isolates or 7 percent of the total results. Deer, elk and moose belong to the Cervidae family of ruminants. The deer/elk source category includes all three ruminants. The deer source category includes white tail and mule deer only. The deer/elk and elk categories contributed 97 isolate matches or 14 percent of total matches. The bovine source category consists of animals in the Bovide family of ruminants. The bovine category includes bison, sheep, goat, cattle, and pronghorn. Bovine contributed 27 matches or 4 percent of total *E. coli* source matches. Feline include cougars, lynx, and domestic house cat. Feline contributed 7 isolate matches or 1 percent of the total.

For this microbial source tracking study, isolated domestic sources of *E. coli* include human, sewage, bovine (livestock), horse, and dog. Domestic sources contributed 116 isolate matches or 17 percent of *E. coli* bacteria (table 3). Wildlife sources comprised the remaining 570 matches or 83 percent (figures 11 and 12). The results from the source tracking analysis indicate dog as the dominate contributor to domestic sources of *E. coli* (47 matches/40%). Sewage sources originate from septic tanks and wastewater effluent, while human sources are from sewer lines or directly from human sources. Sewage isolate matches totaled 31 or 27 percent while human matches totaled 9 or 8 percent.

Sources	# of Matches	% of Total Matches	Fish Creek Basin	Flat Creek Basin
Avian	178	26%	102	76
Rodent	148	22%	74	74
Deer/Elk	80	11%	41	39
Canine	51	7%	17	34
Dog	47	7%	30	17
Unknown	38	5%	19	19
Sewage	31	5%	22	9
Bovine	27	4%	8	19
Elk	17	3%	0	17
Raccoon	14	2%	4	10
Deer	13	2%	6	7
Horse	13	2%	5	8
Human	9	1%	7	2
Bear	8	1%	4	4
Feline	7	1%	1	6
Coyote	5	1%	1	4
TOTAL	**686**	**100%**	**341**	**345**

Table 2. *E. coli* Microbial Source Tracking results for Fish and Flat Creek basins.

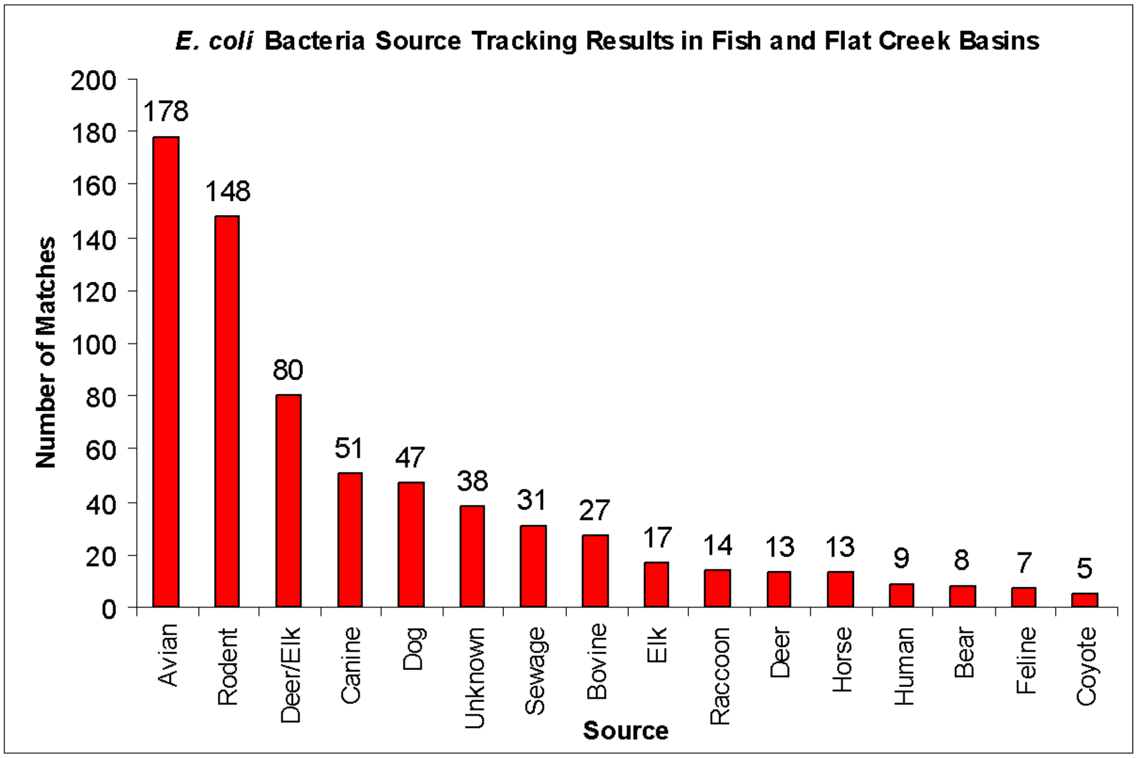


Figure 9. *E. coli* source tracking results for Fish Creek and Flat Creek Basin.

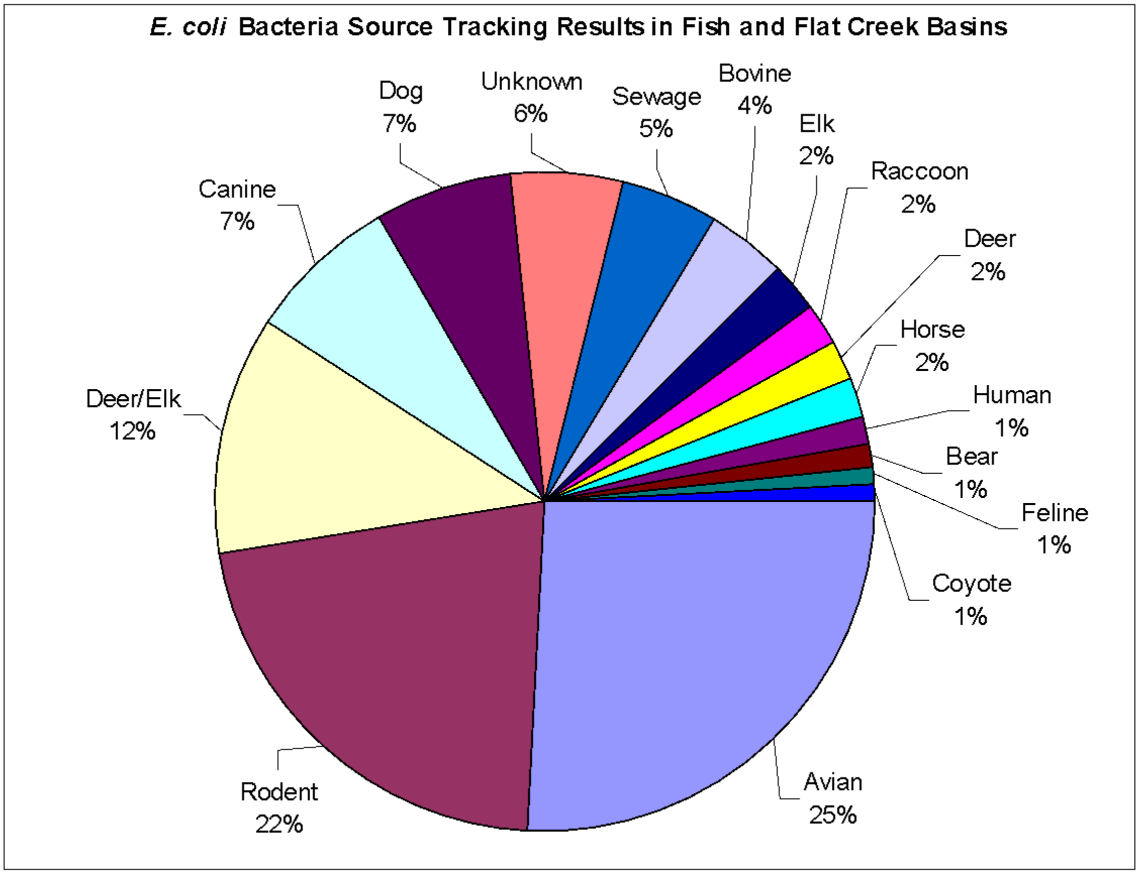


Figure 10. *E. coli* source tracking results by percentage for Fish Creek and Flat Creek Basin.

Species	# of Matches	% of Total Matches	Fish Creek Basin	Flat Creek Basin
Dog	47	7%	30	17
Sewage	31	5%	22	9
Bovine	16	2%	8	8
Horse	13	2%	5	8
Human	9	1%	7	2
TOTAL	**116**	**17%**	**72**	**44**

Note: Bovine at FLRB, CC3 and GC3 were not included in domestic sources

Species	# of Matches	% of Total Matches	Fish Creek Basin	Flat Creek Basin
Wildlife	570	83%	273	297
Domestic	116	17%	72	44
TOTAL	**686**	**100%**	**345**	**341**

Table 3. Domestic sources of *E. coli* for Fish and Flat Creek basins.

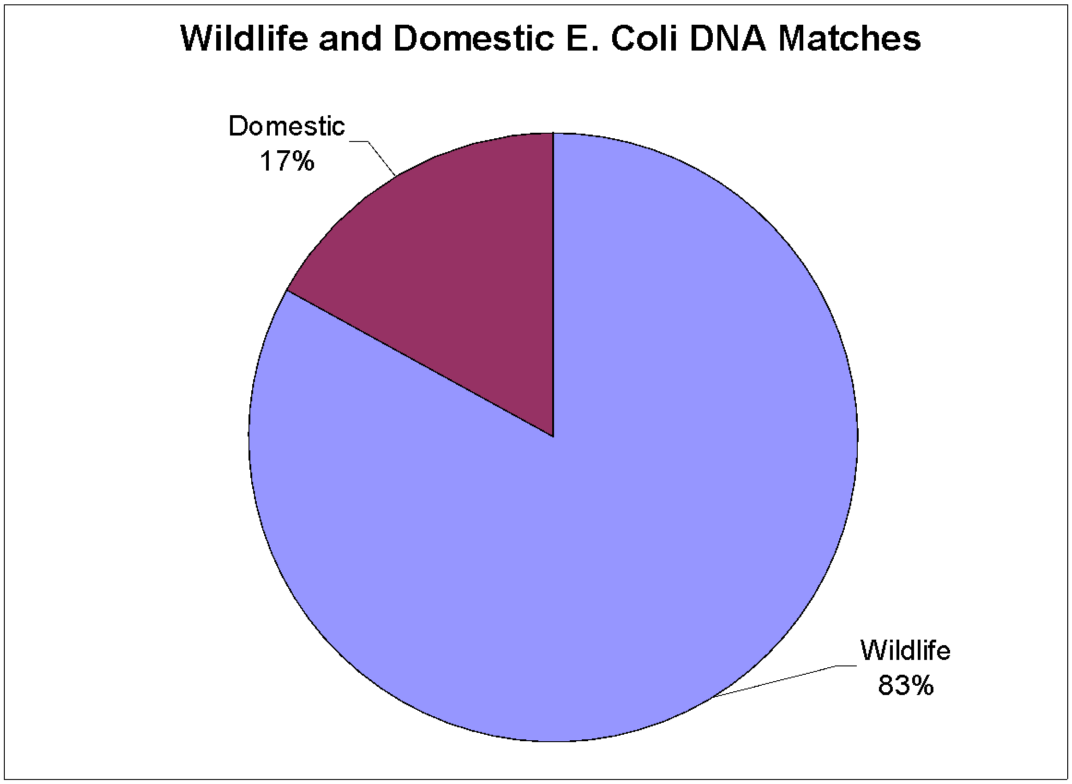


Figure 11. Percentage of wildlife and domestic sources of *E. coli* in Fish and Flat Creek basins.

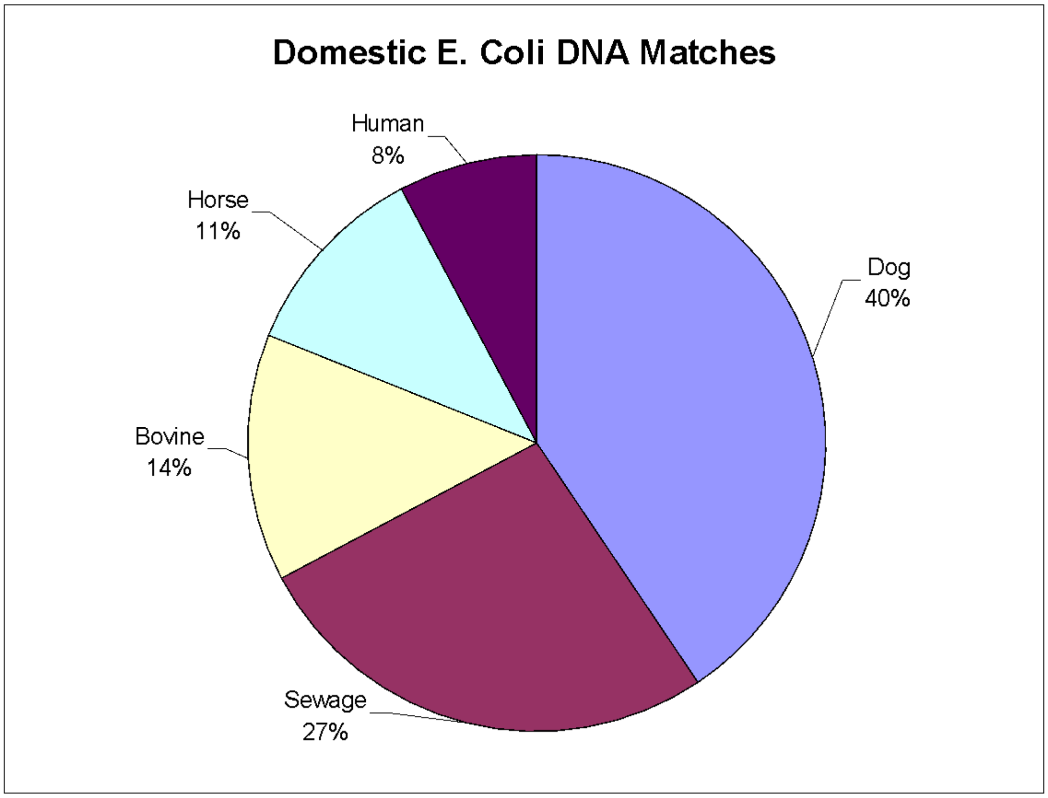


Figure 12. Percentage of domestic sources of *E. coli* in Fish and Flat Creek basins.

Fish Creek Basin

The sampling of five sites on the main stem of Fish Creek resulted in 78 samples with 345 *E. coli* isolate matches (figures 13 and 14). The microbial source tracking results indicate avian (102 isolates/29.9 percent) and rodent (74 isolates/21.7 percent) were the dominant species contributing *E. coli* bacteria during the sampling period. Domestic source matches in the Fish Creek basin total 72 or 21 percent. Dog isolate matches were highest (30 matches/8.8 percent). Human and sewage isolate matches totaled 29 and comprise 8.6 percent of the Fish Creek basin results. All sites with Bovine isolate matches were considered domestic sources. Bison, bighorn sheep, and pronghorn are not known to utilize the lower elevations of Fish Creek. Individual sampling site results are displayed in Appendix A.

Site FC1 is located on the Snake River Ranch approximately one mile upstream of the Lake Creek confluence. Nine flood irrigated pastures totaling 1,299 acres drain to Fish Creek near site FC1 (B. Resor, 2005). The Snake River Ranch provided cattle distribution data for the pastures near site FC1 (table 4). The maximum number of cattle on the pastures near site FC1 was 1,282. Cattle are grazed in the fenced riparian corridor along Fish Creek for short periods during the summer months. No more than one *E. coli* isolate was sourced to bovine during each sampling event.

Flat Creek Basin

The sampling of three sites on the main stem of Flat Creek and two sites on tributaries resulted in 85 samples with 341 *E. coli* isolate matches (fig. 15 and 16). The microbial source tracking results indicate avian (76 isolates/22.0 percent) and rodent (74 isolates/21.4 percent) were the dominant species contributing *E. coli* bacteria during the sampling period. Domestic source matches in the Flat Creek basin total 44 or 13 percent. Dog isolate matches were highest (17 matches/4.9 percent). Human and sewage isolate matches totaled 11 and comprise 3.2 percent of the Flat Creek basin results. Bovine results for sites FLRB, CC3 and GC3 were not included as domestic sources. Domestic bovine are not known to utilize these regions of the Flat Creek basin. Individual sampling site results are displayed in Appendix A.

Relationship of Bacteria Water Quality Standards and *E. coli* Sources

Study results were compared to Wyoming DEQ proposed *E. coli* bacteria standards (table 5). The proposed single-sample maximum concentration standard in Chapter 1 Water Quality Rules and Regulations, Section 27 (c) reads as follows:

> During the recreation season, on all waters designated for primary contact recreation, the following single-sample maximum concentrations of *e.coli* bacteria shall apply:
>
> (i) High use swimming areas -
> 235 organisms per 100 milliliters
> (ii) Moderate full body contact -
> 298 organisms per 100 milliliters
> (iii) Lightly used full body contact -
> 410 organisms per 100 milliliters
> (iv) Infrequently used full body contact -
> 576 organisms per 100 milliliters
>
> The appropriate recreational use category (i through iv above) shall be determined by the administrator as needed, on a case by case basis.

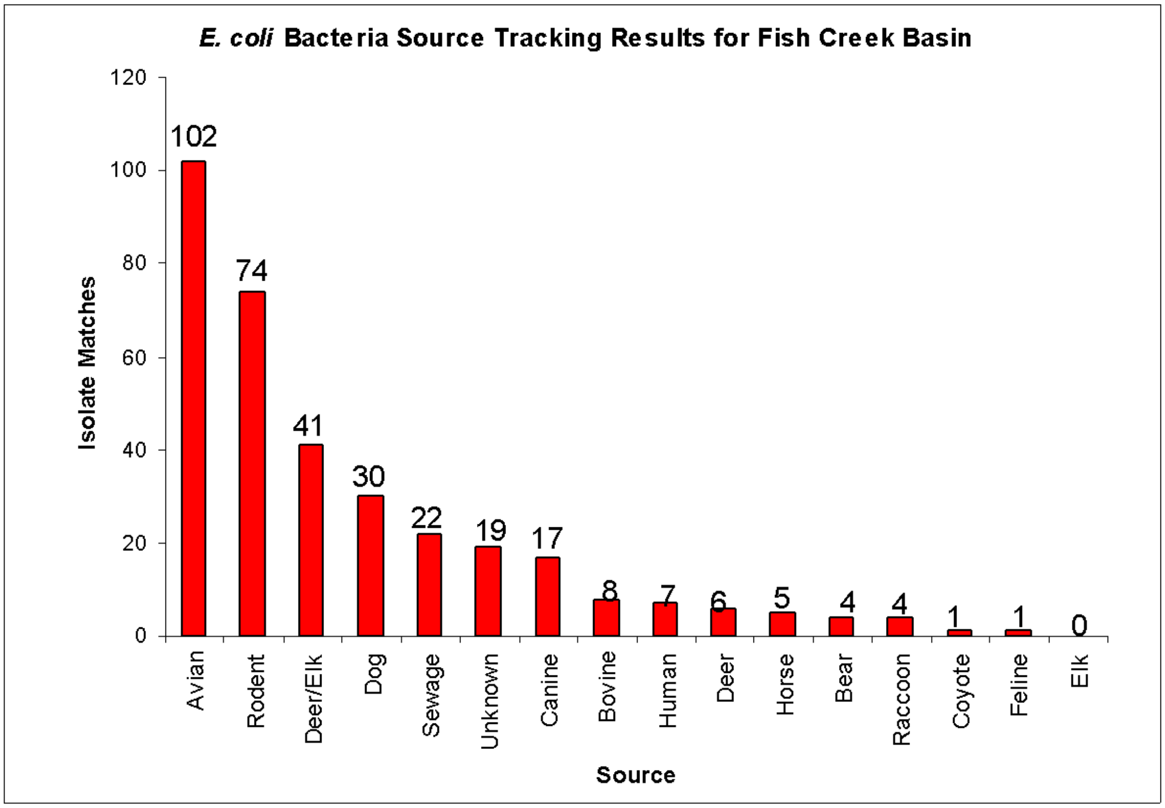
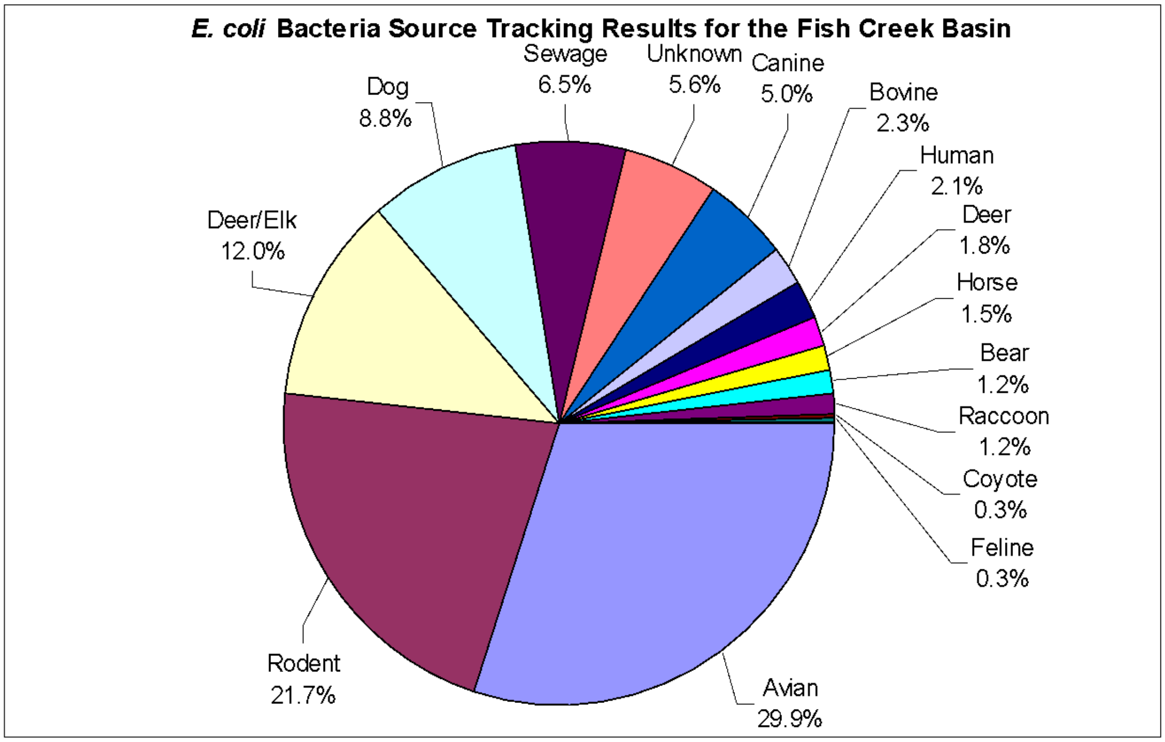


Figure 13. *E. coli* source tracking results for Fish Creek basin.



Figures 14. *E. coli* source tracking results by percentage for Fish Creek Basin.

	# Head	Date	E. Coli Matches	
			Bovine	Other
	1219	06-20-05		
	1219	06-21-05		
	1219	06-22-05		
FC1 - Sample Date	**1219**	**06-23-03**	**0**	**5**
	403	07-22-03		
	400	07-23-03		
	400	07-24-03		
FC1 - Sample Date	**400**	**07-25-03**	**1**	**6**
	400	08-02-03		
	335	08-03-03		
	335	08-04-03		
FC1 - Sample Date	**335**	**08-05-03**	**1**	**4**
	335	08-15-03		
	335	08-16-03		
	335	08-17-03		
FC1 - Sample Date	**335**	**08-18-03**	**1**	**5**
	883	09-01-03		
	1282	09-02-03		
	1282	09-03-03		
FC1 - Sample Date	**1282**	**09-04-03**	**0**	**5**
	0	09-20-03		
	0	09-21-03		
	0	09-22-03		
FC1 - Sample Date	**0**	**09-23-03**	**0**	**5**

Note: No cattle in FC1 area drainage after 09-08-03

Table 4. Number of cattle on pastures upstream of site FC1 during sampling event periods.

Site map number	Date sampled	*E. coli* col/100 mL	Avian	Bovine	Canine	Coyote	Deer/Elk	Dog	Rodent	Unknown
TV1	10-07-03	320	3					1	1	
CC3	06-09-03	510	3			2		1	1	
CC3	07-25-03	368	1				2		1	2
GC4	07-25-03	640		1	6					
GC4	09-23-03	1060	1		3		1			1

Table 5. Water samples exceeding WDEQ moderate full body contact criteria (298 col/100 mL).

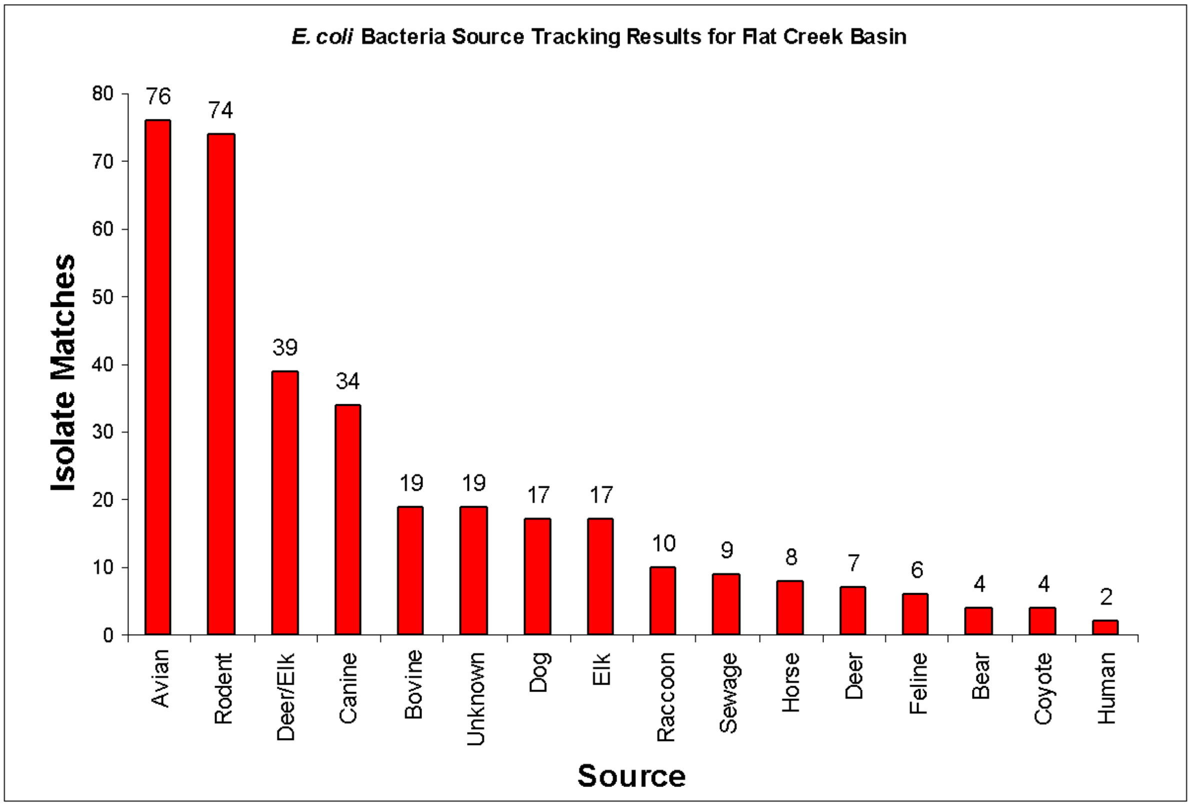


Figure 15. *E. coli* source tracking results for Flat Creek basin.

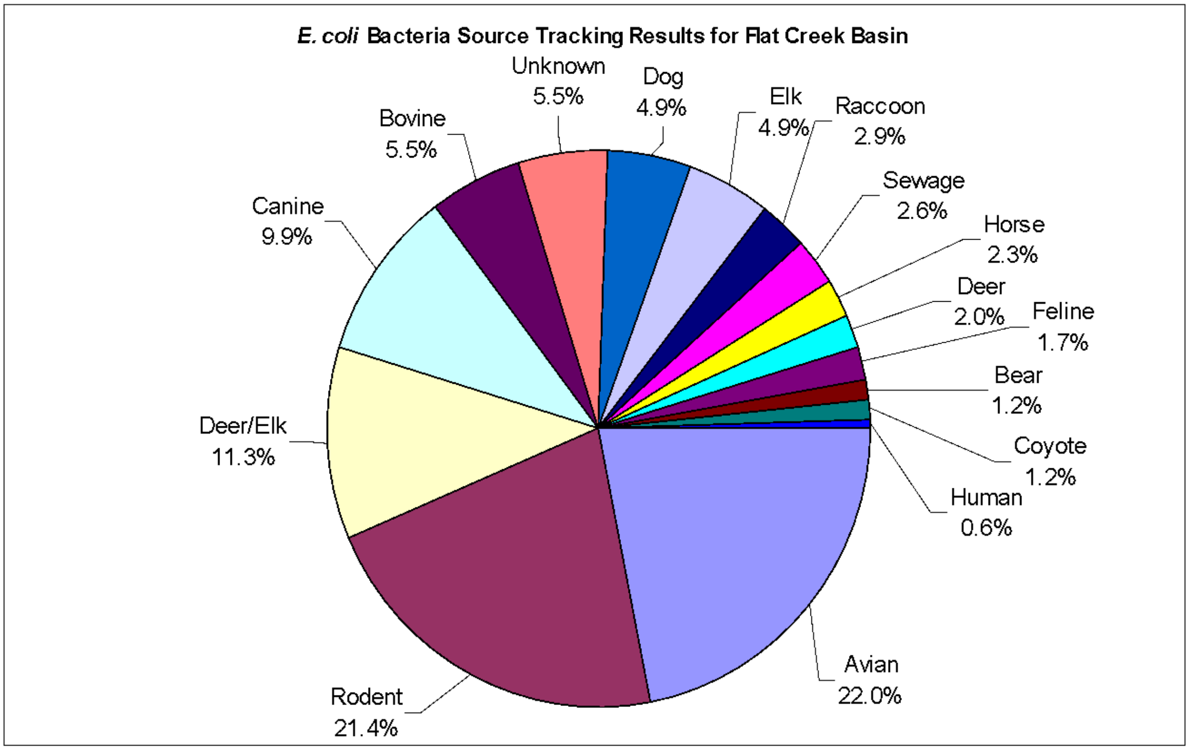


Figure 16. *E. coli* source tracking results by percentage for Flat Creek basin

A recreational use category has not been determined for Fish or Flat Creeks. Based upon summer fishing and float tube usage, the moderate full body contact criteria (298 organisms per 100 mL) will be used for comparisons in this report. Five of the 162 water samples taken during the study period exceeded the moderate full body contact criteria. Two of the five samples contained domestic source matches, domestic dog. No other domestic source matches were associated with water quality standard exceedances.

SUMMARY

In 2002, Fish Creek and Flat Creek basins were chosen as study units for microbial source tracking for *Escherichia coli* fecal-indicator bacteria. Public concern about fecal contamination in surface waters and stream committee guidance led TCD to seek public input and eventually develop a plan to identify the sources of fecal bacteria in the two selected basins.

Twice monthly, from March through November 2003, samples were collected at ten sites. Seventeen sampling events over the nine month period resulted in 162 water samples. The lowest concentration of *E. coli* of >1 colonies/100mL was measured at all sites. The highest concentration of 162 *E. coli* samples was 1060 colonies/100mL for the Game Creek site in the Flat Creek basin (GC4).

Mean concentrations of *E. coli* indicator bacteria at individual sites in the Fish Creek basin varied from 12 to 66 col/100mL. Median concentrations were 3 to 23 col/100 mL. Concentrations ranged from 0 to 320 E. coli col/100mL. One sample, site TV1 October 7, 2003, exceeded WDEQ proposed standard for moderate use recreational contact of 298 col/100mL. Mean concentrations of *E. coli* indicator bacteria at individual sites in the Flat Creek basin varied from 17 to 142 col/100mL. Median concentrations were 1 to 20 col/100 mL. Concentrations ranged from 0 to 1060 *E. coli* col/100mL. Three samples in Game and Cache Creek exceeded WDEQ proposed standard for moderate use recreational contact of 298 col/100mL.

Microbial Source Tracking (MST) results identified 686 *E. coli* isolates matches to specific warm-blooded animal sources. For both basins, 178 isolate matches for avian was the highest and 26 percent of the total. Feline had the fewest isolate matches of 7 and less than 1 percent. Five percent, or 38 isolates, were not matched to any source and were considered unknown. The MST data indicate that both human and non-human sources contribute *E. coli* to surface water in the Fish and Flat creek basins. A higher total percentage of the known isolates are from wildlife sources than from humans, domestic pets, and livestock. Isolates matching human sources totaled 40, about 6 percent of total matches. The highest numbers of domestic sources were dog, about 40 percent of domestic matches.

Wyoming DEQ single-sample standard for moderate full body contact criteria (298 col/ 100 mL) was used for comparisons in this report. Five of the 162 water samples taken during the study period exceeded the moderate full body contact criteria. Two of the five samples contained domestic source matches for domestic dog.

The results from this 2003 study suggest the majority of the fecal contamination in Fish and Flat Creek basins originates from wildlife sources. Domestic sources of fecal contamination were minor, but could pose a human health risk. No particular sampling site or region within the study area showed specific spatial or temporal trends. In order to reduce domestic fecal contamination to surface waters, specific hydrologic paths for fecal contamination should be identified and management actions applied to individual situations. Future development should utilize the most innovative and advanced management practices to prevent domestic sources of fecal contamination from entering all water sources.

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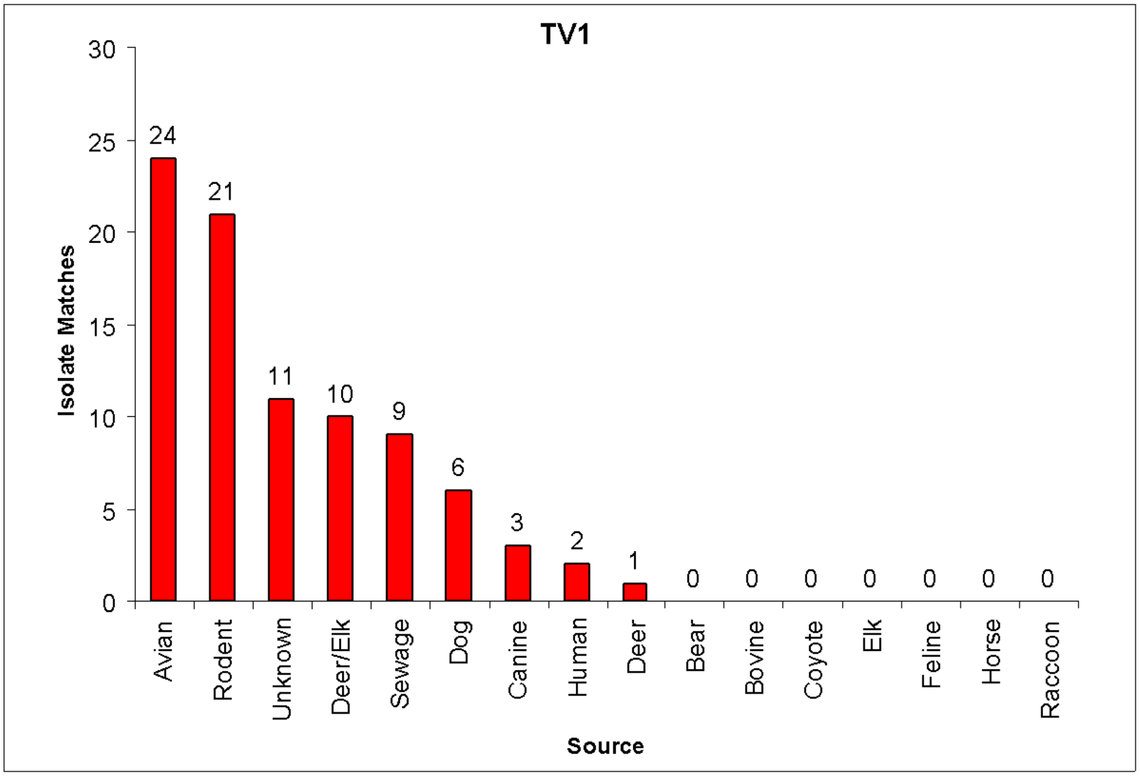
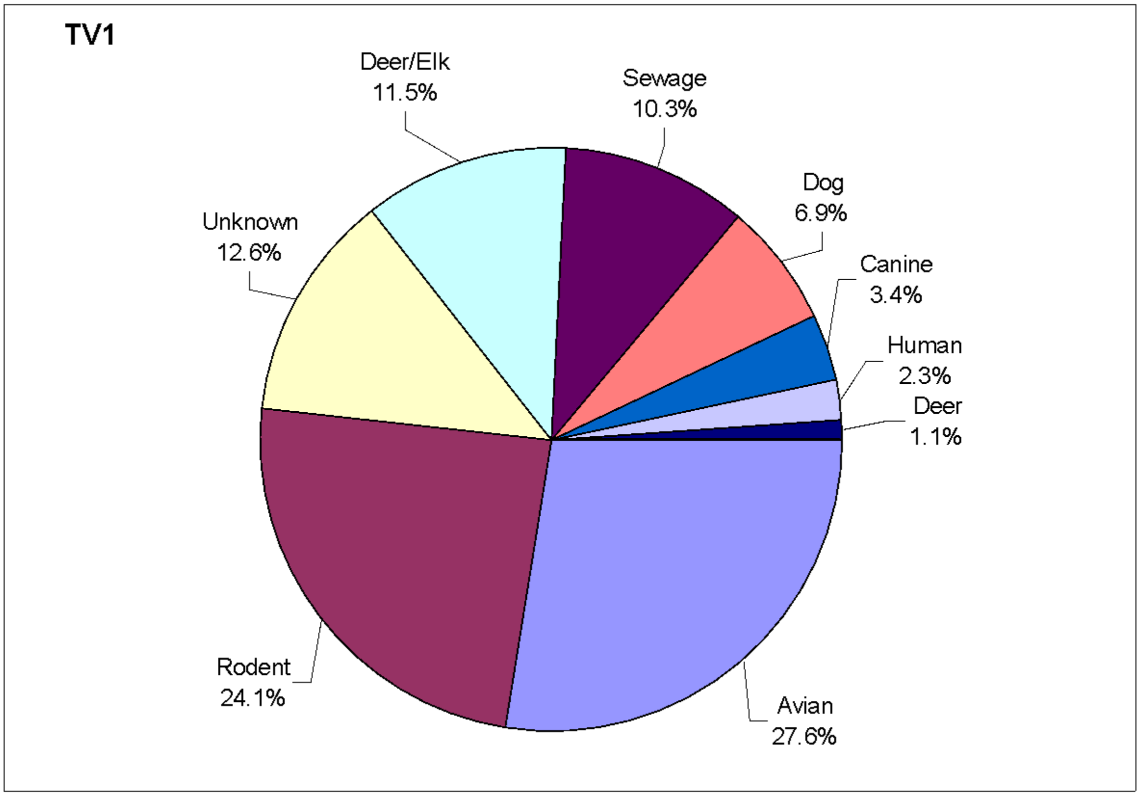
Appendix A

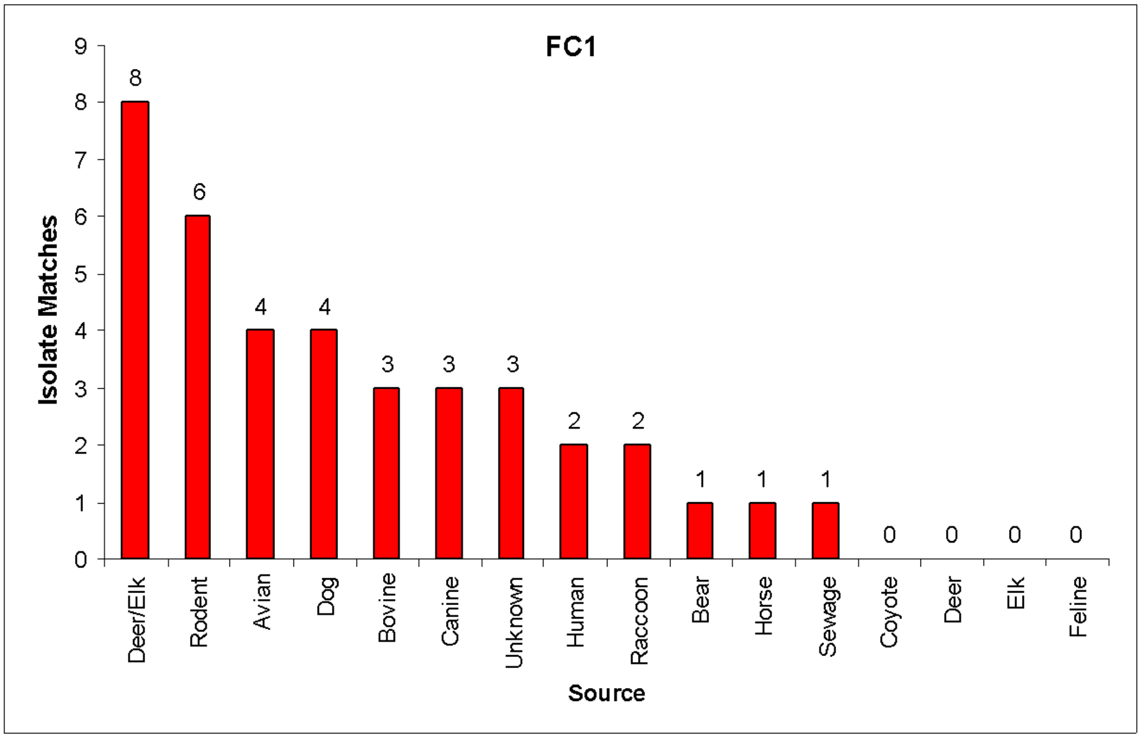
E. coli Microbial Source Tracking Results by Individual Site.

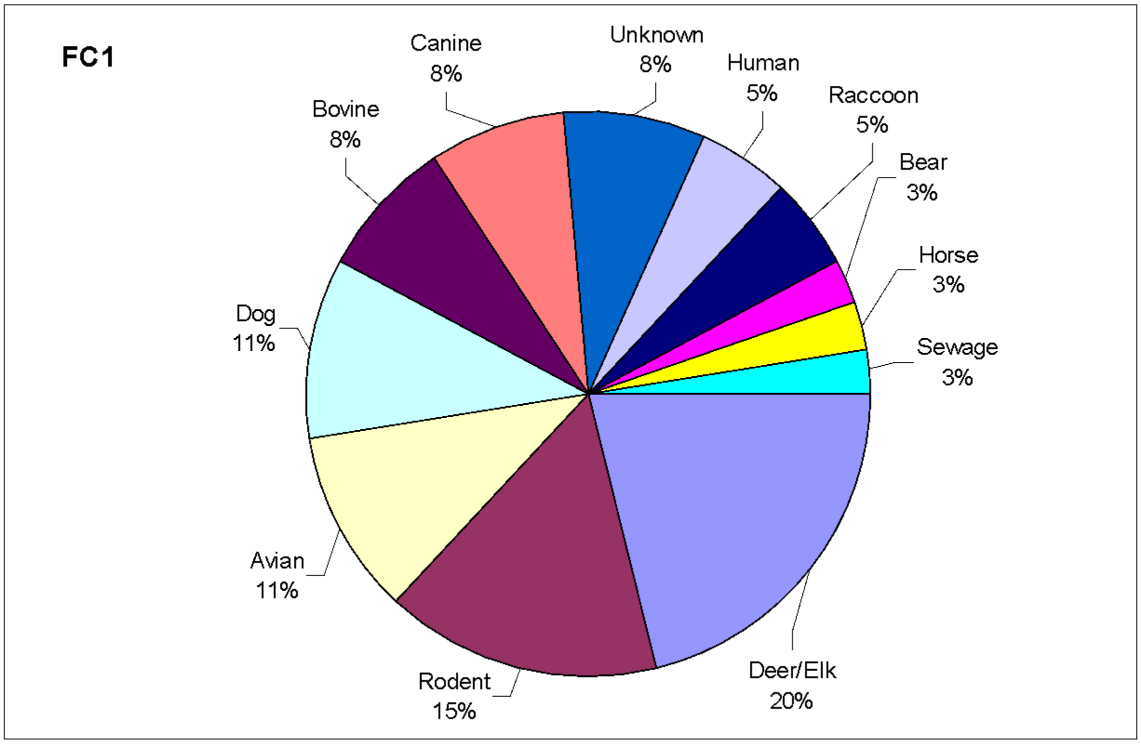
TV1 – Fish Creek



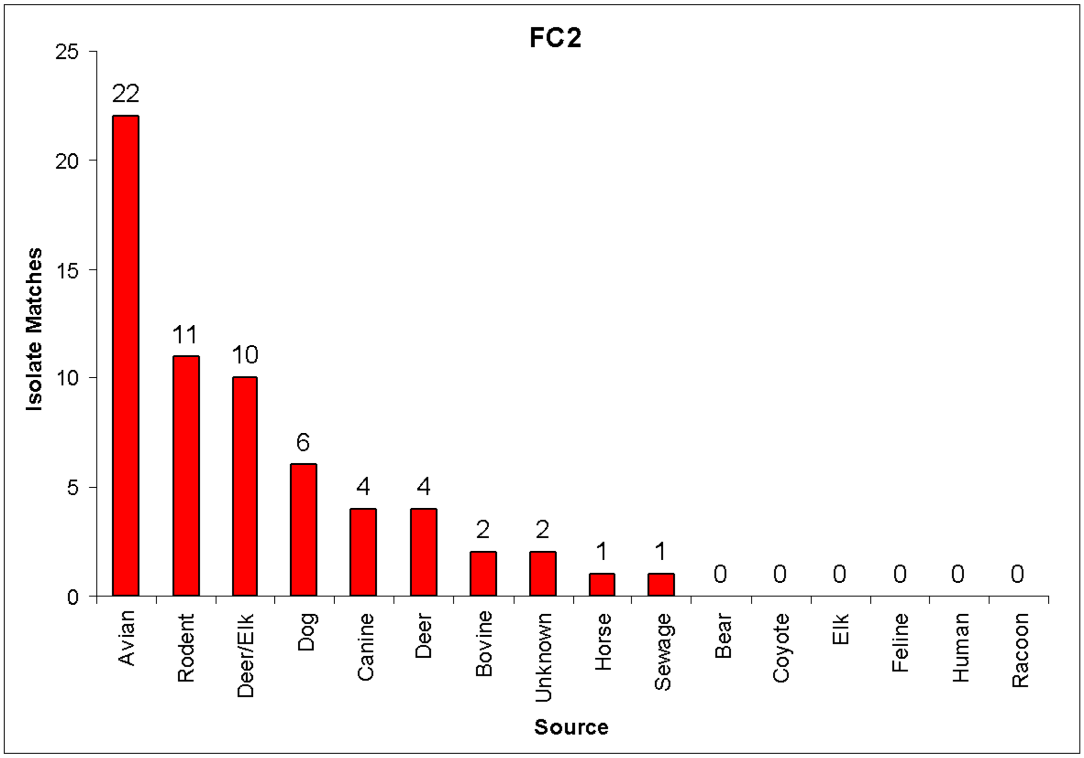


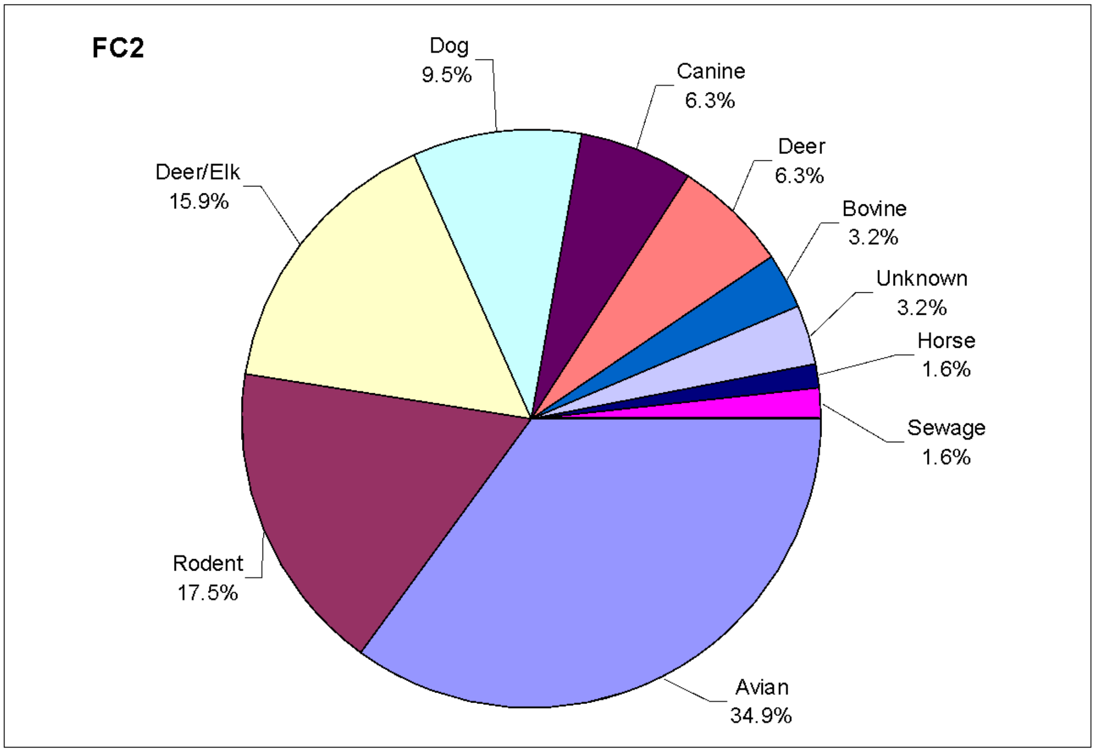
FC1 – Fish Creek



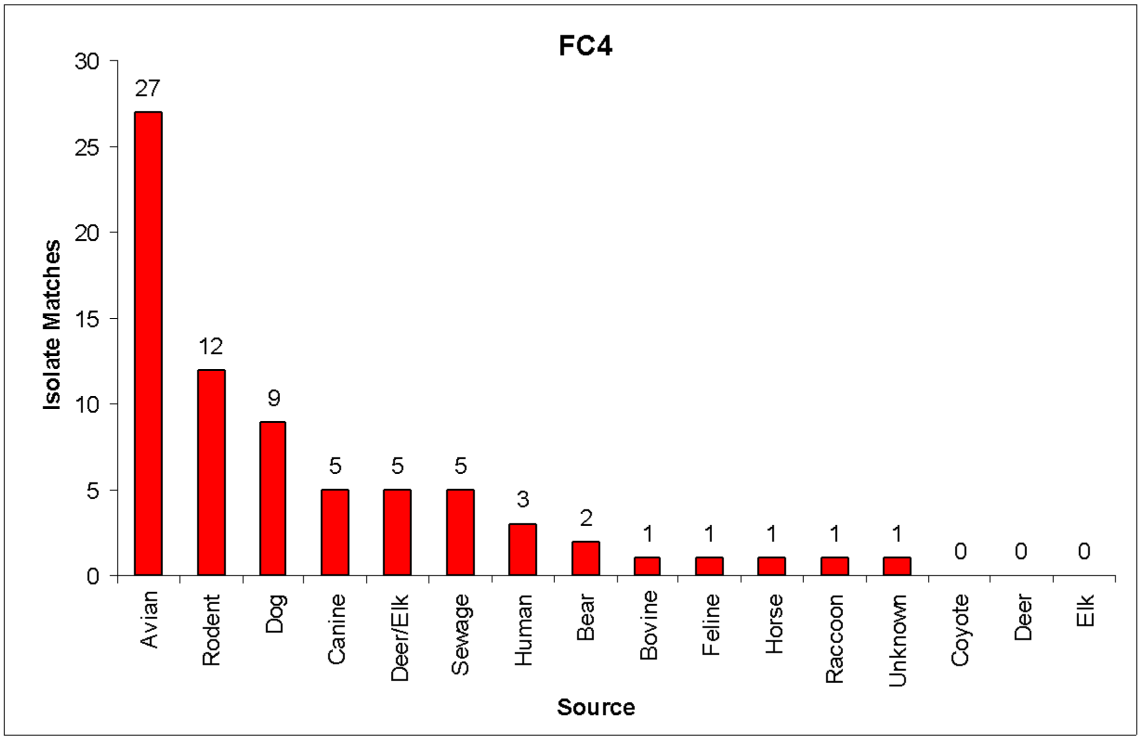


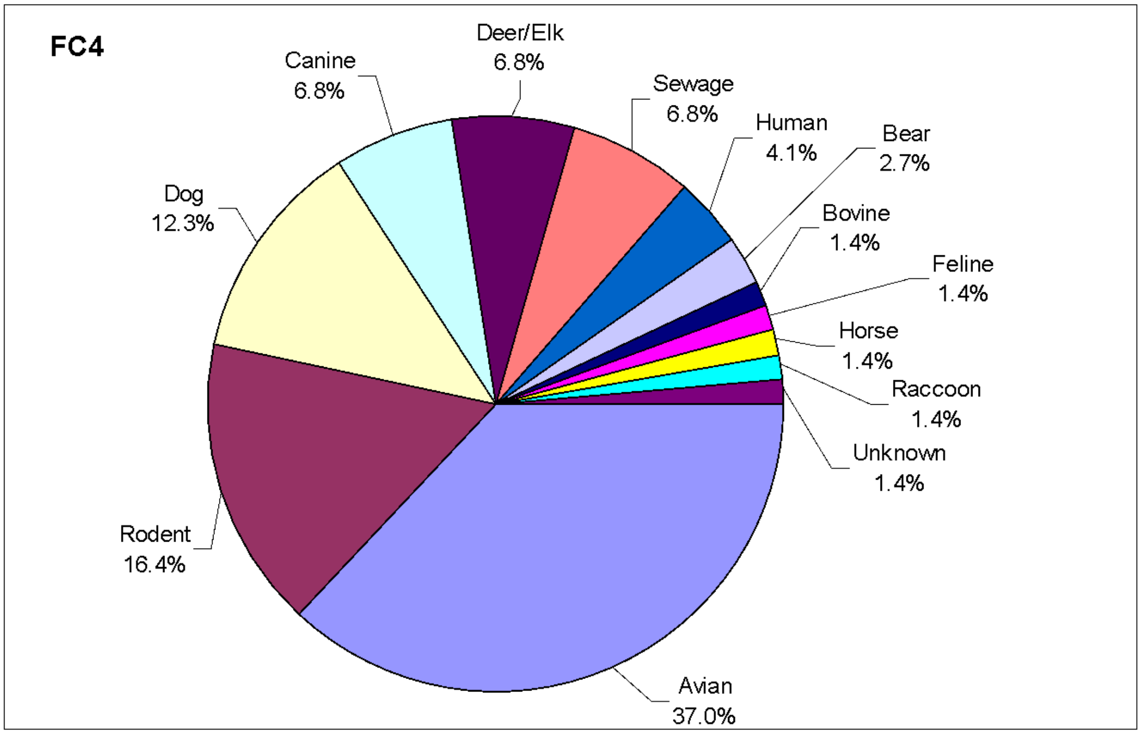
FC2 – Fish Creek



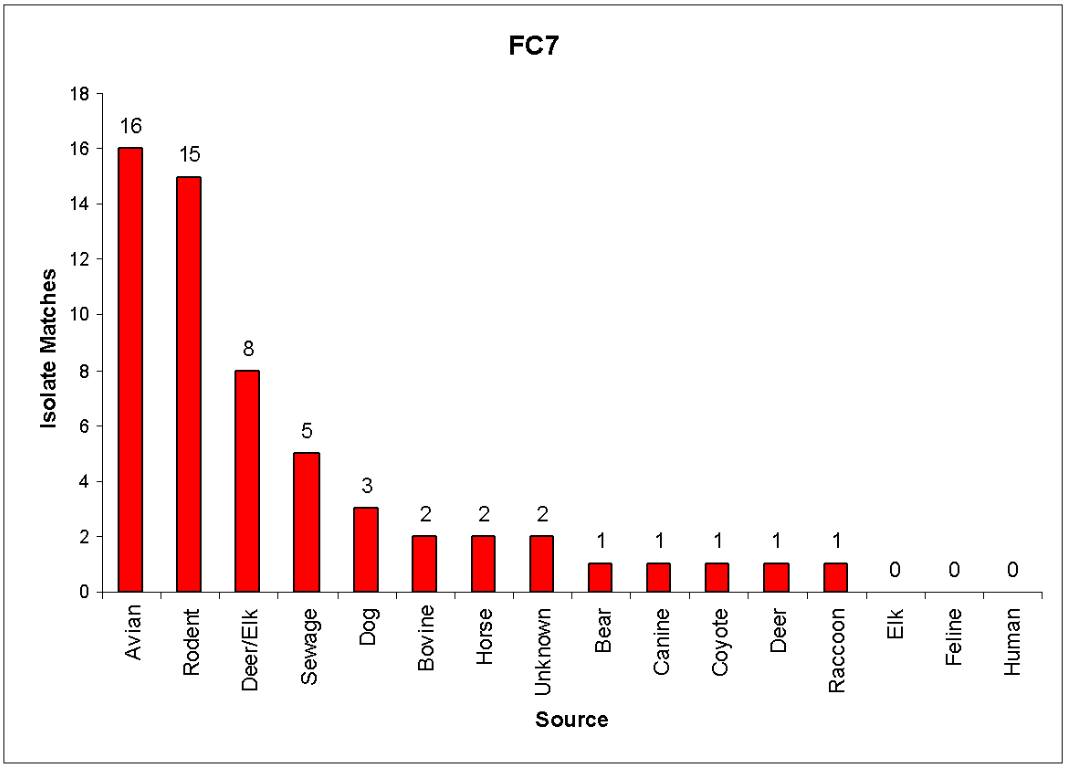


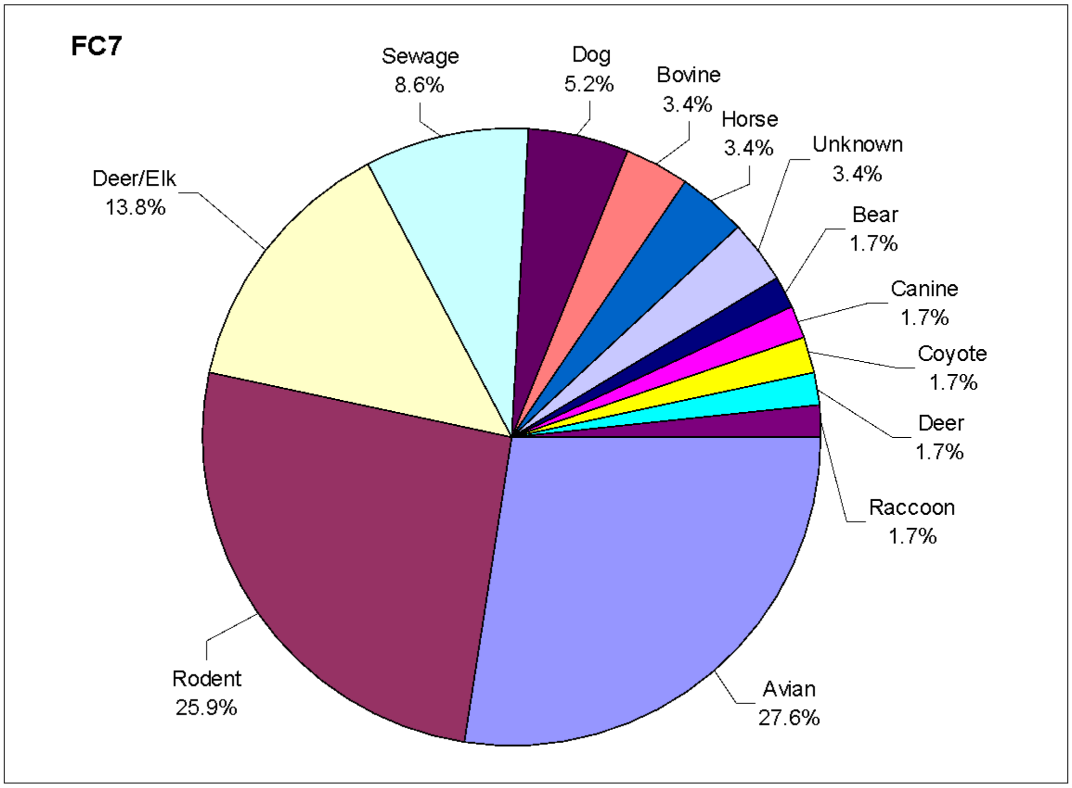
FC4 – Fish Creek



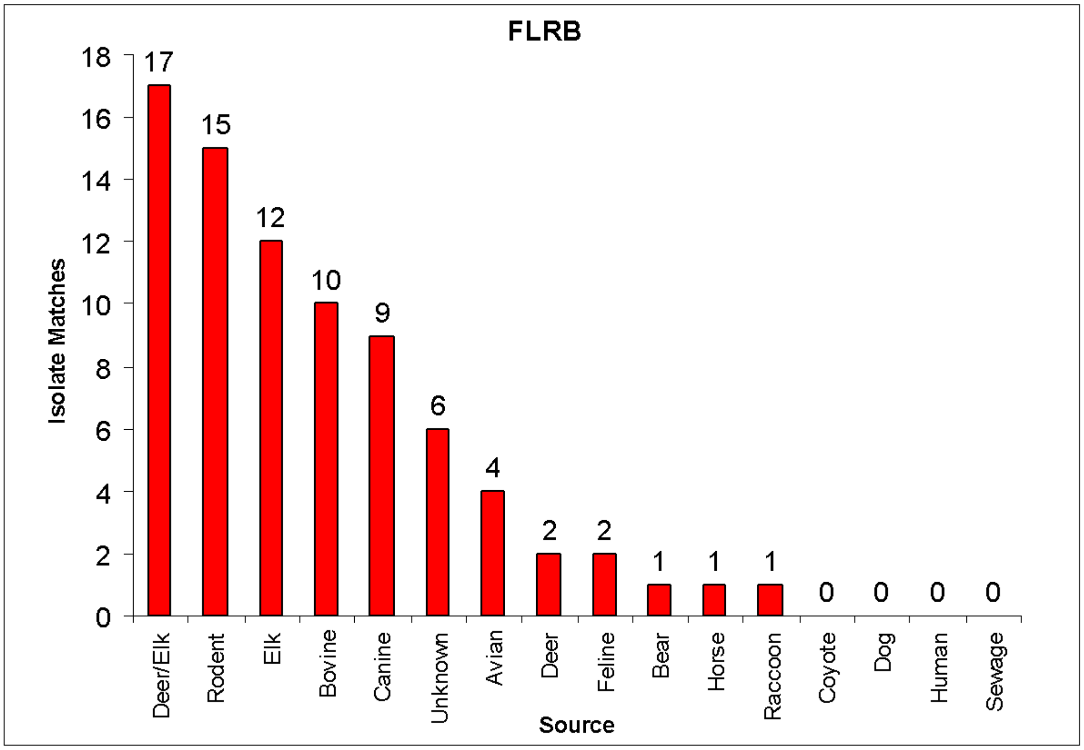


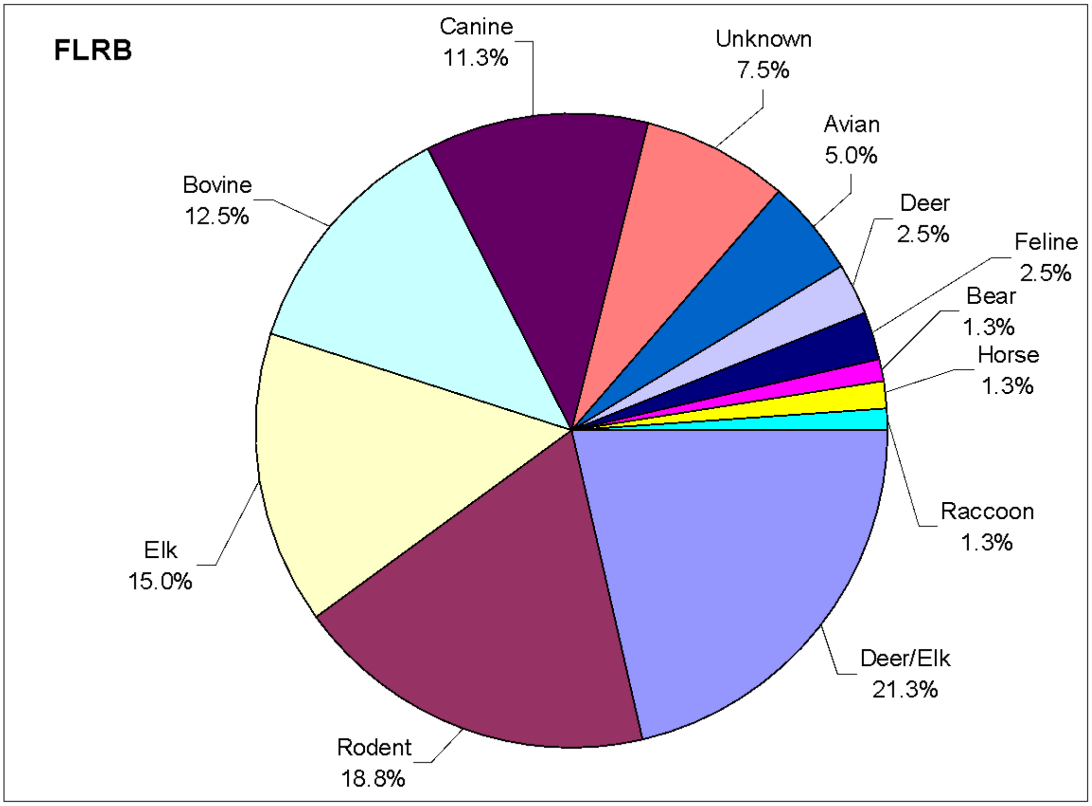
FC7 – Fish Creek



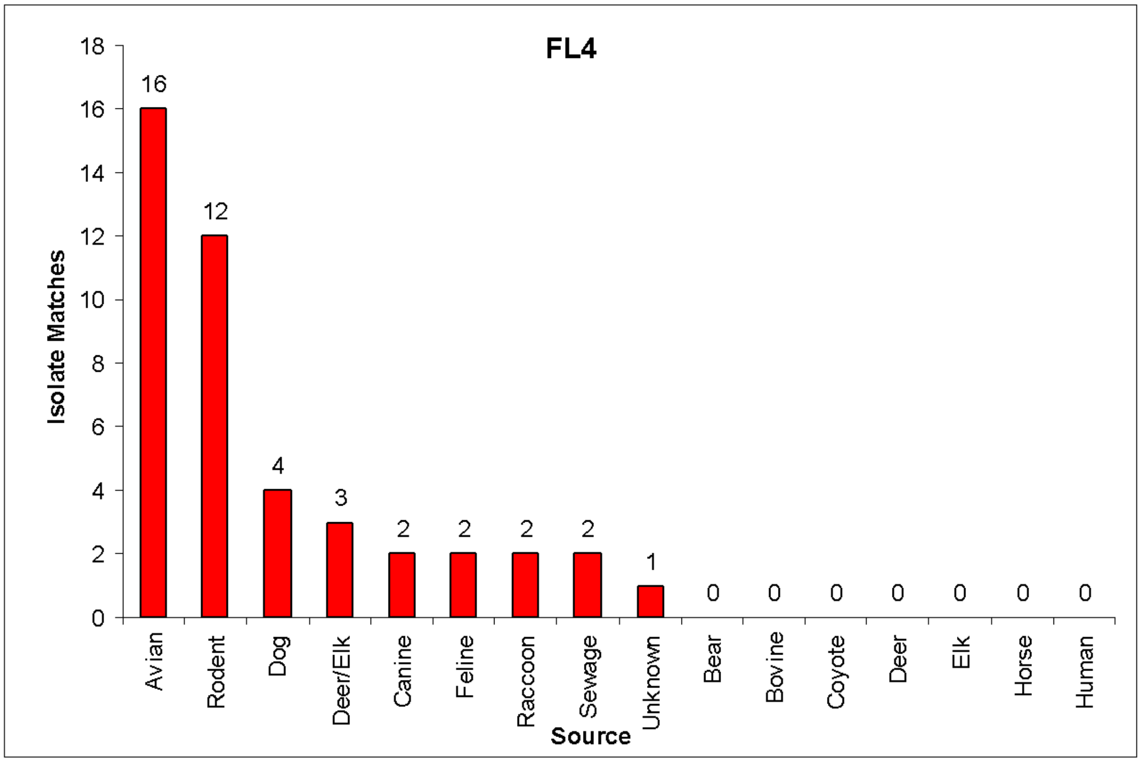


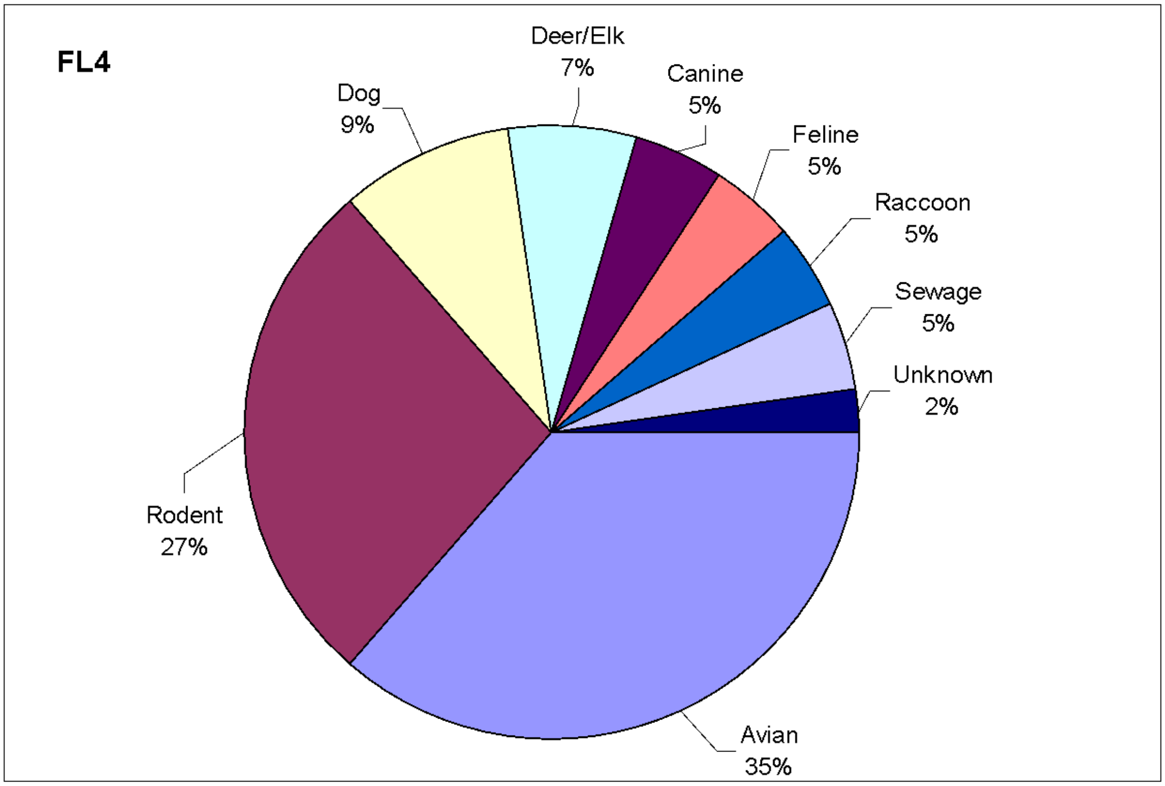
FLRB – Flat Creek



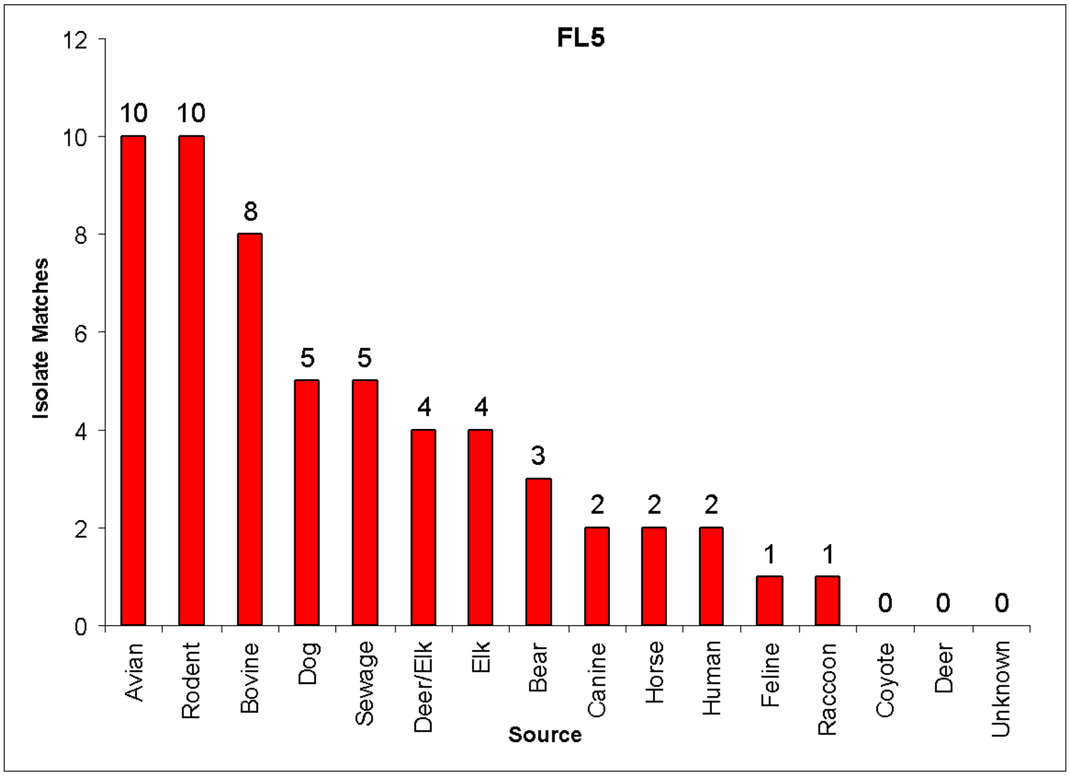


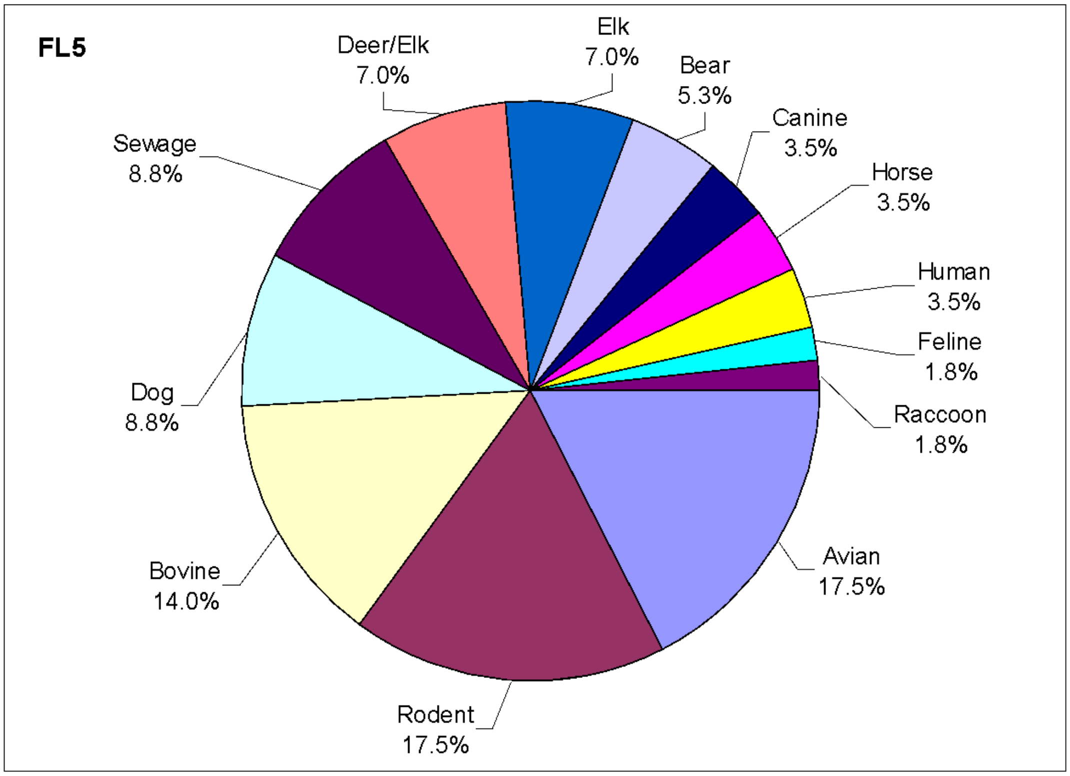
FL4 – Flat Creek



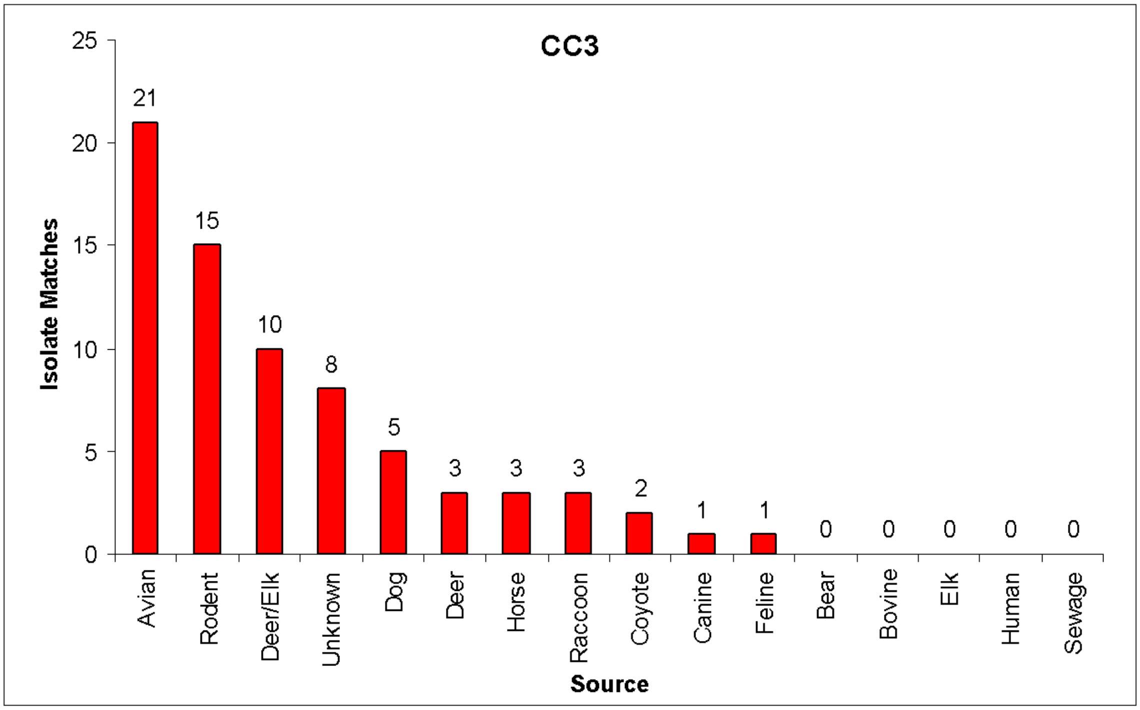


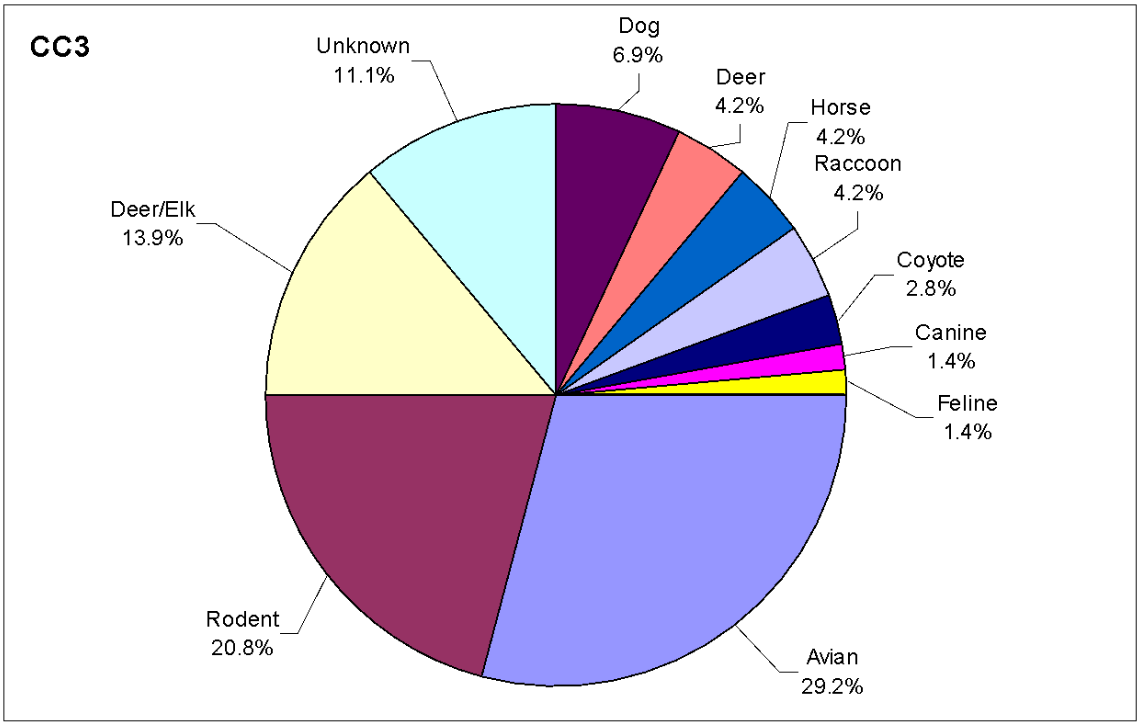
FL5 – Flat Creek





CC3 – Cache Creek





GC4 – Game Creek