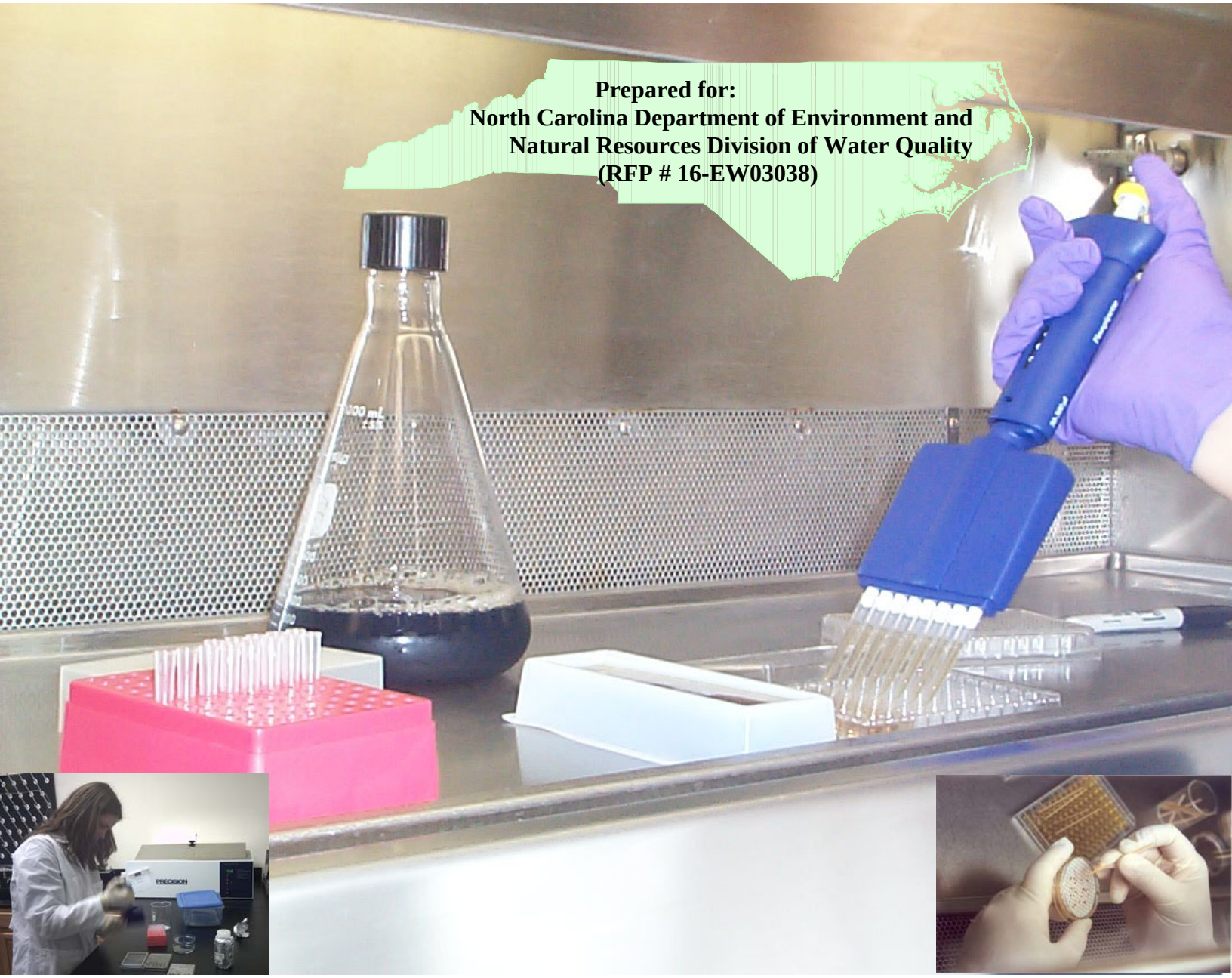
Pathogen Source Assessment for TMDL Development and Implementation in Salem and Muddy Creek Watersheds, Winston-Salem, North Carolina



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1. INTRODUCTION

The North Carolina Department of Environment and Natural Resources-Division of Water Quality (NCDENR-DWQ) has identified the stream segments listed in Table 1.1 and located in Figure 1.1 to be included in a study of Bacterial Source Tracking (BST).

Table 1.1 Location of study for bacteria source tracking project.

River basin	Sub-basin	Stream name	Land use
Yadkin	030704	Muddy Creek	Mixed

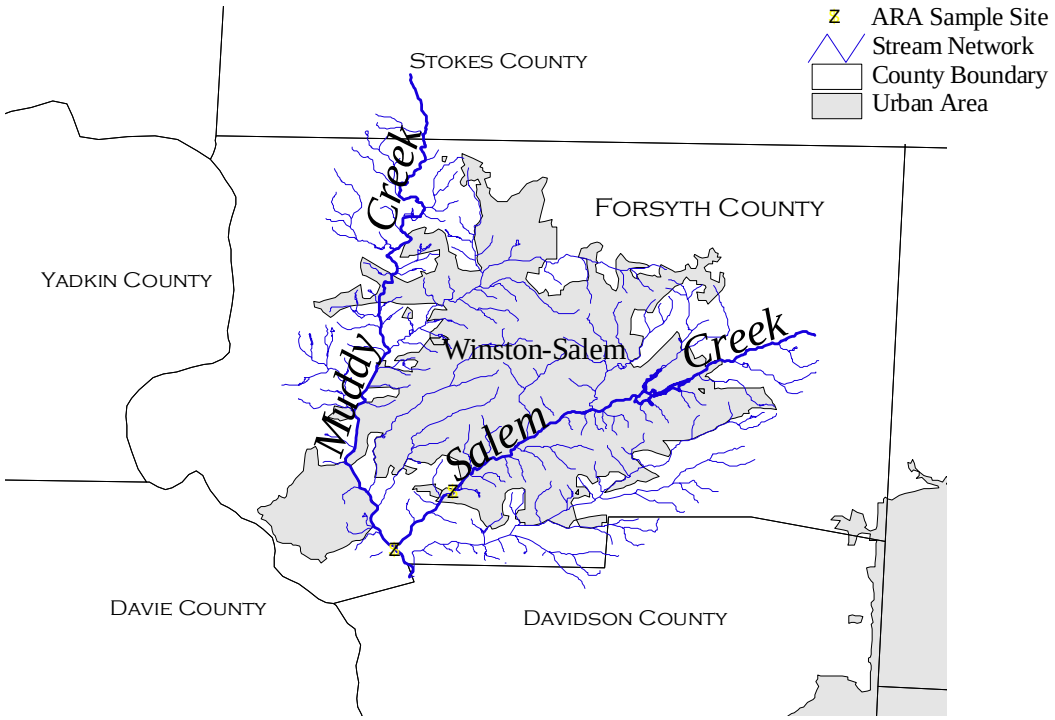

Figure 1.1 Location of Muddy Creek Watershed.

BST methods can be subdivided into three basic groups: Molecular, Biochemical, and Chemical. Molecular (genotype) are typically referred to as "DNA fingerprinting" and are based on the unique genetic makeup of different strains, or subspecies, of fecal bacteria. Biochemical (phenotype) methods are based on an effect of an organism's genes that actively produce a

biochemical substance. The type and quantity of these substances produced is what is actually measured. Chemical methods are based on finding chemical compounds that are associated with human wastewaters, and generally are restricted to determining if sources of pollution are human or not.

Hagedorn's (Hagedorn et al., 1999)[1] Antibiotic Resistance Analysis (ARA) technique was used for this project because it has been demonstrated to be a reliable procedure for confirming the presence of human, livestock, wildlife and pet sources. Compared to DNA fingerprinting, biochemical profiling is much quicker, typically allows for many more isolates to be analyzed (e.g., hundreds per week vs. a few dozen per week for DNA analysis), is more economical, has survived limited court testing, and has undergone rigorous peer review from the scientific community. Additionally, observation of an increased number of isolates allows for an estimate of the relative proportions of the fecal indicator (*e.g.*, *E. coli*) originating from different sources.

[1] Hagedorn, C., S. L. Robinson, J. R. Filtz, S. M. Grubbs, T. A. Angier, and R. B. Reneau, Jr. 1999. Determining Sources of Fecal Pollution in a Rural Virginia Watershed with Antibiotic Resistance Patterns in Fecal Streptococci. *Applied and Environmental Microbiology*. 65.12.5522-5531.

2. APPLYING BST METHODOLOGY

There are many BST methods in use today. The basic premise of all BST methodologies is that there are indicators in receiving waters that can be observed to determine the originating sources of fecal bacteria. Some BST methodologies are developed using a library of known-source samples while others are not dependent on a library. To date, those non-library based methods do not allow quantifying the sources of bacteria. Also, these non-library based methods often use indicators that are not directly related to water quality standards.

All BST methodologies in widespread use today are library-based. This means that a library of fecal samples from known sources is used to determine identifying characteristics of bacteria from specific species or categories of animals. Bacteria in receiving waters are then analyzed to determine if they display any of these identifying characteristics. Individual bacterial isolates (i.e., unique strains of bacteria) that have been collected from receiving waters are examined to determine their most likely source. By examining multiple isolates from a given water sample, an estimate of the proportion of bacteria originating from specific sources can be made.

A four-step process is followed in implementing a BST study. These steps are detailed in the following sections and include:

1) *Defining the problem.*
2) *Choosing a BST method.*
3) *Building the known-source library.*
4) *Collecting and analyzing water samples.*

2.1 Defining the Problem

The first step in any water quality monitoring study is problem definition. This step entails determining the questions that the study is intended to answer. In terms of a BST study, it is important to identify the fecal sources of interest and the level of quantification needed. Depending on the goals of study, the sources of interest may be limited to human vs. non-human or could include many more source categories (e.g., human, poultry, beef cattle, other livestock,

wild geese, and other wildlife). In a watershed with little or no agricultural activity, the emphasis of the study may be on determining human vs. non-human loads, whereas, in a watershed with many different types of animal agriculture, it may be desirable to determine the proportional contribution from humans, wildlife, and each type of domestic animal in production. Additionally, the level of quantification could be coarse (e.g., overall proportional contribution from sources of interest over the study period) or more refined (e.g., proportional contribution during ambient vs. storm conditions, or proportional contribution during each sample event). If influencing public perception is the primary goal, overall proportional contributions may be adequate. However, if the goal of the study is to target implementation efforts, then it would be useful to have more refined data. The decisions in the remaining steps will depend largely on the problem to be addressed, as defined in this step.

2.2 Choosing a BST Method

As mentioned in Section 1, BST methods can be subdivided into three basic groups: Molecular, Biochemical, and Chemical. Molecular (genotype) methods are typically referred to as "DNA fingerprinting" and are based on the unique genetic makeup of different strains, or subspecies, of fecal bacteria. Biochemical (phenotypic) methods, such as ARA, are based on an effect of an organism's genes that actively produce a biochemical substance. Chemical methods, such as fluorescent whitening agents (Gilpin and Saunders, 2005), are based on finding chemical compounds that are associated with human wastewaters, and generally are restricted to determining if sources of pollution are human or not. The choice of BST method will typically be made based on the sources of interest, the level of quantification required, and the cost of the analysis. Increasing refinement of the analysis method in terms of source identification is typically associated with a higher cost. Increasing refinement with regard to the level of quantification is dependent on the number of samples and isolates-per-sample analyzed. Typically, the choice of a BST method requires some compromise between the level of source identification offered by a given method and the level of quantification possible given the budget constraints.

2.3 Building the Known-Source Library

Locally collected known-source libraries are typically needed for library-based BST methods. The existence of geographical differences in source characteristics is well documented, but not well defined. It is typically recommended to collect known-source samples locally, even if an existing library is intended to be used. The locally collected samples can then be used to validate the existing library. The optimal size of the known-source library is dependent on the BST method being used, but the quality of the library is always based on its ability to represent the bacterial population of interest. In order to improve representativeness of the library, known-source samples should be collected from various animal species (including humans) as well as from different individuals from each species in many different locations. If too many samples are collected from one individual or location (*e.g.*, one flock of geese, one farm, or one home) the resulting library may be biased toward the characteristics of that individual or location. Samples should be collected from all animals that have either a large contribution to fecal production in the watershed (*e.g.*, livestock, deer, and humans) or whose fecal production is predominantly in the stream corridor (*e.g.*, aquatic mammals, waterfowl, and raccoon).

The underlying hypothesis for library-based BST methodologies is that certain bacterial types are differentially distributed in the feces of various animals. By way of example, fecal coliform strain "A" is observed 100 times in the course of constructing a library. Eighty occurrences of strain "A" are in cattle feces, five occurrences are in human feces, and fifteen occurrences are in dog feces. This sort of differential strain distribution is observed far more frequently than are strains that appear to be unique to one host, and is ultimately the mechanism that underlies the ability of a known-source library to predict the source of water (unknown source) isolates.

In the library, the data observations are used to construct a predictive model that is used to predict source category (*e.g.*, cow, dog, human) based on the data observed for an individual bacterial isolate. The most elementary test of the predictive power of any library is a self-cross, in which data from known fecal sources that make up the library are used to predict the source of the isolates in that library. If the differential distribution of bacterial strains among host categories was absolute (*i.e.*, strain "A" was found only in cattle feces), all of the isolates analyzed by the self-cross should be assigned to the correct source category (all isolates from cattle feces would be placed in the “Cow” category, and all isolates from dog feces would be

placed in the "Dog" category). The percentage of isolates that are correctly classified in this analysis is referred to as the average rate of correct classification (ARCC). In practice, 100% correct classification rates are almost never observed, particularly in large libraries.

Additional statistical analyses can be applied to determine if the library is representative of the population of concern. A randomization test can be performed to determine if high rates of correct classification are being achieved merely because the library is small and does not represent the diversity in the watershed. The randomization test is performed by randomly assigning source categories to samples and assessing the ARCC for the randomized library. The expected result of randomization of two source categories is an ARCC of 50%, indicating a completely random result; randomization of three source categories is an ARCC of 33.3%, 25% for a four-source categorization, etc. Greater values for the randomized ARCC indicate that the library may be too small to represent the diversity in the watershed. Another test of the library's representativeness is jackknifing. In jackknifing, data from each whole fecal sample are individually withheld during development of the predictive model; the model is then tested for accuracy in predicting the source of the withheld sample.

2.4 Collecting and Analyzing Water Samples

The frequency of sample events, the number of samples collected, and the number of isolates analyzed per sample is dependent on the problem being addressed and the required level of quantification. The frequency of sample events and the number of samples is determined in much the same way as with other water quality monitoring efforts, while determining the number of isolates analyzed per sample is specific to BST studies. All of the sampling and analysis decisions are affected by the level of quantification needed in the study.

As with other water quality monitoring studies, the frequency and number of samples should be adequate to capture the range of climate, hydrologic, and land management conditions that the study is intending to address. Typically, monthly sampling is considered adequate to capture ambient conditions. If seasonal differences or trends are of interest, then a multiple year study will be necessary. In addition, collection of samples during storm events can be used to define differences between ambient conditions and runoff events. If one of the goals of the study is to target implementation efforts, then storm event sampling can be a useful addition to the sampling

plan. If the proportional contribution from a given source increases during storm events, then implementation efforts should be targeted toward source loads that are driven by precipitation. For instance, if the human contribution to fecal bacteria in the stream is low during ambient conditions, but increases dramatically during runoff events, then failing septic systems and combined sewer overflows are likely to be more of a problem than straight pipes discharging directly to the stream, and implementation efforts should be targeted appropriately.

The number of isolates analyzed per sample is dependent on the level of quantification desired for the study. The number of isolates analyzed needs to be high enough to allow for calculating the desired proportions. For instance, if information from each sample is of interest then the number of isolates analyzed per sample should be high enough to allow for a reasonable estimate of the proportional contribution of sources in each sample. Information from 48 isolates per sample is adequate to provide confidence in proportions being calculated. This level of quantification allows for calculating the contribution from each source to the fecal bacteria load measured for each sample. While, as with other monitoring studies, data from individual samples should not be over-emphasized, this level of quantification can be used to look at overall patterns and trends. If information from the study is only going to be used to measure composite loads (e.g., the overall contribution of fecal bacteria from a given source during the entire study period), then fewer isolates per sample can be analyzed.

3. OBJECTIVES

BST was used to identify sources of *E. coli* as well as the relative percentage contribution from source groups (*e.g.*, livestock, wildlife, human and pets). The purpose of the sampling and analysis was to support the development of fecal coliform TMDLs and follow-up implementation strategies to attain water quality goals. The BST analysis will be used in conjunction with a water quality model in the TMDL development process. The specific objectives of the project, as outlined in RFP#16-EW03038, were to:

1. prepare a sampling strategy,
2. build watershed-specific libraries of known sources of *E.coli* bacteria, and
3. analyze and categorize ambient water sources of bacteria.

4. METHODS

Hagedorn's ARA method has been extensively and successfully used by MapTech, and separates fecal sources based on patterns of antibiotic resistance in the *enterococci* or *E. coli*. For this study, *E. coli* was the indicator organism analyzed. The premise of ARA is that fecal bacteria from each source (*e.g.*, human, livestock, wildlife, and pets) will have different resistance patterns to the battery of antibiotics and concentrations used in the analysis. Hagedorn's method for *E. coli* tests each isolate on 28 different combinations of antibiotic type and concentration. Confidence in BST techniques is measured by the level of separation of isolates from known sources, represented as the percentage of isolates that are accurately separated into respective source types (*e.g.*, Average Rate of Correct Classification – ARCC). Additional analyses can be applied to test the specificity of the library. These analyses are discussed further in Section 4 of this document. The ARA method, like other methods (*e.g.*, molecular), requires the collection of source samples from feces of known sources to build a source library. In support of this study, known source samples from the four source classes were collected, analyzed, and entered into known-source libraries.

4.1 Preparation of Sampling Strategy

The basic sampling scheme for ambient water samples was outlined by NCDENR-DWQ. Initially, ambient samples were to be collected from two locations in the Muddy Creek watershed at a fixed frequency of two times per month. The ambient sampling sites included Salem Creek at Elledge WTP in Winston-Salem NC and Muddy Creek at SR2995 (Figure 1). A third station was added on Muddy Creek above the confluence with Salem Creek at SR158. Sampling was initially conducted between July 2003 and February 2004. However, due to a computer malfunction, data from the first four samples were lost. Consequently, four additional samples were collected in July and August 2004. Data from 16 samples per location is reported here.

DWQ or local government personnel collected fecal matter from known sources and shipped samples to MapTech's EDL for analysis and development of a known-source library. Samples were collected with the goal of obtaining 15 viable samples from each of four source categories (*i.e.*, human, livestock, pets, and wildlife) in each watershed (*i.e.*, Muddy and Salem Creeks). A

total of 138 viable samples were collected, yielding 1072 *E. coli* isolates for developing known source libraries.

4.2 Analysis of Known-Source Samples

DWQ or local government personnel collected and labeled each sample and entered the sample information for each site on *Chain of Custody Forms for BST - Source Samples*, provided by the MapTech Team. All samples were packed with ice in insulated coolers at the time of sample collection. After all samples were collected, sampling personnel verified the sample inventory. Samples were delivered to MapTech's EDL by UPS overnight priority. MapTech's EDL personnel inventoried the samples upon receiving.

From each sample, up to 8 isolates were analyzed using BST. Known-source libraries were constructed from 609 isolates (78 samples) collected in the Muddy Creek watershed and 463 isolates (60 samples) collected in the Salem Creek watershed. A predictive model was developed from each library using logistic regression. A known-source library must be large enough to prevent an over-specified fit to the library. However, known-source responses to ARA analyses have been observed to vary geographically. The characteristics of this variance have not been well defined, so regional libraries are typically combined in a stepwise procedure and analyzed to measure the resulting specificity and the predictive accuracy of the combined libraries, as detailed in Section 4 of this document.

4.3 Bacterial Enumerations and BST Analyses

DWQ or local government personnel collected and labeled each sample and entered sample information for each site on *Chain of Custody Forms for Water Quality Samples* provided by the MapTech Team. All samples were packed with ice in insulated coolers at the time of sample collection. After all samples were collected, sampling personnel verified sample inventory. Water quality samples were delivered to MapTech's EDL by UPS overnight priority. MapTech's EDL personnel inventoried the samples upon receiving them.

Samples were received as whole-water samples. All water samples were analyzed for *E. coli* and fecal coliform. BST was run on bacteria isolated from the whole-water samples. Bacteria were analyzed using Hagedorn's ARA methodology, yielding the percentage of isolates classified in each source category (*e.g.*, human, livestock, wildlife, and pets). Up to 48 bacterial isolates were

analyzed per sample, limited only by the number of isolates available from the enumeration process.

5. KNOWN-SOURCE LIBRARY DEVELOPMENT

As discussed in Section 4, a predictive model was developed from each library using logistic regression. The specificity and predictive accuracy of each library was assessed through three analyses. First, the ARCC was calculated for the library. Second, a randomization test was performed by randomly assigning source categories to samples and assessing the ARCC for the randomized library. Twenty-five randomizations were performed and the results averaged. The expected result of randomization is dependent on the number of source categories considered. For example, with four source categories, the expected result is an ARCC of 25%, indicating a completely random result. Alternatively, with two source categories, the expected result is an ARCC of 50%, indicating a completely random result. Greater values for the randomized ARCC indicate a more specified model. Third, a jackknifing routine was conducted, where data from each whole fecal sample were individually withheld during development of the statistical model. The model was then tested for predictive accuracy on the withheld sample. In combining regional libraries, a balance is sought between minimizing the randomized ARCC and maximizing the jackknifed ARCC. A fourth statistic reported for each category in each library is the false-positive rate. This represents the frequency at which bacteria that are not from the source category in question will be falsely placed in the category. This value is used in the analysis of water samples to determine if ratios are significantly different from zero.

Three source groupings were considered in this study (Table 5.1). The groupings increase in refinement from 2 categories (*i.e.*, human vs. non-human) to 4 categories (*i.e.*, human vs. livestock vs. pets vs. wildlife). With increasing refinement, accuracy, as measured by the RCCs decreases.

Table 5.1 Proposed BST Source Library Characterizations.

Source Grouping			Potential Sources
2 Categories	*3 Categories*	*4 Categories*	
Human	Human	Human	Human
Non-Human	Domestic Animals	Pets	Cats Dogs Sewage
		Livestock	Cattle Horses Poultry Goats
	Wildlife	Wildlife	Birds Deer Raccoons Groundhogs Opossum

Tables 5.2 through 5.7 present the results from the initial libraries developed for the Muddy and Salem Creek watersheds. While the basic RCCs tend to be high, the randomized RCCs indicate a significant amount of over fitting (*i.e.*, the libraries are too small). Additionally, the jackknifed RCCs and false-positive rates for the Salem Creek library indicate that the library is not representative enough to give reliable results for the 3 and 4 source category groupings.

Table 5.2 Known-source library statistics for the initial Muddy Creek watershed library with Human/Non-Human source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	Fasle-Positive Rate
Human	92%	70%	75%	13%
Non-Human	88%	71%	82%	8%
Overall	89%	70%	79%	N/A

Table 5.3 Known-source library statistics for the initial Muddy Creek watershed library with Human/Domestic Animal/Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	Fasle-Positive Rate
Human	86%	57%	72%	12%
Domestic	75%	60%	60%	12%
Wildlife	76%	59%	55%	8%
Overall	80%	59%	65%	N/A

Table 5.4 Known-source library statistics for the initial Muddy Creek watershed library with Human/Livestock/Pets/Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	Fasle-Positive Rate
Human	81%	52%	67%	7%
Livestock	70%	51%	49%	13%
Pets	74%	53%	57%	7%
Wildlife	69%	51%	48%	12%
Overall	75%	52%	58%	N/A

Table 5.5 Known-source library statistics for the initial Salem watershed library with Human/Non-Human source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	Fasle-Positive Rate
Human	91%	82%	60%	15%
Non-Human	85%	81%	80%	9%
Overall	86%	81%	76%	N/A

Table 5.6 Known-source library statistics for the initial Salem watershed library with Human/Domestic Animal/Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	Fasle-Positive Rate
Human	89%	66%	58%	9%
Domestic	63%	67%	50%	13%
Wildlife	81%	64%	39%	18%
Overall	73%	66%	49%	N/A

Table 5.7 Known-source library statistics for the initial Salem watershed library with Human/Livestock/Pets/Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	Fasle-Positive Rate
Human	86%	56%	58%	6%
Livestock	48%	56%	28%	15%
Pets	61%	54%	25%	15%
Wildlife	70%	58%	36%	40%
Overall	65%	56%	35%	N/A

Based on the results of analyses on the individual libraries, the libraries were combined. Tables 5.8 through 5.10 present the results from this combined library developed for both the Muddy and Salem Creek watersheds. Combining the two individual libraries improved the results,

particularly for the randomized RCCs. However, the Jackknifed RCCs and false-positive rates still indicate problems with using more than 2 source categories.

Table 5.8 Known-source library statistics for the combined Muddy & Salem Creek watershed library with Human/Non-Human source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	Fasle-Positive Rate
Human	89%	73%	71%	19%
Non-Human	81%	69%	79%	11%
Overall	85%	71%	75%	N/A

Table 5.9 Known-source library statistics for the combined Muddy & Salem Creek watershed library with Human/Domestic Animal/Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	Fasle-Positive Rate
Human	80%	54%	72%	11%
Domestic	69%	53%	62%	23%
Wildlife	61%	55%	40%	13%
Overall	71%	54%	61%	N/A

Table 5.10 Known-source library statistics for the combined Muddy & Salem Creek watershed library with Human/Livestock/Pets/Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	Fasle-Positive Rate
Human	73%	44%	66%	8%
Livestock	56%	43%	37%	20%
Pets	56%	45%	42%	16%
Wildlife	56%	45%	39%	27%
Overall	60%	44%	46%	N/A

Based on these results, MapTech initiated collection of additional non-human samples. Twenty-seven additional source samples were collected in the Muddy and Salem Creek watersheds, yielding 216 *E. coli* isolates. With the addition of these samples, the library was improved (Tables 5.11 through 5.13). The 2-category split (Figure 5.11) was improved, with lower Randomized RCCs, higher Jackknifed RCCs, and a lower False-Positive Rate for the human category. The 3-category split (Figure 5.12) was also improved, with generally more balanced results across categories (*e.g.*, the Jackknifed RCCs are more consistent), lower Randomized RCCs, and lower False-Positive Rates. However, the Jackknifed RCCs and false-positive rates indicate problems with using the 4-category split.

Table 5.11 Known-source library statistics for the updated Muddy & Salem Creek watershed library with Human/Non-Human source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	Fasle-Positive Rate
Human	84%	66%	75%	14%
Non-Human	86%	66%	81%	16%
Overall	86%	66%	79%	N/A

Table 5.12 Known-source library statistics for the updated Muddy & Salem Creek watershed library with Human/Domestic Animal/Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	Fasle-Positive Rate
Human	79%	50%	66%	12%
Domestic	70%	51%	57%	18%
Wildlife	72%	52%	59%	11%
Overall	73%	51%	61%	N/A

Table 5.13 Known-source library statistics for the updated Muddy & Salem Creek watershed library with Human/Livestock/Pets/Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	Fasle-Positive Rate
Human	89%	43%	81%	4%
Livestock	57%	42%	40%	14%
Pets	60%	42%	45%	11%
Wildlife	71%	46%	55%	32%
Overall	70%	43%	57%	N/A

6. RESULTS

The results of the water quality analyses are reported in this section. Fecal coliform enumerations, *E. coli* enumerations, and the results of the BST analyses are reported. The proportions reported are formatted to indicate statistical significance (*i.e.*, **BOLD** numbers indicate a statistically significant result). The statistical significance was determined through two tests. The first was based on the sample size. A z-test was used to determine if the proportion was significantly different from zero (alpha = 0.10). For the second test, the false-positive rate, calculated for each source category was used. A proportion was not considered significantly different from zero unless it was greater than the false-positive rate plus three standard deviations.

In order to capitalize on the higher RCCs for the 2 and 3-category splits, ARA data from water samples were analyzed in a three-step process. First, *E. coli* isolates originating from human vs. non-human sources were identified using the results of the 2-category split. Next, the non-human isolates were divided between domestic animals and wildlife sources using the results of the 3-category split. Finally, domestic animal isolates were divided between livestock and pets using the results of the 4-category split. Through the process of developing the library, we have improved confidence over data presented in preliminary reports. Additionally, ARA results were compared to fluorometry results for six samples. Fluorometry gives a qualitative assessment of the presence of human wastewater (*i.e.*, optical brighteners from detergents) in stream samples. The fluorometric data were in agreement with the ARA data, improving confidence in the results.

Table 6.1 Bacterial Source Tracking results for Muddy Creek at HWY 158.

Station ID	Sample Date	Lab ID	*E. coli* Value	*E. coli* Qual	Fecal Coliform Value	Fecal Coliform Qual	Isolates	Bacteria Source Human	Bacteria Source Livestock	Bacteria Source Pets	Bacteria Source Wildlife
HWY 158	07/01/03	NC2	270		2,500		N/A	N/A	N/A	N/A	N/A
HWY 158	07/15/03	NC5	560		3,600		N/A	N/A	N/A	N/A	N/A
HWY 158	07/30/03	NC8	8,200		16,000		N/A	N/A	N/A	N/A	N/A
HWY 158	08/14/03	NC11	480		3,000		N/A	N/A	N/A	N/A	N/A
HWY 158	09/02/03	NC14	540		6,000		48	**31%**	4%	**50%**	**15%**
HWY 158	09/16/03	NC17	800		340		48	19%	**31%**	**19%**	**31%**
Hwy 158	10/01/03	NC20	140		190		48	**58%**	0%	0%	**42%**
Hwy 158	10/15/03	NC23	1,370		590		48	8%	**46%**	**23%**	**23%**
Hwy 158	11/03/03	NC26	200		130		24	8%	**42%**	4%	**46%**
Hwy 158	11/12/03	NC29	220		280		16	**45%**	12%	**31%**	12%
Hwy 158	12/01/03	NC32	190		310		24	**25%**	4%	**63%**	8%
Hwy 158	12/15/03	NC35	1,000		550		48	**40%**	**21%**	**29%**	10%
Hwy 158	01/05/04	NC38	280		170		16	**69%**	0%	12%	**19%**
Hwy 158	01/20/04	NC41	<1	BDL	50		0	--	--	--	--
Hwy 158	02/02/04	NC44	10		30		2	0%	0%	100%	0%
Hwy 158	02/16/04	NC47	<10	BDL	90		0	--	--	--	--
Hwy 158	07/07/04	NC49	680		260		48	2%	4%	**92%**	2%
Hwy 158	07/21/04	NC53	230		140		24	4%	**21%**	**54%**	**21%**
Hwy 158	08/02/04	NC57	340		30		48	**70%**	10%	12%	8%
Hwy 158	08/16/04	NC60	680		270		48	**29%**	12%	**57%**	2%

BOLD type indicates a statistically significant value. "N/A" indicates that the data is not available. "BDL" indicates that the number of bacterial colonies was below the detection level of the enumeration methodology. "NVI" indicates that there were no viable isolates available for BST analysis.

Table 6.2 Bacterial Source Tracking results for Muddy Creek at Station Q2600000.

Station ID	Sample Date	Lab ID	*E. coli* Value	*E. coli* Qual	Fecal Coliform Value	Fecal Coliform Qual	Isolates	Bacteria Source Human	Bacteria Source Livestock	Bacteria Source Pets	Bacteria Source Wildlife
Q2600000	07/01/03	NC3	370		2,700		N/A	N/A	N/A	N/A	N/A
Q2600000	07/15/03	NC6	600		4,900		N/A	N/A	N/A	N/A	N/A
Q2600000	07/30/03	NC9	8,900		12,000		N/A	N/A	N/A	N/A	N/A
Q2600000	08/14/03	NC12	420		4,000		N/A	N/A	N/A	N/A	N/A
Q2600000	09/02/03	NC15	660		8,500		48	**33%**	**27%**	**40%**	0%
Q2600000	09/16/03	NC18	450		300		48	**69%**	4%	0%	**27%**
Q2600000	10/01/03	NC21	102		400		48	**71%**	12%	0%	**17%**
Q2600000	10/15/03	NC24	1,500		510		48	10%	**29%**	**25%**	**36%**
Q2600000	11/03/03	NC27	310		380		24	**21%**	**29%**	12%	**38%**
Q2600000	11/12/03	NC30	410		4,800		48	15%	**41%**	6%	**38%**
Q2600000	12/01/03	NC33	380		580		48	**29%**	6%	**48%**	**17%**
Q2600000	12/15/03	NC36	900		560		48	**27%**	**25%**	17%	**31%**
Q2600000	01/05/04	NC39	110		280		30	17%	**20%**	**23%**	**40%**
Q2600000	01/20/04	NC42	140		540		18	6%	6%	**33%**	**55%**
Q2600000	02/02/04	NC45	30	NVI	100		0	--	--	--	--
Q2600000	02/16/04	NC48	100		330		13	8%	0%	**84%**	8%
Q2600000	07/07/04	NC51	400		490		48	**21%**	2%	**75%**	2%
Q2600000	07/21/04	NC54	160		430		16	0%	6%	**69%**	**25%**
Q2600000	08/02/04	NC56	390		70		48	16%	0%	**42%**	**42%**
Q2600000	08/16/04	NC58	670		520		48	2%	**38%**	**56%**	4%

BOLD type indicates a statistically significant value. "N/A" indicates that the data is not available. "BDL" indicates that the number of bacterial colonies was below the detection level of the enumeration methodology. "NVI" indicates that there were no viable isolates available for BST analysis.

Table 6.3 Bacterial Source Tracking results for Salem Creek at Station Q2510000.

Station ID	Sample Date	Lab ID	*E. coli* Value	*E. coli* Qual	Fecal Coliform Value	Fecal Coliform Qual	Isolates	Bacteria Source Human	Bacteria Source Livestock	Bacteria Source Pets	Bacteria Source Wildlife
Q2510000	07/01/03	NC1	410		2,400		N/A	N/A	N/A	N/A	N/A
Q2510000	07/15/03	NC4	310		7,000		N/A	N/A	N/A	N/A	N/A
Q2510000	07/30/03	NC7	3,500		5,700		N/A	N/A	N/A	N/A	N/A
Q2510000	08/14/03	NC10	11,000		15,000		N/A	N/A	N/A	N/A	N/A
Q2510000	09/02/03	NC13	2,000		10,000		48	**48%**	17%	8%	**27%**
Q2510000	09/16/03	NC16	650		350		48	8%	**44%**	**31%**	**17%**
Q2510000	10/01/03	NC19	470		3,600		48	**42%**	6%	0%	**52%**
Q2510000	10/15/03	NC22	6,200		5,000		48	2%	**43%**	**38%**	**17%**
Q2510000	11/03/03	NC25	1,800		4,000		48	**25%**	**25%**	**19%**	**31%**
Q2510000	11/12/03	NC28	1,300		7,500		48	**53%**	12%	**27%**	8%
Q2510000	12/01/03	NC31	550		380		48	17%	2%	**31%**	**50%**
Q2510000	12/15/03	NC34	1,700		2,700		48	19%	**31%**	**25%**	**25%**
Q2510000	01/05/04	NC37	900		470		48	**40%**	2%	**27%**	**31%**
Q2510000	01/20/04	NC40	140		4,500		14	0%	14%	14%	**72%**
Q2510000	02/02/04	NC43	40		460		5	0%	20%	0%	80%
Q2510000	02/16/04	NC46	230		580		44	0%	0%	0%	**100%**
Q2510000	07/07/04	NC50	500		640		48	4%	6%	**69%**	**21%**
Q2510000	07/21/04	NC52	100		30		11	**46%**	18%	**36%**	0%
Q2510000	08/02/04	NC55	2,500		<1	BDL	48	2%	0%	**81%**	**17%**
Q2510000	08/16/04	NC59	450		120		48	15%	**48%**	**35%**	2%

BOLD type indicates a statistically significant value. "N/A" indicates that the data is not available. "BDL" indicates that the number of bacterial colonies was below the detection level of the enumeration methodology. "NVI" indicates that there were no viable isolates available for BST analysis.

APPENDIX A

Bacterial Source Tracking Analyses Supplemental Report

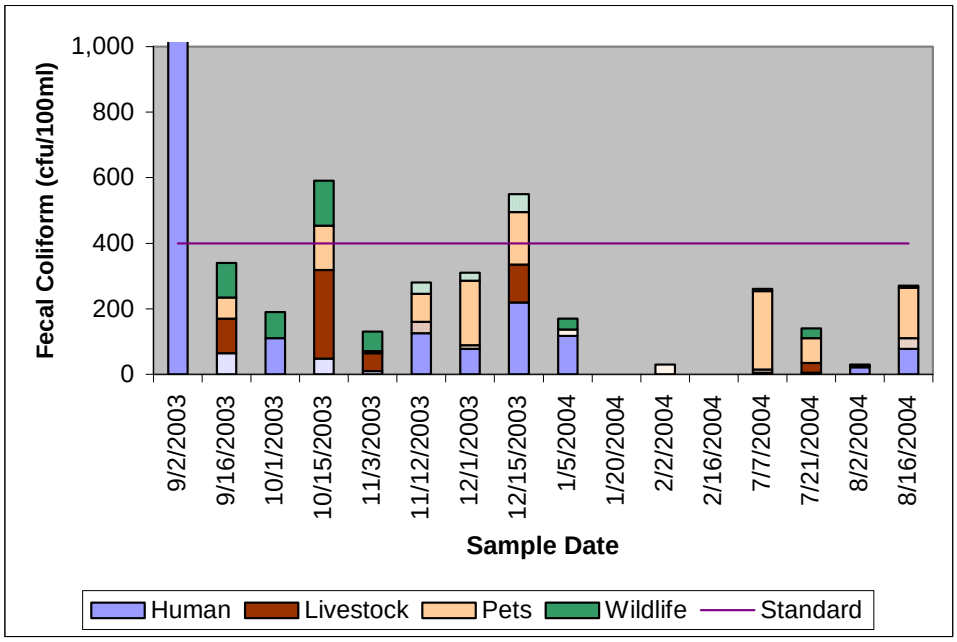

Figure A.1 **Fecal Coliform enumerations with proportional source contributions indicated for Station HWY 158 on Muddy Creek. Fecal coliform enumerations are censored at 1,000 cfu/100 ml to improve resolution on values near the standard. Solid colors indicate statistical significance.**

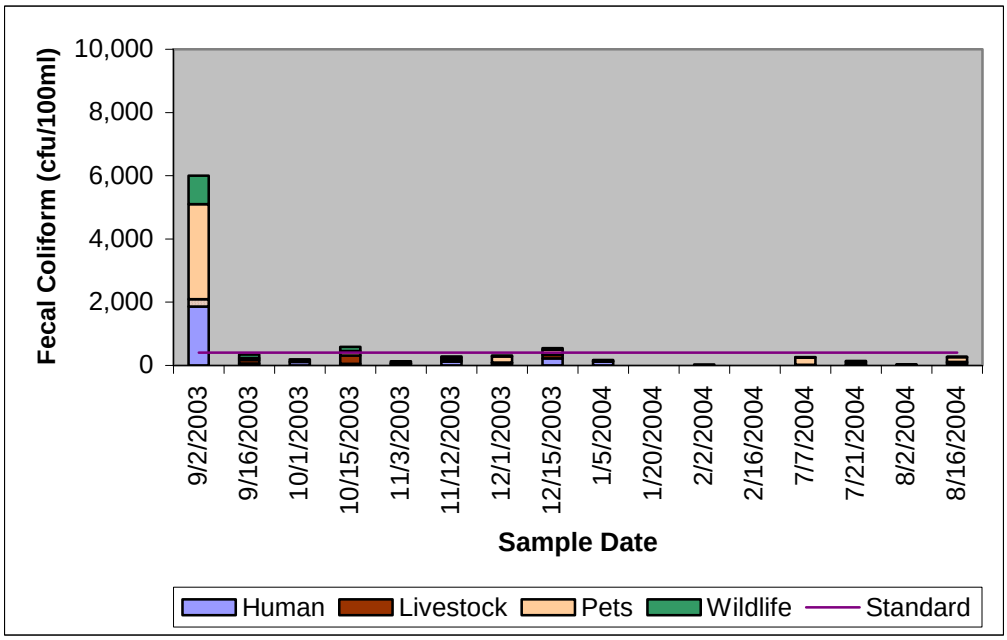

Figure A. 2 **Fecal Coliform enumerations with proportional source contributions indicated for Station HWY 158 on Muddy Creek. Solid colors indicate statistical significance.**

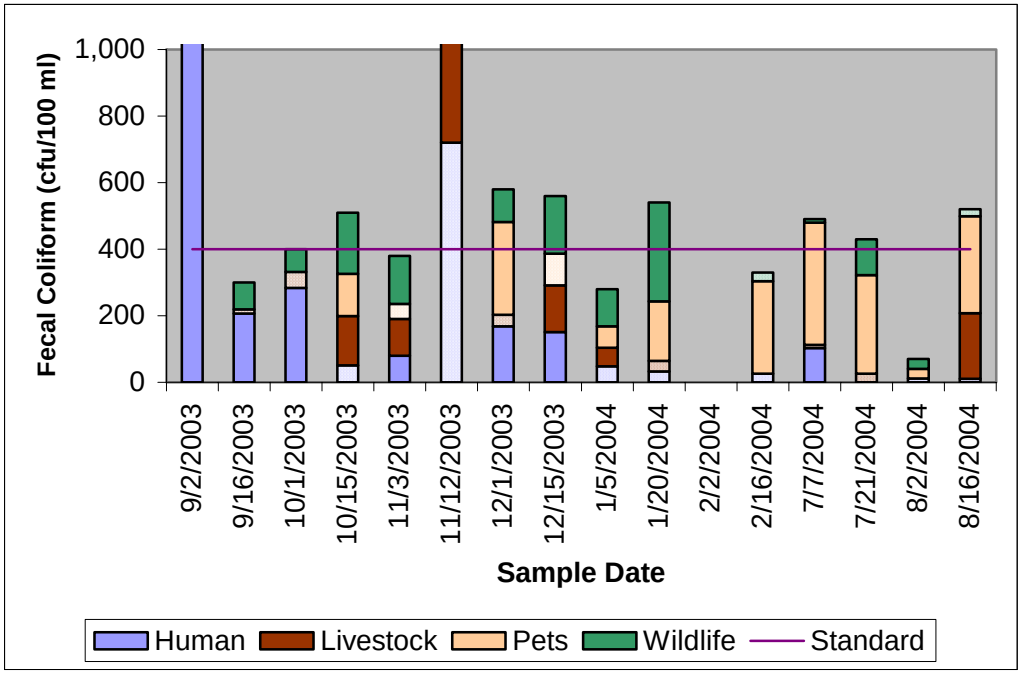

Figure A.3 **Fecal Coliform enumerations with proportional source contributions indicated for Station Q2600000 on Muddy Creek. Fecal coliform enumerations are censored at 1,000 cfu/100 ml to improve resolution on values near the standard. Solid colors indicate statistical significance.**

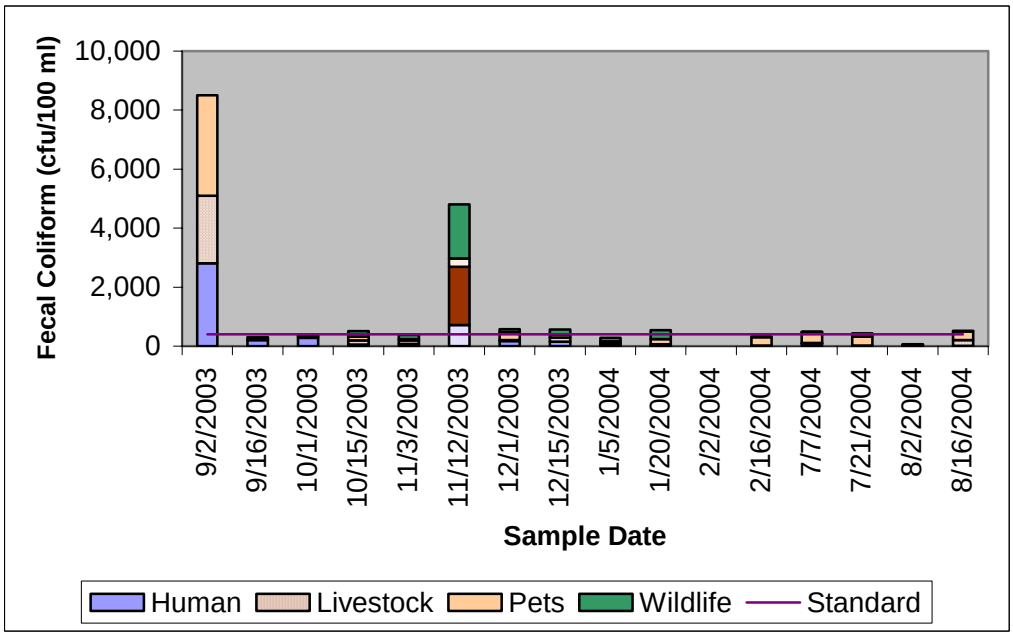

Figure A. 4 **Fecal Coliform enumerations with proportional source contributions indicated for Station Q2600000 on Muddy Creek. Solid colors indicate statistical significance.**

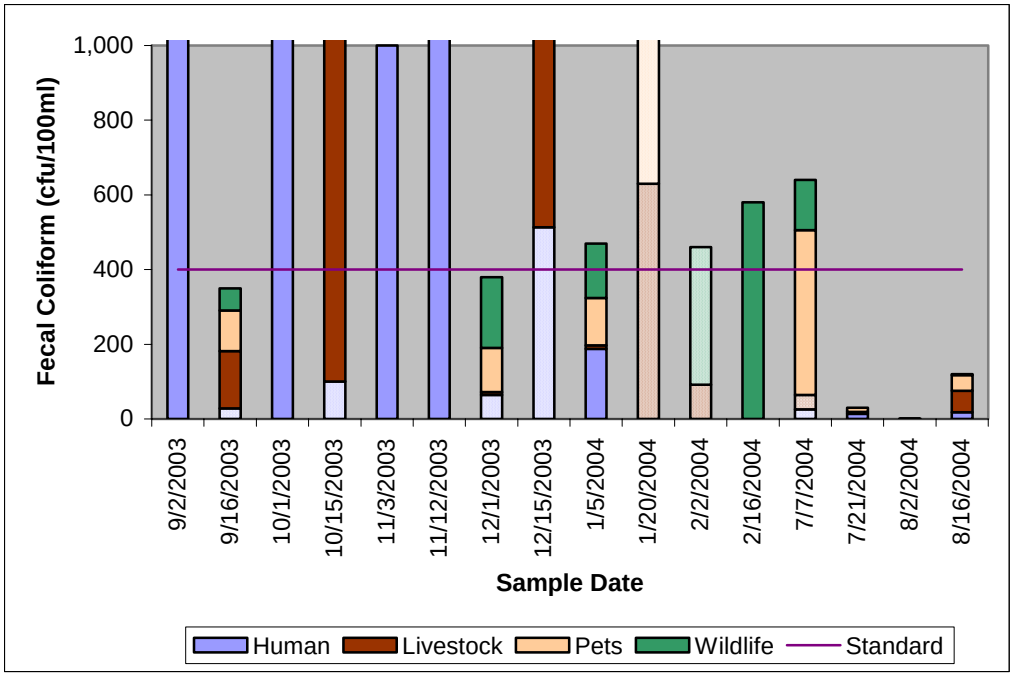

Figure A.5 **Fecal Coliform enumerations with proportional source contributions indicated for Station Q2510000 on Salem Creek. Fecal coliform enumerations are censored at 1,000 cfu/100 ml to improve resolution on values near the standard. Solid colors indicate statistical significance.**

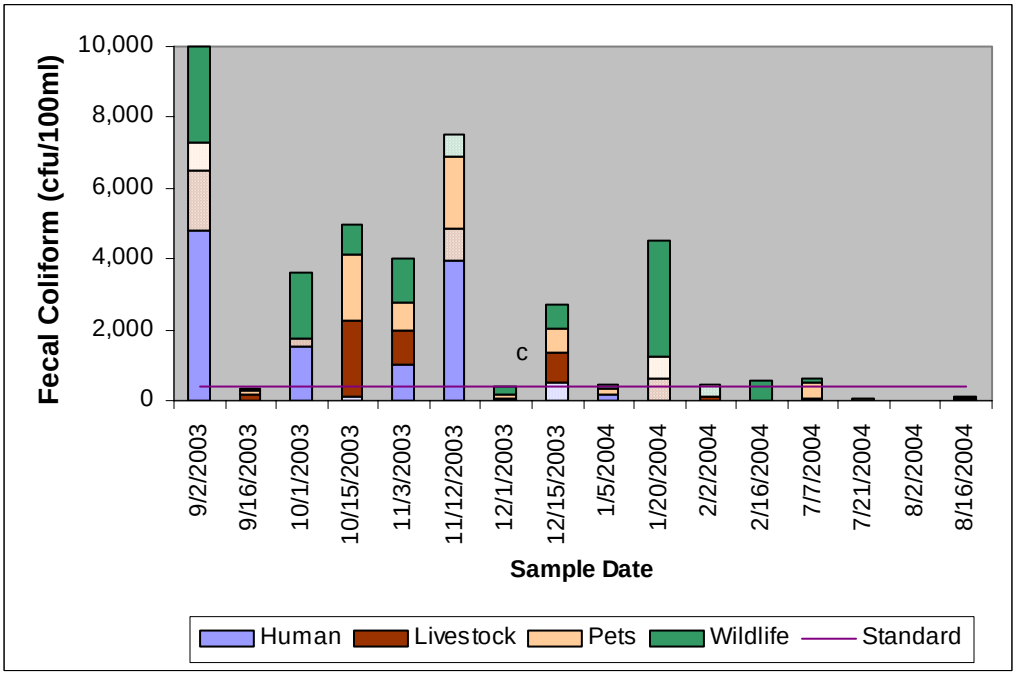

Figure A. 6 **Fecal Coliform enumerations with proportional source contributions indicated for Station Q2510000 on Salem Creek. Solid colors indicate statistical significance.**