Identifying Bacteria Sources in Two North Idaho Streams

Kristin Keith and Bob Steed

Idaho Department of Environmental Quality, Coeur d'Alene Regional Office

September 2010

Introduction

During the summer of 2009, the Coeur d'Alene Regional Office of Idaho Department of Environmental Quality (DEQ) conducted a study on two north Idaho streams to determine probable sources of *Escherichia coli* (*E. coli*) bacteria contamination during the summer recreation months. The State of Idaho monitors surface water for *E. coli* bacteria as an indicator of other pathogens in order to evaluate risk to human health from waterborne disease as a result of fecal contamination from human or other warm-blooded animals. Hauser Creek and Riley Creek were ranked as high-priority streams for the study because they both had downstream recreational use; they are recognized on Idaho's 303(d) list of water quality impaired streams due to violations of Idaho's Water Quality Standards for *E. coli* bacteria, and the source of contamination was unclear from evaluation of landuse types within the watershed.

Section 303(d) of the Clean Water Act requires states to write prescriptions (Total Maximum Daily Loads or TMDLs) to identify pollutant sources and reduce pollutant loading to water bodies that do not meet water quality standards. To accurately characterize the sources of *E. coli* bacteria, a genetic fingerprinting technique was used to determine the animal from which the *E. coli* came. For example, the technique is so accurate that it can differentiate house cat *E. coli* from bobcat and cougar, and house dog *E. coli* from coyote or wolf. The objective of this study was to characterize the source as being human, livestock, pet (dog or cat), or wildlife. Such categorization of data would be helpful in writing meaningful TMDLs, implementing land use management changes to mitigate contamination of the local surface water body, and to minimizing risk to human health. The study was funded by the U.S. Environmental Protection Agency and was done as a partnership with the University of Idaho. Technical assistance was provided by Herrera Environmental Consultants, Inc. (Seattle, WA) and Tetratech, Inc.

Watershed Characteristics

Hauser Creek is located in western Kootenai County, and it is a tributary to Hauser Lake (Figure 1). The Hauser Creek watershed, which includes the Right Fork, Middle Fork, and mainstem Hauser Creek, is 13.9 square miles. Land use in the watershed is primarily undeveloped forest, low-density housing, and a very small percentage of agriculture. Riley Creek is located in Bonner County, Idaho, and it is a tributary to the Pend Oreille River (Figure 1). The Riley Creek watershed is 16.5 square miles, and land use in the watershed is primarily undeveloped forest, low-density housing, and a very small percentage of agriculture.
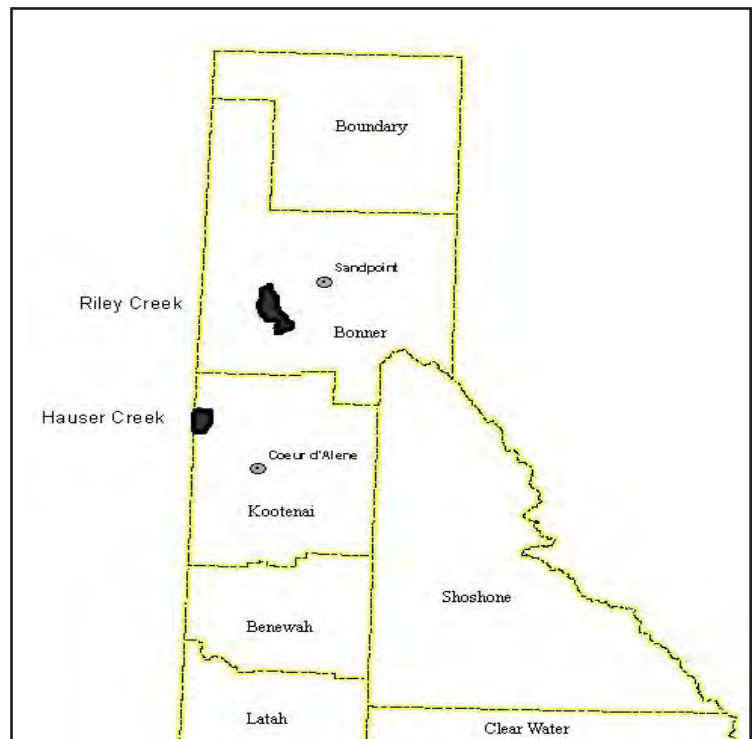

Figure 1. Location of Riley Creek and Hauser Creek Watersheds

Water Quality Monitoring

Water samples were collected by DEQ and the University of Idaho at five stream monitoring stations located within the project watersheds: 1) upper Hauser Creek above the confluence with Right Fork and Middle Fork Hauser Creek; 2) Right Fork Hauser Creek (RF Hauser Creek); 3) lower Hauser Creek, 4) upper Riley Creek, and 5) lower Riley Creek (for monitoring locations see Figures 2 & 3). Upper Hauser Creek and upper Riley Creek were chosen because they were thought to be above the area of human-effected impact to the creek and would be representative of background *E. coli* concentrations in the watersheds. Four grab samples were collected from each station approximately every two weeks between June 1 and the end of September, 2009 – a time period where recreational activities occur in the Creeks. To capture delivery of *E. coli* from storm runoff, samples were collected within 24 hours following a rain event (greater than 0.2 inches within 24 hours). A total of 10 sample events occurred during the study — two of which were following a rain events within both project watersheds. For use in the TMDL loading analysis for *E. coli* flow data was also collected during each sample event.
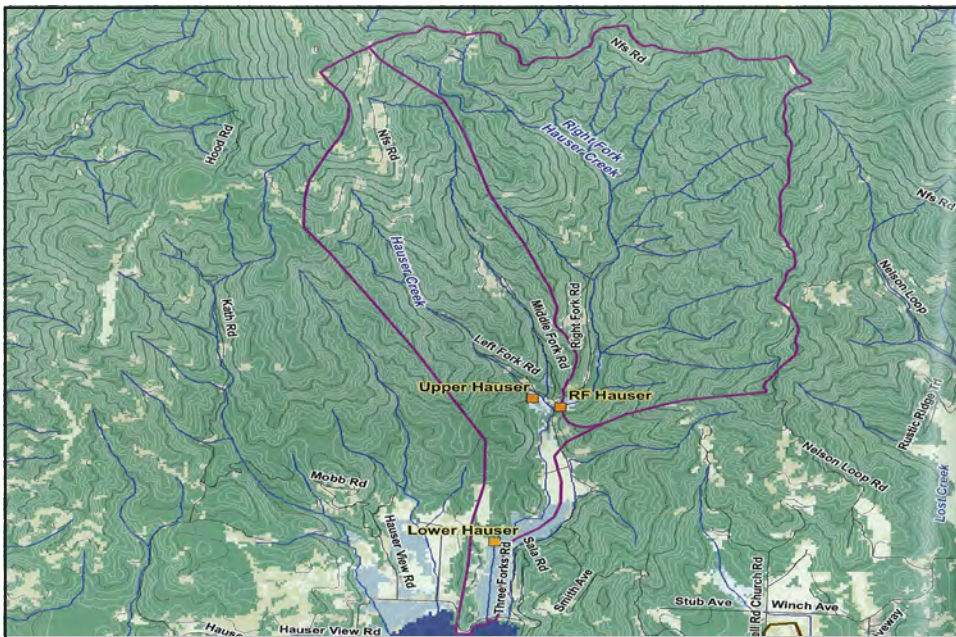
Figure 2. Location of Water Quality Sampling Sites in the Hauser Creek Watershed
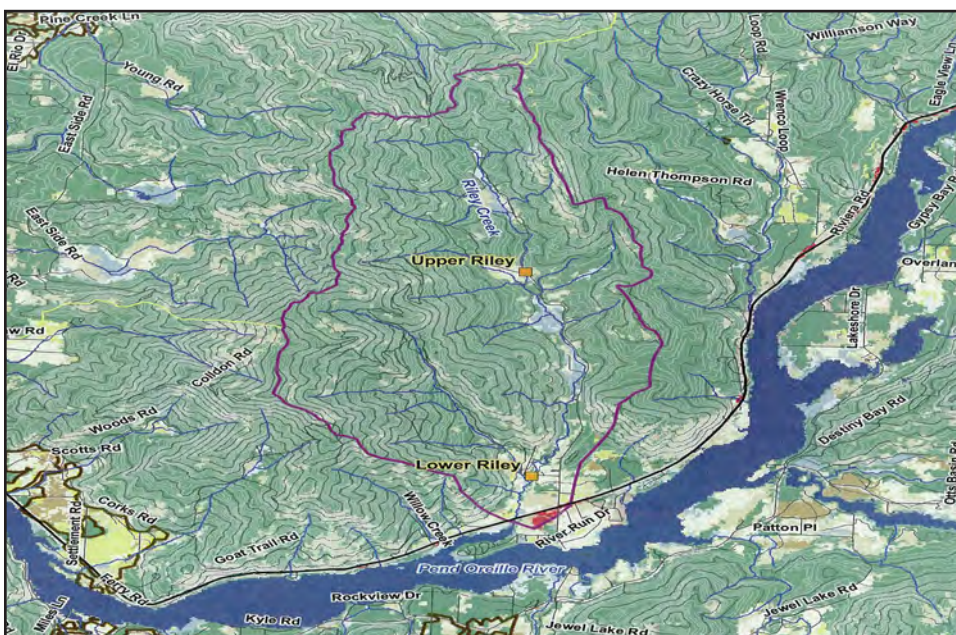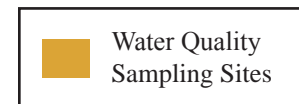
Figure 3. Location of Water Quality Sampling Sites in the Riley Creek Watershed

E. coli Enumeration and DNA Analysis

Within 8 hours of sample collection, *E. coli* enumeration to count individual organisms within the water sample was completed at the University of Idaho, Coeur d'Alene laboratory. At the same laboratory, bacteria culture plates were used to isolate individual *E. coli* colonies. To maximize detection of *E. coli* sources, 12 *E. coli* colonies were isolated from water samples from each date at each sample site (for 120 isolates total). *E. coli* isolates were sent to the Institute for Environmental Health in Lake Forest Park (IEH), WA for Pulse Field Gel Electrophoresis, which matches the *E. coli* DNA to DNA from a known source from a DNA library. To enhance the DNA library and maximize probability of matching the isolates with a known source, fecal samples were collected from each watershed and sent to IEH.

Results

Idaho's Water Quality Standard for a single sample collected within swimmable (primary contact) waters is 406 *E. coli* organisms per 100 milliliters (expressed as Colony Forming Units or CFU/100 ml). This standard applies to Hauser Creek and RF Hauser Creek, where primary contact is a beneficial use. Idaho's Water Quality Standard for a single sample collected within wadeable (non-swimmable, secondary contact) waters is 526 CFU/100 ml. This standard applies to Riley Creek, where secondary contact is a beneficial use. When comparing the distribution of *E. coli* enumeration results to Idaho's standard(s) for a single sample maximum, only the outliers in upper Riley Creek, and RF Hauser Creek exceeded the standard(s). However, mean *E. coli* counts on upper Hauser Creek (446 MPN/100 ml), lower Hauser Creek (1216 MPN/100 ml), and almost the entire interpercentile range (middle 50% of the data) of counts on lower Hauser Creek exceeded the standard (Figure 4 and 5).
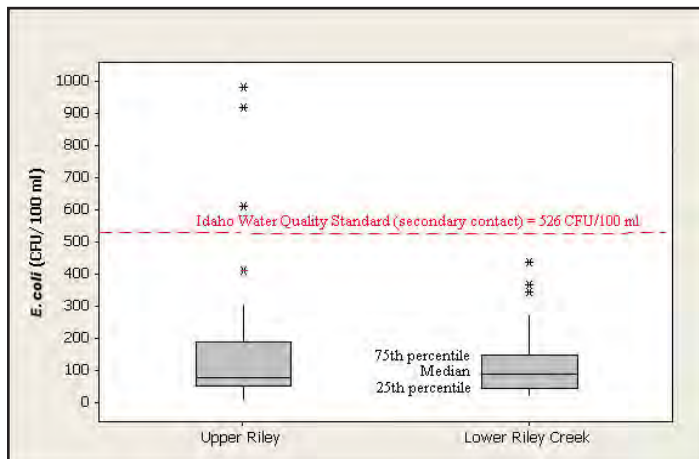

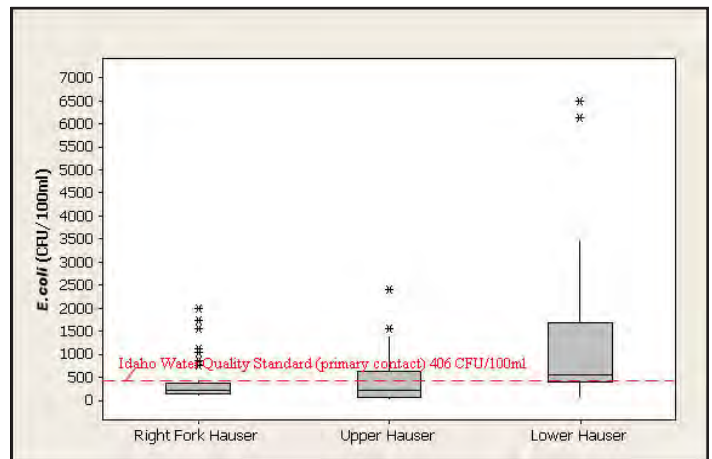Figure 4. Boxplot of *E. coli* Counts in Riley Creek

Figure 5. Boxplot of *E. coli* Counts in Upper Hauser, Right Fork Hauser, and Lower Hauser Creeks

Flow observed was typical of snowmelt hydrographs in north Idaho — with high flows in the spring, then decreasing steadily to base flow in late summer/fall. Typically, a rise in stream flow can be observed in the hydrograph following a rain event as a result of infiltration and runoff processes; however, this hydrologic response was not observed on the project streams following the two rain events. In general, flow decreased in the project streams as the summer months progressed (Figure 6 & 7).
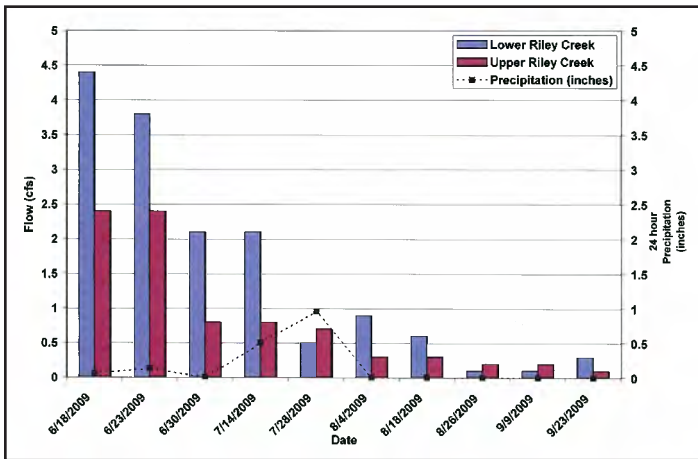

Figure 6. Flow versus Precipitation Graph of Riley Creek
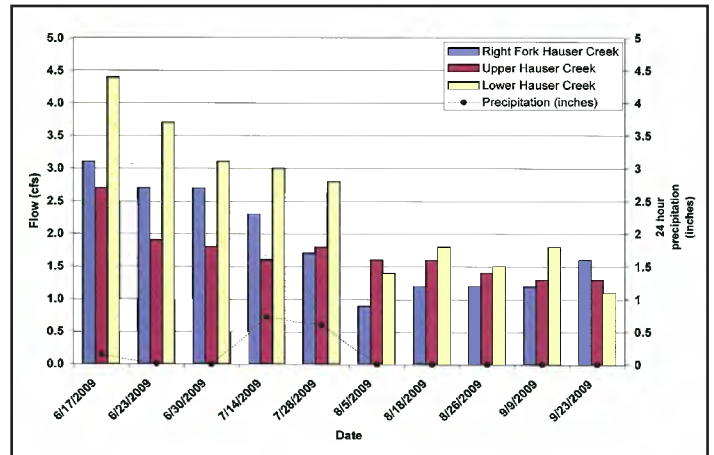
Figure 7. Flow versus Precipitation Graph of Hauser Creek

Since no hydrologic response was observed following the two rain events on either of the creeks, one would not expect *E. coli* counts to increase in the project streams following the rain events. However, *E. coli* counts did increase on Riley Creek following the rain events (Figure 8). Although *E. coli* counts did increase significantly in the Hauser Creek streams, the increase was not in response to a rain event (Figure 9).

Figure 8. Riley Creek Average *E. coli* Concentration

Figure 9. Hauser Creek Average *E. coli* Concentration

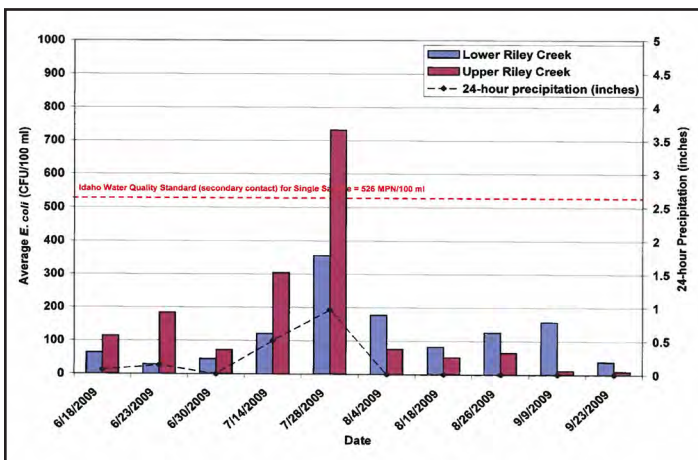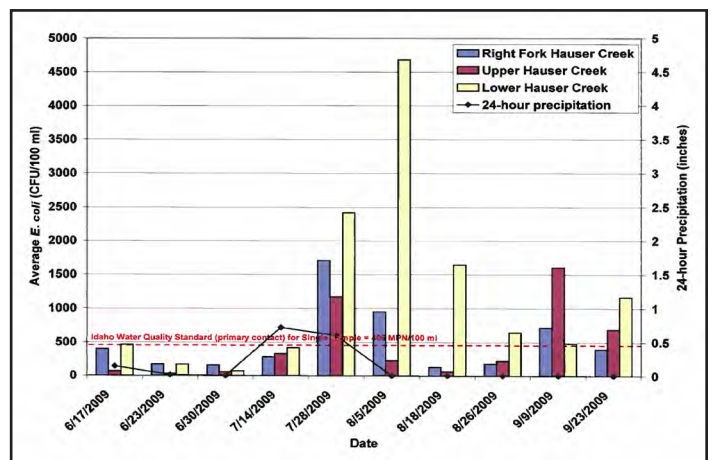Results of the genetic fingerprinting analysis of *E. coli* colonies isolated from water samples were varied: when combining isolate data across all sample dates for each water body, sources of *E. coli* were from a diversity of animals, each present as a very low percentage of the total. No animal was a dominant source in either watershed (Figures 10 & 11). "Unknown" sources were *E. coli* isolates that could not be matched to any source contained within the DNA library at IEH.
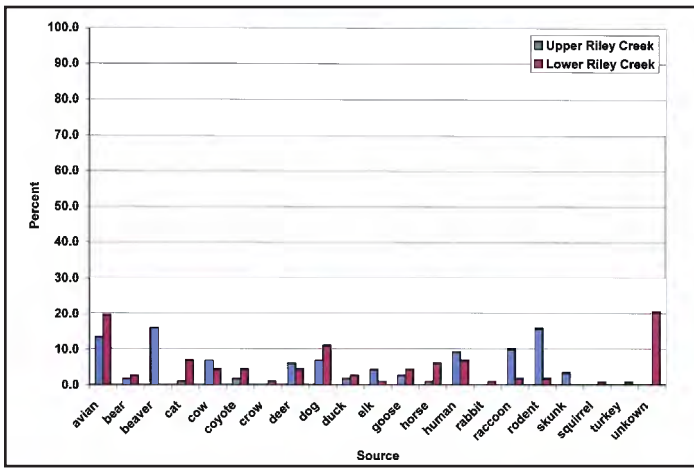
Figure 10. Riley Creek Watershed Total Percentage of Source Isolates
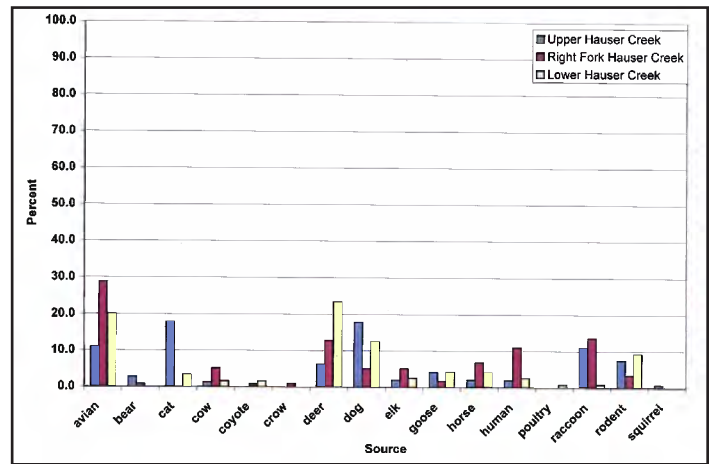
Figure 11. Hauser Creek Watershed Total Percentage of Source Isolates

When combining the individual animals into larger categories (wildlife, pet, livestock, and human), wildlife was the predominant source of *E. coli* in the water bodies — exceeding 50 percent of the total isolates collected in the Hauser Creek watershed and exceeding 40 percent of the total isolates in Riley Creek (Figure 12).

Figure 12. Summary of Percentages of *E. Coli* Sources in Hauser and Riley Creeks

On days when Idaho's single sample *E. coli* standard(s) was exceeded, genetic fingerprinting analysis revealed no dominant wildlife source on the majority of days (Table 1).

Table 1. *E. coli* colony isolates on days where Idaho's water quality standard was exceeded.

Lower Hauser Creek

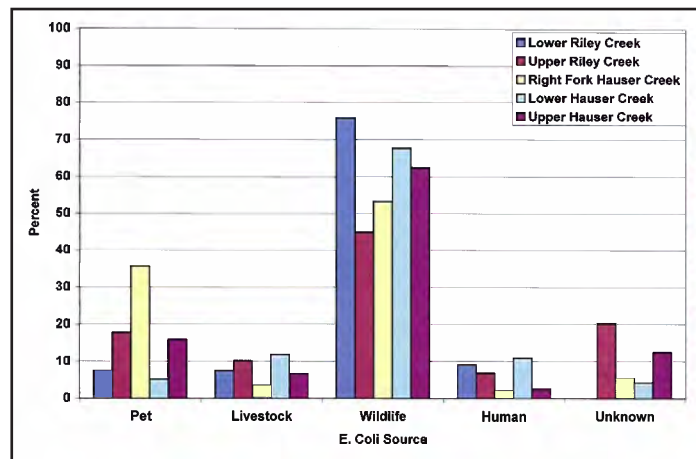|	avian	bear	beaver	cat	cow	coyote	dog	deer	elk	goose	horse	human	racoon	rodent	turkey	unknown	total isolates
6/17/2010				3				5		1		1				2	12
7/14/2010	1			1		1	1	1	1	1			1	4			12
7/28/2010					1		5	1			1	1		1		2	12
8/5/2010	5						2				2			1		2	12
8/18/2010	3					1	5			1				2			12
8/26/2010	3				1		1	1			1			1	1	3	12
9/9/2010	2							4	1		1	1				3	12
9/23/2010	1						1	10									12

Right Fork Hauser Creek

	avian	bear	beaver	cat	cow	coyote	dog	deer	elk	goose	horse	human	racoon	rodent	turkey	unknown	total isolates
7/28/2010	4						3				3	1		1			12
8/5/2010	10	1			1												12
9/9/2010	2							2	4		2	1	1				12

Upper Hauser Creek

	avian	bear	beaver	cat	cow	coyote	dog	deer	elk	goose	horse	human	racoon	rodent	turkey	unknown	total isolates
7/28/2010							5	1		2				4			12
9/9/2010							6	2			1			1		2	12
9/23/2010															12		12

Upper Riley Creek

	avian	bear	beaver	cat	cow	coyote	dog	deer	elk	goose	horse	human	racoon	rodent	turkey	unknown	total isolates
7/28/2010	1		6								1	1	2	1			12

* Idaho's water quality standard was not exceeded on lower Riley Creek during the project period.

Genetic fingerprinting concluded that greater than 10 percent of the total *E. coli* colonies isolated for the sample period were from dogs in upper Hauser, lower Hauser, and lower Riley Creeks, and cats were almost 20 percent in upper Hauser Creek.   In addition, there were two days on lower Hauser Creek when Idaho's primary contact water quality standard for *E. coli* was exceeded, during which dogs were the source of over 40 percent of the isolates. Horses and cattle each did not exceed 10 percent of the total *E. coli* isolates in the sample period; however, horses were greater than 15 percent of the *E. coli* isolates from RF Hauser Creek when Idaho's standard was exceeded. Although humans made up 11 percent of the total *E. coli* colonies isolated within the project period on RF Hauser Creek, only one *E. coli* colony was isolated from water samples collected on days when the water quality standard was exceeded.

Discussion

The primary objectives of this study were to identify the sources of bacterial contamination in Hauser and Riley Creeks, determine the bacterial loadings within each stream, and calculate the load reductions necessary to meet bacteria water quality standards. Categorical sources of *E. coli* could then be managed accordingly: load reductions prescribed by the TMDL for human sources would be implemented under the guidance of Idaho Panhandle Health Department; livestock owners would be educated and directed to agency programs to help with the cost of implementing landuse changes to protect water quality in nearby water bodies. Should the issue be from pets, all property owners would be educated about the necessity to clean up pet waste, and wildlife sources would be considered natural background. Knowledge of the sources of *E. coli* is critical to implementing land use management changes to mitigate contamination of the local surface water body, and to minimizing risk to human health.

Results of the genetic fingerprinting analysis of water samples were unexpected, and they present a challenge in terms of calculating TMDL load reductions necessary to meet water quality standards. Idaho's water quality standard for *E. coli* was exceeded in three of the project streams, including upper Riley and upper Hauser Creeks — streams DEQ thought were not impacted by landuse within the watershed and would be at background conditions. Water quality standards were never exceeded in lower Riley Creek, where evidence suggesting a failing septic was visible upstream. On all project streams, wildlife was overwhelmingly the dominant source of *E. coli* bacteria to the streams. If DEQ were to assign this source a load reduction in a TMDL instead of identifying it as natural background, it would not be feasible, as there was no animal that was a dominant offender in either of the watersheds. Although humans made up 11 percent of the total *E. coli* colonies isolated within the project period, human was not present in significant quantities on days when the standard was exceeded. Therefore, while educational outreach would be worthwhile in both Hauser Creek and Riley Creek watersheds, it may not be reasonable to prescribe a load reduction in a TMDL that necessitates action by Panhandle Health. In upper Hauser Creek, *E. coli* from pets (dogs and cats) and horses within the RF Hauser Creek watershed may be a concern. Prescribing TMDL load reductions to these sources and action to reduce these sources could reduce the *E. coli* loads in the creeks. However, with the continued presence of wildlife in the area, *E. coli* may still be present above water quality standards after such reductions.

Determining the source of *E. coli* on Hauser and Riley Creeks using the genetic fingerprinting technique was time and labor intensive. Lessons learned from the project are many. However, DEQ staff collectively learned the cost and effort expended on this project was not worth the information gained for writing an effective TMDL prescription to reduce *E. coli* numbers in the two watersheds. The initial tendency from such a project would be to easily conclude that animals "X", "Y", and "Z" are the sources of *E. coli* to the impaired streams. However, individual animal *E. coli* sources detected on days that exceeded water quality standards were not consistent and, for the most part, were not in quantities that are statistically reproducible. Therefore, it is reasonable to conclude the only certainty in the data was that wildlife is the dominant *E. coli* source in the Hauser Creek and Riley Creek watersheds, and that we did not take enough samples to rule out additional sources. The Clean Water Act requires a TMDL on water quality-impaired streams; however, the low abundance and frequency observed in non-wildlife sources (such as human, livestock, and pets) would likely equate to too much uncertainty in a TMDL meant to prescribe reductions in *E. coli* to numbers below Idaho's water quality standard. Therefore, DEQ decided to write a watershed-wide TMDL for Hauser and Riley Creeks with prescribed load reductions required to meet their respective water quality criteria; however, the load reductions will not be based on land-use, nor will they specify *E. coli* sources.