Pathogen Source Assessment for TMDL Development and Implementation in North Carolina Piedmont and Coastal Plain Watersheds

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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). As indicated in Table 1.1, this project was conducted over a two-year time period, with four watersheds monitored in the first year and the remaining five watersheds monitored in the second.

Table 1.1 Location and year of study for bacteria source tracking project.

River basin	Sub-basin	Stream name	Year 1	Year 2	Land use
Cape Fear	030602	North Buffalo Creek		x	Urban
	030605	Northeast Creek	x		Urban
Catawba	030834	Little Sugar Creek	x		Urban
	030837	Crowders Creek		x	Mixed
Neuse	030402	Pigeon House Branch	x		Urban
Tar-Pamlico	030305	Chicod Creek	x		Rural
Yadkin	030704	Grants Creek		x	Mixed
	030706	Fourth Creek		x	Mixed

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 (phenotypic) 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

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[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.

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.

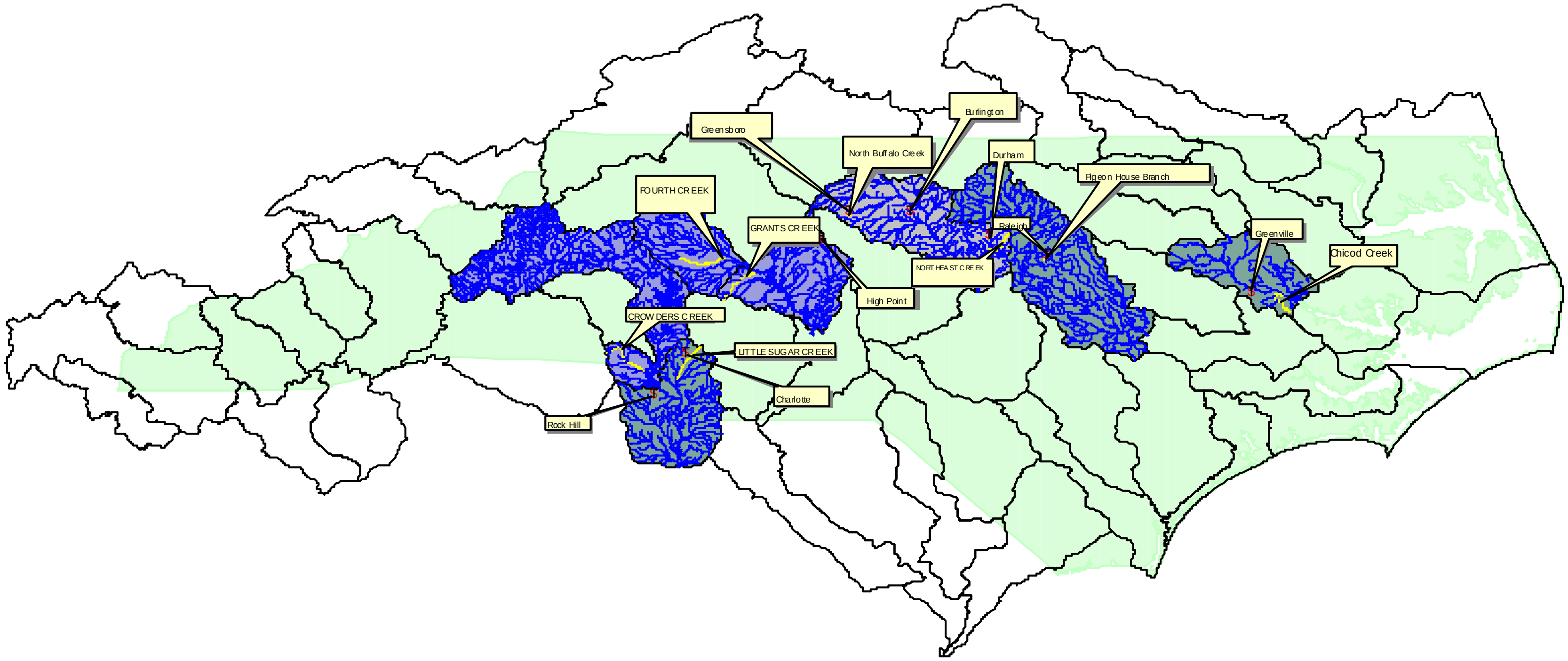

Figure 1.1 Location of streams monitored during the study.

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 species0 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

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-EW03032, were to:

1. prepare a sampling strategy for Year 1 and Year 2 watersheds,
2. build watershed-specific libraries of known sources of *E. coli* bacteria,
3. analyze and categorize ambient water sources of bacteria., and
4. compare libraries of urban watersheds to determine cross-applicability.

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 5 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. Ambient samples were to be collected from two to three locations in each watershed at a fixed frequency of two times per month by DWQ or local government staff. Some adjustments were made due to scheduling conflicts. The number of stations selected in each watershed and the number of samples targeted are identified in Table 4.1. The locations of ambient sampling sites are provided in Table 4.2. The first year's sampling was initially conducted between July 2003 and January 2004, with the initial sampling date staggered by watershed. However, due to a computer malfunction, BST data from the samples collected prior to August 15, 2003 were lost. Consequently, MapTech personnel collected additional samples in July and August 2004.

DWQ, local government, or MapTech personnel collected fecal matter from known sources and shipped samples to MapTech's Environmental Diagnostics Laboratory (EDL) for analysis and development of a known-source library. Samples were collected with the goal of obtaining 60 viable samples from appropriate source categories for each watershed (*e.g.*, human, livestock, pets, and wildlife). Samples were intended to be evenly distributed among the source categories in each watershed. A total of 426 viable samples were collected, yielding 3401 *E. coli* isolates for developing known source libraries.

Table 4.1 Final sampling schedule for each watershed in the study.

Stream name	Number of Stations	Number of Samples	First Scheduled Sample	Final Scheduled Sample	Additional Samples (MapTech)
North Buffalo Creek	3	12	8/23/04	1/25/05	--
Northeast Creek	2	12	4/13/04	7/29/04	--
Little Sugar Creek	3	14	7/30/03	1/14/04	2004: 8/2, 8/16
Crowders Creek	1	8	6/23/04	10/4/04	--
Pigeon House Branch	2	17	7/15/03	1/21/04	2004: 7/21, 8/1, 8/16
Chicod Creek	3	12	8/18/03	1/21/04	--
Grants Creek	1	9	6/23/04	10/20/04	--
Fourth Creek	1	9	6/23/04	10/20/04	--

Table 4.2 Location of ambient water quality monitoring stations.

Watershed	Waterbody	Station ID	Latitude (Dec Deg)	Longitude (Dec Deg)
North Buffalo Cr.	North Buffalo Cr.	Aycock	36.01250	-79.81330
North Buffalo Cr.	North Buffalo Cr.	Rankin Mill	36.00361	-79.70830
North Buffalo Cr.	North Buffalo Cr.	Summit	36.10552	-79.76463
Northeast Cr.	Northeast Cr.	O'Kelly Chapel Rd	35.85000	-78.93300
Northeast Cr.	Northeast Cr.	Sedwick Rd	35.88330	-78.88330
Little Sugar Cr.	Little Sugar Cr.	MC29	35.16030	-80.84920
Little Sugar Cr.	Briar Cr.	MC31	35.15820	-80.84880
Little Sugar Cr.	Little Sugar Cr.	MC49A	35.08490	-80.88280
Crowders Cr.	Crowders Cr.	CROWDH2O2	35.15856	-81.18040
Pigeon House Br.	Pigeon House Br.	0208732544	35.79389	-78.64250
Pigeon House Br.	Pigeon House Br.	ONC-site 3	35.80639	-78.61444
Chicod Cr.	Chicod Cr.	CHICOD 06450000	35.56153	-77.23086
Chicod Cr.	Chicod Cr.	CHICOD1	35.49853	-77.16980
Chicod Cr.	Chicod Cr.	CHICOD2	35.53336	-77.18817
Grants Cr.	Grants Cr.	GRANTSH2O2	35.70694	-80.43650
Fourth Cr.	Fourth Cr.	4thH2O3	35.76775	-80.72190

4.2 Analysis of Known-Source Samples

DWQ, local government, or MapTech 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 ARA. Known-source libraries were constructed from isolates collected in the study watersheds (Table 4.3). 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.

Table 4.3 Known-source library development for BST project.

Library Name	Number of Samples Collected	Number of Viable Samples	Number of Viable Isolates	Source Categories Collected[1]
North Buffalo Creek	68	56	448	HPW
Northeast Creek	61	55	440	HLPW
Little Sugar Creek	82	59	472	HPW
Crowders Creek	86	63	504	HLPW
Pigeon House Branch	76	57	449	HPW
Chicod Creek	65	61	488	HDW
Fourth & Grants Creek	85	75	600	HLPW

[1] "HPW" = Human/Pets/Wildlife, "HLPW" = Human/Livestock/Pets/Wildlife, "HDW" = Human/Domestic Animals/Wildlife

4.3 Bacterial Enumerations and BST Analyses

DWQ, local government, or MapTech 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. In highly urbanized watersheds (*i.e.*, North Buffalo Creek, Little Sugar Creek, and Pigeon House Branch), where little or no livestock impacts were anticipated, no livestock source samples were collected and no more than 3 categories were considered (*i.e.*, human, wildlife, and domestic animals – pets). Similarly, in Chicod Creek, a rural watershed, where impacts from pet waste were anticipated to be minimal, no more than 3 categories were considered (*i.e.*, human, wildlife, and domestic animals).

Table 5.1 Proposed BST Source Library Characterizations.

Source Grouping			Potential Sources
2 Categories	*3 Categories*	*4 Categories*	
Human	Human	Human	Sewage (Failing Septic Systems, Straight Pipes, Sewer Overflows, Exfiltration) Biosolids
Non-Human	Domestic Animals	Pets	Cats Dogs Sewage (Flushed Pet Waste)
		Livestock	Cattle Horses Poultry Goats etc.
	Wildlife	Wildlife	Birds Deer Raccoons Groundhogs Opossum etc.

5.1 Initial Known-Source Library Development

Tables 5.2 through 5.18 present the results from the initial libraries developed for the Piedmont and Coastal Plains watersheds. While the basic RCCs tend to be high, the randomized RCCs indicate a significant potential for over fitting (*i.e.*, the libraries are too small). Additionally, the jackknifed RCCs and false-positive rates for some of the libraries indicate that the libraries are 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 North Buffalo Creek watershed library with Human / Non-Human source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	79%	68%	57%	20%
Non-Human	80%	67%	69%	21%
Overall	79%	67%	63%	N/A

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

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	78%	63%	48%	11%
Domestic	70%	60%	38%	21%
Wildlife	61%	63%	38%	13%
Overall	70%	62%	41%	N/A

Table 5.4 Known-source library statistics for the initial Northeast Creek watershed library with Human / Non-Human source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	83%	70%	55%	14%
Non-Human	86%	70%	73%	17%
Overall	84%	70%	64%	N/A

Table 5.5 Known-source library statistics for the initial Northeast Creek watershed library with Human / Domestic Animal / Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	78%	59%	48%	11%
Domestic	70%	65%	38%	21%
Wildlife	61%	60%	38%	13%
Overall	70%	62%	41%	N/A

Table 5.6 Known-source library statistics for the initial Northeast Creek watershed library with Human / Livestock / Pets / Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	75%	60%	28%	7%
Livestock	94%	61%	22%	6%
Pets	69%	59%	22%	13%
Wildlife	56%	61%	9%	9%
Overall	73%	61%	20%	N/A

Table 5.7 Known-source library statistics for the initial Little Sugar Creek watershed library with Human / Non-Human source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	85%	70%	59%	17%
Non-Human	83%	70%	75%	15%
Overall	84%	70%	67%	N/A

Table 5.8 Known-source library statistics for the initial Little Sugar Creek watershed library with Human / Domestic Animal / Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	77%	62%	47%	9%
Domestic	74%	62%	56%	13%
Wildlife	83%	62%	55%	12%
Overall	78%	62%	53%	N/A

Table 5.9 Known-source library statistics for the initial Crowders Creek watershed library with Human / Non-Human source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	91%	68%	57%	19%
Non-Human	81%	68%	66%	9%
Overall	86%	68%	61%	N/A

Table 5.10 Known-source library statistics for the initial Crowders Creek watershed library with Human / Domestic Animal / Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	84%	58%	50%	11%
Domestic	85%	56%	71%	8%
Wildlife	85%	58%	58%	4%
Overall	85%	57%	59%	N/A

Table 5.11 Known-source library statistics for the initial Crowders Creek watershed library with Human / Livestock / Pets / Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	81%	53%	37%	9%
Livestock	76%	54%	58%	7%
Pets	95%	52%	67%	4%
Wildlife	79%	53%	54%	3%
Overall	83%	53%	54%	N/A

Table 5.12 Known-source library statistics for the initial Pigeon House Branch watershed library with Human / Non-Human source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	78%	70%	70%	22%
Non-Human	78%	70%	67%	22%
Overall	78%	70%	68%	N/A

Table 5.13 Known-source library statistics for the initial Pigeon House Branch watershed library with Human / Domestic Animal / Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	77%	62%	64%	9%
Domestic	93%	63%	80%	3%
Wildlife	84%	63%	43%	11%
Overall	85%	63%	62%	N/A

Table 5.14 Known-source library statistics for the initial Chicod Creek watershed library with Human / Non-Human source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	89%	63%	68%	17%
Non-Human	78%	62%	69%	11%
Overall	83%	63%	68%	N/A

Table 5.15 Known-source library statistics for the initial Chicod Creek watershed library with Human/Domestic Animal / Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	71%	53%	52%	13%
Domestic	77%	54%	56%	14%
Wildlife	73%	55%	49%	13%
Overall	74%	54%	52%	N/A

Table 5.16 Known-source library statistics for the initial Grants Creek / Fourth Creek watersheds library with Human / Non-Human source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	91%	63%	N/A	5%
Non-Human	91%	62%	N/A	14%
Overall	91%	63%	N/A	N/A

Table 5.17 Known-source library statistics for the initial Grants Creek / Fourth Creek watersheds library with Human / Domestic Animal / Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	94%	53%	80%	8%
Domestic	82%	54%	67%	5%
Wildlife	88%	50%	69%	5%
Overall	88%	52%	72%	N/A

Table 5.18 Known-source library statistics for the initial Grants Creek / Fourth Creek watersheds library with Human / Livestock / Pets / Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	95%	46%	87%	3%
Livestock	88%	46%	69%	2%
Pets	95%	47%	68%	3%
Wildlife	87%	47%	63%	3%
Overall	91%	47%	72%	N/A

5.2 Combined Regional Known-Source Libraries

Based on the results of analyses on the individual libraries, the libraries were combined to improve confidence in analysis of water samples. Combinations were determined based on geographical proximity and statistical analysis of the resulting library. Where appropriate, libraries developed for Virginia watersheds were considered. Table 5.19 presents the results from the combined library of known sources from the Chicod Creek watershed and sources collected from a similar coastal region in Virginia. Table 5.20 presents the results of the combined Northeast Creek and Pigeon House Branch library. Table 5.21 presents the results of the combined library of the "western" watersheds (Little Sugar Creek, Crowders Creek, Grants Creek, Fourth Creek, and North Buffalo Creek) with three known source categories (human, domestic animal, wildlife). This combined library was used to analyze water samples from Little Sugar Creek and North Buffalo Creek. Table 5.22 presents the results from the combined library of the western watersheds with four known source categories (human, pets, livestock, wildlife) developed for the Crowders, Grants, and Fourth Creeks watersheds. Combining individual libraries improved the results, particularly for the randomized RCCs.

Table 5.19 Known-source library statistics for the combined Chicod Creek watershed and Virginia regional library with Human / Domestic Animal / Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	64%	47%	50%	18%
Domestic	65%	43%	55%	15%
Wildlife	63%	47%	31%	22%
Overall	64%	45%	49%	N/A

Table 5.20 Known-source library statistics for the combined Northeast Creek & Pigeon House Branch watersheds library with Human / Domestic Animal / Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	70%	51%	60%	14%
Domestic	61%	52%	50%	13%
Wildlife	64%	52%	45%	25%
Overall	65%	52%	52%	N/A

Table 5.21 Known-source library statistics for the western-watersheds library with Human / Domestic Animal / Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	71%	42%	65%	17%
Domestic	52%	45%	47%	21%
Wildlife	62%	42%	52%	20%
Overall	62%	43%	55%	N/A

Table 5.22 Known-source library statistics for the western-watersheds library with Human / Livestock/ Pets / Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	63%	36%	58%	12%
Livestock	51%	35%	38%	20%
Pets	40%	36%	29%	15%
Wildlife	54%	36%	45%	16%
Overall	52%	36%	43%	N/A

5.3 Analysis of Urban Watersheds

Known-source data from all urban watersheds were combined to explore the possibility of a statewide library for urban watersheds. The results of analyses on the urban-watersheds library are reported in Tables 5.23 and 5.24. The urban-watersheds library did not perform as well as the regional libraries. Based on this analysis, it appears that geographic proximity is a better criteria than land use similarity in combining known-source libraries.

Table 5.23 Known-source library statistics for the combined urban-watersheds library with Human / Non-Human source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	64%	59%	60%	31%
Non-Human	69%	59%	65%	36%
Overall	67%	60%	63%	N/A

Table 5.24 Known-source library statistics for the combined urban-watersheds library with Human / Domestic Animal / Wildlife source categories.

Source	RCC	Randomized RCC	Jackknifed RCC	False-Positive Rate
Human	62%	46%	49%	20%
Domestic	56%	47%	50%	19%
Wildlife	52%	45%	44%	26%
Overall	57%	46%	48%	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.

ARA results were compared to fluorometry results for nineteen 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.

6.1 North Buffalo Creek

There were three stations monitored in the North Buffalo Creek watershed. The Aycock and Rankin Mill stations showed similar characteristics, with a persistently significant contribution of bacteria from wildlife. Human and domestic animal sources were occasionally significant, but much less frequently than wildlife. The Summit station showed different characteristics from the first two stations. At this station, while wildlife sources were still persistent, human and domestic animal sources were also persistent at this station.

6.2 Northeast Creek

There were two stations monitored in the Northeast Creek watershed. The O’Kelly Chapel Road station showed a persistently significant contribution of bacteria from human sources. Wildlife and domestic animal sources were also significant, but to a lesser degree than human sources. The Sedwick Road station showed different characteristics from the O’Kelly Chapel Road station. At this station, wildlife sources were the most persistent, while human and domestic animal sources were present to a lesser degree.

6.3 Little Sugar Creek

There were three stations monitored in the Little Sugar Creek watershed (MC29, MC29a, and MC31). All of these stations showed similar characteristics, with a persistently significant contribution of bacteria from all three source categories. Wildlife sources tend to dominate, followed by domestic animal sources, then human sources. However, the differences between source contributions are subtle.

6.4 Crowders Creek

There was one station monitored in the Crowders Creek watershed (CROWDH202). Livestock and wildlife sources were persistently significant. Human and pet source contributions were rarely significant.

6.5 Pigeon House Branch

There were two stations monitored in the Pigeon House Branch watershed (0208732544, and ONC-Site 3). Both of these stations showed a persistently significant contribution of bacteria from human sources. Wildlife and domestic animal sources were occasionally significant, but much less frequently than human sources.

6.6 Chicod Creek

There were three stations monitored in the Chicod Creek watershed (CHICOD 06450000, CHICOD 1, and CHICOD 2). The CHICOD 06450000 and CHICOD 2 stations showed similar characteristics, with significant contributions of bacteria from all sources. Human sources were the least persistent of the three source categories, and livestock sources tended to dominate at CHICOD 06450000. At CHICOD 1, *E. coli* counts tended to be lower than at the other two stations and the contribution from human sources was not significant. The persistence of significant contributions from wildlife and domestic animals was comparable at this station.

6.7 Grants Creek

There was one station monitored in the Grants Creek watershed (GRANTSH202). Human source contributions were not significant. Livestock and wildlife sources were the most persistently significant of the four source categories, with the wildlife contribution tending to be slightly greater than the livestock contribution.

6.8 Fourth Creek

There was one station monitored in the Fourth Creek watershed (4thH203). Human source contributions were not significant at this station. Pet and livestock contributions were occasionally significant. Wildlife source contributions were the most persistently significant of the four source categories, with the wildlife contribution tending to be greater than the livestock or pet contribution. It may be significant to note, however, that livestock and pet sources contributed approximately half of the load at the time when the highest *E. coli* value was measured.

Table 6.1 Bacterial Source Tracking results for North Buffalo Creek (Aycock).

			E. coli		Fecal Coliform			Bacteria Source		
Station ID	**Sample Date**	**Lab ID**	**Value**	**Qual**	**Value**	**Qual**	**Isolates**	**Human**	**Domestic**	**Wildlife**
Aycock	8/23/04	2NC127	230		160		26	0%	4%	**96%**
Aycock	8/30/04	2NC133	6000		6900		48	6%	**56%**	**38%**
Aycock	9/14/04	2NC141	800		570		48	2%	0%	**98%**
Aycock	9/27/04	2NC145	6900		6300		48	17%	4%	**79%**
Aycock	10/5/04	2NC151	760		470		48	**27%**	**56%**	17%
Aycock	10/26/04	2NC156	700		410		48	0%	**60%**	**40%**
Aycock	11/8/04	2NC159	800		540		48	**69%**	12%	19%
Aycock	11/22/04	2NC162	231		320		48	15%	4%	**81%**
Aycock	12/6/04	2NC165	173		200		48	2%	0%	**98%**
Aycock	12/28/04	2NC168	134		210		48	2%	6%	**92%**
Aycock	1/18/05	2NC171	226		310		48	**46%**	4%	**50%**
Aycock	1/25/05	2NC174	72		80		38	8%	**37%**	**55%**

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 North Buffalo Creek (Rankin Mill).

			E. coli		Fecal Coliform			Bacteria Source		
Station ID	**Sample Date**	**Lab ID**	**Value**	**Qual**	**Value**	**Qual**	**Isolates**	**Human**	**Domestic**	**Wildlife**
Rankin Mill	8/23/04	2NC129	650		250		48	6%	4%	**90%**
Rankin Mill	8/30/04	2NC135	7100		1	BDL	48	19%	4%	**77%**
Rankin Mill	9/14/04	2NC139	240		130		32	**69%**	19%	12%
Rankin Mill	9/27/04	2NC146	320		380		40	15%	15%	**70%**
Rankin Mill	10/5/04	2NC152	110		130		11	**27%**	9%	**64%**
Rankin Mill	10/26/04	2NC158	160		270		16	**44%**	**56%**	0%
Rankin Mill	11/8/04	2NC161	600		330		48	15%	12%	**73%**
Rankin Mill	11/22/04	2NC164	110		190		48	2%	4%	**94%**
Rankin Mill	12/6/04	2NC167	100		90		16	12%	0%	**88%**
Rankin Mill	12/28/04	2NC170	10		40		8	0%	0%	100%
Rankin Mill	1/18/05	2NC173	16		50		9	11%	11%	**78%**
Rankin Mill	1/25/05	2NC176	1		20		1	0%	0%	100%

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 North Buffalo Creek (Summit).

			E. coli		Fecal Coliform			Bacteria Source		
Station ID	**Sample Date**	**Lab ID**	**Value**	**Qual**	**Value**	**Qual**	**Isolates**	**Human**	**Domestic**	**Wildlife**
Summit	8/23/04	2NC128	550		130		48	19%	10%	**71%**
Summit	8/30/04	2NC134	7500		6800		48	17%	**29%**	**54%**
Summit	9/14/04	2NC140	200		380		47	**29%**	12%	**59%**
Summit	9/27/04	2NC147	1300		670		48	**27%**	**58%**	15%
Summit	10/5/04	2NC153	600		260		48	**44%**	**54%**	2%
Summit	10/26/04	2NC157	680		420		48	**46%**	**23%**	**31%**
Summit	11/8/04	2NC160	340		270		28	7%	**50%**	**43%**
Summit	11/22/04	2NC163	269		290		48	**31%**	4%	**65%**
Summit	12/6/04	2NC166	338		460		48	10%	2%	**88%**
Summit	12/28/04	2NC169	210		260		48	12%	6%	**82%**
Summit	1/18/05	2NC172	226		220		48	**47%**	15%	**38%**
Summit	1/25/05	2NC175	138		40		48	21%	**29%**	**50%**

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 6.4 Bacterial Source Tracking results for Northeast Creek (O'Kelly Chapel Rd).

			E. coli		Fecal Coliform			Bacteria Source		
Station ID	**Sample Date**	**Lab ID**	**Value**	**Qual**	**Value**	**Qual**	**Isolates**	**Human**	**Domestic**	**Wildlife**
O'Kelly Chapel Rd	4/13/04	4NC2	1100		220		48	**23%**	10%	**67%**
O'Kelly Chapel Rd	4/20/04	4NC4	30		20		5	40%	40%	20%
O'Kelly Chapel Rd	4/28/04	4NC6	230		40		28	**64%**	4%	**32%**
O'Kelly Chapel Rd	5/4/04	4NC8	410		50		48	**63%**	4%	**33%**
O'Kelly Chapel Rd	5/10/04	4NC10	220		60		23	**66%**	4%	30%
O'Kelly Chapel Rd	5/17/04	4NC12	60		1	BDL	6	0%	0%	100%
O'Kelly Chapel Rd	5/24/04	4NC14	700		100		48	10%	**63%**	27%
O'Kelly Chapel Rd	6/2/04	4NC16	80		50		7	0%	57%	43%
O'Kelly Chapel Rd	6/8/04	4NC18	530		60		48	**56%**	**21%**	23%
O'Kelly Chapel Rd	6/15/04	4NC20	54		20		32	**22%**	16%	**62%**
O'Kelly Chapel Rd	6/23/04	4NC22	1700		200		48	**23%**	0%	**77%**
O'Kelly Chapel Rd	7/29/04	4NC24	1900		50		48	**73%**	6%	21%

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 6.5 Bacterial Source Tracking results for Northeast Creek (Sedwick Rd).

			E. coli		Fecal Coliform			Bacteria Source		
Station ID	**Sample Date**	**Lab ID**	**Value**	**Qual**	**Value**	**Qual**	**Isolates**	**Human**	**Domestic**	**Wildlife**
Sedwick Rd	4/13/04	4NC1	1400		90		48	**86%**	2%	12%
Sedwick Rd	4/20/04	4NC3	50		30		3	0%	0%	100%
Sedwick Rd	4/28/04	4NC5	360		100		40	**55%**	0%	**45%**
Sedwick Rd	5/4/04	4NC7	590		40		48	**83%**	0%	17%
Sedwick Rd	5/10/04	4NC9	310		50		34	9%	3%	**88%**
Sedwick Rd	5/17/04	4NC11	110		20		12	0%	**50%**	**50%**
Sedwick Rd	5/24/04	4NC13	700		70		48	10%	**78%**	12%
Sedwick Rd	6/2/04	4NC15	650		60		48	4%	**48%**	**48%**
Sedwick Rd	6/8/04	4NC17	140		120		15	0%	**53%**	**47%**
Sedwick Rd	6/15/04	4NC19	72		30		48	**23%**	12%	**65%**
Sedwick Rd	6/23/04	4NC21	3300		80		#N/A	#N/A	#N/A	#N/A
Sedwick Rd	7/29/04	4NC23	1700		1300		48	**44%**	8%	**48%**

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 6.6 Bacterial Source Tracking results for Little Sugar Creek (MC29).

Station ID	Sample Date	Lab ID	*E. coli* Value	*E. coli* Qual	Fecal Coliform Value	Fecal Coliform Qual	Isolates	Bacteria Source Human	Bacteria Source Domestic	Bacteria Source Wildlife
MC29	7/30/03*	2NC5	5300		12000		#N/A	#N/A	#N/A	#N/A
MC29	8/13/03*	2NC10	4000		15000		#N/A	#N/A	#N/A	#N/A
MC29	8/26/03	2NC18	450		4800		48	8%	**69%**	23%
MC29	9/10/03	2NC26	340		440		48	19%	**79%**	2%
MC29	9/24/03	2NC34	570		350		48	**67%**	4%	**29%**
MC29	10/21/03	2NC42	300		370		24	4%	**96%**	0%
MC29	10/28/03	2NC50	750		370		48	23%	12%	**65%**
MC29	11/4/03	2NC55	170		200		18	**33%**	**22%**	**45%**
MC29	12/1/03	2NC66	140		120		16	0%	6%	**94%**
MC29	12/16/03*	2NC76	680		360		48	8%	**75%**	17%
MC29	12/29/03	2NC79	50		30		6	33%	33%	34%
MC29	1/14/04	2NC90	550		140		48	**63%**	6%	**31%**
MC29	8/2/04	2NC111	3500		1	BDL	48	**42%**	2%	**56%**
MC29	8/16/04	2NC124	340		380		40	**28%**	12%	**60%**

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.

*Sampling conducted during elevated stream flow conditions.

Table 6.7 Bacterial Source Tracking results for Little Sugar Creek (MC31).

			E. coli		Fecal Coliform			Bacteria Source		
Station ID	**Sample Date**	**Lab ID**	**Value**	**Qual**	**Value**	**Qual**	**Isolates**	**Human**	**Domestic**	**Wildlife**
MC31	7/30/03*	2NC6	5700		16000		#N/A	#N/A	#N/A	#N/A
MC31	8/13/03*	2NC11	2500		9000		#N/A	#N/A	#N/A	#N/A
MC31	8/26/03	2NC19	500		3800		48	0%	**81%**	19%
MC31	9/10/03	2NC27	360		270		48	**84%**	10%	6%
MC31	9/24/03	2NC35	420		300		48	**38%**	**52%**	10%
MC31	10/21/03	2NC43	180		250		24	12%	**80%**	8%
MC31	10/28/03	2NC51	400		210		48	0%	10%	**90%**
MC31	11/4/03	2NC56	200		230		22	5%	**27%**	**68%**
MC31	12/1/03	2NC67	170		110		24	0%	**67%**	**33%**
MC31	12/16/03*	2NC77	800		590		48	25%	10%	**65%**
MC31	12/29/03	2NC80	20		130		2	0%	50%	50%
MC31	1/14/04	2NC91	30		30		3	0%	0%	100%
MC31	8/2/04	2NC113	900		1	BDL	48	**33%**	12%	**55%**
MC31	8/16/04	2NC126	670		340		48	15%	**70%**	15%

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.
*Sampling conducted during elevated stream flow conditions.

Table 6.8 Bacterial Source Tracking results for Little Sugar Creek (MC49A).

			E. coli		Fecal Coliform			Bacteria Source		
Station ID	**Sample Date**	**Lab ID**	**Value**	**Qual**	**Value**	**Qual**	**Isolates**	**Human**	**Domestic**	**Wildlife**
MC49A	7/30/03*	2NC7	5900		13000		#N/A	#N/A	#N/A	#N/A
MC49A	8/13/03*	2NC12	2700		11000		#N/A	#N/A	#N/A	#N/A
MC49A	8/26/03	2NC20	410		4000		48	6%	12%	**82%**
MC49A	9/10/03	2NC28	250		400		30	**80%**	13%	7%
MC49A	9/24/03	2NC36	530		220		48	**33%**	**40%**	27%
MC49A	10/21/03	2NC44	430		450		48	**41%**	21%	**38%**
MC49A	10/28/03	2NC52	5000		1200		48	25%	**29%**	**46%**
MC49A	11/4/03	2NC57	250		250		24	0%	0%	**100%**
MC49A	12/1/03	2NC68	330		140		46	24%	**30%**	**46%**
MC49A	12/16/03*	2NC78	300		220		39	19%	12%	**69%**
MC49A	12/29/03	2NC81	100		70		15	20%	**27%**	**53%**
MC49A	1/14/04	2NC92	230		160		24	**29%**	**46%**	25%
MC49A	8/2/04	2NC112	2400		1	BDL	48	21%	10%	**69%**
MC49A	8/16/04	2NC125	430		330		48	8%	**48%**	**44%**

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.
*Sampling conducted during elevated stream flow conditions.

Table 6.9 Bacterial Source Tracking results for Crowders Creek (CROWDH202).

			E. coli		Fecal Coliform			Bacteria Source			
Station ID	**Sample Date**	**Lab ID**	**Value**	**Qual**	**Value**	**Qual**	**Isolates**	**Human**	**Livestock**	**Pets**	**Wildlife**
CROWDH2O2	6/23/04*	2NC98	1200		250		48	0%	**48%**	**19%**	**33%**
CROWDH2O2	7/6/04	2NC103	570		210		48	0%	8%	10%	**82%**
CROWDH2O2	7/20/04	2NC104	600		340		48	0%	15%	4%	**81%**
CROWDH2O2	8/11/04	2NC119	260		140		37	**22%**	0%	**73%**	5%
CROWDH2O2	8/24/04	2NC132	390		260		48	0%	0%	4%	**96%**
CROWDH2O2	9/8/04*	2NC136	6000		3100		48	4%	**40%**	12%	**44%**
CROWDH2O2	9/20/04	2NC144	640		420		48	2%	**54%**	6%	**38%**
CROWDH2O2	10/4/04	2NC150	1300		480		48	2%	**25%**	**56%**	17%

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.
*Sampling conducted during elevated stream flow conditions.

Table 6.10 Bacterial Source Tracking results for Pigeon House Branch (0208732544).

			E. coli		Fecal Coliform			Bacteria Source		
Station ID	**Sample Date**	**Lab ID**	**Value**	**Qual**	**Value**	**Qual**	**Isolates**	**Human**	**Domestic**	**Wildlife**
208732544	7/15/03	2NC1	790		8000		#N/A	#N/A	#N/A	#N/A
208732544	7/29/03	2NC3	700		2700		#N/A	#N/A	#N/A	#N/A
208732544	8/6/03	2NC8	13000		18000		#N/A	#N/A	#N/A	#N/A
208732544	8/19/03	2NC16	1800		20000		48	**75%**	**19%**	6%
208732544	9/2/03	2NC21	2000		18000		48	**83%**	15%	2%
208732544	9/15/03	2NC29	400		90		48	**75%**	**19%**	6%
208732544	10/8/03	2NC40	5700		2700		48	**73%**	**27%**	0%
208732544	10/21/03	2NC48	750		500		48	8%	10%	**82%**
208732544	11/3/03	2NC53	670		380		48	**42%**	**42%**	16%
208732544	11/17/03	2NC61	10		1	BDL	1	0%	100%	0%
208732544	12/3/03	2NC69	780		360		48	**75%**	0%	25%
208732544	12/10/03	2NC74	90		60		8	50%	25%	25%
208732544	1/6/04	2NC88	3400		4800		48	**40%**	17%	**43%**
208732544	1/21/04	2NC93	30		90		2	0%	50%	50%
208732544	7/21/04	2NC107	1100		6600		48	**60%**	2%	**38%**
208732544	8/1/04	2NC109	4600		4700		48	**29%**	10%	**61%**
208732544	8/16/04	2NC122	1500		3000		48	**73%**	**25%**	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 6.11 Bacterial Source Tracking results for Pigeon House Branch (ONC-Site 3).

			E. coli		Fecal Coliform			Bacteria Source		
Station ID	**Sample Date**	**Lab ID**	**Value**	**Qual**	**Value**	**Qual**	**Isolates**	**Human**	**Domestic**	**Wildlife**
ONC-site 3	7/15/03	2NC2	600		7800		#N/A	#N/A	#N/A	#N/A
ONC-site 3	7/29/03	2NC4	760		4200		#N/A	#N/A	#N/A	#N/A
ONC-site 3	8/6/03	2NC9	2600		8800		#N/A	#N/A	#N/A	#N/A
ONC-Site 3	8/19/03	2NC17	1200		10000		48	**84%**	10%	6%
ONC-Site 3	9/2/03	2NC22	1000		11000		48	**61%**	**27%**	12%
ONC-Site 3	9/15/03	2NC30	560		290		48	**98%**	0%	2%
ONC-Site 3	10/8/03	2NC41	8400		3200		48	**21%**	**69%**	10%
ONC-site 3	10/21/03	2NC49	760		480		48	**63%**	**27%**	10%
ONC-site 3	11/3/03	2NC54	2000		1800		48	**35%**	**57%**	8%
ONC-site 3	11/17/03	2NC62	200		200		24	**38%**	0%	**62%**
ONC-site 3	12/3/03	2NC70	410		340		40	**100%**	0%	0%
ONC-site 3	12/10/03	2NC75	1	BDL	1	BDL	NVI	NVI	NVI	NVI
ONC-site 3	1/6/04	2NC89	2900		330		48	15%	15%	**70%**
ONC-site 3	1/21/04	2NC94	20		70		2	0%	0%	100%
ONC-site 3	7/21/04	2NC108	1000		260		48	**19%**	2%	**79%**
ONC-site 3	8/1/04	2NC110	1100		2000		48	**46%**	8%	**46%**
ONC-site 3	8/16/04	2NC123	420		140		40	0%	**35%**	**65%**

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 6.12 Bacterial Source Tracking results for Chicod Creek (CHICOD 06450000).

			E. coli		Fecal Coliform			Bacteria Source		
Station ID	**Sample Date**	**Lab ID**	**Value**	**Qual**	**Value**	**Qual**	**Isolates**	**Human**	**Domestic**	**Wildlife**
CHICOD 06450000	8/18/03	2NC15	100		2000		48	15%	0%	**85%**
CHICOD 06450000	9/3/03	2NC25	75		4300		16	**100%**	0%	0%
CHICOD 06450000	9/16/03	2NC33	60		70		8	12%	38%	50%
CHICOD 06450000	10/7/03	2NC39	250		220		28	8%	**46%**	**46%**
CHICOD 06450000	10/21/03	2NC47	340		260		48	4%	**56%**	**40%**
CHICOD 06450000	11/5/03	2NC60	250		340		24	**29%**	**42%**	**29%**
CHICOD 06450000	11/17/03	2NC65	340		250		33	**27%**	**55%**	18%
CHICOD 06450000	12/3/03	2NC73	250		190		28	**25%**	**50%**	25%
CHICOD 06450000	12/16/03*	2NC84	#N/A		#N/A		56	23%	12%	**65%**
CHICOD 06450000	1/6/04	2NC87	290		250		32	**53%**	**38%**	9%
CHICOD 06450000	1/21/04	2NC97	50		20		3	33%	0%	67%

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.

*Sampling conducted during elevated stream flow conditions.

Table 6.13 Bacterial Source Tracking results for Chicod Creek (CHICOD1).

			E. coli		Fecal Coliform			Bacteria Source		
Station ID	**Sample Date**	**Lab ID**	**Value**	**Qual**	**Value**	**Qual**	**Isolates**	**Human**	**Domestic**	**Wildlife**
CHICOD1	8/18/03	2NC13	30		490		16	**38%**	0%	**62%**
CHICOD1	9/3/03	2NC23	80		5600		16	6%	**25%**	**69%**
CHICOD1	9/16/03	2NC31	350		150		32	22%	**38%**	**40%**
CHICOD1	10/7/03	2NC37	50		60		7	14%	72%	14%
CHICOD1	10/21/03	2NC45	10		420		7	14%	86%	0%
CHICOD1	11/5/03	2NC58	180		370		24	8%	0%	**92%**
CHICOD1	11/17/03	2NC63	40		10		3	33%	33%	34%
CHICOD1	12/3/03	2NC71	50		20		16	12%	**76%**	12%
CHICOD1	12/16/03*	2NC82	#N/A		#N/A		30	3%	**57%**	**40%**
CHICOD1	1/6/04	2NC85	60		30		13	8%	**84%**	8%
CHICOD1	1/21/04	2NC95	1	BDL	40		0	NVI	NVI	NVI

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.

*Sampling conducted during elevated stream flow conditions.

Table 6.14 Bacterial Source Tracking results for Chicod Creek (CHICOD 2).

			E. coli		**Fecal Coliform**			**Bacteria Source**		
Station ID	**Sample Date**	**Lab ID**	**Value**	**Qual**	**Value**	**Qual**	**Isolates**	**Human**	**Domestic**	**Wildlife**
CHICOD2	8/18/03	2NC14	110		3000		48	0%	2%	**98%**
CHICOD2	9/3/03	2NC24	270		2900		32	**38%**	16%	**46%**
CHICOD2	9/16/03	2NC32	50		110		8	25%	25%	50%
CHICOD2	10/7/03	2NC38	30		50		3	0%	67%	33%
CHICOD2	10/21/03	2NC46	300		250		32	**25%**	**34%**	**41%**
CHICOD2	11/5/03	2NC59	190		120		44	**30%**	**23%**	**47%**
CHICOD2	11/17/03	2NC64	30		1080		4	0%	25%	75%
CHICOD2	12/3/03	2NC72	300		210		16	**38%**	**31%**	**31%**
CHICOD2	12/16/03*	2NC83	#N/A		#N/A		48	23%	10%	**67%**
CHICOD2	1/6/04	2NC86	800		350		48	12%	**65%**	23%
CHICOD2	1/21/04	2NC96	50		20		0	NVI	NVI	NVI

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.
*Sampling conducted during elevated stream flow conditions.

Table 6.15 Bacterial Source Tracking results for Grants Creek (GRANTSH202).

			E. coli		Fecal Coliform			Bacteria Source			
Station ID	**Sample Date**	**Lab ID**	**Value**	**Qual**	**Value**	**Qual**	**Isolates**	**Human**	**Livestock**	**Pets**	**Wildlife**
GRANTSH2O2	6/23/04*	2NC100	270		200		34	3%	**26%**	**32%**	**39%**
GRANTSH2O2	7/6/04	2NC101	900		190		48	0%	12%	15%	**73%**
GRANTSH2O2	7/20/04	2NC106	420		330		48	6%	**40%**	**33%**	**21%**
GRANTSH2O2	8/11/04	2NC121	230		50		26	0%	**54%**	**31%**	15%
GRANTSH2O2	8/24/04	2NC130	260		5000		30	0%	**23%**	7%	**70%**
GRANTSH2O2	9/8/04*	2NC137	4300		500		48	0%	**33%**	6%	**61%**
GRANTSH2O2	9/20/04	2NC142	630		330		48	0%	**53%**	**35%**	12%
GRANTSH2O2	10/4/04	2NC148	200		450		48	0%	10%	12%	**78%**
GRANTSH2O2	10/20/04	2NC154	500		220		48	8%	19%	**61%**	12%

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.

*Sampling conducted during elevated stream flow conditions.

Table 6.16 Bacterial Source Tracking results for Fourth Creek (4thH203).

			E. coli		Fecal Coliform			Bacteria Source			
Station ID	**Sample Date**	**Lab ID**	**Value**	**Qual**	**Value**	**Qual**	**Isolates**	**Human**	**Livestock**	**Pets**	**Wildlife**
4thH2O3	6/23/04*	2NC99	3900		1	BDL	48	16%	0%	8%	**76%**
4thH2O3	7/6/04	2NC102	600		290		48	0%	15%	6%	**79%**
4thH2O3	7/20/04	2NC105	310		110		32	3%	3%	3%	**91%**
4thH2O3	8/11/04	2NC120	290		110		40	0%	0%	0%	**100%**
4thH2O3	8/24/04	2NC131	620		140		48	0%	12%	6%	**82%**
4thH2O3	9/8/04*	2NC138	5000		100		48	0%	**31%**	**21%**	**48%**
4thH2O3	9/20/04	2NC143	760		380		48	0%	6%	**65%**	**29%**
4thH2O3	10/4/04	2NC149	400		180		44	9%	**50%**	5%	**36%**
4thH2O3	10/20/04	2NC155	440		100		46	4%	15%	**33%**	**48%**

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.

*Sampling conducted during elevated stream flow conditions.

APPENDIX A

Bacterial Source Tracking Analyses Supplemental Report

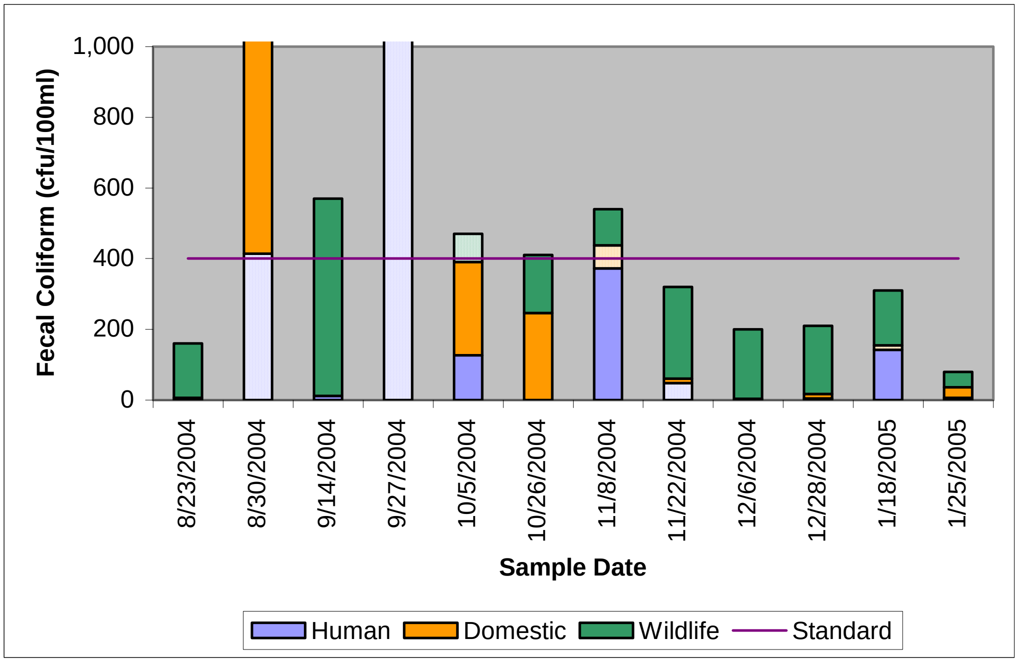

Figure A. 1 **Fecal Coliform enumerations with proportional source contributions indicated for Station Aycock on North Buffalo Creek. Fecal coliform enumerations are censored at 1,000 cfu/100 ml to improve resolution on values near the standard. Solid colors indicate statistical significance.**

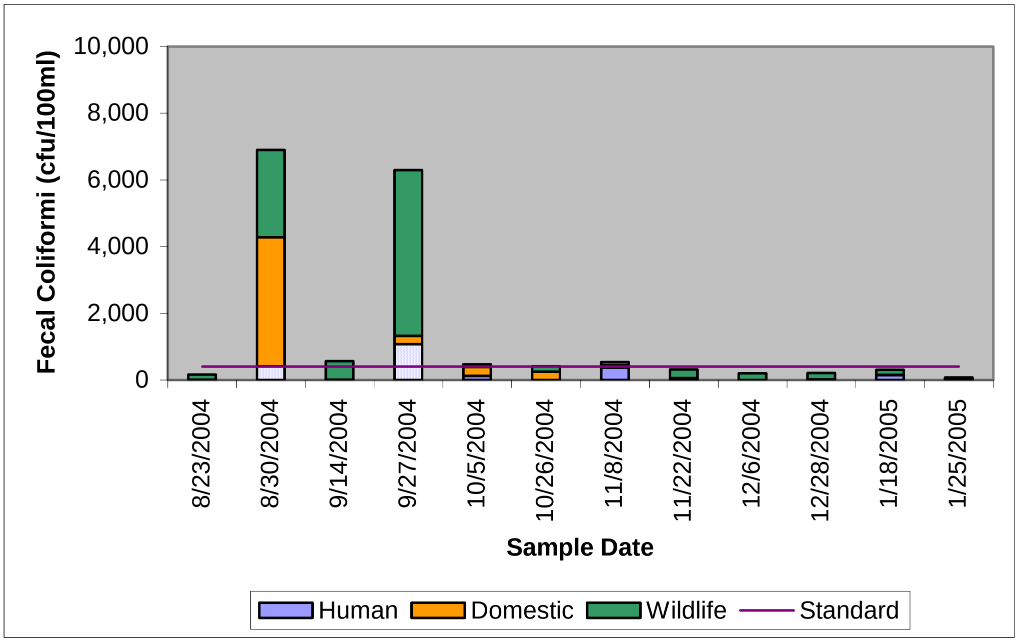

Figure A. 2 **Fecal Coliform enumerations with proportional source contributions indicated for Station Aycock on North Buffalo Creek. Solid colors indicate statistical significance.**

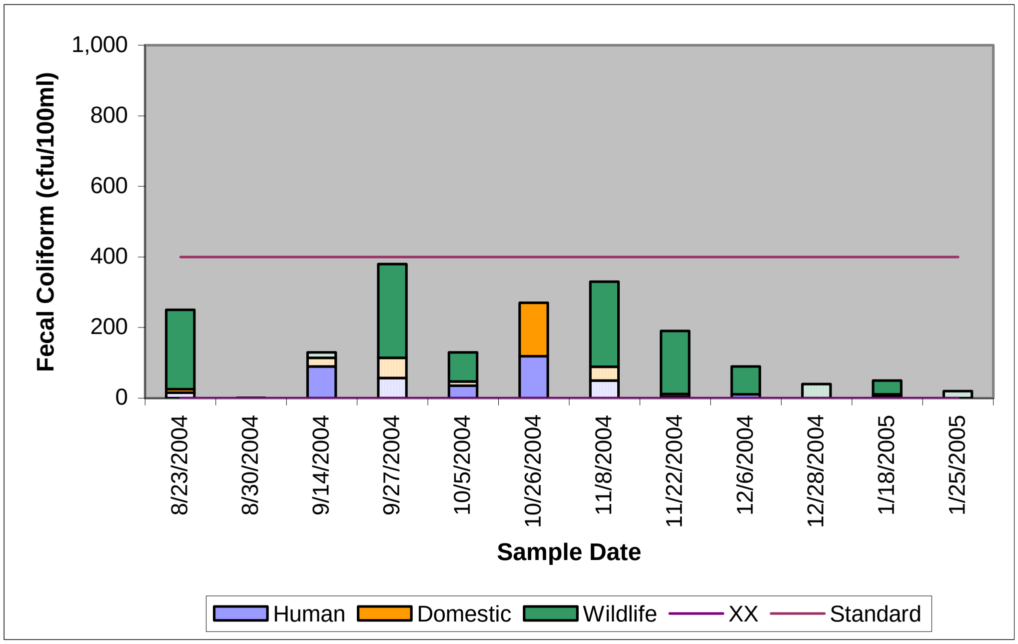

Figure A. 3 **Fecal Coliform enumerations with proportional source contributions indicated for Station Rankin Mill on North Buffalo Creek. Fecal coliform enumerations are censored at 1,000 cfu/100 ml to improve resolution on values near the standard. Solid colors indicate statistical significance.**

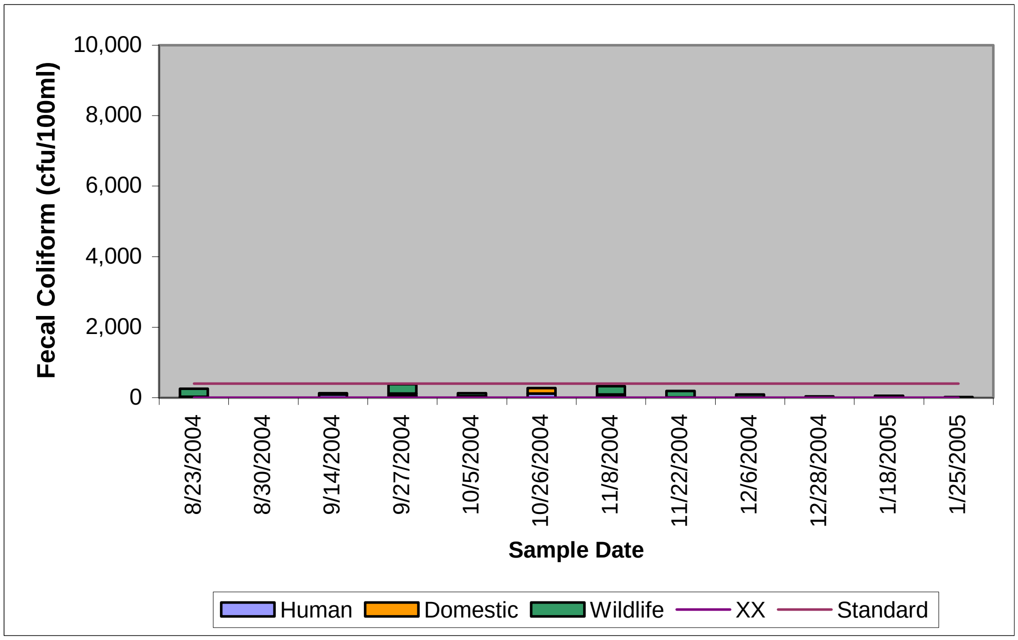

Figure A. 4 **Fecal Coliform enumerations with proportional source contributions indicated for Station Rankin Mill on North Buffalo Creek. Solid colors indicate statistical significance.**

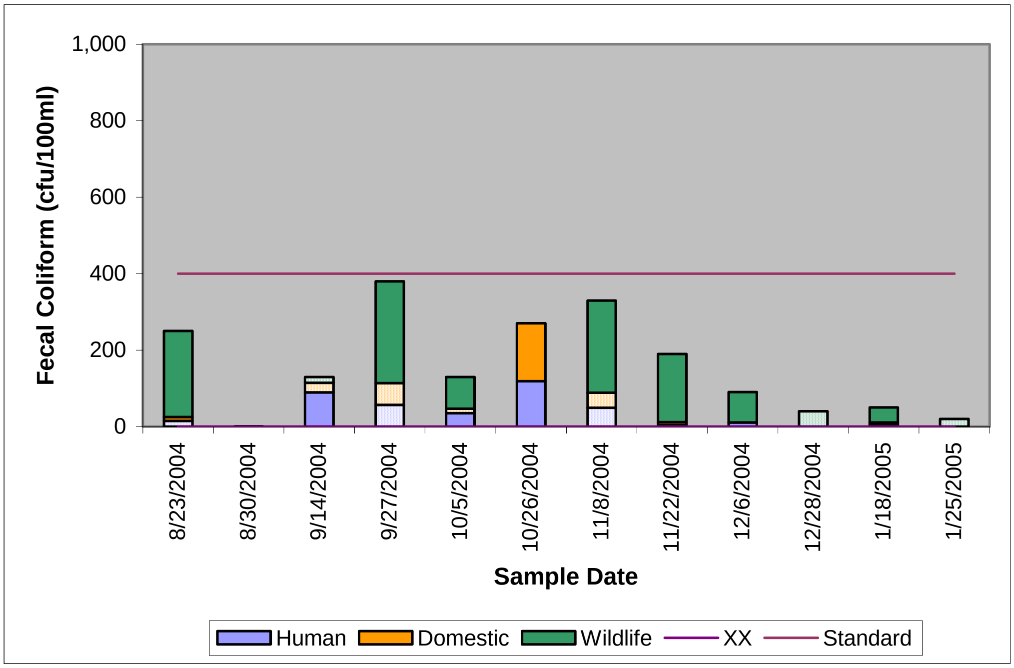

Figure A. 5 **Fecal Coliform enumerations with proportional source contributions indicated for Station Summit on North Buffalo Creek. Fecal coliform enumerations are censored at 1,000 cfu/100 ml to improve resolution on values near the standard. Solid colors indicate statistical significance.**

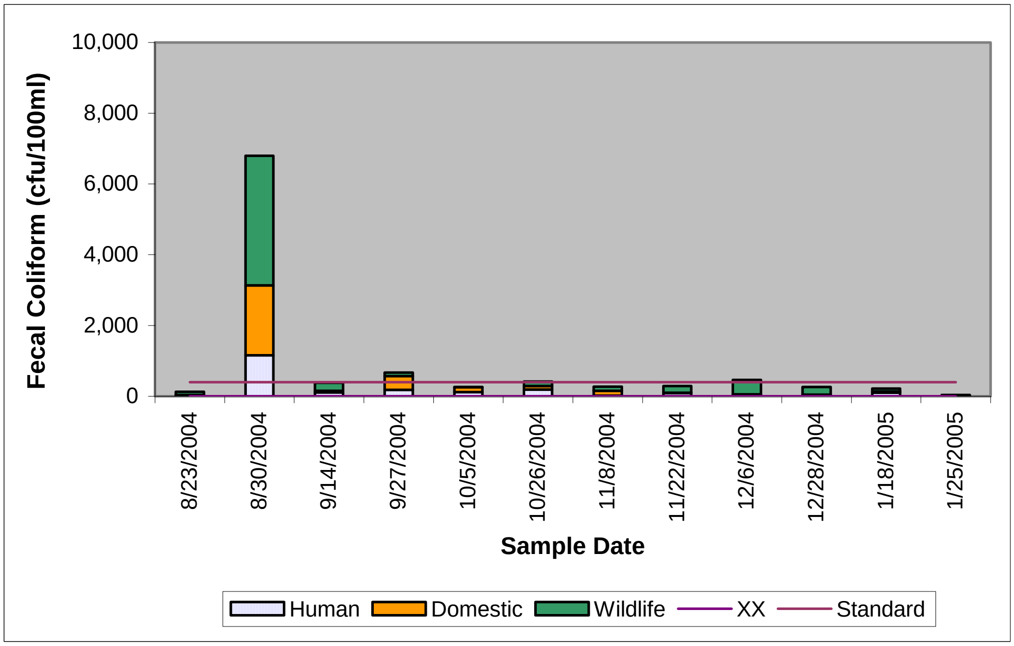

Figure A. 6 **Fecal Coliform enumerations with proportional source contributions indicated for Station Summit on North Buffalo Creek. Solid colors indicate statistical significance.**

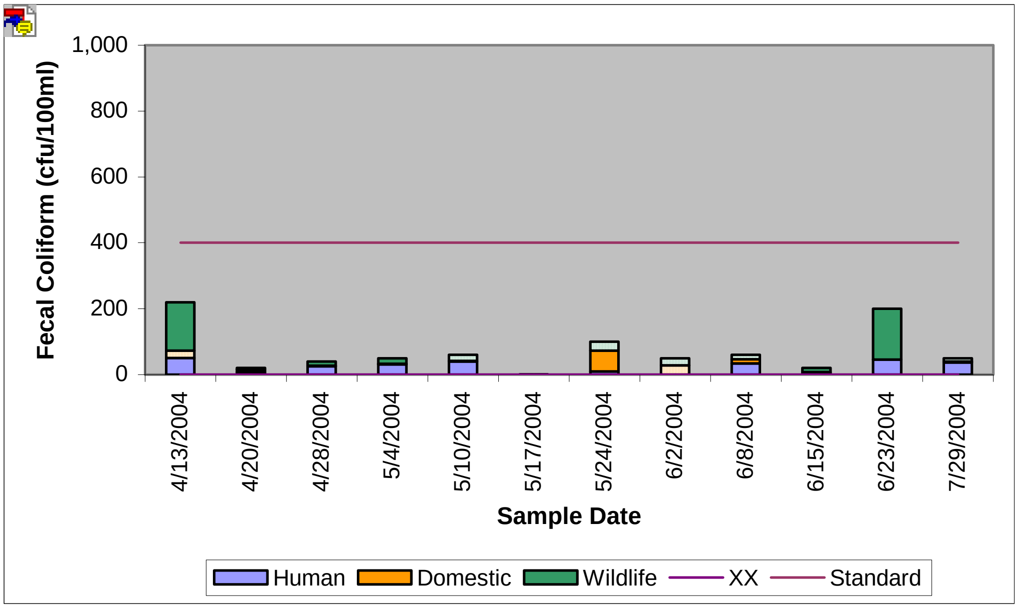

Figure A. 7 **Fecal Coliform enumerations with proportional source contributions indicated for Station O'Kelly on Northeast Creek. Fecal coliform enumerations are censored at 1, 000 cfu/100 ml to improve resolution on values near the standard. Solid colors indicate statistical significance.**

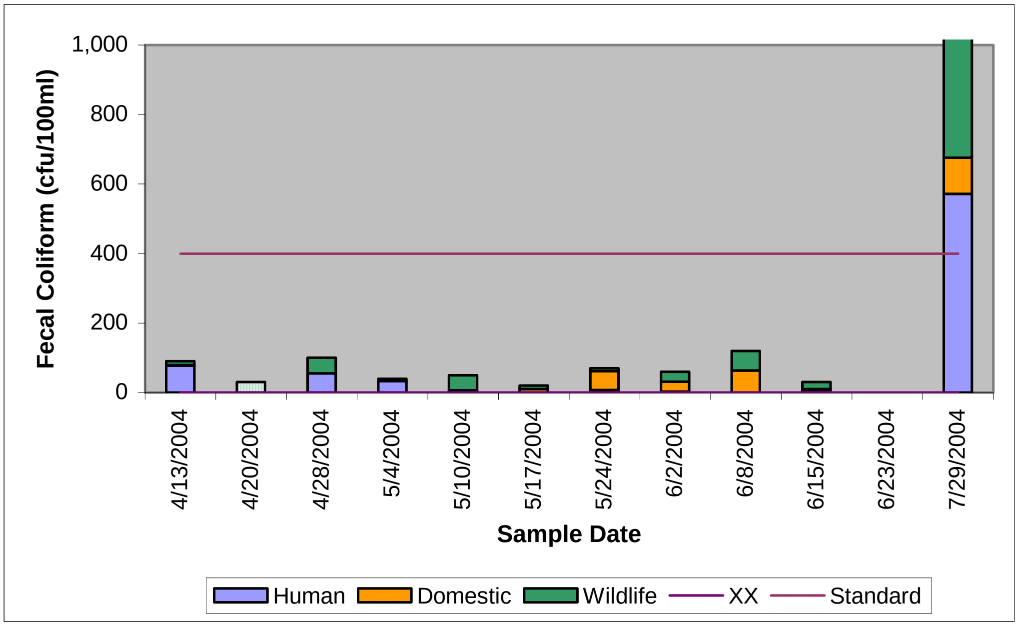

Figure A. 8 **Fecal Coliform enumerations with proportional source contributions indicated for Station Sedwick Road on Northeast Creek. Fecal coliform enumerations are censored at 1, 000 cfu/100 ml to improve resolution on values near the standard. Solid colors indicate statistical significance.**

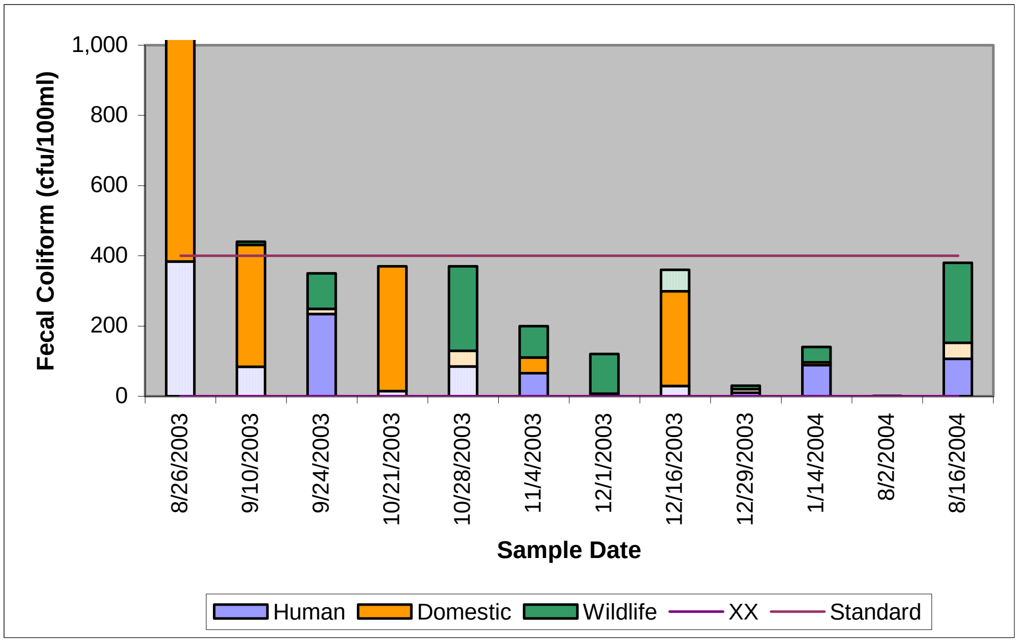

Figure A. 9 **Fecal Coliform enumerations with proportional source contributions indicated for Station MC29 on Little Sugar Creek. Fecal coliform enumerations are censored at 1, 000 cfu/100 ml to improve resolution on values near the standard. Solid colors indicate statistical significance.**

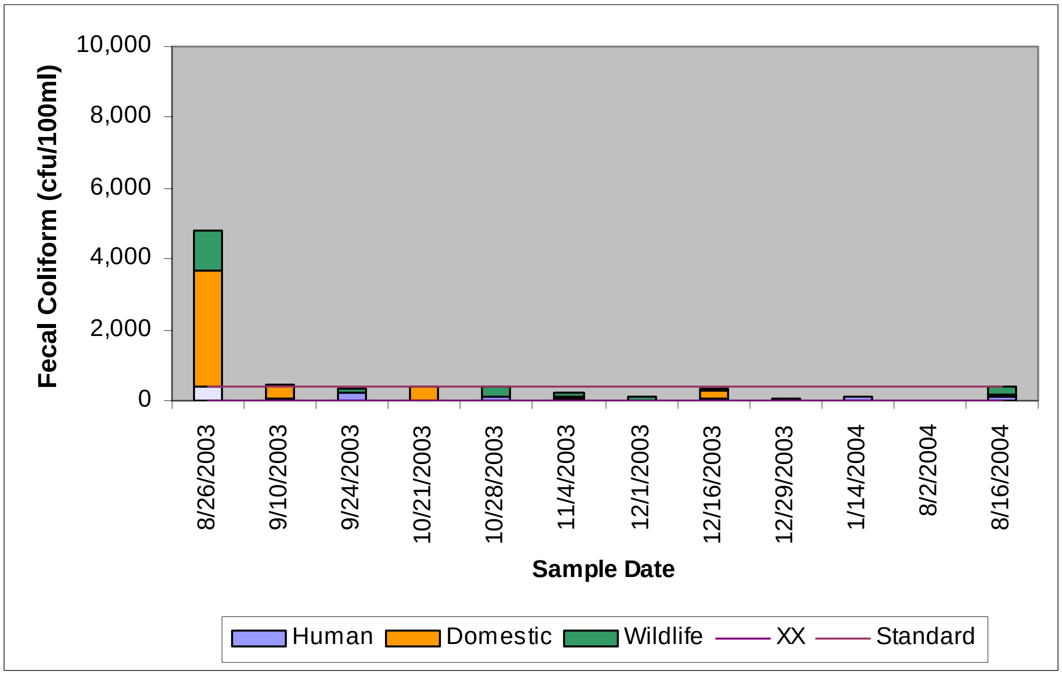

Figure A. 10 **Fecal Coliform enumerations with proportional source contributions indicated for Station MC29 on Little Sugar Creek. Solid colors indicate statistical significance.**

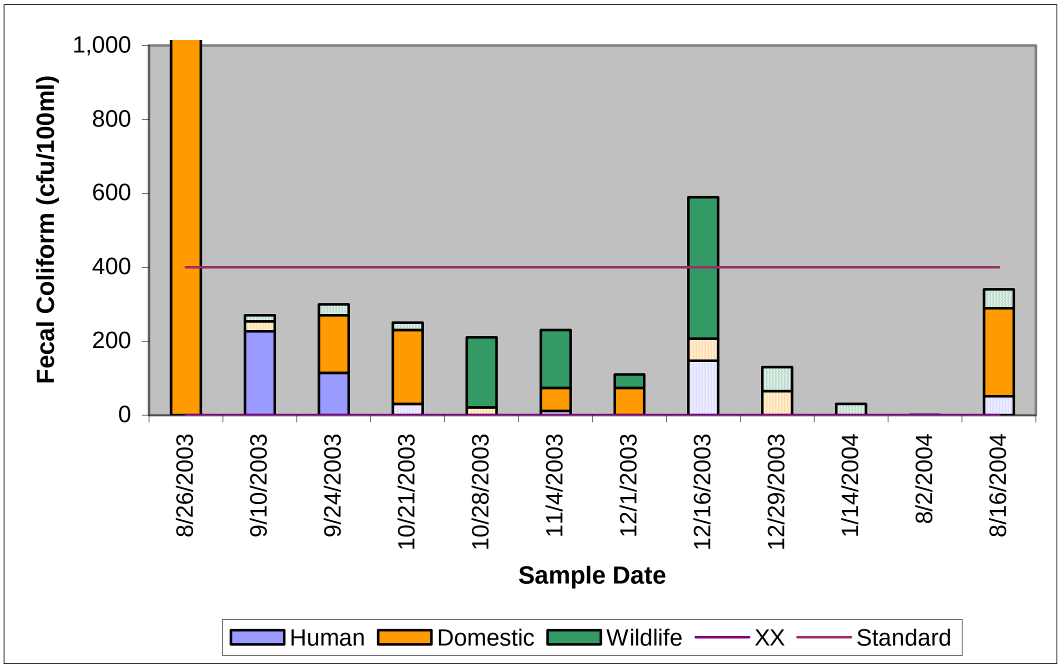

Figure A. 11 **Fecal Coliform enumerations with proportional source contributions indicated for Station MC31 on Little Sugar Creek. Fecal coliform enumerations are censored at 1, 000 cfu/100 ml to improve resolution on values near the standard. Solid colors indicate statistical significance.**

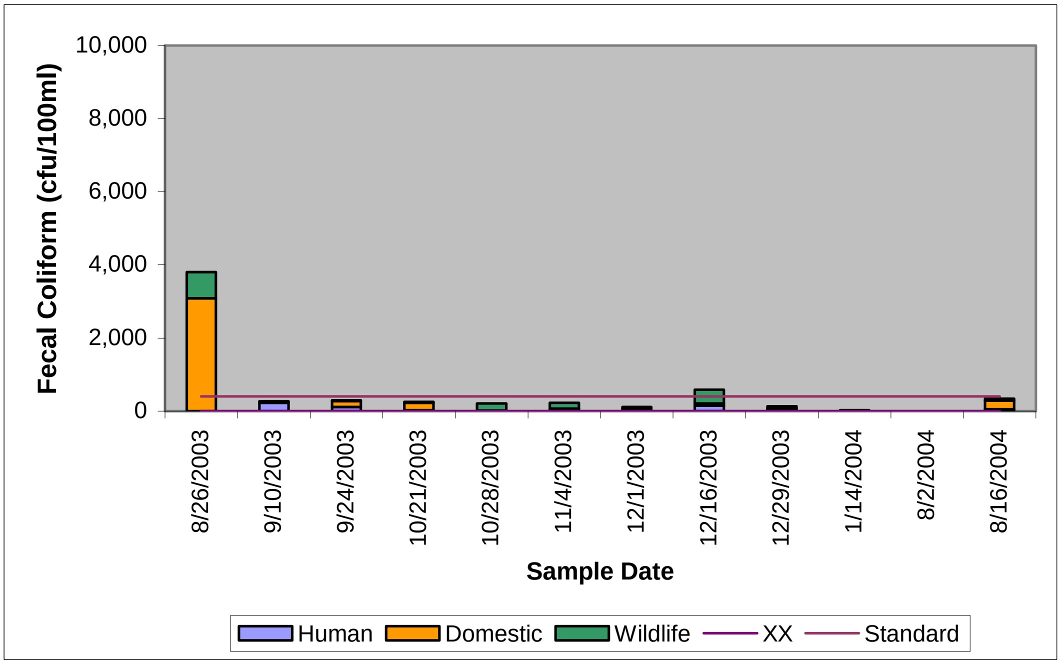

Figure A. 12 **Fecal Coliform enumerations with proportional source contributions indicated for Station MC31 on Little Sugar Creek. Solid colors indicate statistical significance.**

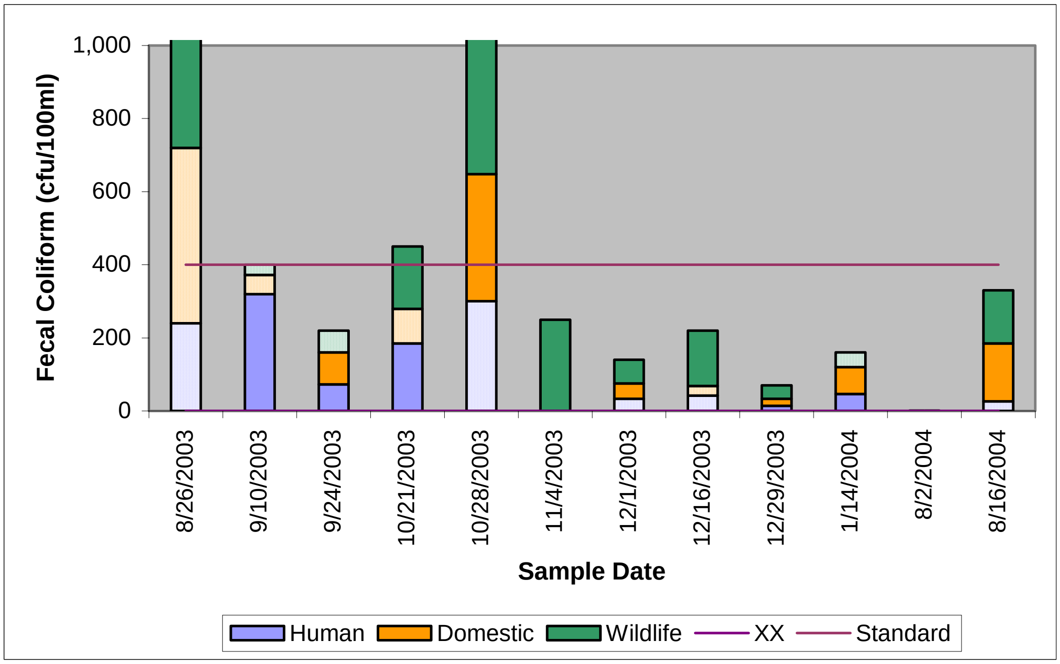

Figure A. 13 **Fecal Coliform enumerations with proportional source contributions indicated for Station MC49A on Little Sugar Creek. Fecal coliform enumerations are censored at 1,000 cfu/100 ml to improve resolution on values near the standard. Solid colors indicate statistical significance.**

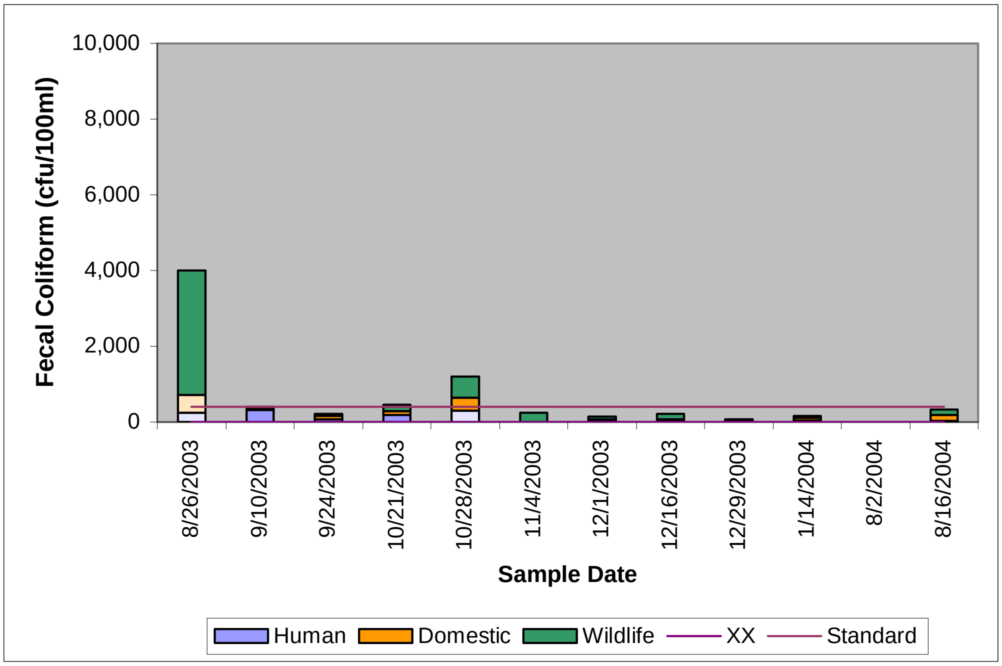

Figure A. 14 **Fecal Coliform enumerations with proportional source contributions indicated for Station MC49A on Little Sugar Creek. Solid colors indicate statistical significance.**

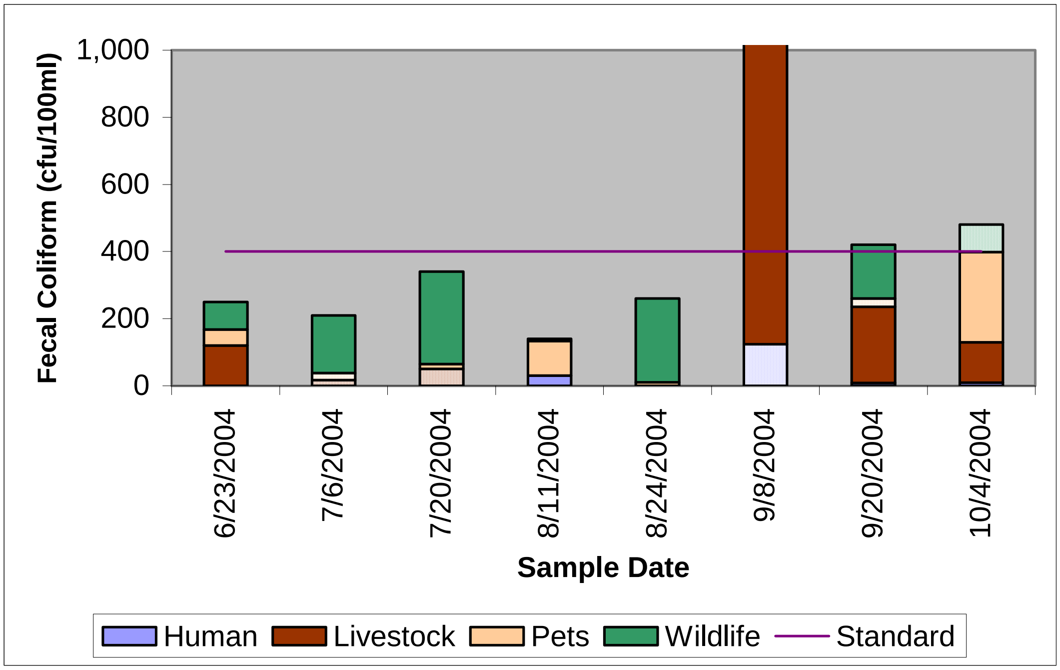

Figure A. 15 **Fecal Coliform enumerations with proportional source contributions indicated for Station CROWDDH202 on Crowders Creek. Fecal coliform enumerations are censored at 1, 000 cfu/100 ml to improve resolution on values near the standard. Solid colors indicate statistical significance.**

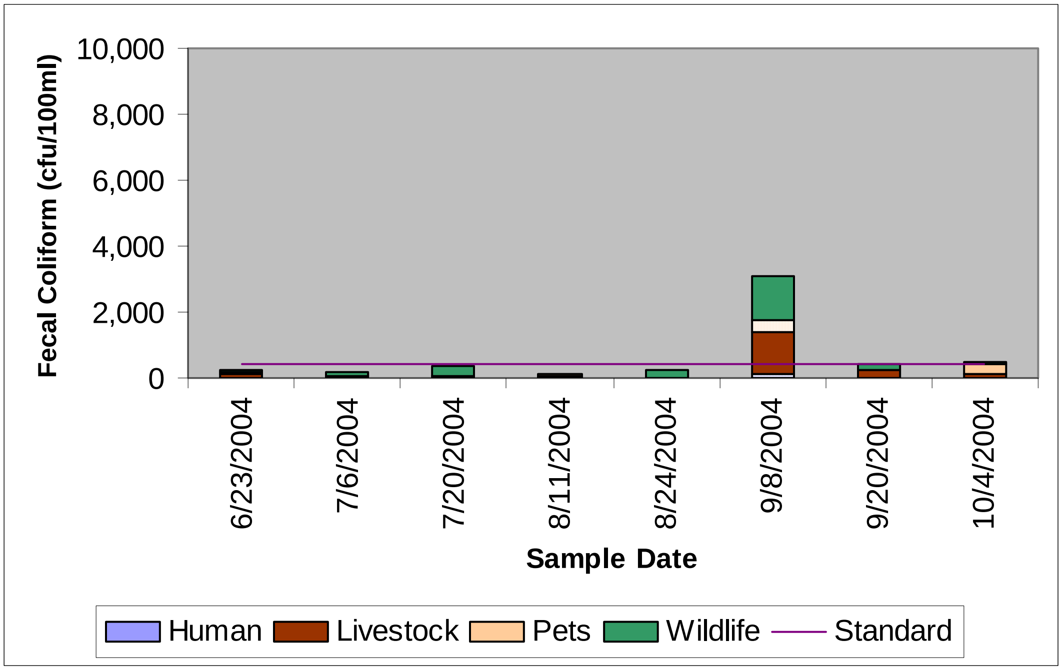

Figure A. 16 **Fecal Coliform enumerations with proportional source contributions indicated for Station CROWDDH202 on Crowders Creek. Solid colors indicate statistical significance.**

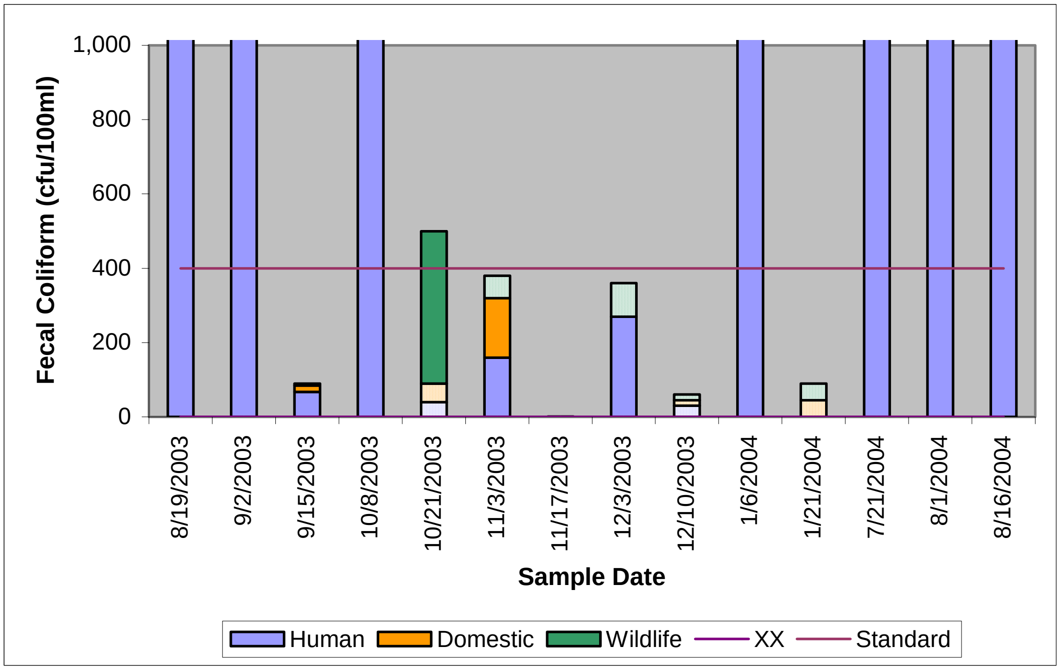

Figure A. 17 **Fecal Coliform enumerations with proportional source contributions indicated for Station 0208732544 on Pigeon House Branch. Fecal coliform enumerations are censored at 1, 000 cfu/100 ml to improve resolution on values near the standard. Solid colors indicate statistical significance.**

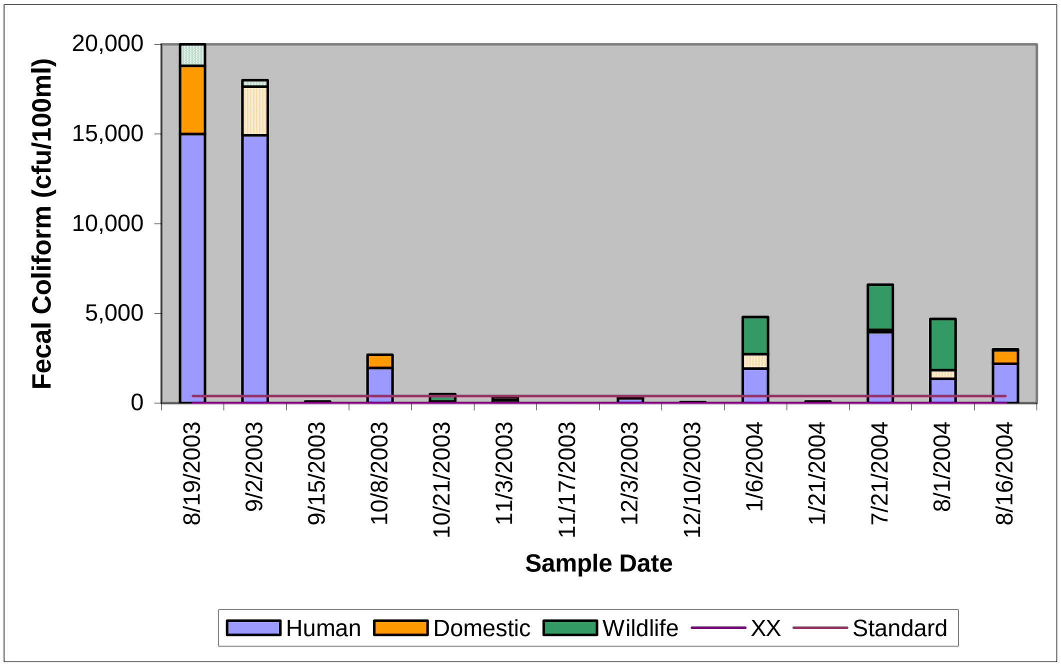

Figure A. 18 **Fecal Coliform enumerations with proportional source contributions indicated for Station 0208732544 on Pigeon House Branch. Solid colors indicate statistical significance.**

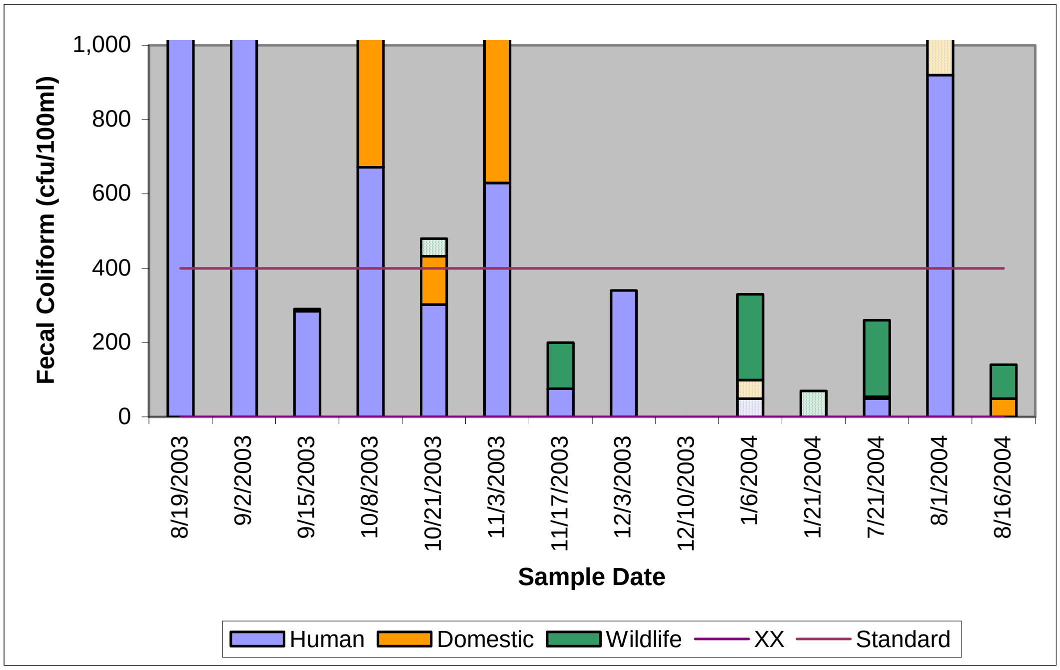

Figure A. 19 **Fecal Coliform enumerations with proportional source contributions indicated for Station ONC-Site 3 on Pigeon House Branch. Fecal coliform enumerations are censored at 1, 000 cfu/100 ml to improve resolution on values near the standard. Solid colors indicate statistical significance.**

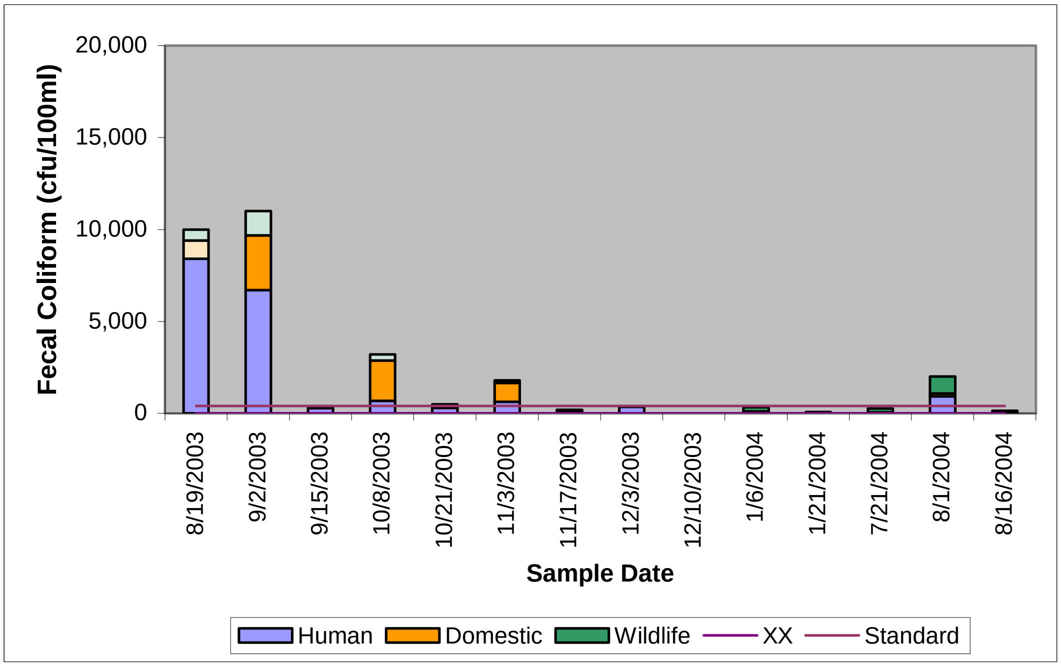

Figure A. 20 **Fecal Coliform enumerations with proportional source contributions indicated for Station ONC-Site 3 on Pigeon House Branch. Solid colors indicate statistical significance.**

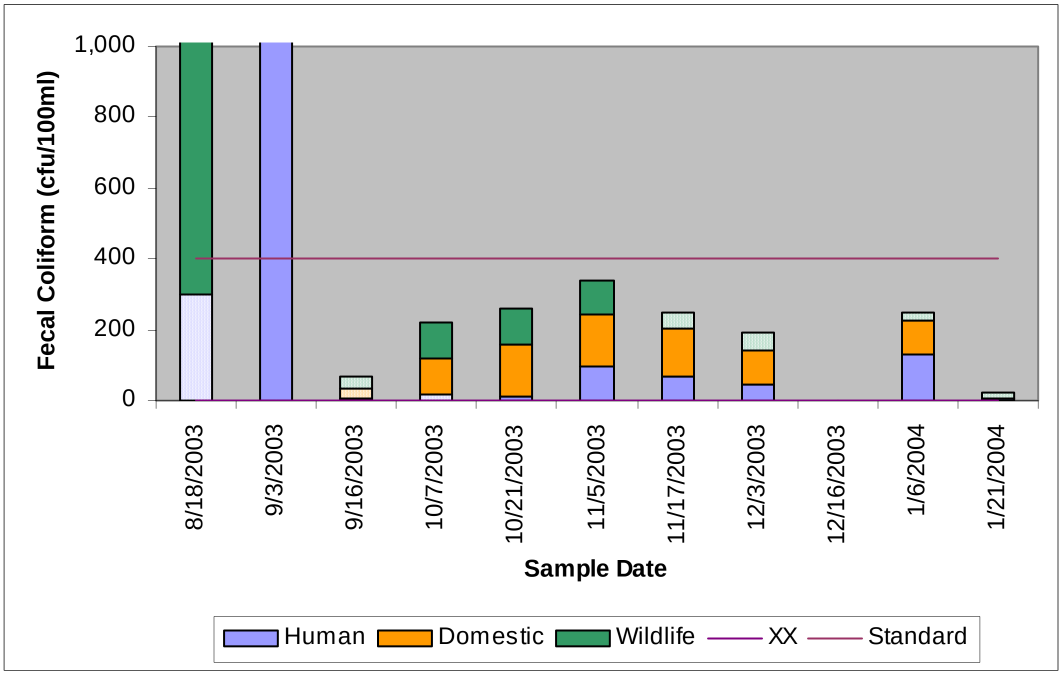

Figure A. 21 **Fecal Coliform enumerations with proportional source contributions indicated for Station CHICOD 06450000 on Chicod Creek. Fecal coliform enumerations are censored at 1, 000 cfu/100 ml to improve resolution on values near the standard. Solid colors indicate statistical significance.**

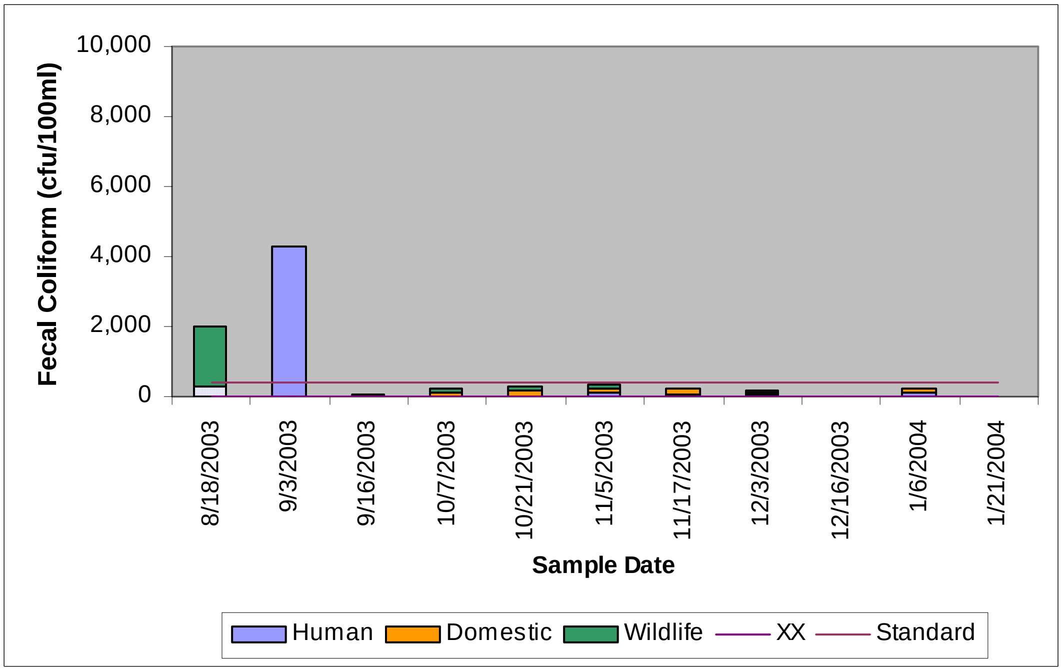

Figure A. 22 **Fecal Coliform enumerations with proportional source contributions indicated for Station CHICOD 06450000 on Chicod Creek. Solid colors indicate statistical significance.**

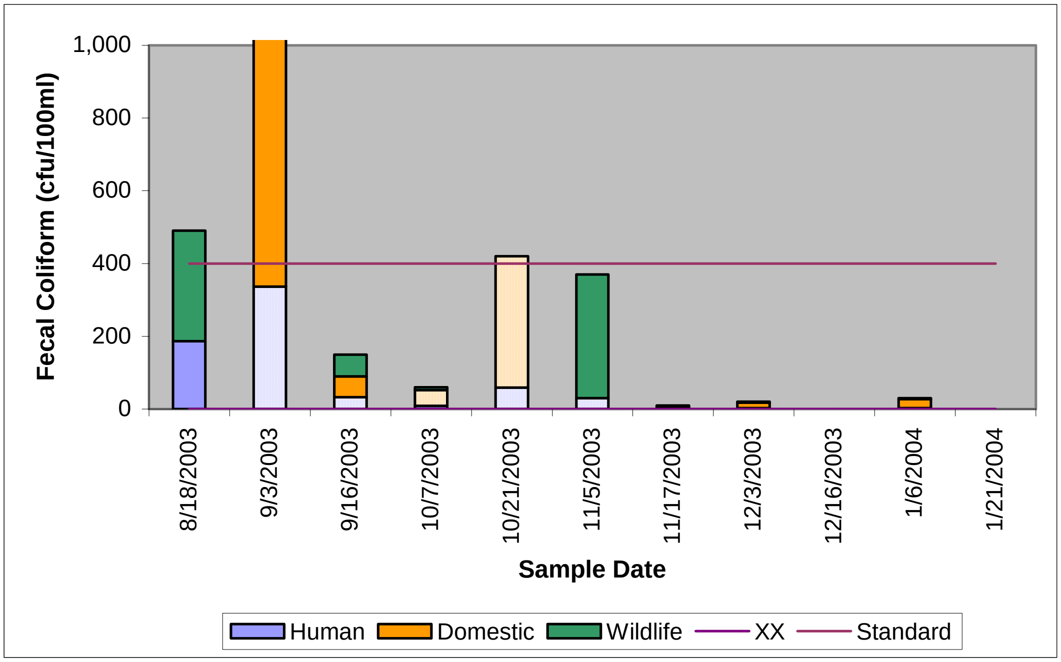

Figure A. 23 **Fecal Coliform enumerations with proportional source contributions indicated for Station CHICOD1 on Chicod Creek. Fecal coliform enumerations are censored at 1, 000 cfu/100 ml to improve resolution on values near the standard. Solid colors indicate statistical significance.**

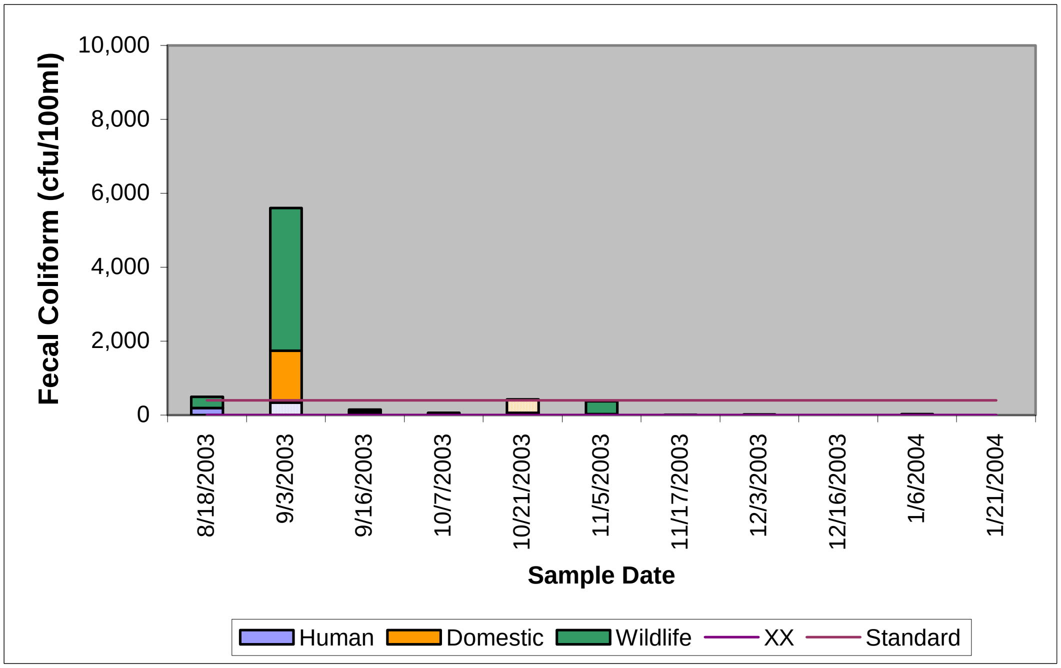

Figure A. 24 **Fecal Coliform enumerations with proportional source contributions indicated for Station CHICOD1 on Chicod Creek. Solid colors indicate statistical significance.**

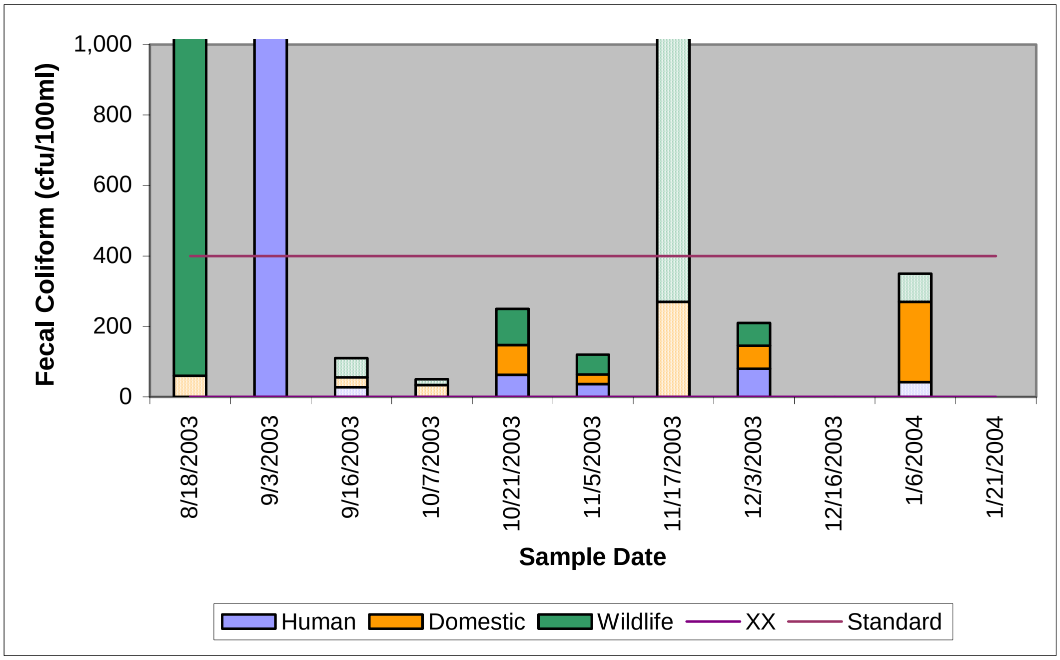

Figure A. 25 **Fecal Coliform enumerations with proportional source contributions indicated for Station CHICOD 2 on Chicod Creek. Fecal coliform enumerations are censored at 1, 000 cfu/100 ml to improve resolution on values near the standard. Solid colors indicate statistical significance.**

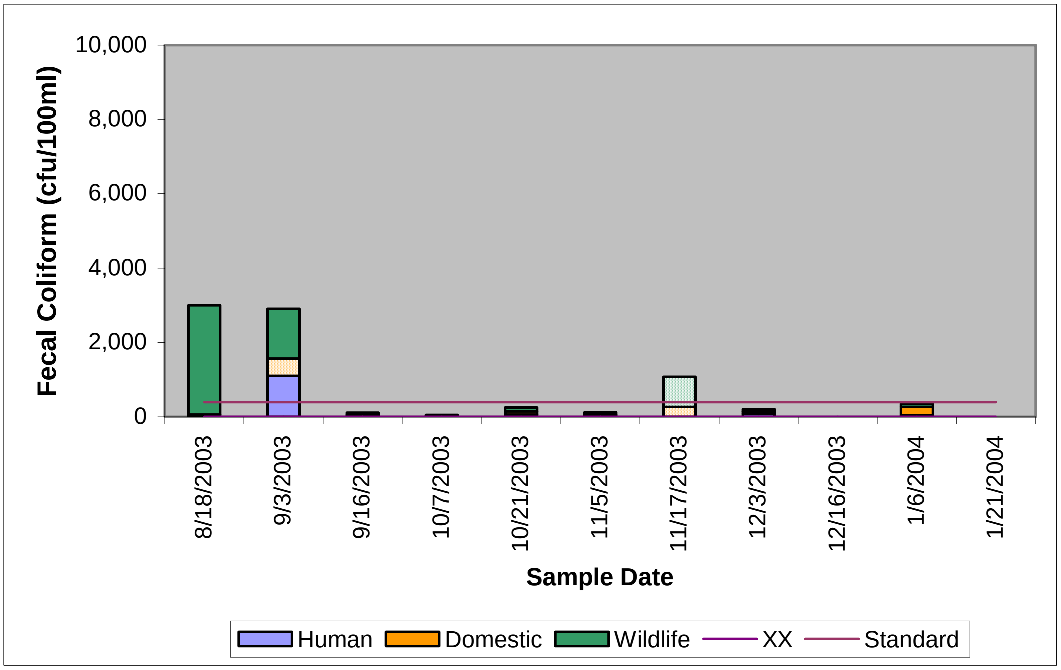

Figure A. 26 **Fecal Coliform enumerations with proportional source contributions indicated for Station CHICOD2 on Chicod Creek. Solid colors indicate statistical significance.**

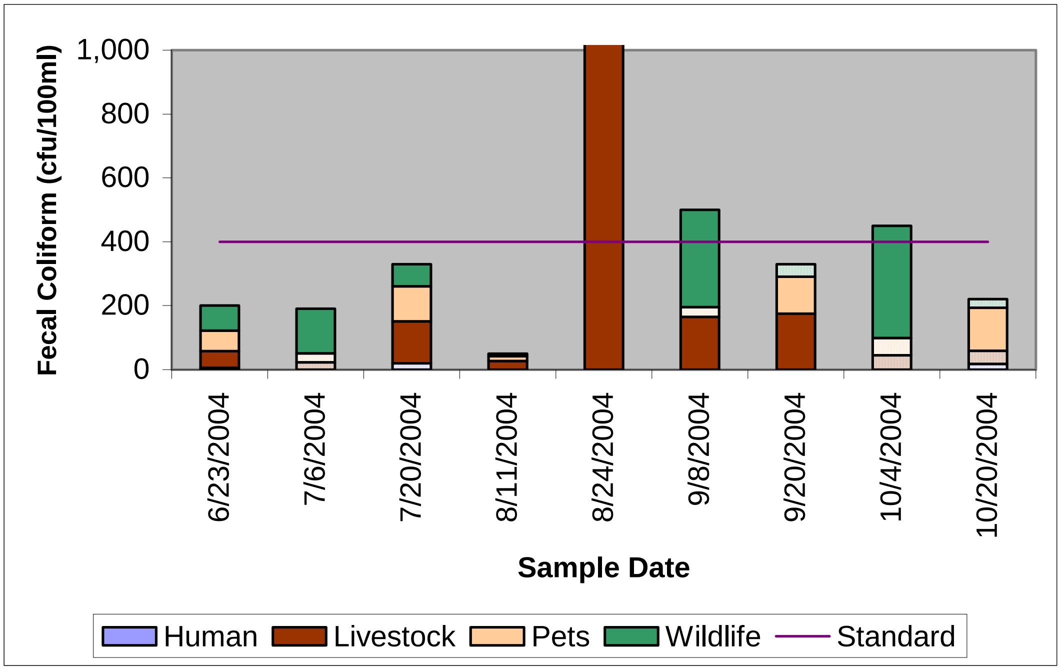

Figure A. 27 **Fecal Coliform enumerations with proportional source contributions indicated for Station GRANTSH202 on Grants Creek. Fecal coliform enumerations are censored at 1,000 cfu/100 ml to improve resolution on values near the standard. Solid colors indicate statistical significance.**

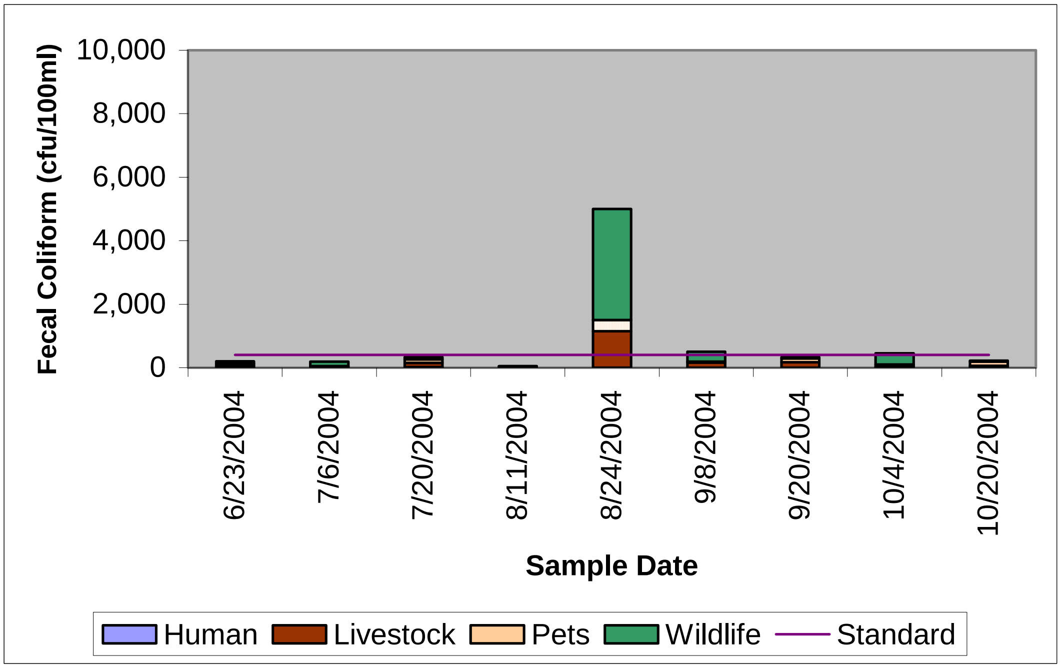

Figure A. 28 **Fecal Coliform enumerations with proportional source contributions indicated for Station GRANTSH202 on Grants Creek. Solid colors indicate statistical significance.**

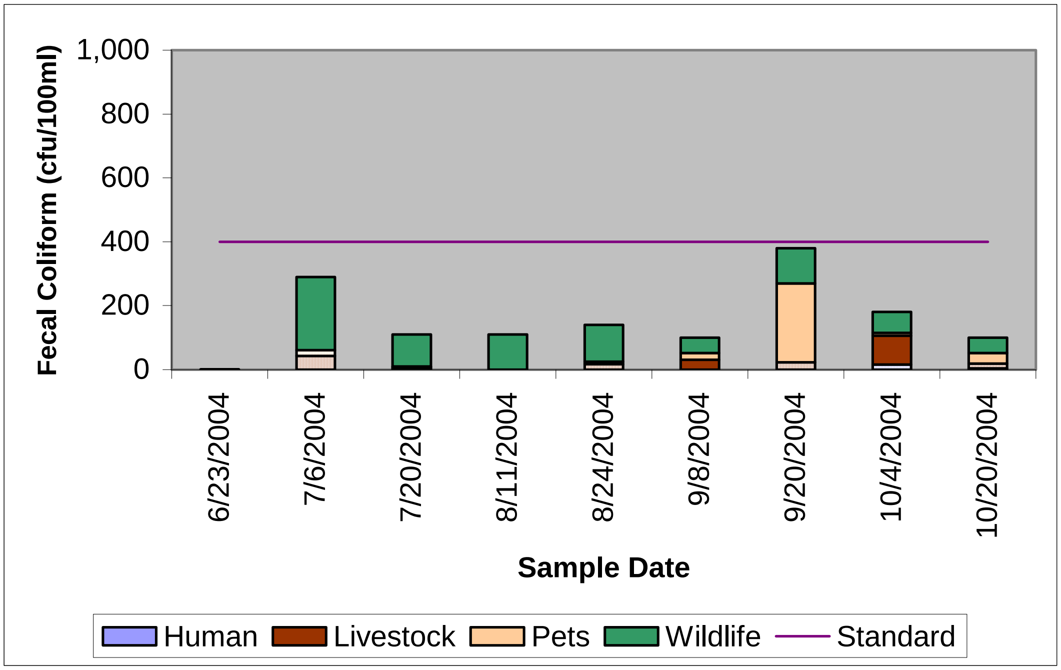

Figure A. 29 **Fecal Coliform enumerations with proportional source contributions indicated for Station 4thH203 on Fourth Creek. Fecal coliform enumerations are censored at 1, 000 cfu/100 ml to improve resolution on values near the standard. Solid colors indicate statistical significance.**