Total Maximum Daily Loads of Fecal Bacteria for the Antietam Creek Basin in Washington County, Maryland

FINAL

MARYLAND
DEPARTMENT OF THE ENVIRONMENT
1800 Washington Boulevard, Suite 540
Baltimore MD 21230-1718

Submitted to:

Water Protection Division
U.S. Environmental Protection Agency, Region III
1650 Arch Street
Philadelphia, PA 19103-2029

September 2009

EPA Submittal Date: June 18, 2008
EPA Approval Date: October 8, 2009

This page deliberately left blank.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

ARCC	Average rates of correct classification
ARA	Antibiotic Resistance Analysis
BMP	Best Management Practice
BST	Bacteria Source Tracking
cfs	Cubic Feet per Second
CFR	Code of Federal Regulations
CFU	Colony Forming Units
COMAR	Code of Maryland Regulations
CSO	Combined Sewer Overflow
CSS	Combined Sewer System
CWA	Clean Water Act
DNR	Department of Natural Resources
EPA	Environmental Protection Agency
GIS	Geographic Information System
LA	Load Allocation
MACS	Maryland Agricultural Cost Share Program
MDE	Maryland Department of the Environment
MDP	Maryland Department of Planning
MGD	Millions of Gallons per Day
ml	Milliliter(s)
MOS	Margin of Safety
MPN	Most Probable Number
MPR	Maximum Practicable Reduction
MS4	Municipal Separate Storm Sewer System
MST	Microbial Source Tracking
NPDES	National Pollutant Discharge Elimination System
NRCS	National Resources Conservation Service
RCC	Rates of Correct Classification
RESAC	Mid-Atlantic Regional Earth Science Applications Center
SSO	Sanitary Sewer Overflows
SW	Stormwater
STATSGO	State Soil Geographic Database
TMDL	Total Maximum Daily Load
USGS	United States Geological Survey
WQIA	Water Quality Improvement Act
WLA	Wasteload Allocation
WQLS	Water Quality Limited Segment
WWTP	Wastewater Treatment Plant

EXECUTIVE SUMMARY

This document, upon approval by the U.S. Environmental Protection Agency (EPA), establishes a Total Maximum Daily Load (TMDL) for fecal bacteria in the Antietam Creek watershed (MD basin number 02-14-05-02). Section 303(d) of the federal Clean Water Act (CWA) and the EPA's implementing regulations direct each state to identify and list waters, known as water quality limited segments (WQLSs), in which current required controls of a specified substance are inadequate to achieve water quality standards. For each WQLS, states are required to either establish a Total Maximum Daily Load (TMDL) of the specified substance that the waterbody can receive without violating water quality standards or demonstrate that water quality standards are being met.

The Maryland Department of the Environment (MDE) has identified Antietam Creek and its tributaries in the State of Maryland's 303(d) List as impaired by low dissolved oxygen, nutrients, and sediments (all listed in 1996), fecal bacteria (listed in 2002) and impacts to biological communities (listed in 2002). Antietam Creek and its tributaries have been designated as Use IV-P (Recreational Trout Waters and Public Water Supply). Beaver Creek, Marsh Run, Little Antietam Creek and their tributaries have been designated as Use III-P (Nontidal Cold Water and Public Water Supply). See Code of Maryland Regulations (COMAR) 26.08.02.08Q. This document proposes to establish a TMDL for fecal bacteria in the Antietam Creek watershed that will allow for attainment of the beneficial use designation of primary contact recreation. A TMDL for Biochemical Oxygen Demand, addressing the low dissolved oxygen listing, was approved by EPA in 2002. The listings for nutrients, sediments and impacts to biological communities will be addressed in separate TMDL documents. MDE monitored the Antietam Creek watershed from 2002-2003 for fecal bacteria. A data solicitation for fecal bacteria was conducted by MDE in 2003, and all readily available data from the past five years were considered.

For this TMDL analysis, the Antietam Creek watershed has been divided into nine subwatersheds. For convenience, each subwatershed will be referenced by the downstream bacteria monitoring station's name and location. The nine subwatersheds are ANT0366 (Antietam Creek in Rocky Forge), ANT0277 (Antietam Creek at Marsh Run), MRS0000 (Marsh Run), ANT0223 (Antietam Creek in Funkstown), ANT0132 (Antietam Creek in Devil's Backbone Park), BEC0001 (Beaver Creek), LAS0004 (Little Antietam Creek in Keedysville), ANT0044 (Antietam Creek at Burnside Bridge) and ANT0002 (Antietam Creek in Antietam). The pollutant loads set forth in this document are for these nine subwatersheds. To establish baseline and allowable pollutant loads for this TMDL, a flow duration curve approach was employed, using bacteria data from MDE and flow strata estimated from United States Geological Survey (USGS) daily flow monitoring. The sources of fecal bacteria are estimated at nine representative stations in the Antietam Creek watershed where samples were collected for one year. Multiple antibiotic resistance analysis (ARA) source tracking was used to determine the relative proportion of domestic (pets and human associated animals), human (human waste), livestock (agriculture-related animals), and wildlife (mammals and waterfowl) source categories.

The baseline load is estimated from current monitoring data using a long-term geometric mean and weighting factors from the flow duration curve. The TMDL for fecal bacteria entering the

Antietam Creek watershed is established after considering three different hydrological conditions: high flow and low flow annual conditions, and an average seasonal condition (the period between May 1st and September 30th when water contact recreation is more prevalent). The allowable load quantified by the TMDL is reported in units of Most Probable Number (MPN)/day and represents a long-term load estimated over a variety of hydrological conditions.

Two scenarios were developed, with the first assessing if attainment of current water quality standards could be achieved by applying maximum practicable reductions (MPRs), and the second applying higher reductions than MPRs. Scenario solutions were based on an optimization method where the objective was to minimize the overall risk to human health, assuming that the risk varies over the four bacteria source categories. In all nine subwatersheds, it was estimated that water quality standards could not be attained with MPRs; thus, higher reductions were applied.

The MD 8-digit Antietam Creek Total Baseline Load is 5,793,581 billion MPN *E. coli*/year. This baseline load consists of upstream loads generated outside the assessment unit (i.e., the MD 8-digit watershed): a Pennsylvania Upstream Baseline (BL_{PA}) of 2,324,273 billion MPN *E. coli*/year, plus loads generated within the assessment unit: a MD 8-digit Antietam Creek Baseline Load Contribution of 3,469,308 billion MPN *E. coli*/year. The baseline loads are summarized in the following table:

MD 8-Digit Antietam Creek Fecal Bacteria Baseline Loads (Billion MPN *E. coli*/year)

Total Baseline Load	=	**Upstream Baseline Load[1] (BL_{PA})**	+	**MD 8-digit Antietam Creek Baseline Load Contribution**
5,793,581	=	2,324,273	+	3,469,308

[1] Although the upstream baseline load is reported here as a single value, it could include point and nonpoint sources.

The MD 8-digit Antietam Creek TMDL of fecal bacteria consists of an annual average allocation attributed to loads generated outside the assessment unit: a Pennsylvania Upstream Load Allocation (LA_{PA}), plus allocations attributed to loads generated within the assessment unit: a MD 8-digit Antietam Creek TMDL Contribution.

The MD 8-digit Antietam Creek TMDL Contribution, representing the sum of individual TMDLs for the nine subwatersheds or portions thereof within MD, is distributed between a load allocation (LA_{ANT}) for nonpoint sources and waste load allocations (WLA_{ANT}) for point sources. Point sources include National Pollutant Discharge Elimination System (NPDES) wastewater treatment plants (WWTPs) and NPDES regulated stormwater (SW) discharges, including county and municipal separate storm sewer systems (MS4s). The margin of safety (MOS) has been incorporated using a conservative assumption by estimating the loading capacity of the stream based on a water quality endpoint concentration more stringent than the applicable MD water quality standard criterion. The *E. coli* water quality criterion concentration was reduced by 5%, from 126 MPN/100ml to 119.7 MPN/100ml.

The MD 8-digit Antietam Creek TMDL of fecal bacteria is presented in the following table:

MD 8-Digit Antietam Creek Fecal Bacteria TMDL (Billion MPN *E. coli*/year)

TMDL	=	LA			+	WLA			+	MOS
		LA_{PA}[1]	+	LA_{ANT}		SW WLA_{ANT}	+	WWTP WLA_{ANT}		
382,109	=	121,716	+	189,808	+	47,810	+	22,775	+	Incorporated

Upstream Load Allocation

MD 8-digit Antietam Creek TMDL Contribution (260,393)

[1] Although the upstream load is reported here as a single value, it could include point and nonpoint sources.

The LA_{PA}, accounting for portions of subwatersheds located in Pennsylvania, is determined to be necessary in order to meet MD water quality standards in the MD portion of the Antietam Creek watershed. The LA_{PA} represents a reduction of approximately 95% from the PA baseline load of 2,324,273 billion MPN *E. coli*/year. The MD 8-digit TMDL Contribution (260,393 billion MPN *E. coli*/year) represents a reduction of approximately 92% from the MD 8-digit Baseline Load Contribution of 3,469,308 billion MPN *E. coli*/year.

Pursuant to recent EPA guidance (US EPA 2006a), maximum daily load (MDL) expressions of the long-term annual average TMDLs are also provided, as shown in the following table:

MD 8-Digit Antietam Creek Fecal Bacteria MDL Summary (Billion MPN *E. coli*/day)

MDL	=	LA			+	WLA			+	MOS
		LA_{PA}[1]	+	LA_{ANT}		SW WLA_{ANT}	+	WWTP WLA_{ANT}		
11,192	=	3,742	+	5,381	+	1,874	+	194	+	Incorporated

Upstream MDL

MD 8-digit Antietam Creek MDL Contribution (7,449)

Once EPA has approved a TMDL, MDE intends for the required reductions to be implemented in an iterative process that first addresses those sources most severely impacting water quality and posing the greatest risks to human health, with consideration given to ease and cost of implementation. In addition, follow-up monitoring plans will be established to track progress and to assess the implementation efforts. As previously stated, water quality standards cannot be attained in any of the nine subwatersheds using the MPR scenario. MPRs may not be sufficient in subwatersheds where wildlife is a significant component or where very high reductions of fecal bacteria loads are required to meet water quality standards. In these cases, it is expected that the MPR scenario will be the first stage of TMDL implementation. Progress will be made through the iterative implementation process described above, and the situation will be reevaluated in the future.

1.0 INTRODUCTION

This document, upon approval by the U.S. Environmental Protection Agency (EPA), establishes a Total Maximum Daily Load (TMDL) for fecal bacteria in the Antietam Creek watershed (MD basin number 02-14-05-02). Section 303(d)(1)(C) of the federal Clean Water Act (CWA) and the U.S. Environmental Protection Agency's (EPA) implementing regulations direct each state to develop a TMDL for each impaired water quality limited segment (WQLS) on the Section 303(d) List, taking into account seasonal variations and a protective margin of safety (MOS) to account for uncertainty. A TMDL reflects the total pollutant loading of the impairing substance a waterbody can receive and still meet water quality standards.

TMDLs are established to achieve and maintain water quality standards. A water quality standard is the combination of a designated use for a particular body of water and the water quality criteria designed to protect that use. Designated uses include activities such as swimming, drinking water supply, and shellfish propagation and harvest. Water quality criteria consist of narrative statements and numeric values designed to protect the designated uses. Criteria may differ among waters with different designated uses.

Antietam Creek and its tributaries have been identified in the State of Maryland's 303(d) List as impaired by low dissolved oxygen, nutrients, and sediments (all listed in 1996), fecal bacteria (listed in 2002) and impacts to biological communities (listed in 2002). Antietam Creek and its tributaries have been designated as Use IV-P (Recreational Trout Waters and Public Water Supply). Beaver Creek, Marsh Run, Little Antietam Creek and their tributaries have been designated as Use III-P (Nontidal Cold Water and Public Water Supply). See Code of Maryland Regulations (COMAR) 26.08.02.08Q. This document proposes to establish a TMDL for fecal bacteria in the Antietam Creek watershed that will allow for attainment of the beneficial use designation of primary contact recreation. A TMDL for Biochemical Oxygen Demand, addressing the low dissolved oxygen listing, was approved by EPA in 2002. The listings for nitrogen, sediments and impacts to biological communities will be addressed in separate TMDL documents. MDE monitored the Antietam Creek watershed from 2002-2003 for fecal bacteria. A data solicitation for fecal bacteria was conducted by MDE in 2003, and all readily available data from the past five years were considered.

Fecal bacteria are microscopic single-celled organisms (primarily fecal coliform and fecal streptococci) found in the wastes of warm-blooded animals. Their presence in water is used to assess the sanitary quality of water for body-contact recreation, for consumption of molluscan bivalves (shellfish), and for drinking water. Excessive amounts of fecal bacteria in surface water used for recreation are known to indicate an increased risk of pathogen-induced illness to humans. Infections due to pathogen-contaminated recreation waters include gastrointestinal, respiratory, eye, ear, nose, throat, and skin diseases (US EPA 1986).

In 1986, EPA published "Ambient Water Quality Criteria for Bacteria," in which three indicator organisms were assessed to determine their correlation with swimming-associated illnesses. Fecal coliform, *E. coli* and enterococci were the indicators used in the analysis. Fecal coliform bacteria are a subgroup of total coliform bacteria and *E. coli* bacteria are a subgroup of fecal coliform bacteria. Most *E. coli* are harmless and are found in great quantities in the intestines of people and warm-blooded animals. However, certain pathogenic strains may cause illness. Enterococci are a subgroup of bacteria in the fecal streptococcus group. Fecal coliform, *E. coli* and enterococci can all be classified as fecal bacteria. The results of the EPA study demonstrated that fecal coliform showed less correlation to swimming-associated gastroenteritis than did either *E. coli* or enterococci.

Based on EPA's guidance (US EPA 1986), adopted by Maryland in 2004, the State has revised the bacteria water quality criteria and it is now based on water column limits for either *E. coli* or enterococci. Because multiple monitoring datasets are available within this watershed for various pathogen indicators, the general term fecal bacteria will be used to refer to the impairing substance throughout this document. The TMDL will be based on the pathogen indicator organisms specified in MD's current bacteria water quality criteria, either *E. coli* or enterococci. The indicator organism used in the Antietam Creek TMDL analysis was *E. coli*.

2.0 SETTING AND WATER QUALITY DESCRIPTION

2.1 General Setting

Location

The Antietam Creek watershed is located in both Maryland (MD) and Pennsylvania (PA) with a drainage area of 291 square miles (186,166 acres). The majority (64%) of the watershed is in Washington County, MD with portions in Franklin and Adams Counties in PA (See Figure 2.1.1).

The main metropolitan area, Hagerstown, is centrally located along the western edge of the watershed. Antietam Creek and its tributaries flow through several small towns including Mount Alto and Waynesboro in Pennsylvania and Smithsburg, Boonsboro and Sharpsburg in Maryland. The headwaters of Antietam Creek originate south of Waynesboro, PA with the confluence of East and West Branch Antietam Creek. It continues flowing southwest past Hagerstown, MD then through Antietam National Battlefield in Sharpsburg, and empties into the Potomac River near the town of Antietam**.**

The tributaries of Antietam Creek include, West Branch Antietam Creek, East Branch Antietam Creek, Little Antietam Creek (north), Marsh Run, Hamilton Run, Landis Spring Branch, Beaver Creek, Little Antietam Creek (south), and Sharmans Branch. The East and West Branches of Antietam Creek are located almost entirely in PA. Marsh Run flows through both PA and MD. The other tributaries are located entirely in MD.

Geology/Soils

Antietam Creek is located in the Valley and Ridge physiographic region. The watershed lies in an area known as the Great Valley, also called the Hagerstown Valley. The geology of this valley is comprised of Cambrian and Ordovician limestone and dolomite (Edwards 1981). This carbonate bedrock is the reason for the valley's karst landscape. A characteristic of karst terrains is the loss of surface water to the subsurface (Reger 2007).

The overlying soils are composed predominantly of the Hagerstown-Duffield-Clarksburg soil association. This association is comprised of deep and very deep, well drained soils formed from limestone bedrock. The Hagerstown and Duffield series have moderate permeability and for the Clarksburg series permeability is slow to moderately slow (NRCS 2004). Spatial distributions for each soil series are shown in Figure 2.1.2.

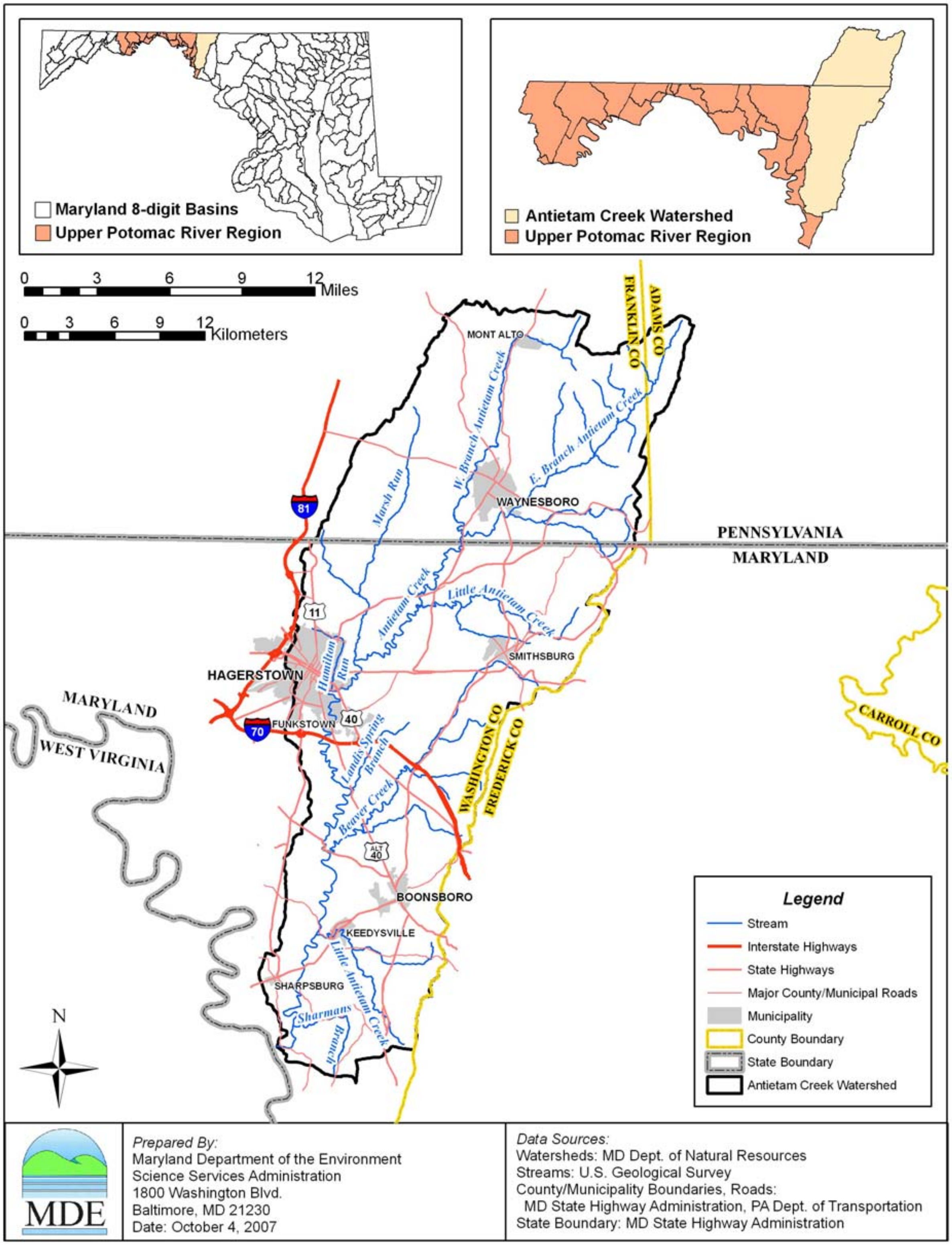


Figure 2.1.1: Location Map of the Antietam Creek Watershed

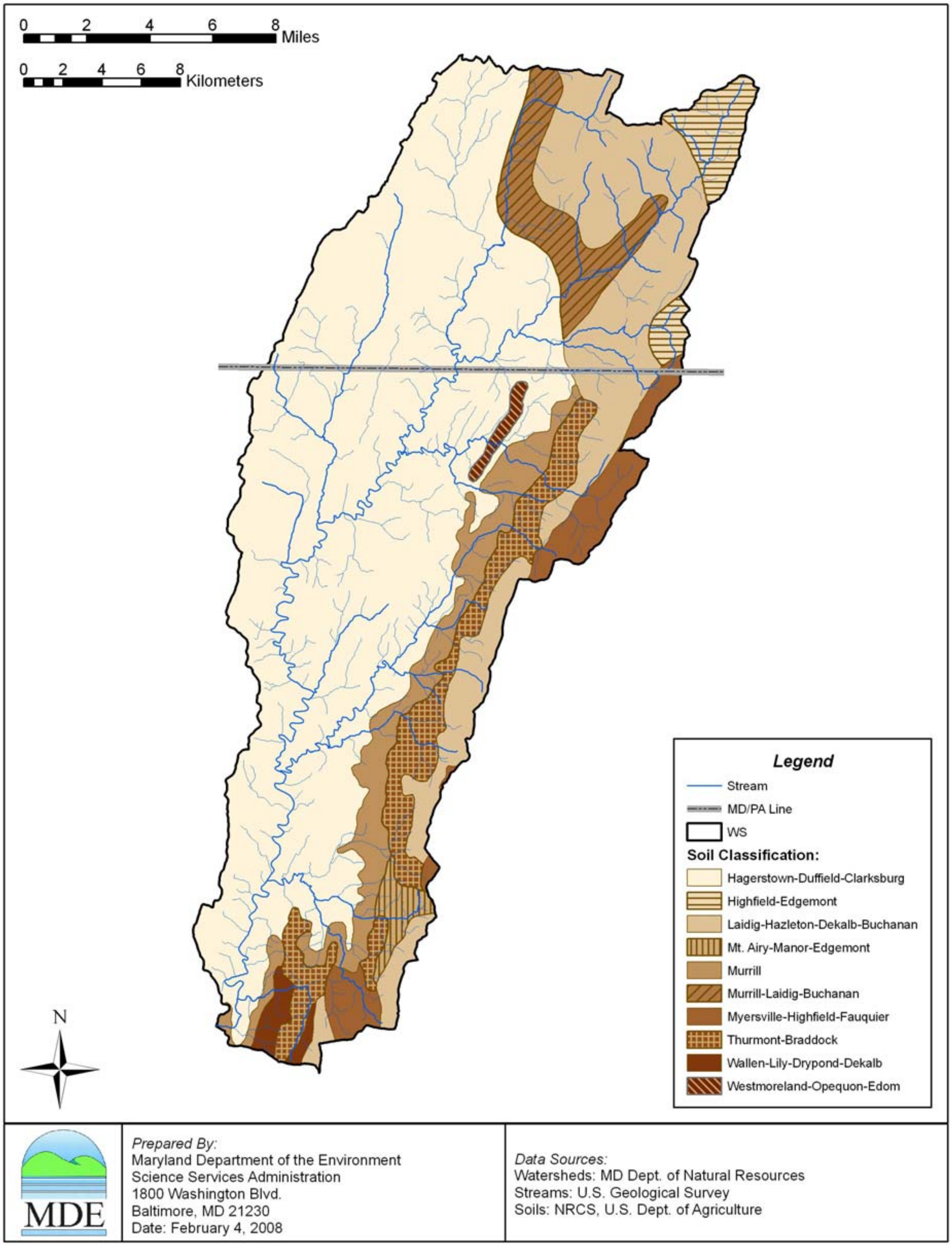


Figure 2.1.2: General Soil Associations in the Antietam Creek Watershed

Land Use

The Antietam Creek watershed covers an area of 186,166 acres in MD and PA. Based on the 2002 Maryland Department of Planning (MDP) land use/land cover data, MD's portion of the watershed is primarily cropland with significant forest and urban land as well. Regional Earth Science Application Center (RESAC) land use/land cover was used to estimate the land use for the PA portion of the watershed. RESAC shows that the PA portion is largely forest and cropland.

The forested areas are mostly in the eastern portion of the watershed where South Mountain State Park, Greenbrier State Park and Washington Monument State Park are located. The two major urban areas of the watershed are Hagerstown, MD and Waynesboro, PA.

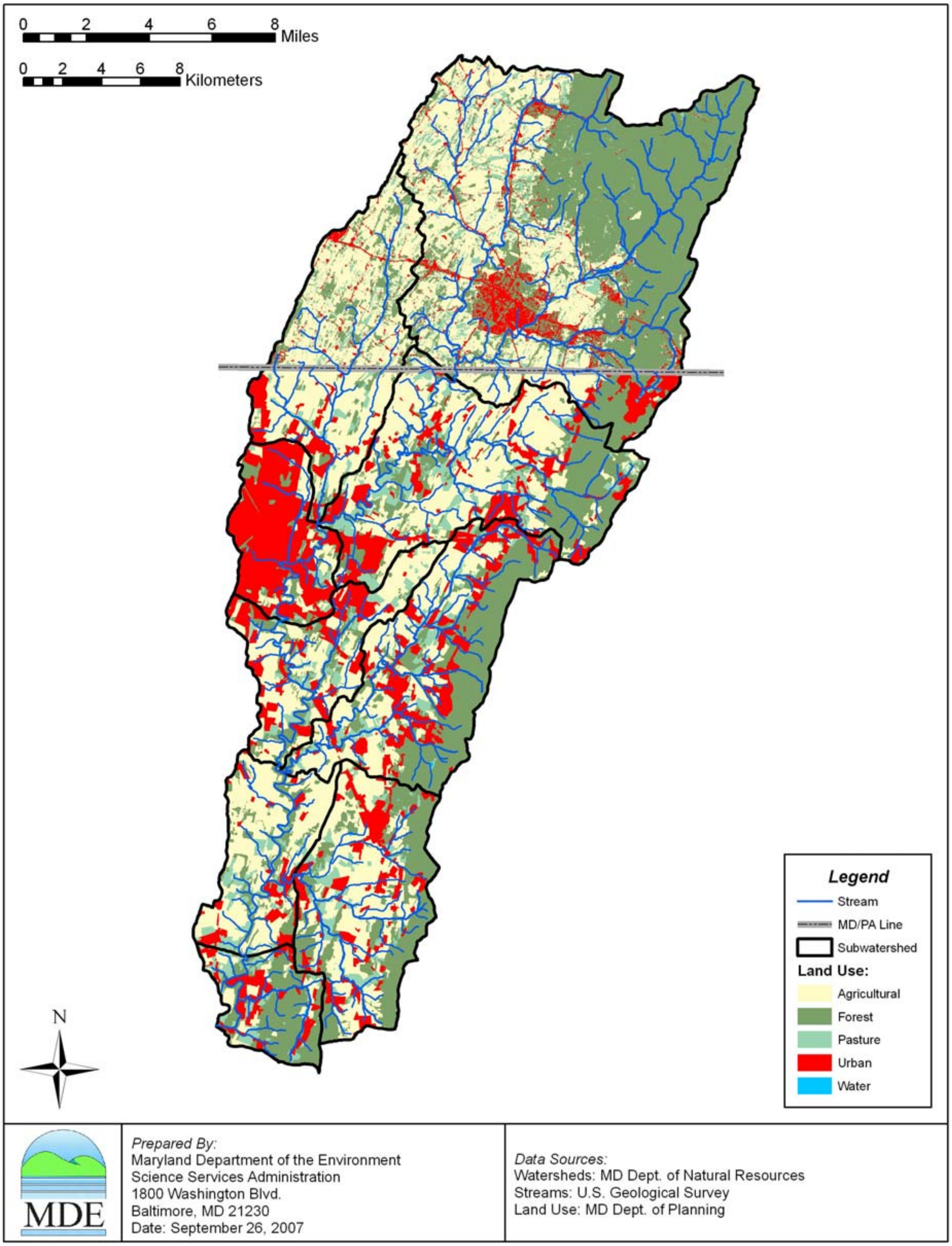
The land use acreage and percentage distribution is shown in Table 2.1.1, and spatial distributions for each land use are shown in Figure 2.1.3. Table 2.1.2 shows the land use percentage distribution for each of the nine subwatersheds considered in the analysis. Note that the subwatersheds are identified by the MDE monitoring stations located in the mainstem of the river and its main tributaries, and are listed by order of flow from upstream to downstream.

Table 2.1.1: Land Use Percentage Distribution for the Antietam Creek Watershed

Land Type	**Maryland Area**		**Pennsylvania Area**		***Total***	
	Acres	**%**	**Acres**	**%**	**Acres**	**%**
Agricultural	46,414	39.1	25,873	38.3	72,287	38.8
Forest	34,400	29.0	31,487	46.6	65,887	35.4
Urban	26,835	22.6	4,755	7.0	31,590	17.0
Pasture	10,826	9.1	5,395	8.0	16,221	8.7
Water	145	0.1	36	0.1	181	0.1
Total	**118,620**	**100**	**67,546**	**100**	**186,166**	**100**

Table 2.1.2: Land Use Percentage Distribution for the Antietam Creek Watershed

Station / Subwatershed	Land Use Area (%)				
	Agricultural	Forest	Urban	Pasture	Water
ANT0366 / Antietam Creek in Rocky Forge	32.8	51.5	8.6	7.0	0.1
ANT0277 / Antietam Creek at Marsh Run	43.3	28.7	16.7	11.3	0.1
MRS0000 / Marsh Run	64.6	15.4	11.1	8.9	0.0
ANT0223 / Antietam Creek in Funkstown	11.6	12.1	71.8	4.3	0.1
ANT0132 / Antietam Creek in Devil's Backbone Park	48.5	17.0	24.1	10.3	0.1
BEC0001 / Beaver Creek	29.5	40.8	21.1	8.5	0.2
LAS0004 / Little Antietam Creek in Keedysville	39.3	35.2	16.0	9.3	0.1
ANT0044 / Antietam Creek at Burnside Bridge	59.9	18.5	10.7	10.9	0.0
ANT0002 / Antietam Creek in Antietam	14.1	53.1	19.8	12.8	0.2



Prepared By:
Maryland Department of the Environment
Science Services Administration
1800 Washington Blvd.
Baltimore, MD 21230
Date: September 26, 2007

Data Sources:
Watersheds: MD Dept. of Natural Resources
Streams: U.S. Geological Survey
Land Use: MD Dept. of Planning

Figure 2.1.3: Land Use of the Antietam Creek Watershed

Population

The total population in the Antietam Creek watershed is estimated to be 113,162 people. Figure 2.1.4 illustrates the population density in the watershed. The human population and the number of households were estimated based on a weighted average from the 2000 Census GIS Block Groups and the 2002 MDP Land Use Land Cover and the RESAC for PA. Since the boundaries of the watershed differ from the boundaries of the block groups, residential land use data were used to extract the necessary areas of the Census block groups. The residential density designations used for this estimation are shown in Table 2.1.3 and were based on MDP and RESAC information.

Table 2.1.3: Number of Dwellings Per Acre

Land Use Code	Dwellings Per Acre
Low Density Residential	1
Medium Density Residential	5
High Density Residential	8

Based on these densities and the population data from the census block groups the population for each subwatershed was estimated and is presented in Table 2.1.4.

Table 2.1.4: Total Population per Subwatershed in the Antietam Creek Watershed

Station / Subwatershed	Population
ANT0366 / Antietam Creek in Rocky Forge	28,227
ANT0277 / Antietam Creek at Marsh Run	10,625
MRS0000 / Marsh Run	6,512
ANT0223 / Antietam Creek in Funkstown	42,886
ANT0132 / Antietam Creek in Devil's Backbone Park	9,656
BEC0001 / Beaver Creek	6,013
LAS0004 / Little Antietam Creek in Keedysville	6,086
ANT0044 / Antietam Creek at Burnside Bridge	1,851
ANT0002 / Antietam Creek in Antietam	1,306
Total	**113,162**

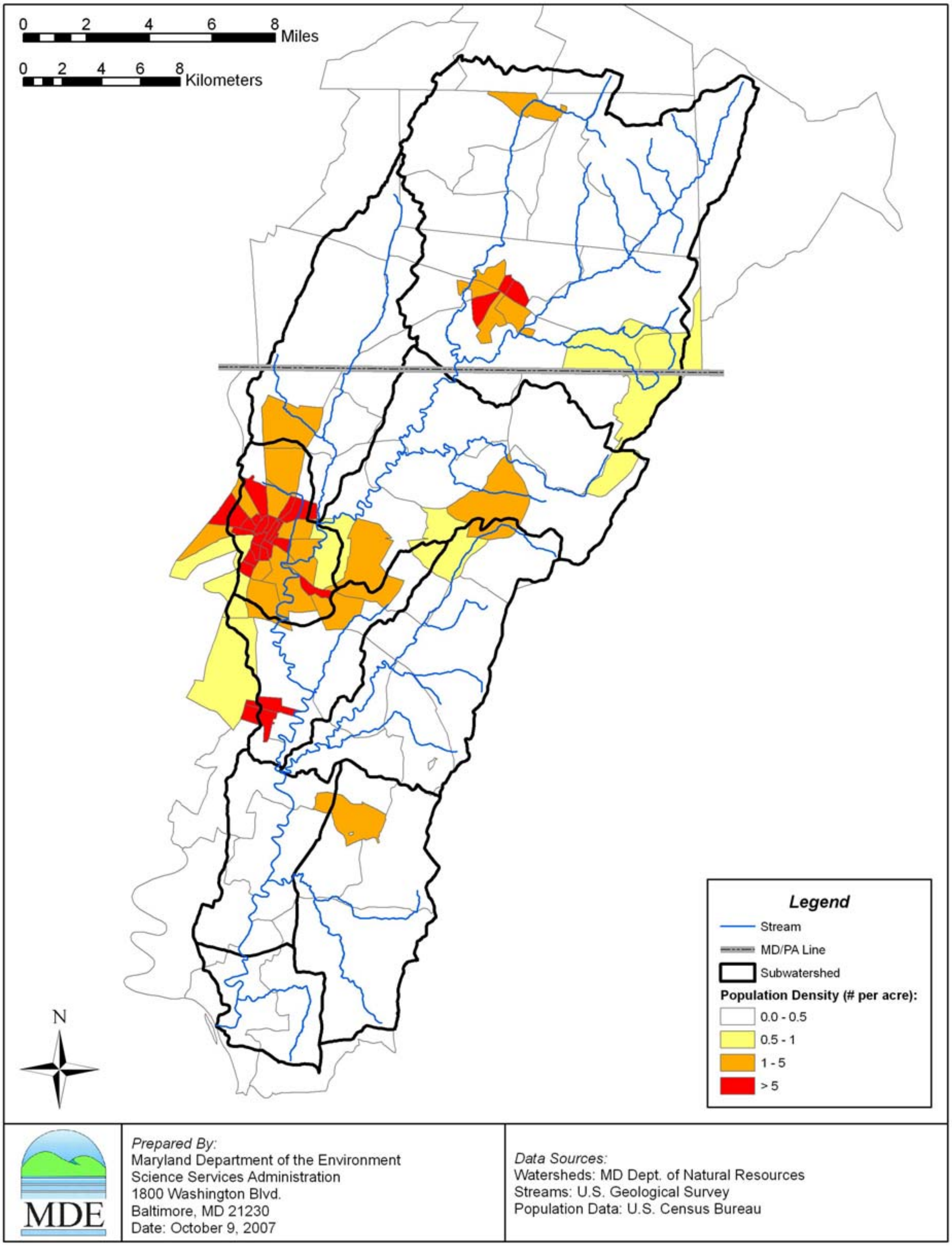


Figure 2.1.4: Population Density in the Antietam Creek Watershed

2.2 Water Quality Characterization

EPA's guidance document, "Ambient Water Quality Criteria for Bacteria" (1986), recommended that states use *E. coli* (for fresh water) or enterococci (for fresh or salt water) as pathogen indicators. Fecal bacteria, *E. coli*, and enterococci were assessed as indicator organisms for predicting human health impacts. A statistical analysis found that the highest correlation to gastrointestinal illness was linked to elevated levels of *E. coli* and enterococci in fresh water (enterococci in salt water).

Pursuant to EPA's guidance, Maryland has adopted the new indicator organisms, *E. coli* and enterococci, for the protection of public health in Use I, II, and IV waters. These bacteria listings were originally assessed using fecal coliform bacteria. The analysis was based on a geometric mean of the monitoring data, where the result had to be less than or equal to 200 MPN/100ml. From EPA's analysis (US EPA 1986), this fecal coliform geometric mean target equates to an approximate risk of 8 illnesses per 1,000 swimmers at fresh water beaches and 19 illnesses per 1,000 swimmers at marine beaches (enterococci only), which is consistent with MDE's revised Use I bacteria criteria. Therefore, the original 303(d) List fecal coliform listings can be addressed using the refined bacteria indicator organisms to ensure that risk levels are acceptable.

<u>Bacteria Monitoring</u>

Table 2.2.1 lists the historical monitoring data for the Antietam Creek watershed. MDE conducted monitoring sampling at nine stations in the Antietam Creek watershed from October 2002 through October 2003. The United States Geological Survey (USGS) gage station 01619500, located near Sharpsburg, MD, was used in deriving the surface water flow. The locations of these stations are shown in Tables 2.2.2 to 2.2.4 and in Figure 2.2.1. Observations recorded from the nine MDE monitoring stations are provided in Appendix A.

Bacteria counts are highly variable which is typical due to the nature of bacteria and their relationship to flow. The *E. coli* counts for the nine stations ranged between 10 and 24,190 MPN/100 ml.

Table 2.2.1: Historical Monitoring Data in the Antietam Creek Watershed

Organization	Date	Design	Summary
DNR	01/1986 through 04/1998	Fecal Coliform*	ANT0044: Antietam Creek just below Burnside Bridge near Sharpsburg, MD
MDE	10/2002 through 10/2003	*E. coli*	9 stations 2 samples per month
MDE	10/2002 through 10/2003	BST (*Enterococcus)*	9 stations 1 sample per month

*Only *E. coli* was used for this analysis.

Table 2.2.2: Location of DNR TREND Monitoring Station in the Antietam Creek Watershed

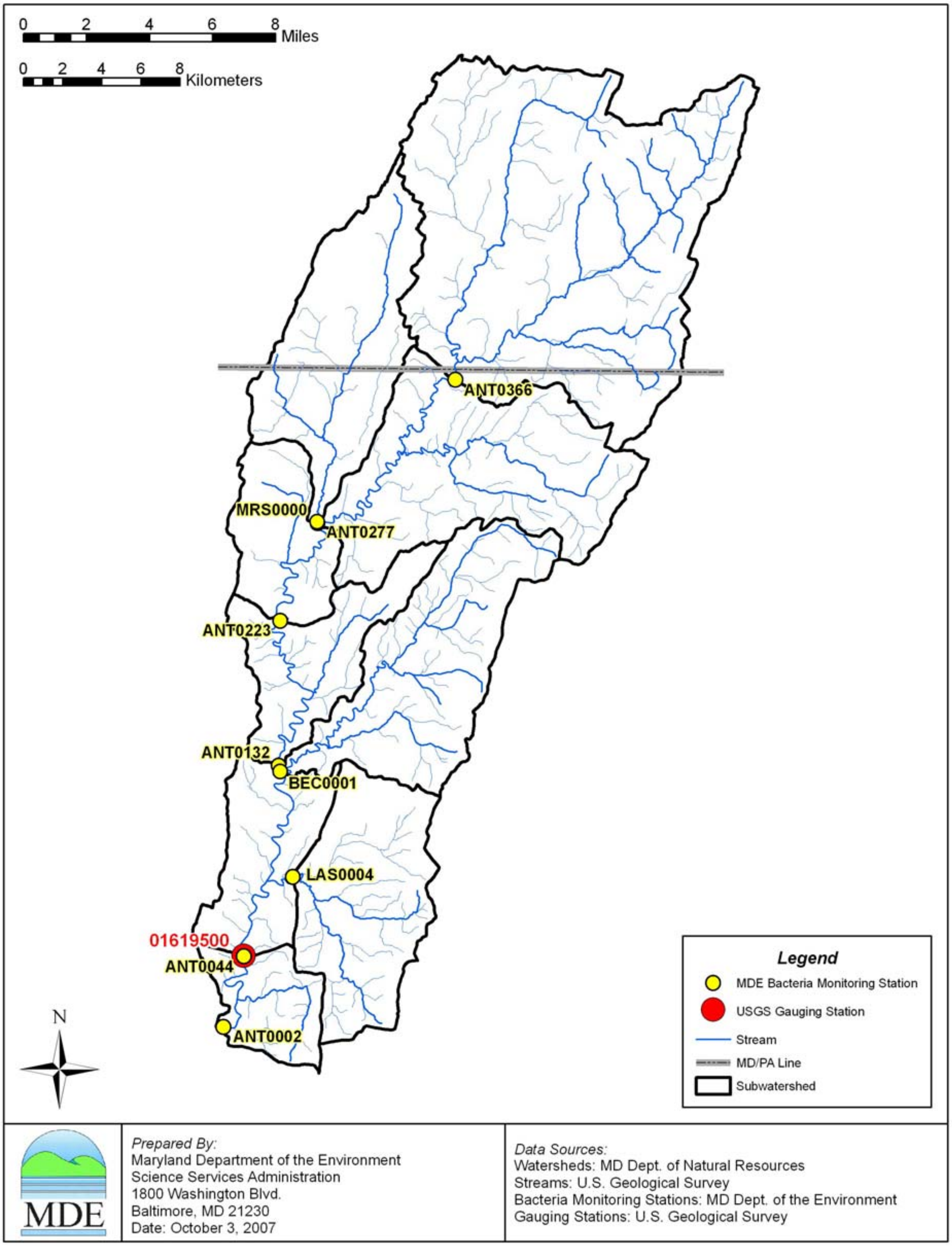
Station	Tributary	Latitude	Longitude
ANT0044	Antietam Creek	39° 27' 1.3"	77° 43' 54.0"

Table 2.2.3: Locations of MDE Monitoring Stations in the Antietam Creek Watershed

Tributary	Station	Observation Period	Total Observations	Latitude	Longitude
Antietam Creek (in Rocky Forge, MD)	ANT0366	2002 - 2003	26	39° 42' 58.4"	77° 36' 24.5"
Antietam Creek (upstream of Marsh Run)	ANT0277	2002 - 2003	24	39° 38' 58.6"	77° 41' 18.0"
Marsh Run (at confluence with Antietam Creek)	MRS0000	2002 - 2003	26	39° 39' 00.5"	77° 41' 19.9"
Antietam Creek (in Funkstown, MD)	ANT0223	2002 - 2003	26	39° 36' 15.4"	77° 42' 35.3"
Antietam Creek (in Devil's Backbone Park)	ANT0132	2002 - 2003	26	39° 32' 15.7"	77° 42' 35.3"
Beaver Creek (at confluence with Antietam Creek)	BEC0001	2002 - 2003	26	39° 32' 05.6"	77° 42' 32.5"
Little Antietam Creek (in Keedysville, MD)	LAS0004	2002 - 2003	26	39° 29' 10.9"	77° 42' 04.7"
Antietam Creek (at Burnside Bridge)	ANT0044	2002 - 2003	26	39° 26' 58.6"	77° 43' 46.4"
Antietam Creek (in Antietam, MD)	ANT0002	2002 - 2003	26	39° 25' 01.3"	77° 44' 31.5"

Table 2.2.4: Location of USGS Gauging Station in Antietam Creek Watershed

Site Number	Observation Period Used	Total Observations	Latitude	Longitude
01619500	1981-2006	9,131	39° 26' 59.2"	77° 43' 48.7"



Prepared By:
Maryland Department of the Environment
Science Services Administration
1800 Washington Blvd.
Baltimore, MD 21230
Date: October 3, 2007

Data Sources:
Watersheds: MD Dept. of Natural Resources
Streams: U.S. Geological Survey
Bacteria Monitoring Stations: MD Dept. of the Environment
Gauging Stations: U.S. Geological Survey

Figure 2.2.1: Monitoring Stations and Subwatersheds in the Antietam Creek Watershed

2.3 Water Quality Impairment

Designated Uses and Water Quality Standard

The Maryland water quality standards Surface Water Use Designation for Beaver Creek, Marsh Run, Little Antietam Creek and their tributaries is Use III-P (Nontidal Cold Water and Public Water Supply). Antietam Creek and its other tributaries have been designated as Use IV-P (Recreational Trout Waters and Public Water Supply). (COMAR 26.08.02.08Q) The Antietam Creek watershed was listed on Maryland's 303(d) List as impaired by fecal bacteria in 2002, due to elevated fecal coliform concentrations detected at the DNR TREND monitoring station ANT0044, which showed a geometric mean of 296 MPN/100ml.

Water Quality Criteria

The State water quality standard for bacteria (*E. coli*) used in this study is as follows:

Table 2.3.1: Bacteria Criteria Values
(Source: COMAR 26.08.02.03-3 Water Quality Criteria Specific to Designated Uses; Table 1)

Indicator	Steady State Geometric Mean Indicator Density
Freshwater	
E. coli	126 MPN/100ml

Interpretation of Bacteria Data for General Recreational Use

The relevant portion (for freshwater) of the listing methodology pursuant to the 2006 Integrated 303(d) List for all Use Waters - Water Contact Recreation and Protection of Aquatic Life is as follows:

Recreational Waters

A steady-state geometric mean will be calculated with available data where there are at least five representative sampling events. The data shall be from samples collected during steady-state conditions and during the beach season (Memorial Day through Labor Day) to be representative of the critical condition. If the resulting steady-state geometric mean is greater than 126 *E. coli* MPN/100 ml in freshwater, the waterbody will be listed as impaired. If fewer than five representative sampling events for an area being assessed are available, data from the previous two years will be evaluated in the same way. The single sample maximum criterion applies only to beaches and is to be used for closure and advisory decisions based on short term exceedances of the geometric mean portion of the standard.

Water Quality Assessment

Bacteria water quality impairment in Antietam Creek was assessed by comparing both the annual and the seasonal (May 1st –September 30th) steady-state geometric means of *E. coli* concentrations with the water quality criterion. Graphs illustrating these results can be found in Appendix B.

The steady-state condition is defined as unbiased sampling targeting average flow conditions and/or equally sampling or providing for unbiased sampling of high and low flows. The 1986 EPA criteria document assumed steady-state flow in determining the risk at various bacterial concentrations, and therefore the chosen criterion value also reflects steady-state conditions (EPA 1986). The steady-state geometric mean condition can be estimated either by monitoring design or more practically by statistical analysis as follows:

1. A stratified monitoring design is used where the number of samples collected is proportional to the duration of high flows, mid flows and low flows within the watershed. This sample design allows a geometric mean to be calculated directly from the monitoring data without bias.

2. Routine monitoring typically results in samples from varying hydrologic conditions (i.e., high flows, mid flows and low flows) where the numbers of samples are not proportional to the duration of those conditions. Averaging these results without consideration of the sampling conditions results in a biased estimate of the steady-state geometric mean. The potential bias of the steady-state geometric means can be reduced by weighting the samples results collected during high flow, mid flow and low flow regimes by the proportion of time each flow regime is expected to occur. This ensures that the high flow and low flow conditions are proportionally balanced.

3. If (1) the monitoring design was not stratified based on flow regime or (2) flow information is not available to weight the samples accordingly, then a geometric mean of sequential monitoring data can be used as an estimate of the steady-state geometric mean condition for the specified period.

A routine monitoring design was used to collect bacteria data in the Antietam Creek watershed. To estimate the steady-state geometric mean, the monitoring data were first reviewed by plotting the sample results versus their corresponding daily flow duration percentile. Graphs illustrating these results can be found in Appendix B.

To calculate the steady-state geometric mean with routine monitoring data, a conceptual model was developed by dividing the daily flow frequency for the stream segment into strata that are representative of hydrologic conditions. A conceptual continuum of flows is illustrated in Figure 2.3.1.

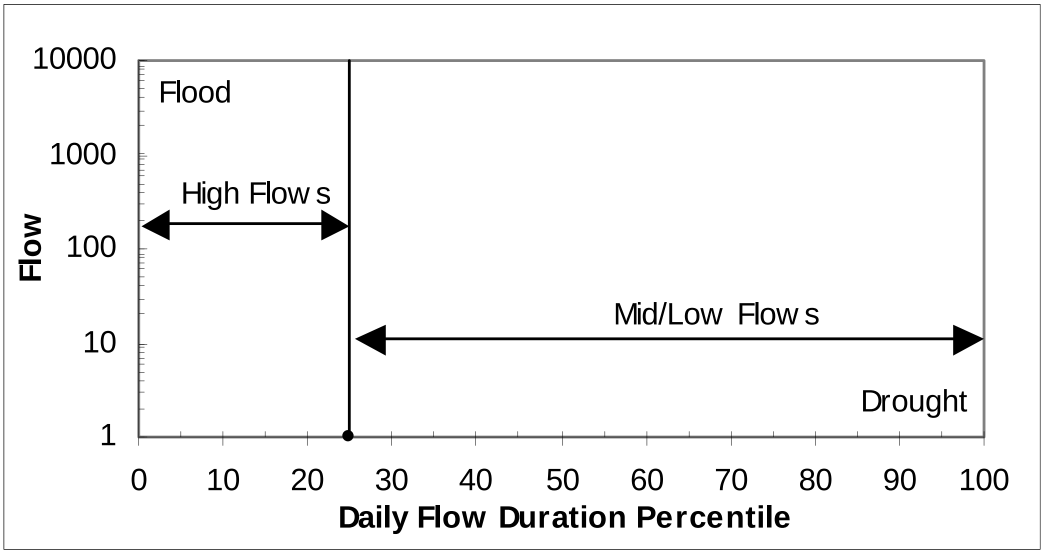


Figure 2.3.1: Conceptual Diagram of Flow Duration Zones

During high flows, a significant portion of the total stream flow is from surface flow contributions. Low flow conditions represent periods with minimal rainfall and surface runoff. There is typically a transitional mid flow period between the high and low flow durations, representative of varying contributions of surface flow inputs that result from differing rainfall volumes and antecedent soil moisture conditions. The division of the entire flow regime into strata enables the estimation of a less biased geometric mean from routine monitoring data that more closely approaches steady-state. Based on flow data of USGS gage 01619500 it was determined that the long-term average daily flow corresponds to a daily flow duration of 32.2%. Hence for this analysis it is defined that flows greater than the 32.2 percentile flow represent high flows, and flows lower than the 32.2 percentile flow represent mid/low flows. A detailed method of how the flow strata were defined is presented in Appendix B.

Factors for estimating a steady-state geometric mean are based on the frequency of each flow stratum. The weighting factor accounts for the proportion of time that each flow stratum represents. The weighting factors for an average hydrological year used in the Antietam Creek TMDL analysis are presented in Table 2.3.2.

Table 2.3.2: Weighting Factors for Average Hydrology Year Used for Estimation of Geometric Means in the Antietam Creek Watershed

Flow Duration Zone	Duration Interval	Weighting Factor
High Flows	0 – 32.2%	0.322
Mid/Low Flows	32.2 – 100%	0.678

Bacteria enumeration results for samples within a specified stratum will receive their corresponding weighting factor. The steady-state geometric mean is calculated as follows:

$$M = \sum_{i=1}^{2} M_i * W_i \qquad (1)$$

where,

$$M_i = \frac{\sum_{j=1}^{n_i} \log_{10}(C_{i,j})}{n_i} \qquad (2)$$

M = log weighted mean
M_i = log mean concentration for stratum i
W_i = proportion of stratum i
$C_{i,j}$ = concentration for sample j in stratum i
n_i = number of samples in stratum

Finally, the steady-state geometric mean concentration is estimated using the following equation:

$$C_{gm} = 10^M \qquad (3)$$

where,

C_{gm} = Steady-state geometric mean concentration

Tables 2.3.3 and 2.3.4 present the maximum and minimum concentrations and the geometric means by stratum, and the overall steady-state geometric mean for the Antietam Creek subwatersheds for the annual and seasonal (May 1st –September 30th) periods. For the seasonal period, insufficient samples fell in the low flow zone. As such, for the seasonal analysis, only the overall geometric mean for the period was applied.

Table 2.3.3: Antietam Creek Watershed Annual Steady-State Geometric Means by Flow Stratum per Subwatershed

Station / Tributary	Flow Stratum	Number of Samples	*E. coli* Minimum Concentration (MPN/100ml)	*E. coli* Maximum Concentration (MPN/100ml)	Annual Steady State Geometric Mean (MPN/100ml)	Annual Weighted Geometric Mean (MPN/100ml)
ANT0366 Antietam Creek in Rocky Forge	**High**	17	190	14,140	1,160	741
	Low	9	17	4,570	599	
ANT0277 Antietam Creek at Marsh Run	**High**	16	200	24,190	966	402
	Low	8	23	2,610	265	
MRS0000 Marsh Run	**High**	17	90	24,190	981	487
	Low	9	7	1,790	349	
ANT0223 Antietam Creek in Funkstown	**High**	17	130	24,190	915	259
	Low	9	10	1,080	142	
ANT0132 Antietam Creek in Devil’s Backbone Park	**High**	17	30	24,190	631	228
	Low	9	30	3,650	140	
BEC0001 Beaver Creek	**High**	17	80	10,460	557	384
	Low	9	60	1,550	321	
LAS0004 Little Antietam Creek in Keedysville	**High**	17	110	12,030	715	296
	Low	9	20	860	195	
ANT0044 Antietam Creek at Burnside Bridge	**High**	17	120	24,190	622	185
	Low	9	20	360	104	
ANT0002 Antietam Creek in Antietam	**High**	17	70	24,190	507	155
	Low	9	10	290	88	

Table 2.3.4: Antietam Creek Watershed Seasonal Period (May 1 - September 30) Steady-State Geometric Mean per Subwatershed

Station / Tributary	Number of Samples	*E. coli* Minimum Concentration (MPN/100ml)	*E. coli* Maximum Concentration (MPN/100ml)	Seasonal Steady State Geometric Mean (MPN/100ml)
ANT0366 Antietam Creek in Rocky Forge	12	640	14,140	2,288
ANT0277 Antietam Creek at Marsh Run	12	390	24,190	1,579
MRS0000 Marsh Run	12	660	24,190	2,311
ANT0223 Antietam Creek in Funkstown	12	450	24,190	1,904
ANT0132 Antietam Creek in Devil's Backbone Park	12	160	24,190	1,337
BEC0001 Beaver Creek	12	400	10,460	1,246
LAS0004 Little Antietam Creek in Keedysville	12	360	12,030	1,378
ANT0044 Antietam Creek at Burnside Bridge	12	170	24,190	1,087
ANT0002 Antietam Creek in Antietam	12	150	24,190	1,037

2.4 Source Assessment

Nonpoint Source Assessment

Nonpoint sources of fecal bacteria do not have one discharge point but occur over the entire length of a stream or waterbody. During rain events, surface runoff transports water and fecal bacteria over the land surface and discharges to the stream system. This transport is dictated by rainfall, soil type, land use, and topography of the watershed. Many types of nonpoint sources introduce fecal bacteria to the land surface, including the manure spreading process, direct deposition from livestock during the grazing season, and excretions from pets and wildlife. The deposition of non-human fecal bacteria directly to the stream occurs when livestock or wildlife have direct access to the waterbody. Nonpoint source contributions from human sources generally arise from failing septic systems and their associated drain fields or leaking infrastructure (i.e., sewer systems). The entire Antietam Creek watershed in MD is covered by a general Phase II NPDES Municipal Separate Storm Sewer System (MS4) permit for Washington County, Hagerstown, and Smithsburg. Therefore, contributions from domestic animal and human sources will be categorized under point sources as part of a Stormwater (SW) Waste Load Allocation (WLA_{ANT}). The presence of agricultural land use is significant in the watershed, and sources associated with it (i.e., livestock) contribute to the load allocation (LA_{ANT}) in this analysis. Wildlife contributions will be distributed between WLAs and LAs due to the presence of wildlife in both developed and undeveloped areas of the watershed.

Sewer Systems

The MD Antietam Creek watershed is serviced by both sewer systems and septic systems. Sewer systems are present in the towns of Cascade, Smithsburg, Hagerstown, Funkstown, Boonsboro, Keedysville and Sharpsburg.

Septic Systems

On-site disposal (septic) systems are located throughout the Antietam Creek watershed. Table 2.4.1 presents the number of septic systems per subwatershed. Figure 2.4.1 displays the areas that are serviced by sewers and the locations of the septic systems in MD.

Table 2.4.1: Septic Systems and Households per Subwatershed in the Antietam Creek Watershed in MD

Station / Subwatershed	Septic Systems	Households
ANT0366 / Antietam Creek in Rocky Forge	128	692
ANT0277 / Antietam Creek at Marsh Run	2,383	3,958
MRS0000 / Marsh Run	846	1,480
ANT0223 / Antietam Creek in Funkstown	506	18,387
ANT0132 / Antietam Creek in Devil's Backbone Park	765	1,738
BEC0001 / Beaver Creek	2,017	2,201
LAS0004 / Little Antietam Creek in Keedysville	1,213	2,250
ANT0044 / Antietam Creek at Burnside Bridge	419	681
ANT0002 / Antietam Creek in Antietam	453	478
Total	**8,730**	**31,865**

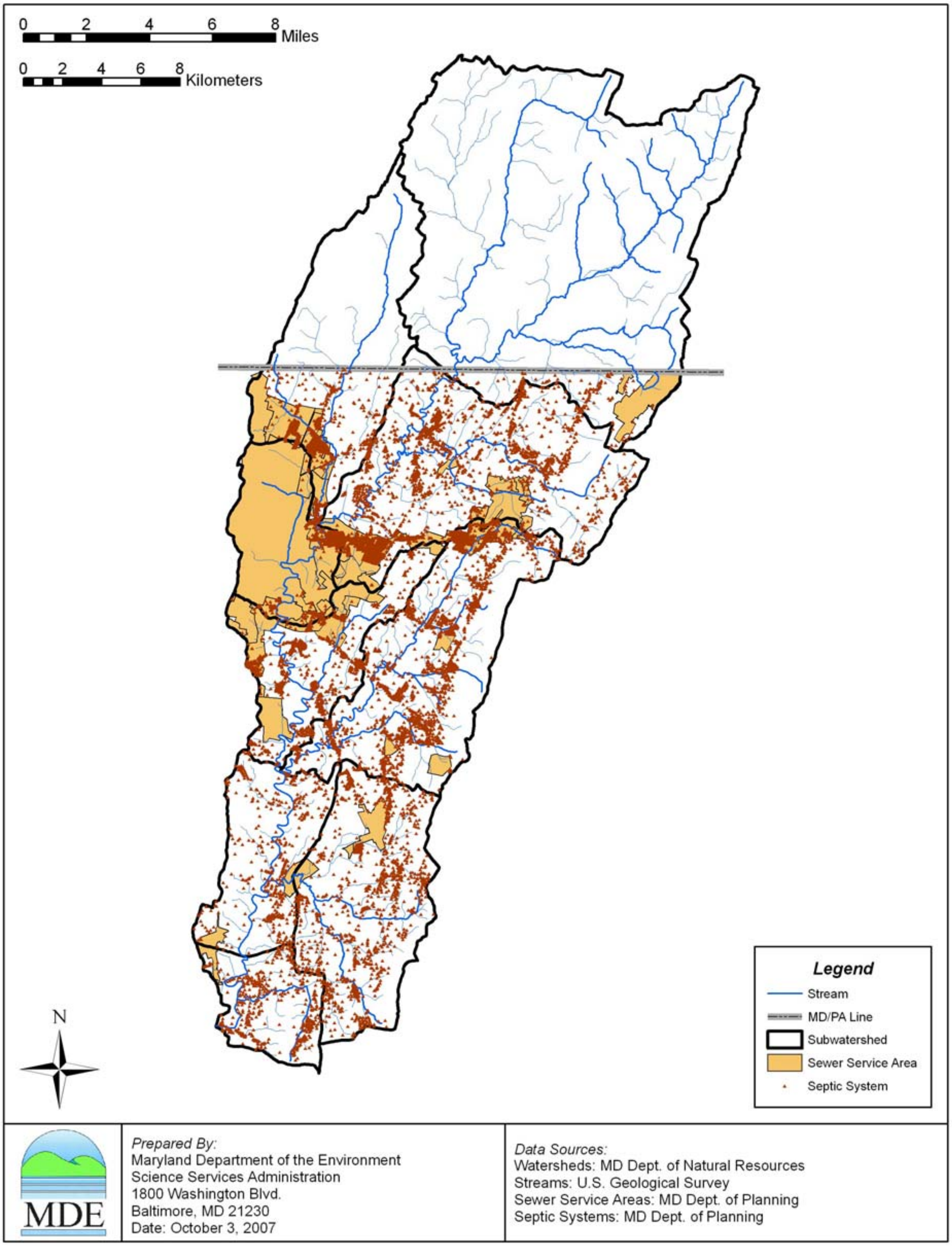


Figure 2.4.1: Sanitary Sewer Service Areas and Septic Locations in the MD 8-digit Antietam Creek Watershed

Point Source Assessment

There are two broad types of National Pollutant Discharge Elimination System (NPDES) permits considered in this analysis, individual and general. Both types of permits include industrial and municipal categories. Individual permits are issued for industrial and municipal WWTPs and Phase I municipal separate storm sewer systems (MS4s). MDE general permits have been established for surface water discharges from: Phase II and other MS4 entities, surface coal mines, mineral mines, quarries, borrow pits, ready-mix concrete, asphalt plants, seafood processors, hydrostatic testing of tanks and pipelines, marinas, concentrated animal feeding operations, and stormwater associated with industrial activities.

NPDES Regulated Stormwater

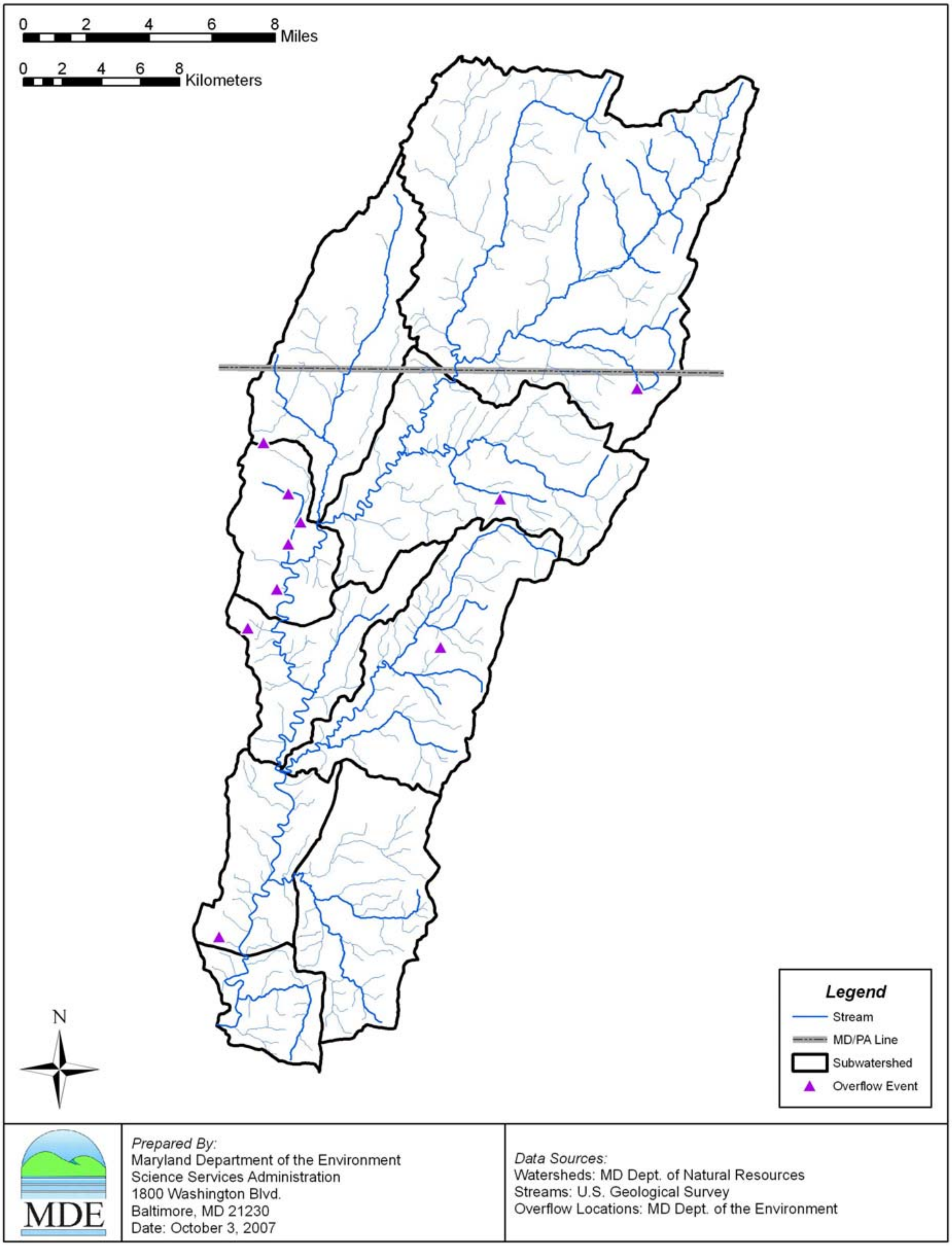
Bacteria sources associated with MS4s are considered point sources. Stormwater runoff is an important source of water pollution, including bacterial pollution. An MS4 is a conveyance or system of conveyances (roads with drainage systems, municipal streets, catch basins, curbs, gutters, ditches, man-made channels, storm drains) designed or used for collecting or conveying stormwater and delivering it to a waterbody. MS4 programs are designed to reduce the amount of pollution that enters a waterbody from storm sewer systems to the maximum extent practicable.

Maryland's portion of the Antietam Creek watershed is located entirely in Washington County. Washington County, along with Hagerstown and Smithsburg is covered by a general Phase II NPDES MS4 permit (permit # MDR055500). Bacteria loads associated with these MS4s are therefore included in the Stormwater (SW) WLA_{ANT} of this TMDL, which also encompasses any other NPDES regulated Phase I and Phase II stormwater discharges in the watershed, including state and federal entities.

Sanitary Sewer Overflows

Sanitary Sewer Overflows (SSOs) occur when the capacity of a separate sanitary sewer is exceeded. There are several factors that may contribute to SSOs from a sewerage system, including pipe capacity, operations and maintenance effectiveness, sewer design, age of system, pipe materials, geology and building codes. SSOs are prohibited by the facilities' permits, and must be reported to MDE's Water Management Administration in accordance with COMAR 26.08.10 to be addressed under the State's enforcement program.

There were a total of 19 SSOs reported to MDE between October 2002 and October 2003 in the Antietam Creek watershed. Approximately 35 million gallons of untreated or partially treated wastewater from SSOs were discharged through various waterways (surface water, groundwater, sanitary sewers, etc.). Figure 2.4.2 shows the locations where SSOs occurred in the MD portion of the watershed between October 2002 and October 2003.



Prepared By:
Maryland Department of the Environment
Science Services Administration
1800 Washington Blvd.
Baltimore, MD 21230
Date: October 3, 2007

Data Sources:
Watersheds: MD Dept. of Natural Resources
Streams: U.S. Geological Survey
Overflow Locations: MD Dept. of the Environment

Figure 2.4.2: Sanitary Sewer Overflow Areas in the MD 8-digit Antietam Creek Watershed

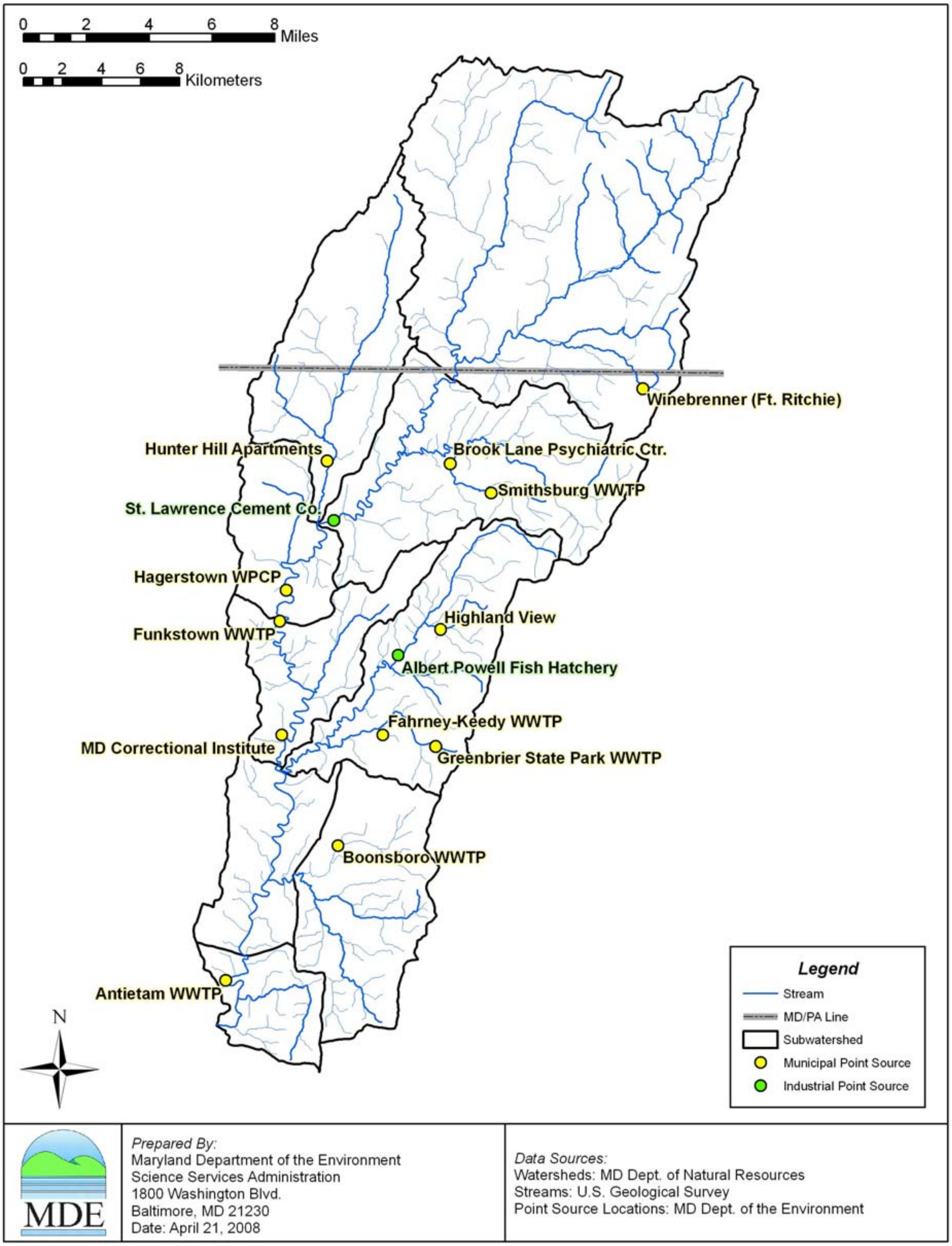
Municipal and Industrial Wastewater Treatment Plants (WWTPs)

Wastewater treatment plants are designed to treat wastewater before it is discharged to a stream or river. The goals of wastewater treatment are to protect the public health, protect aquatic life, and to prevent harmful substances from entering the environment.

Based on MDE's point source permitting information, there are twelve active municipal NPDES permitted point source facilities with permits regulating the discharge of fecal bacteria in the Antietam Creek watershed. These twelve facilities combined treat approximately 9.8 MGD (million gallons per day). There are also two industrial facilities in the Antietam Creek watershed with NPDES permits regulating the discharge of fecal bacteria. These two facilities combined discharge approximately 0.5 MGD. Table 2.4.2 lists these fourteen facilities. Figure 2.4.3 shows their location in the watershed.

Table 2.4.2: NPDES Permit Holders Regulated for Fecal Bacteria Discharge in the MD 8-digit Antietam Creek Watershed

Facility	NPDES Permit No.	County / Subwatershed	Average Flow (MGD)	Fecal Coliform Concentration Annual AVG (MPN/100ml)	Fecal Coliform Load (Billion MPN/day)
Funkstown WWTP	MD0020362	Washington / ANT0132	0.108	2.33	0.010
Highland View Academy WWTP	MD0024627	Washington / BEC0001	0.011	1.40	0.001
Brook Lane Psychiatric Center WWTP	MD0053198	Washington / ANT0277	0.009	11.08	0.004
Smithsburg WRF	MD0024317	Washington / ANT0277	0.243	8.46	0.078
Hagerstown WPCP	MD0021776	Washington / ANT0223	7.679	25.23	7.334
Boonsboro WTF	MD0020231	Washington / LAS0004	0.449	2.38	0.040
Hunter Hill Apartments WWTP	MD0022926	Washington / MRS0000	0.010	5.05	0.002
Antietam WRF	MD0062308	Washington / ANT0002	0.110	8.85	0.037
Winebrenner WRF	MD0003221	Washington / ANT0366	0.181	4.54	0.031
MD Correctional Institute WWTP	MD0023957	Washington / ANT0132	0.998	2.00	0.076
Fahrney-Keedy Memorial Home WWTP	MD0053066	Washington / BEC0001	0.026	3.10	0.003
Greenbrier State Park WWTP	MD0023868	Washington / BEC0001	0.014	1.60	0.001
Albert Powell Fish Hatchery	MD0054054	Washington / BEC0001	0.153	1.00	0.006
St. Lawrence Cement Co.	MD0002151	Washington / ANT0277	0.369	2.00	0.028



Prepared By:
Maryland Department of the Environment
Science Services Administration
1800 Washington Blvd.
Baltimore, MD 21230
Date: April 21, 2008

Data Sources:
Watersheds: MD Dept. of Natural Resources
Streams: U.S. Geological Survey
Point Source Locations: MD Dept. of the Environment

Figure 2.4.3: Permitted Point Sources Discharging Fecal Bacteria in the MD 8-digit Antietam Creek Watershed

Bacteria Source Tracking

Bacteria source tracking (BST) was used to identify the relative contributions of different sources of bacteria to in-stream water samples. BST monitoring was conducted at nine stations in the Antietam Creek watershed, where samples were collected once per month for a one-year duration. Sources are defined as domestic (pets and human associated animals), human (human waste), livestock (agricultural animals), and wildlife (mammals and waterfowl). To identify sources, samples are collected within the watershed from known fecal sources, and the patterns of antibiotic resistance of these known sources are compared to isolates of unknown bacteria from ambient water samples. Details of the BST methodology and data can be found in Appendix C.

An accurate representation of the expected contribution of each source at each station is estimated by using a stratified weighted mean of the identified sample results. The weighting factors are based on the $\log_{10}$ of the bacteria concentration and the percent of time that represents the high stream flow or low stream flow (see Appendix B). The procedure for calculating the stratified weighted mean of the sources per monitoring station is as follows:

1. Calculate the percentage of isolates per source per each sample date (S).
2. Calculate an initial weighted percentage (MS) of each source per flow strata (high/low). The weighting is based on the $\log_{10}$ bacteria concentration for the water sample.
3. Adjust the weighted percentage based on the classification of known sources.
4. The final weighted mean source percentage, for each source category, is based on the proportion of time in each flow duration zone.

The weighted mean for each source category is calculated using the following equations:

$$MS_l = \sum_{i=1}^{2} MS_{i,l} * W_i \qquad (4)$$

where,

$$MS_{i,l} = \sum_{k=1}^{5} \frac{A_{l,k} * IMS_{i,k}}{P_k} \qquad (5)$$

where,

$$IMS_{i,k} = \frac{\sum_{j=1}^{n_i} \log_{10}(C_{i,j}) * S_{i,j,k}}{\sum_{j=1}^{n_i} \log_{10}(C_{i,j})} \qquad (6)$$

and where,

MS_l = weighted mean proportion of isolates of source *l*
$MS_{i,l}$ = adjusted weighted mean proportion of isolates for source *l* in stratum *i*
$IMS_{i,k}$ = initial weighted mean proportion of isolates for source *k* in stratum *i*

W_i = proportion covered by stratum i
$A_{l,k}$ = number of known source l isolates initially predicted as source k
P_k = number of total known isolates initially predicted as source k
i = stratum
j = sample
k = source category (1=human, 2=domestic, 3=livestock, 4=wildlife, 5=unknown)
l = final source category (1=human, 2=domestic, 3=livestock, 4=wildlife)
$C_{i,j}$ = concentration for sample j in stratum i
$S_{i,j,k}$ = proportion of isolates for sample j, of source k in stratum i
n_i = number of samples in stratum i

The complete distributions of the annual and seasonal periods source loads are listed in Tables 2.4.3 and 2.4.4. Details of the BST data and tables with the BST analysis results can be found in Appendix C.

In the seasonal period, only two bacteria samples fell in the low flow category for all subwatersheds; therefore, a distribution by flow stratum was not calculated due to an insufficient number of samples. For the seasonal analysis, a distribution of all samples was calculated and applied.

Table 2.4.3: Distribution of Fecal Bacteria Source Loads in the Antietam Creek Watershed for the Average Annual Period

Station	Flow Stratum	% Domestic Animals	% Human	% Livestock	% Wildlife
ANT0366	**High**	22.0	20.7	32.8	24.5
	Low	25.7	20.4	30.1	23.7
	Weighted	24.5	20.5	31.0	24.0
ANT0277	**High**	27.0	15.3	28.9	28.8
	Low	18.9	33.9	28.9	18.3
	Weighted	21.5	27.9	28.9	21.7
MRS0000	**High**	25.6	17.5	33.0	23.9
	Low	23.9	11.3	35.1	29.7
	Weighted	24.5	13.3	34.4	27.8
ANT0223	**High**	17.1	24.8	33.0	25.1
	Low	21.9	20.9	33.2	24.0
	Weighted	20.4	22.1	33.2	24.3
ANT0132	**High**	16.0	18.6	34.0	31.3
	Low	13.4	18.4	36.3	31.8
	Weighted	14.3	18.5	35.6	31.7
BEC0001	**High**	15.3	20.6	32.5	31.6
	Low	14.1	13.1	38.4	34.4
	Weighted	14.5	15.5	36.5	33.5
LAS0004	**High**	22.3	12.7	34.4	30.6
	Low	30.6	18.1	26.4	24.9
	Weighted	27.9	16.4	29.0	26.7
ANT0044	**High**	16.3	18.5	34.7	30.5
	Low	25.4	25.1	25.4	24.1
	Weighted	22.5	23.0	28.4	26.2
ANT0002	**High**	17.5	21.3	32.6	28.6
	Low	23.0	20.1	29.8	27.2
	Weighted	21.2	20.5	30.7	27.6

Table 2.4.4: Distribution of Fecal Bacteria Source Loads in the Antietam Creek Watershed for the Seasonal Period (May 1st – September 30th)

Station	% Domestic Animals	% Human	% Livestock	% Wildlife
ANT0366	26.6	17.0	30.8	25.5
ANT0277	28.7	13.2	27.9	30.2
MRS0000	35.5	13.8	27.2	23.5
ANT0223	21.5	21.2	31.8	25.5
ANT0132	19.9	15.2	32.6	32.3
BEC0001	16.8	18.8	30.4	34.0
LAS0004	25.7	13.2	29.7	31.5
ANT0044	17.6	18.4	34.5	29.5
ANT0002	17.7	16.9	32.0	33.4

3.0 TARGETED WATER QUALITY GOAL

The overall objective of the fecal bacteria TMDL set forth in this document is to establish the loading caps needed to assure attainment of water quality standards in the MD 8-digit Antietam Creek watershed. These standards are described fully in Section 2.3, "Water Quality Impairment."

4.0 TOTAL MAXIMUM DAILY LOADS AND SOURCE ALLOCATION

4.1 Overview

This section provides an overview of the non-tidal fecal bacteria TMDL development, with a discussion of the many complexities involved in estimating bacteria concentrations, loads and sources. The second section presents the analysis framework and how the hydrological, water quality and BST data are linked together in the TMDL process. The third section describes the analysis for estimating a representative geometric mean fecal bacteria concentration and baseline loads. This analysis methodology is based on available monitoring data and is specific to a free-flowing stream system. The fourth section addresses the critical condition and seasonality. The fifth section presents the margin of safety. The sixth section discusses annual average TMDL loading caps and how maximum daily loads are estimated. The seventh section presents TMDL scenario descriptions. The eighth section presents the load allocations. Finally, in section nine, the TMDL equation is summarized.

To be most effective, the TMDL provides a basis for allocating loads among the known pollutant sources in the watershed so that appropriate control measures can be implemented and water quality standards achieved. By definition, the TMDL is the sum of the individual waste load allocations (WLAs) for point sources and load allocations (LAs) for nonpoint sources and natural background sources. A margin of safety (MOS) is also included and accounts for the uncertainty in the analytical procedures used for water quality modeling, as well as the limits in scientific and technical understanding of water quality in natural systems. Although this formulation suggests that the TMDL be expressed as a load, the Code of Federal Regulations (40 CFR 130.2(i)) states that the TMDL can be expressed in terms of “mass per time, toxicity or other appropriate measure.”

For many reasons, bacteria are difficult to simulate in water quality models. They reproduce and die off in a non-linear fashion as a function of many environmental factors, including temperature, pH, turbidity (UV light penetration) and settling. They occur in concentrations that vary widely (i.e., over orders of magnitude) and an accurate estimation of source inputs is difficult to develop. Finally, limited data are available to characterize the effectiveness of any program or practice at reducing bacteria loads (Schueler 1999).

Bacteria concentrations, determined through laboratory analysis of in-stream water samples for bacteria indicators (e.g., enterococci), are expressed in either colony forming units (CFU) or most probable number (MPN) of colonies. The first method (US EPA 1985) is a direct estimate of the bacteria colonies (Method 1600). The second method is a statistical estimate of the number of colonies (ONPG MUG Standard Method 9223B, AOAC 991.15). Sample results indicate the extreme variability in the total bacteria counts (see Appendix A). The distribution of the sample results tends to be lognormal, with a strong positive skew of the data. Estimating loads of constituents that vary by orders of magnitude can introduce much uncertainty and result in large confidence intervals around the final results.

Estimating bacteria sources can also be problematic due to the many assumptions required and limited available data. Lack of specific numeric and spatial location data for several source categories, from failing septic systems to domestic animals, livestock, and wildlife populations, can create many potential uncertainties in traditional water quality modeling. For this reason, MDE applies an analytical method combined with the bacteria source tracking described above for the calculation of this TMDL.

4.2 Analytical Framework

This TMDL analysis uses flow duration curves to identify flow intervals that are used as indicators of hydrological conditions (i.e., annual average and critical conditions). This analytical method, combined with water quality monitoring data and BST, provides reasonable results (Cleland 2003), a better description of water quality than traditional water quality modeling, and also meets TMDL requirements.

In brief, baseline loads are estimated first for each subwatershed by using bacteria monitoring data and long-term flow data. These baseline loads are divided into four bacteria source categories, using the results of BST analysis. Next, the percent reduction required to meet the water quality criterion in each subwatershed is estimated from the observed bacteria concentrations after determining the critical condition and accounting for seasonality. Critical condition and seasonality are determined by assessing annual and seasonal hydrological conditions for high flow and low flow periods. Finally, TMDLs for each subwatershed are estimated by applying these percent reductions.

Figure 4.2.1 illustrates how the hydrological (flow duration curve), water quality and BST data are linked together for the TMDL development.

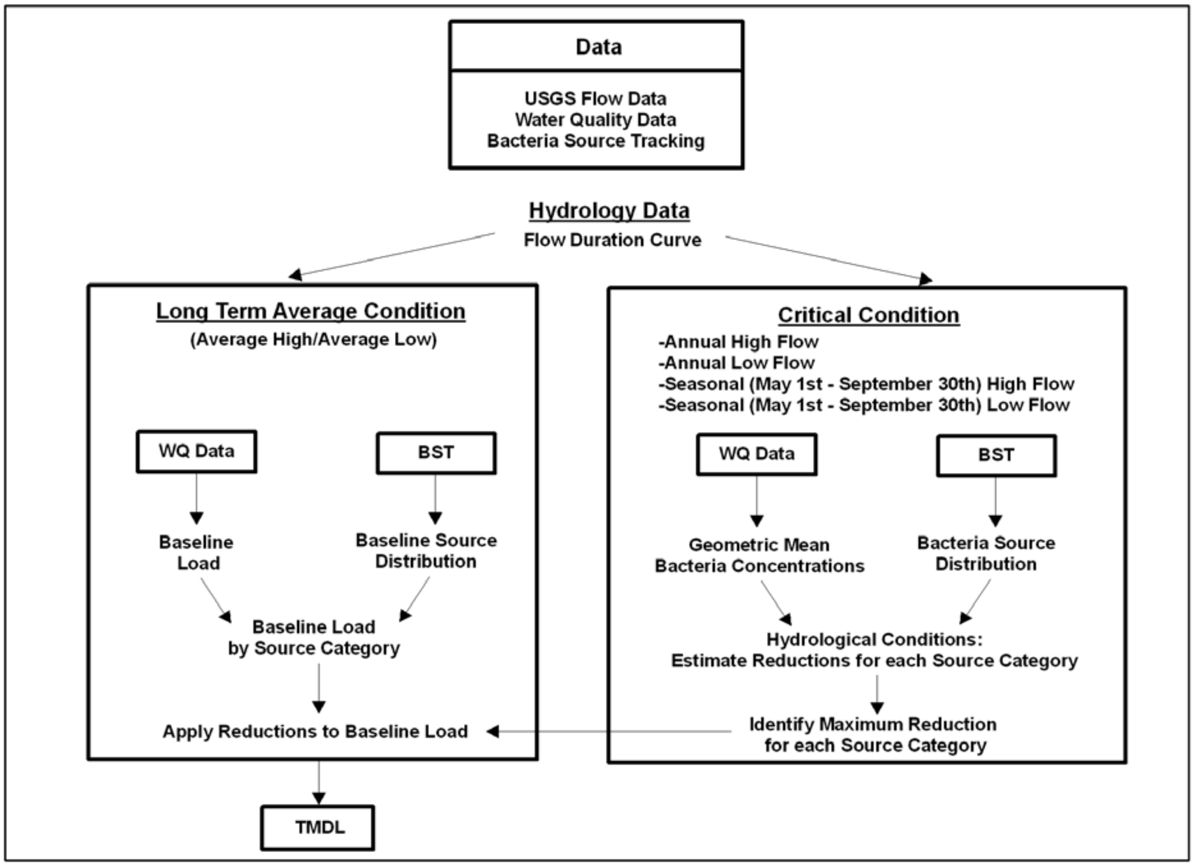


Figure 4.2.1: Diagram of the Non-tidal Bacteria TMDL Analysis Framework

4.3 Estimating Baseline Loads

Baseline loads are estimated for all subwatersheds of Antietam Creek, including, for computational purposes, those totally or partially located in PA. Baseline loads estimated in this TMDL analysis are reported as long-term average annual loads. These loads are estimated using geometric mean concentrations and bias correction factors (calculated from bacteria monitoring data) and daily average flows (estimated from long-term flow data).

The geometric mean concentration is calculated from the log transformation of the raw data. Statistical theory tells us that when back-transformed values are used to calculate average daily loads or total annual loads, the loads will be biased low (Richards 1998). To avoid this bias, a factor should be added to the log-concentration before it is back-transformed. There are several methods of determining this bias correction factor, ranging from parametric estimates resulting from the theory of the log-normal distribution to non-parametric estimates using a bias correction factor [Ferguson 1986; Cohn et al. 1989; Duan 1983]. There is much literature on the applicability and results from these various methods with a summary provided in Richards (1998). Each has advantages and conditions of applicability. A non-parametric estimate of the bias correction factor (Duan 1983) was used in this TMDL analysis.

With calculated geometric means and arithmetic means for each flow stratum, the bias correction factors are estimated as follows:

$$F_{1i} = A_i/C_i \qquad (6)$$

where,

F_{1i} = bias correction factor for stratum *i*
A_i = long term annual arithmetic mean for stratum *i*
C_i = long term annual geometric mean for stratum *i*

Daily average flows are estimated for each flow stratum using the watershed area ratio approach, since nearby long-term monitoring data are available.

The loads for each stratum are estimated as follows:

$$L_i = Q_i * C_i * F_{1i} * F_2 \qquad (7)$$

where,

L_i = daily average load (Billion MPN/day) at monitoring station for stratum *i*
Q_i = daily average flow (cfs) for stratum *i*
C_i = geometric mean for stratum *i*
F_{1i} = bias correction factor for stratum *i*
F_2 = unit conversion factor (0.0245)

Finally, for each subwatershed, the baseline load is estimated as follows:

$$L = \sum_{i=1}^{2} L_i * W_i \qquad (8)$$

where,

L = daily average load at station (MPN/day)
W_i = proportion of stratum *i*

In the Antietam Creek watershed, weighting factors of 0.322 for high flow and 0.678 for low/mid flows were used to estimate the annual baseline load expressed as Billion MPN *E. coli*/year.

Estimating Subwatershed Loads

Subwatersheds with more than one monitoring station were subdivided into unique watershed segments, thus allowing individual load and reduction targets to be determined for each. In the Antietam Creek watershed, five stations have upstream monitoring stations, as listed in Table

4.3.1. In these five cases, the subwatershed is differentiated by adding the extension “sub” to the name of the downstream monitoring station. For example, ANT0277sub refers only the area and load between stations ANT0277 and ANT0366, while ANT0277 refers to the cumulative area draining to that station. There are a total of nine subwatersheds considered in this analysis corresponding to the nine monitoring stations.

Table 4.3.1: Subdivided Watersheds in the Antietam Creek Watershed

Station / Tributary	Upstream Station(s)
ANT0277sub / Antietam Creek at Marsh Run	ANT0366
ANT0223sub / Antietam Creek in Funkstown	ANT0277, MRS0000
ANT0132sub / Antietam Creek in Devil's Backbone Park	ANT0223
ANT0044sub / Antietam Creek at Burnside Bridge	ANT0132, BEC0001, LAS0004
ANT0002sub / Antietam Creek in Antietam	ANT0044

Bacteria loads and concentrations from these subwatersheds could include transported loads from their upstream subwatersheds. However, for the purposes of this TMDL, the bacteria concentration measured at each monitoring station is assumed to be representative of that corresponding subwatershed and independent of flow from upstream subwatersheds. For example, the load transported from upstream station ANT0366 is not considered in the estimation of the load from the subwatershed ANT0277sub. Instead the bacteria concentration measured at station ANT0277 is assigned to that subwatershed.

This assumption is necessary due to a particular phenomenon, an example of which is observed in the subwatersheds of ANT0132sub and ANT0002sub. For each of these subwatersheds, bacteria loadings from upstream subwatersheds are significantly greater than the loads measured at the downstream stations. This occurrence indicates that the bacteria loads are significantly diminished as they are transported downstream. Bacteria are either dying off quickly, possibly due to environmental conditions such as extreme pH levels, elevated chlorine concentrations, etc., or are being diverted due to the Karst geology present in the area. Given this phenomenon, the measured concentration at each station is attributed solely to the immediate subwatershed. This will result in a slightly conservative estimate of bacteria loads, but will also allow a more consistent methodology throughout the watershed than applying unpredictable upstream loads.

Source estimates from the BST analysis are completed for each station and are based on the contribution from the upstream watershed. Given the uncertainty of in-stream bacteria processes and the complexity involved in back-calculating an accurate source transport factor, the sources for the subwatersheds defined in Table 4.3.1 were assigned from the analysis of their downstream stations.

Results of the baseline load calculations, including subwatersheds partially located in PA, are presented in Table 4.3.2.

Table 4.3.2: Baseline Loads Calculations

Subwatershed	**Area** (mi²)	**High Flow** (Unit Flow = 2.18 cfs/mi²)		**Low Flow** (Unit Flow = 0.63 cfs/mi²)		**Baseline *E. coli* Load** (Billion MPN/year)
		Average Flow (cfs)	***E. coli* Concentration. (MPN/100ml)**	**Average Flow (cfs)**	***E. coli* Concentration (MPN/100ml)**	
ANT0366[1]	94.2	205.3	1160	59.1	599	1,972,068
ANT0277sub[1]	42.2	91.9	966	26.4	265	904,110
MRS0000[1]	31.7	69.1	981	19.9	349	859,730
ANT0223sub	15.1	32.8	915	9.4	142	307,558
ANT0132sub	17.8	38.8	631	11.2	140	410,245
BEC0001	33.7	73.4	557	21.1	321	367,446
LAS0004	28.4	61.8	715	17.8	195	424,550
ANT0044sub	17.2	37.5	622	10.8	104	308,398
ANT0002sub	10.6	23.1	507	6.7	88	239,477

[1] Subwatersheds partially located in Pennsylvania

Baseline loads for subwatersheds located in both MD and PA were estimated using the ratios of the areas of the MD and PA portions to the total area of the subwatershed. The total baseline load for all subwatersheds or portions thereof located in MD is estimated as 3,469,308 billion MPN *E.coli*/year. The total baseline load for the portions of subwatersheds located in PA is 2,324,273 billion MPN E. coli/year.

4.4 Critical Condition and Seasonality

Federal regulations (40 CFR 130.7(c)(1)) require TMDLs to take into account critical conditions for stream flow, loading, and water quality parameters. The intent of this requirement is to ensure that the water quality of the waterbody is protected during times when it is most vulnerable.

For this TMDL the critical condition is determined by assessing annual and seasonal hydrological conditions for high flow and low flow periods. Seasonality is assessed as the time period when water contact recreation is expected, specifically May 1st through September 30th. For this TMDL analysis, the average hydrological condition over a 25-year period has been estimated as 32.2% high flow and 67.8% low flow as defined in Appendix B. Using the definition of a high flow condition as occurring when the daily flow duration interval is less than 32.2% and a low flow condition as occurring when the daily flow duration interval is greater than 32.2%, critical hydrological condition can be estimated by the percent of high or low flows during a specific period.

Using long term flow data from USGS station 01619500, critical condition and seasonality has been determined by assessing various hydrological conditions to account for seasonal and annual averaging periods.

The critical condition requirement is met by determining the maximum reduction per bacteria source that satisfies all hydrological conditions and meets the water quality standard, thereby minimizing the risk to water contact recreation. It is assumed that the reduction applied to a bacteria source category will be constant through all conditions.

The bacteria monitoring data for all stations located in the Antietam Creek watershed cover a sufficient temporal span (at least one year) to estimate annual conditions. However, sufficient data were not available for the seasonal period to consider high flow and low flow conditions. Since only two samples were taken during low flow conditions, a geometric mean cannot be established for that condition. Therefore, an average geometric mean and average flow were used for the seasonal analysis.

The four conditions listed in Table 4.4.1 were used to account for the critical condition.

Table 4.4.1: Hydrological Conditions Used to Account for Critical Condition and Seasonality

Hydrological Condition		Averaging Period	Water Quality Data Used	Fraction High Flow	Fraction Low Flow	Condition Period
Annual	Average	365 days	All	0.322	0.678	Long-Term Average
	Wet (High Flow)	365 days	All	1.000	0	Jan. 1996 – Jan. 1997
	Dry (Low Flow)	365 days	All	0	1.000	Apr. 2001 – Apr. 2002
Seasonal	Average	May 1st – Sept. 30th	May 1st – Sept. 30th	N/A	N/A	Long-Term Average For May – Sept. Period

The reductions of fecal bacteria required to meet water quality standards in each subwatershed of the Antietam Creek watershed are shown in Table 4.4.2. For computational purposes, the calculations include those subwatersheds partially located in PA.

Table 4.4.2: Required Fecal Bacteria Reductions (by Hydrological Condition per Subwatershed) to Meet Water Quality Standards

Station / Tributary	Hydrological Condition		Domestic Animals %	Human %	Livestock %	Wildlife %
ANT0366[1] Antietam Creek in Rocky Forge	Annual	Average	98.0	96.9	98.0	39.9
		Wet	98.0	96.9	98.0	64.9
		Dry	98.0	96.9	98.0	23.2
	Seasonal	Average	98.0	96.9	98.0	86.0
	Maximum Source Reduction		**98.0**	**96.9**	**98.0**	**86.0**
ANT0277sub[1] Antietam Creek at Marsh Run	Annual	Average	71.2	98.0	95.5	0.0
		Wet	98.0	98.0	98.0	61.9
		Dry	34.9	98.0	52.3	0.0
	Seasonal	Average	98.0	98.0	98.0	79.5
	Maximum Source Reduction		**98.0**	**98.0**	**98.0**	**79.5**
MRS0000[1] Marsh Run	Annual	Average	98.0	98.0	98.0	16.9
		Wet	98.0	98.0	98.0	55.3
		Dry	84.8	98.0	98.0	0.0
	Seasonal	Average	98.0	98.0	98.0	84.5
	Maximum Source Reduction		**98.0**	**98.0**	**98.0**	**84.5**
ANT0223sub Antietam Creek in Funkstown	Annual	Average	48.6	79.5	79.2	0.0
		Wet	98.0	79.5	98.0	72.1
		Dry	0.0	79.5	0.0	0.0
	Seasonal	Average	98.0	79.5	98.0	96.6
	Maximum Source Reduction		**98.0**	**79.5**	**98.0**	**96.6**

ANT0132sub Antietam Creek in Devil's Backbone Park	Annual	Average	28.8	95.9	71.8	0.0
		Wet	98.0	95.9	98.0	45.1
		Dry	0.0	79.3	0.0	0.0
	Seasonal	Average	98.0	95.9	98.0	77.5
	Maximum Source Reduction		**98.0**	**95.9**	**98.0**	**77.5**
BEC0001 Beaver Creek	Annual	Average	98.0	95.0	98.0	12.2
		Wet	98.0	95.0	98.0	38.3
		Dry	90.0	95.0	98.0	0.0
	Seasonal	Average	98.0	95.0	98.0	77.3
	Maximum Source Reduction		**98.0**	**95.0**	**98.0**	**77.3**
LAS0004 Little Antietam Creek in Keedysville	Annual	Average	75.1	98.0	77.8	0.0
		Wet	98.0	98.0	98.0	49.9
		Dry	39.0	98.0	33.5	0.0
	Seasonal	Average	98.0	98.0	98.0	76.7
	Maximum Source Reduction		**98.0**	**98.0**	**98.0**	**76.7**
ANT0044sub Antietam Creek at Burnside Bridge	Annual	Average	23.1	95.0	29.0	0.0
		Wet	98.0	98.0	98.0	41.5
		Dry	0.0	0.0	0.0	0.0
	Seasonal	Average	98.0	98.0	98.0	67.5
	Maximum Source Reduction		**98.0**	**98.0**	**98.0**	**67.5**
ANT0002sub Antietam Creek in Antietam	Annual	Average	4.7	95.0	6.9	0.0
		Wet	98.0	98.0	98.0	22.4
		Dry	0.0	0.0	0.0	0.0
	Seasonal	Average	98.0	98.0	98.0	69.4
	Maximum Source Reduction		**98.0**	**98.0**	**98.0**	**69.4**

4.5 Margin of Safety

A margin of safety (MOS) is required as part of this TMDL in recognition of the many uncertainties in the understanding and simulation of bacteriological water quality in natural systems and in statistical estimates of indicators. As mentioned in Section 4.1, it is difficult to estimate stream loadings for fecal bacteria due to the variation in loadings across sample locations and time. Load estimation methods should be both precise and accurate to obtain the true estimate of the mean load. Refined precision in the load estimation is due to using a stratified approach along the flow duration intervals, thus reducing the variation in the estimates. Moreover, Richards (1998) reports that averaging methods are generally biased, and the bias increases as the size of the averaging window increases. Finally, accuracy in the load estimation is based on minimal bias in the final result when compared to the true value.

Based on EPA guidance, the MOS can be achieved through two approaches (EPA 1991). One approach is to reserve a portion of the loading capacity as a separate term in the TMDL (i.e., TMDL = LA + WLA + MOS). The second approach is to incorporate the MOS as conservative assumptions used in the TMDL analysis. The second approach was used for this TMDL by estimating the loading capacity of the stream based on a reduced (more stringent) water quality criterion concentration. The *E. coli* water quality criterion concentration was reduced by 5%, from 126 *E. coli* MPN/100ml to 119.7 *E. coli* MPN/100ml.

4.6 Scenario Descriptions

Source Distribution

The final bacteria source distribution and corresponding baseline loads are derived from the source proportions listed in Table 2.4.3. For the purposes of the TMDL analysis and allocations, the percentage of sources identified as “unknown” was removed and redistributed proportionally among the known sources to total 100%. The source distribution and baseline loads used in the TMDL scenarios are presented in Table 4.6.1. As stated in Section 4.3, the source distributions for subwatersheds ANT0277sub, ANT0223sub, ANT0132sub, ANT0044sub, and ANT0002sub, were based on the sources identified at stations ANT0277, ANT0223, ANT0132, ANT0044, and ANT0002, respectively.

Table 4.6.1: Bacteria Source Distributions and Corresponding Baseline Loads Used in the Annual Average TMDL Analysis

Subwatershed	Domestic		Human		Livestock		Wildlife		Total Load (Billion *E. coli* MPN/year)
	%	Load (Billion *E. coli* MPN/year)	%	Load (Billion *E. coli* MPN/year)	%	Load (Billion *E. coli* MPN/year)	%	Load (Billion *E. coli* MPN/year)	
ANT0366[1]	24.5	484,049	20.5	404,455	31.0	610,915	24.0	472,648	1,972,068
ANT0277sub[1]	21.5	194,701	27.9	252,210	28.9	261,021	21.7	196,178	904,110
MRS0000[1]	24.5	210,279	13.3	114,298	34.4	295,804	27.8	239,350	859,730
ANT0223sub	20.4	62,662	22.1	68,018	33.2	102,017	24.3	74,861	307,558
ANT0132sub	14.3	58,492	18.5	75,817	35.6	145,989	31.7	129,947	410,245
BEC0001	14.5	53,361	15.5	56,947	36.5	134,094	33.5	123,044	367,446
LAS0004	27.9	118,612	16.4	69,479	29.0	122,947	26.7	113,511	424,550
ANT0044sub	22.5	69,295	23.0	70,840	28.4	87,501	26.2	80,762	308,398
ANT0002sub	21.2	50,817	20.5	49,034	30.7	73,421	27.6	66,205	239,477

[1] Subwatersheds partially located in Pennsylvania

First Scenario: Fecal Bacteria Practicable Reduction Targets

The maximum practicable reduction (MPR) for each of the four source categories is listed in Table 4.6.2. These values are based on review of the available literature and best professional judgment. It is assumed that human sources would potentially have the highest risk of causing gastrointestinal illness and therefore should have the highest reduction. If a domestic WWTP is located in the upstream watershed, this is considered when estimating applicable reductions so as to not violate the permitted loads. For this reason, in the subwatersheds of ANT0366, ANT0223sub, ANT0132sub and BEC0001 the human source reduction had to be limited in order to accommodate the permitted loads of the WWTPs. The domestic animal category includes sources from pets (e.g., dogs) and the MPR is based on an estimated success of education and outreach programs.

Table 4.6.2: Maximum Practicable Reduction Targets

Max Practicable Reduction per Source	**Human**	**Domestic**	**Livestock**	**Wildlife**
	95%	75%	75%	0%
Rationale	(a) Direct source inputs. (b) Human pathogens more prevalent in humans than animals. (c) Enteric viral diseases spread from human to human.[1]	Target goal reflects uncertainty in effectiveness of urban BMPs[2] and is also based on best professional judgment	Target goal based on sediment reductions from BMPs[3] and best professional judgment	No programmatic approaches for wildlife reduction to meet water quality standards. Waters contaminated by wild animal wastes offer a public health risk that is orders of magnitude less than that associated with human waste.[4]

[1] Health Effects Criteria for Fresh Recreational Waters. EPA-600/1-84-004. U.S. Environmental Protection Agency, Washington, DC. EPA. 1984.
[2] Preliminary Data Summary of Urban Storm Water Best Management Practices. EPA-821-R-99-012. U.S. Environmental Protection Agency, Washington, DC. EPA. 1999.
[3] Agricultural BMP Descriptions as Defined for The Chesapeake Bay Program Watershed Model. Nutrient Subcommittee Agricultural Nutrient Reduction Workshop. EPA. 2004.
[4] Environmental Indicators and Shellfish Safety. 1994. Edited by Cameron, R., Mackeney and Merle D. Pierson, Chapman & Hall.

As previously stated, these maximum practicable reduction targets are based on the available literature and best professional judgment. There is much uncertainty with estimated reductions from best management practices (BMPs). The BMP efficiency for bacteria reduction ranged from –6% to +99% based on a total of 10 observations (US EPA 1999). The MPR to agricultural lands was based on sediment reductions identified by EPA (US EPA 2004).

The practicable reduction scenario was developed based on an optimization analysis whereby a subjective estimate of risk was minimized and constraints were set on maximum reduction and allowable background conditions. Risk was defined on a scale of one to five, where it was assumed that human sources had the highest risk (5), domestic animals and livestock next (3), and wildlife the lowest (1) (See Table 4.6.2). The model was defined as follows:

$$\text{Risk Score} = \text{Min} \sum_{i=1}^{4} P_j * W_j \qquad (9)$$

where,

$$P_j = \frac{(1 - R_i) * Pb_j}{1 - TR} \qquad (10)$$

and,

$$TR = \frac{C - C_{cr}}{C} \qquad (11)$$

Therefore the risk score can be represented as:

$$Risk\ Score = Min \sum_{i=1}^{4} \left[\frac{(1 - R_j) * Pb_j}{(1 - \frac{C - Ccr}{C})} * W_j \right] \qquad (12)$$

where,

i = hydrological condition
j = bacteria source category =human, domestic animal, livestock and wildlife
P_j = % of each source category (human, domestic animals, livestock and wildlife) in final allocation
W_j = weight of risk per source category = 5, 3 or 1
R_j = percent reduction applied by source category (human, domestic animals, livestock and wildlife) for the specified hydrological condition (variable)
Pb_j = original (baseline) percent distribution by source category (variable)
TR = total reduction (constant within each hydrological condition) = Target reduction
C = in-stream concentration
Ccr = water quality criterion

The model is subject to the following constraints:

$C = Ccr$
$0 \leq R_{human} \leq 95\%$
$0 \leq R_{pets} \leq 75\%$
$0 \leq R_{livestock} \leq 75\%$
$R_{wildlife} = 0$
$P_j \geq 1\%$

In all nine subwatersheds, the constraints of this scenario could not be satisfied, indicating there was not a practicable solution. A summary of the first scenario analysis results is presented in Table 4.6.3.

Table 4.6.3: Maximum Practicable Reduction Scenario Results

Subwatershed	Applied Reductions				Total Reduction %	Target Reduction %	Achievable?
	Domestic %	Human %	Livestock %	Wildlife %			
ANT0366[1]	75.0	95.0	75.0	0.0	61.1	94.9	No
ANT0277sub[1]	75.0	95.0	75.0	0.0	64.3	94.0	No
MRS0000[1]	75.0	95.0	75.0	0.0	56.8	94.2	No
ANT0223sub	75.0	79.5	75.0	0.0	57.7	93.6	No
ANT0132sub	75.0	95.0	75.0	0.0	54.9	91.1	No
BEC0001	75.0	95.0	75.0	0.0	53.0	90.6	No
LAS0004	75.0	95.0	75.0	0.0	58.2	92.3	No
ANT0044sub	75.0	95.0	75.0	0.0	60.0	90.0	No
ANT0002sub	75.0	95.0	75.0	0.0	58.4	90.1	No

[1] Subwatersheds partially located in Pennsylvania

Second Scenario: Fecal Bacteria Reductions Higher than MPRs

The TMDL must specify load allocations that will meet the water quality standards. In the practicable reduction targets scenario, none of the subwatersheds could meet water quality standards based on MPRs.

To further develop the TMDL, a second scenario was analyzed in which the constraints on the MPRs were relaxed. In these subwatersheds, the percentage reduction from baseline loads for all sources including wildlife was allowed to increase, as necessary to achieve water quality standards, up to a maximum of 98%. A similar optimization procedure as before was used to minimize risk. Again, the objective is to minimize the sum of the risk for all conditions while meeting the scenario reduction constraints. The model was defined in the same manner as considered in the practicable reduction scenario but subject to the following constraints:

$$
\begin{aligned}
C &= Ccr \\
0 \leq R_{human} &\leq 98\% \\
0 \leq R_{pets} &\leq 98\% \\
0 \leq R_{livestock} &\leq 98\% \\
0 \leq R_{wildlife} &\leq 98\% \\
P_j &\geq 1\%
\end{aligned}
$$

The constraint for human source reduction for subwatersheds ANT0366, ANT0223sub, ANT0132sub and BEC0001 was changed not to be greater than 96.9%, 79.5%, 95.9% and 95.0% respectively, in order to maintain the permitted loads of WWTPs.

A summary of the results of this second scenario analysis is presented in Table 4.6.4.

Table 4.6.4: Reduction Results Based on Optimization Model Allowing Up to 98% Reduction

Subwatershed	Applied Reductions				Total Reduction %	Target Reduction %
	Domestic %	Human %	Livestock %	Wildlife %		
ANT0366[1]	98.0	96.9	98.0	86.0	94.9	94.9
ANT0277sub[1]	98.0	98.0	98.0	79.5	94.0	94.0
MRS0000[1]	98.0	98.0	98.0	84.5	94.2	94.2
ANT0223sub	98.0	79.5	98.0	96.6	93.6	93.6
ANT0132sub	98.0	95.9	98.0	77.5	91.1	91.1
BEC0001	98.0	95.0	98.0	77.3	90.6	90.6
LAS0004	98.0	98.0	98.0	76.7	92.3	92.3
ANT0044sub	98.0	98.0	98.0	67.5	90.0	90.0
ANT0002sub	98.0	98.0	98.0	69.4	90.1	90.1

[1] Subwatersheds partially located in Pennsylvania

4.7 TMDL Loading Caps

The TMDL loading cap is an estimate of the assimilative capacity of the monitored watershed. Estimation of the TMDL requires knowledge of how bacteria concentrations vary with flow rate or the flow duration interval. This relationship between concentration and flow is established using the strata defined by the flow duration curve.

The TMDL loading caps are provided in billion MPN *E. coli*/day. These loading caps are for the nine subwatersheds located upstream of their respective monitoring stations: ANT0366, ANT0277sub, MRS0000, ANT0223sub, ANT0132sub, BEC0001, LAS0004, ANT0044sub, and ANT0002sub. Loading caps for subwatersheds of Antietam Creek partially located in PA were included in the TMDL scenario. A TMDL summary for the entire Antietam Creek watershed will include an upstream load allocation (LA_{PA}) for the portion of the watershed located in PA to indicate estimated loads necessary to meet MD water quality standards in the MD 8-digit assessment unit for the Antietam Creek watershed.

Annual Average TMDL

As explained in the sections above, the annual average TMDL loading caps are estimated by first determining the baseline or current condition loads for each subwatershed and the associated geometric mean from the available monitoring data. This annual average baseline load is estimated using the geometric mean concentration and the long-term annual average daily flow for each flow stratum. The loads from these two strata are then weighted to represent average conditions (see Table 4.3.1), based on the proportion of each stratum, to estimate the total long-term loading rate.

Next, the percent reduction required to meet the water quality criterion is estimated from the observed bacteria concentrations accounting for the critical conditions (See Section 4.4). A reduction in concentration is proportional to a reduction in load; thus the TMDL is equal to the current baseline load multiplied by one minus the required reduction. This reduction, estimated as explained in Section 4.4, represents the maximum reduction per source that satisfies all hydrological conditions in each subwatershed, and that is required to meet water quality standards.

$$\text{TMDL Loading Cap} = L_b * (1 - R) \qquad (13)$$

where,

L_b = current or baseline load estimated from monitoring data
R = reduction required from baseline to meet water quality criterion.

The annual average bacteria TMDL loading caps for the subwatersheds, including those partially located in PA, are shown in Tables 4.7.1 and 4.7.2.

Table 4.7.1: Annual Average TMDL Loading Caps

Subwatershed	*E. coli* Baseline Load (Billion MPN/year)	Long-Term Average *E. coli* TMDL Load (Billion MPN/year)	% Target Reduction
ANT0366[1]	1,972,068	100,268	94.9
ANT0277sub[1]	904,110	54,285	94.0
MRS0000[1]	859,730	49,530	94.2
ANT0223sub	307,558	19,793	93.6
ANT0132sub	410,245	36,472	91.1
BEC0001	367,446	34,572	90.6
LAS0004	424,550	32,632	92.3
ANT0044sub	308,398	30,837	90.0
ANT0002sub	239,477	23,720	90.1
Total	**5,793,581**	**382,109**	**93.4**

[1] Subwatersheds partially located in Pennsylvania

Table 4.7.2: Annual Average TMDL Loading Caps by Source Category

Subwatershed	Domestic		Human		Livestock		Wildlife		Total Load (Billion *E. coli* MPN/year)
	%	Load (Billion *E. coli* MPN/year)	%	Load (Billion *E. coli* MPN/year)	%	Load (Billion *E. coli* MPN/year)	%	Load (Billion *E. coli* MPN/year)	
ANT0366[1]	9.7	9,681	12.3	12,372	12.2	12,218	65.8	65,997	100,268
ANT0277sub[1]	7.2	3,894	9.3	5,044	9.6	5,220	73.9	40,126	54,285
MRS0000[1]	8.5	4,206	4.6	2,286	11.9	5,916	74.9	37,122	49,530
ANT0223sub	6.3	1,253	70.4	13,928	10.3	2,040	13.0	2,571	19,793
ANT0132sub	3.2	1,170	8.6	3,132	8.0	2,920	80.2	29,250	36,472
BEC0001	3.1	1,067	8.2	2,839	7.8	2,682	80.9	27,984	34,572
LAS0004	7.3	2,372	4.3	1,390	7.5	2,459	80.9	26,412	32,632
ANT0044sub	4.5	1,386	4.6	1,417	5.7	1,750	85.2	26,285	30,837
ANT0002sub	4.3	1,016	4.1	981	6.2	1,468	85.4	20,255	23,720

[1] Subwatersheds partially located in Pennsylvania

Maximum Daily Loads

Recent EPA guidance (US EPA 2006a) recommends that maximum daily load (MDL) expressions of long-term annual average TMDLs should also be provided as part of the TMDL analysis and report. Selection of an appropriate method for translating a TMDL based on a longer time period into one using a daily time period requires decisions regarding 1) the level of resolution, and 2) the level of protection. The level of resolution pertains to the amount of detail used in specifying the maximum daily load. The level of protection represents how often the maximum daily load (MDL) is expected to be exceeded. Draft EPA/TetraTech guidance on daily loads (Limno-Tech 2007) provides three categories of options for both level of resolution and level of protection, and discusses these categories in detail.

For the Antietam Creek MDLs, a "representative daily load" option was selected as the level of resolution, and a value "that will be exceeded with a pre-defined probability" was selected as the level of protection. In these options, the MDLs are two single daily loads that correspond to the two flow strata, with an upper bound percentile that accounts for the variability of daily loads. The upper bound percentile and the MDLs were estimated following EPA's "*Technical Support Document for Water Quality-Based Toxics Control*" (1991 TSD) (EPA 1991b); and "*Approaches*

For Developing a Daily Load Expression for TMDLs Computed for Longer Term Averages" (EPA 2006b).

There are three steps to the overall process of estimating these MDLs. First, all the data available from each monitoring station are examined together by stratum and the percentile rank of the highest observed concentration (for each stratum at each station) is computed. The highest computed percentile rank is the upper bound percentile to be used in estimating the MDLs.

Secondly, the long-term annual average TMDL (see Table 4.7.1) concentrations are estimated for both high-flow and low-flow strata. This is conducted for each station using a statistical methodology (the "Statistical Theory of Rollback," or "STR," described more fully in Appendix D).

Third, based on the estimated long-term average (LTA) TMDL concentrations, the MDL for each flow stratum at each station is estimated using the upper boundary percentile computed in the first step above. Finally, MDLs are computed from these MDL concentrations and their corresponding flows.

Results of the fecal bacteria MDL analysis for the Antietam Creek subwatersheds, including for computational purposes those partially located in PA, are shown in Table 4.7.3.

Table 4.7.3: Antietam Creek Watershed Maximum Daily Loads Summary

Subwatershed	**Flow Stratum**	**Maximum Daily Load (Billion *E. coli* MPN/day)**	
		by Stratum	**Weighted by Stratum**
ANT0366[1]	High Low	5,264 1,834	2,939
ANT0277sub[1]	High Low	3,264 401	1,323
MRS0000[1]	High Low	3,971 703	1,755
ANT0223sub	High Low	1,724 90	616
ANT0132sub	High Low	4,293 104	1,453
BEC0001	High Low	1,899 211	755
LAS0004	High Low	2,545 97	886
ANT0044sub	High Low	2,349 19	769
ANT0002sub	High Low	2,126 17	696

[1] Subwatersheds partially located in Pennsylvania

See Appendix D for a more detailed explanation of the procedure for obtaining these daily loads.

4.8 TMDL Allocations

The MD 8-digit Antietam Creek fecal bacteria TMDL is composed of the following components:

$$\text{TMDL} = \text{LA}_{\text{PA}} + \text{LA}_{\text{ANT}} + \text{WLA}_{\text{ANT}} + \text{MOS} \qquad (14)$$

where,

LA_{PA} = Pennsylvania Load Allocation
LA_{ANT} = MD Antietam Creek Load Allocation
WLA_{ANT} = MD Antietam Creek Waste Load Allocation
MOS = Margin of Safety

The TMDL allocations for the MD 8-digit Antietam Creek basin include a load allocation (LA_{ANT}) for certain nonpoint sources, and waste load allocations (WLA_{ANT}) for point sources including WWTPs and NPDES-regulated stormwater discharges. The Stormwater (SW) WLA_{ANT} includes any nonpoint source loads deemed to be transported and discharged by regulated stormwater systems. An explanation of the distribution of nonpoint source loads and point source loads to the LA_{ANT} and to the SW-WLA_{ANT} and WWTP-WLA_{ANT} is provided in the subsections that follow.

In addition to these allocation categories for the MD 8-digit watershed, the MD Antietam Creek TMDL includes an upstream load allocation for the portion of the watershed located in PA (LA_{PA}). The LA_{PA} was calculated using the ratios of the areas of the watershed in MD and in PA to the total area of the watershed, and is presented as a "lump-sum" upstream load comprising all bacteria source categories. The portion of the watershed in PA includes 92% of subwatershed ANT0366, 59% of MRS0000, and 1% of ANT0277sub. The LA_{PA}, determined to be necessary in order to meet MD water quality standards in the MD 8-digit Antietam Creek basin, will not be distributed between nonpoint sources (LA) and point sources (WLA).

The margin of safety (MOS) is explicit and is incorporated in the analysis using a conservative assumption; it is not specified as a separate term. The assumption is that a 5% reduction of the criterion concentration established by MD to meet the applicable water quality standard will result in more conservative allowable loads of fecal bacteria, and thus provide the MOS. The final loads are based on average hydrological conditions, with reductions estimated based on critical hydrological conditions. The load reduction scenario results in load allocations that will achieve water quality standards. The State reserves the right to revise these allocations provided such revisions are consistent with the achievement of water quality standards.

Bacteria Source Categories and Allocation Distributions

The bacteria sources are grouped into four categories that are also consistent with divisions for various management strategies. The categories are human, domestic animal, livestock and wildlife. TMDL allocation rules are presented in Table 4.8.1. This table identifies how the TMDL will be allocated among the LA_{ANT} (those nonpoint sources or portions thereof not transported and discharged by stormwater systems) and the WLA_{ANT} (point sources including WWTPs, and NPDES regulated stormwater discharges). Only the final LA_{ANT} or WLA_{ANT} is reported in this TMDL. Note that the assignment of a small allowable human load to the Stormwater WLA is in consideration of the possible presence of such loads in the watershed beyond the reach of the sanitary sewer systems. The term "allowable load" means the load that the waterbody can assimilate and still meet water quality standards.

Table 4.8.1: Potential Source Contributions for TMDL Allocation Categories in the Antietam Creek Watershed in MD

Source Category	TMDL Allocation Categories		
	LA	WLA	
		WWTP	Stormwater
Human		X	X
Domestic			X
Livestock	X		
Wildlife	X		X

* These allocations apply only to the portion of the watershed in MD. The upstream load allocation attributed to PA includes all four bacteria source categories in one single load.

LA_{ANT}

All four bacteria source categories could potentially contribute to nonpoint source loads. For human sources, if the watershed has no MS4s or other NPDES-regulated Phase I or Phase II stormwater discharges, the nonpoint source contribution is estimated by subtracting any WWTP and/or CSO loads from the TMDL human load, and is then assigned to the LA. However, in watersheds covered by NPDES-regulated stormwater permits, any such nonpoint sources of human bacteria (i.e., beyond the reach of the sanitary sewer systems) are assigned to the SW-WLA (see below). There are twelve municipal and two industrial wastewater treatment facilities with NPDES permits regulating the discharge of fecal bacteria in the Antietam Creek watershed. There are no subwatersheds with assigned NPDES CSO WLA.

Livestock loads are all assigned to the LA_{ANT}. Domestic animals (pets) loads are assigned to the LA in watersheds with no MS4s or other NPDES-regulated stormwater systems. Since the entire Antietam Creek watershed is covered by NPDES MS4 permits, bacteria loads from domestic animal sources are assigned to the SW-WLA_{ANT} in all nine subwatersheds of Antietam Creek. However, wildlife sources will be distributed between the LA_{ANT} and the SW- WLA_{ANT} based on a ratio of the amount of pervious area in non-urban land to pervious area in urban land.

WLA$_{ANT}$

NPDES Regulated Stormwater

EPA's guidance document, "Establishing Total Maximum Daily Load (TMDL) Wasteload Allocations (WLAs) for Storm Water Sources and NPDES Permit Requirements Based on Those WLAs" (November 2002), advises that all individual and general NPDES Phase I and Phase II stormwater permits are point sources subject to WLA assignment in the TMDL. The document acknowledges that quantification of rainfall-driven nonpoint source loads is uncertain, stating that available data and information usually are not detailed enough to determine WLAs for NPDES-regulated stormwater discharges on an outfall-specific basis; therefore, the EPA guidance allows the stormwater WLA to be expressed as an aggregate allotment. Thus, in watersheds with existing NPDES-regulated stormwater permits, domestic animal bacteria loads are grouped together into a single SW-WLA along with other potential nonpoint source loads such as human and wildlife loads. This allowable human load in the SW-WLA$_{ANT}$ is estimated by subtracting any WWTP and CSO loads (if present) from the total allowable (TMDL) human load. (There are twelve municipal and three industrial wastewater treatment facilities with NPDES permits regulating the discharge of fecal bacteria in the Antietam Creek watershed. There are no NPDES CSO permits in the watershed.) The SW-WLA$_{ANT}$ wildlife load is estimated as explained above. In watersheds with no existing NPDES-regulated stormwater permits, these loads will be included in the LA.

The jurisdictions within the MD portion of the Antietam Creek watershed, Washington County, Hagerstown, and Smithsburg, are covered by the general Phase II MS4 program regulations. Based on EPA's guidance, the SW-WLA$_{ANT}$ is presented as one combined load for the entire land area of each subwatershed. The SW-WLA$_{ANT}$ category encompasses any other Phase I and Phase II NPDES-regulated stormwater discharges in the watershed, including State and federal entities. The SW-WLA$_{ANT}$ includes loads from sources such as leaks from broken sanitary infrastructure and failing septic systems, which may be transported through the storm drain system. These loads may be more effectively controlled through other management programs, but at this time such components cannot be determined separately. As stormwater assessment and/or other program monitoring efforts result in a more refined source assessment, MDE reserves the right to revise the current SW-WLA$_{ANT}$, provided the revisions are consistent with achieving water quality standards. Upon approval of the TMDL, "NPDES-regulated municipal and small construction storm water discharges effluent limits should be expressed as BMPs or other similar requirements, rather than as numeric effluent limits" (US EPA 2002a). The SW-WLA$_{ANT}$ distribution in Washington County is presented in Table 4.8.2.

Table 4.8.2: Annual Average Stormwater Allocations in MD

Subwatershed	Washington County $SW\text{-}WLA_{ANT}$ (Billion MPN *E. coli*/year)
ANT0366[1]	1,094
ANT0277sub[1]	13,156
MRS0000[1]	3,626
ANT0223sub	2,719
ANT0132sub	7,021
BEC0001	4,801
LAS0004	5,804
ANT0044sub	4,745
ANT0002sub	4,844
Total	**47,810**

[1] MD portion of the subwatershed only.

Municipal and Industrial WWTPs

As explained in the source assessment section above, there are fourteen NPDES permitted point source facilities with permits regulating the discharge of fecal bacteria in the Antietam Creek watershed in MD. The WLA for each WWTP is estimated using the design flow of the plant stated in the facility's NPDES permit and the *E. coli* criterion of 126 MPN/100ml. Bacteria loads assigned to the WWTPs are allocated as the $WWTP\text{-}WLA_{ANT}$.

4.9 Summary

The long-term annual average TMDL and TMDL allocations are presented in Table 4.9.1. Table 4.9.2 presents the maximum daily loads for the subwatersheds or portions thereof within the Antietam Creek MD 8-digit basin.

Table 4.9.1: MD 8-Digit Antietam Creek Watershed Annual Average TMDL

Subwatershed	**Total Allocation**	**LA_{ANT}**	**$SW\text{-}WLA_{ANT}$**	**$WWTP\text{-}WLA_{ANT}$**
	(Billion MPN *E. coli* /year)			
ANT0366[1]	8,466	6,327	1,094	1,045
ANT0277sub[1]	53,525	39,768	13,156	601
MRS0000[1]	20,376	16,726	3,626	24
ANT0223sub	19,793	3,147	2,719	13,927
ANT0132sub	36,472	26,317	7,021	3,134
BEC0001	34,572	26,934	4,801	2,838
LAS0004	32,632	25,906	5,804	923
ANT0044sub	30,837	26,092	4,745	0
ANT0002sub	23,720	18,592	4,844	284
MD Total	260,393	189,808	47,810	22,775
PA Upstream Load	121,716			
***TMDL*[2]**	**382,109**			

[1] MD portion of the subwatershed only.
[2] The MOS is incorporated.

Table 4.9.2: MD 8-digit Antietam Creek Watershed Maximum Daily Loads

Subwatershed	Total Allocation	LA_{ANT}	$SW\text{-}WLA_{ANT}$	$WWTP\text{-}WLA_{ANT}$
	(Billion MPN *E. coli* /day)			
ANT0366[1]	248	185	54	9
ANT0277sub[1]	1,304	969	330	5
MRS0000[1]	722	593	129	0.2
ANT0223sub	616	98	399	119
ANT0132sub	1,453	1,048	378	27
BEC0001	755	588	143	24
LAS0004	886	703	175	8
ANT0044sub	769	651	118	0
ANT0002sub	696	546	148	2
MD Total	7,449	5,381	1,874	194
PA Upstream Load	3,742			
TOTAL	**11,192**			

[1] MD portion of the subwatershed only.

The long-term annual average fecal bacteria TMDL summary for the MD 8-digit Antietam Creek watershed is presented in Table 4.9.3. Note that the upstream PA load allocation (LA$_{PA}$) is determined to be necessary in order to meet MD water quality standards in the MD portion of the Antietam Creek watershed. Although reported here as a single value, it may include both point and nonpoint sources.

Table 4.9.3: MD 8-Digit Antietam Creek Watershed Annual Average TMDL Summary

Billion MPN *E. coli*/year										
TMDL	=	**LA**			+	**WLA**			+	**MOS**
		LA$_{PA}$[1]	+	**LA$_{ANT}$**		**SW WLA$_{ANT}$**	+	**WWTP WLA$_{ANT}$**		
382,109	=	121,716	+	189,808	+	47,810	+	22,775	+	Incorpo-rated

Upstream Load Allocation

MD 8-digit Antietam Creek TMDL Contribution (260,393)

The maximum daily loads of fecal bacteria for the MD 8-digit Antietam Creek watershed, including the PA upstream load, are summarized in Table 4.9.4.

Table 4.9.4: MD 8-Digit Antietam Creek Watershed MDL Summary

Billion MPN *E. coli*/day										
MDL	=	**LA**			+	**WLA**			+	**MOS**
		LA$_{PA}$[1]	+	**LA$_{ANT}$**		**SW WLA$_{ANT}$**	+	**WWTP WLA$_{ANT}$**		
11,192	=	3,742	+	5,381	+	1,874	+	194	+	Incorpo-rated

Upstream MDL

MD 8-digit Antietam Creek MDL Contribution (7,449)

In certain watersheds, the goal of meeting water quality standards may require very high reductions that are not achievable with current technologies and management practices. In this situation, where there is no feasible TMDL scenario, MPRs are increased to provide estimates of the reductions required to meet water quality standards. In the subwatersheds of Antietam Creek, water quality standards cannot be achieved with the maximum practicable reduction rates specified in Table 4.6.3. The TMDLs shown in Tables 4.9.1 and 4.9.2 represent reductions from current bacteria loadings that are beyond practical reductions. In cases where such high reductions are required to meet standards, it is expected that the first stage of implementation will be to carry out the MPR scenario.

5.0 ASSURANCE OF IMPLEMENTATION

Section 303(d) of the Clean Water Act and current EPA regulations require reasonable assurance that the TMDL load and wasteload allocations can and will be implemented. In the Antietam Creek watershed, the TMDL analysis indicates that, for all nine subwatersheds, the reduction of fecal bacteria loads from all sources including wildlife are beyond the MPR targets. These MPR targets were defined based on a literature review of BMPs effectiveness and assuming a zero reduction for wildlife sources. Antietam Creek and its tributaries may not be able to attain water quality standards. The fecal bacteria load reductions required to meet water quality criteria in the nine subwatersheds of Antietam Creek are not feasible by implementing effluent limitations and cost-effective, reasonable BMPs to nonpoint sources. Therefore, MDE proposes a staged approach to implementation beginning with the MPR scenario, with regularly scheduled follow-up monitoring to assess the effectiveness of the implementation plan.

Additional reductions will be achieved through the implementation of BMPs; however, the literature reports considerable uncertainty concerning the effectiveness of BMPs in treating bacteria. As an example, pet waste education programs have varying results based on stakeholder involvement. Additionally, the extent of wildlife reduction associated with various BMPs methods (e.g., structural, non-structural, etc.) is uncertain. Therefore, MDE intends for the required reductions to be implemented in an iterative process that first addresses those sources with the largest impact on water quality and human health risk, with consideration given to ease of implementation and cost. The iterative implementation of BMPs in the watershed has several benefits: tracking of water quality improvements following BMP implementation through follow-up stream monitoring; providing a mechanism for developing public support through periodic updates on BMP implementation; and helping to ensure that the most cost-effective practices are implemented first.

Potential funding sources for implementation include the Maryland’s Agricultural Cost Share Program (MACS), which provides grants to farmers to help protect natural resources, and the Environmental Quality and Incentives Program, which focuses on implementing conservation practices and BMPs on land involved with livestock and production. Though not directly linked, it is assumed that the nutrient management plans from the Water Quality Improvement Act of 1998 (WQIA) will have some reduction of bacteria from manure application practices.

Implementation and Wildlife Sources

It is expected that in some waters for which TMDLs will be developed, the bacteria source analysis indicates that after controls are in place for all anthropogenic sources, the waterbody will meet water quality standards. However, while neither Maryland nor EPA is proposing the elimination of wildlife to allow for the attainment of water quality standards, managing the overpopulation of wildlife remains an option for state and local stakeholders.

After developing and implementing, to the maximum extent possible, a reduction goal based on the anthropogenic sources identified in the TMDL, Maryland anticipates that implementation to reduce the controllable non-point sources may also reduce some wildlife inputs to the waters.

6.0 PUBLIC PARTICIPATION

Stakeholders were informed by an October 18, 2007 MDE mailing of a notice of intent to develop a fecal bacteria TMDL for the non-tidal Antietam Creek basin. The notice letters provided MDE contact information and offered upon request an informational briefing on the proposed TMDL. In response to a request by Washington County officials, a briefing and presentation on the TMDL was provided by MDE on March 20, 2008, at the Washington County Departments of Planning and Engineering.

A public notice of intent to establish the Antietam Creek fecal bacteria TMDL, announcing the opening and closing dates of the formal 30-day Public Comment Period, was published in the Washington County Herald-Mail. The notice was also sent to MDE's stakeholder distribution list for the Antietam Creek watershed and all other interested parties. All were invited to send written comments on the draft TMDL to MDE. The public notice announced the availability of the draft TMDL document, copies of which were placed in the Hagerstown, Boonsboro, and Smithsburg Branches of the Washington County Free Library. The public notice also provided information on how to access the draft TMDL documents on MDE's website.

All written comments received by the close of the comment period are recorded and formally responded to in a Comment Response Document (CRD), to be included in the draft final TMDL documentation package submitted to EPA for the Agency's approval. Receipt of each set of comments is acknowledged by MDE, either by letter or email to comment authors. Following EPA approval of the TMDL, the responses are made available when the CRD is posted on MDE's website, together with the final approved TMDL documentation. The CRD is also mailed to stakeholders, including all those who sent comments to MDE, along with an approval notification letter.

REFERENCES

Cameron, R., Mackeney and Merle D. Pierson, eds. 1994. Environmental Indicators and Shellfish Safety. Chapman & Hall.

Code of Federal Regulations, 40 CFR 130.2(h), 40 CFR 130.7(c)(1). Website http://www.access.gpo.gov/nara/cfr/waisidx_04/40cfr130_04.html, last visited 06/24/06.

Code of Maryland Regulations, 26.08.02.03-3, and 26.08.02.08. Website http://www.dsd.state.md.us/comar, last visited 10/17/07.

Code of Maryland Regulations, 26.08.10. Website http://www.dsd.state.md.us/comar, last visited 07/29/06.

Cohn, T.A., L.L. DeLong, E.J. Gilroy, R.M. Hirsch, and D.K. Wells. 1989. Estimating Constituent Loads. Water Resources Research 25: 937-942.

Duan, N. 1983. Smearing Estimate: A Nonparametric Retransformation method. Journal of the American Statistical Association 78:605-610.

Easton, J. H., M. M. Lalor, J. J. Gauthier and R. E. Pitt. 2001. Pathogen Decay in Urban Streams. In: AWRA Annual Spring Specialty Conference Proceedings: Water Quality Monitoring and Modeling, American Water Resources Association, San Antonio, TX, pp. 169-174.

Edwards, Jonathan Jr. 1981. A Brief Description of the Geology of Maryland. Maryland Geological Survey.

Ferguson, R.I. 1986. River Loads Underestimated by Rating Curves. Water Resources Research 22: 74-76.

Maryland Department of Planning. 2002. 2002 Land Use, Land Cover Map Series.

————. 2003. Estimates of Septic Systems. Baltimore: Maryland Department of Planning, Comprehensive Planning Unit.

Maryland Department of the Environment. 2002. 2002 List of Impaired Surface Waters [303(d) List] and Integrated Assessment of Water Quality in Maryland.

————. 2004. 2004 FINAL List of Impaired Surface Waters [303(d) List] and Integrated Assessment of Water Quality in Maryland.

————. 2006. 2006 List of Impaired Surface Waters [303(d) List] and Integrated Assessment of Water Quality in Maryland.

Natural Resources Conservation Service. 2004. United States Department of Agriculture. Official Soil Series Descriptions [Online WWW]. Available URL: "http://soils.usda.gov/technical/classification/osd/index.html" [Accessed 4 February 2008].

————. U.S. General Soil Map (STATSGO) for Maryland [Online WWW] Available URL: "http://soildatamart.nrcs.usda.gov" [Accessed 29 January 2008].

————. U.S. General Soil Map (STATSGO) for Pennsylvania [Online WWW] Available URL: "http://soildatamart.nrcs.usda.gov" [Accessed 4 February 2008]

Reger, James P. 2007. Foundation Engineering Problems and Hazards in Karst Terranes. Maryland Geological Survey. "http://www.mgs.md.gov/esic/fs/fs11.html" [Accessed 12 October 2007].

Richards, R.P. 1998. Estimation of pollutant loads in rivers and streams: A guidance document for NPS programs. Project report prepared under Grant X998397-01-0, U.S. Environmental Protection Agency, Region VIII, Denver. 108 p.

Schueler, T. 1999. Microbes and Urban Watersheds. Watershed Protection Techniques. 3(1): 551-596.

U.S. Department of Commerce. 2000. United States Census Bureau's GIS Coverage. Washington DC: US Bureau of the Census.

U.S. Environmental Protection Agency. 1984. Health Effects Criteria for Fresh Recreational Waters. EPA-600/1-84-004.

————. 1985. Test Methods for Eschericichia coli and Enterococci in Water by the Membrane Filter Procedure. EPA600/4-85-076. Washington, D.C. NTIS PB86-158052.

————. 1986. Ambient Water Quality Criteria for Bacteria-1986. EPA-440/5-84-002.

————. 1991a. Guidance for Water Quality-Based Decisions: The TMDL Process. EPA 440/4-91-001.

————. 1991b. Technical Support Document for Water Quality-Based Toxics Control (1991 TSD). Washington, D.C.

————. 1999. Preliminary Data Summary of Urban Storm Water Best Management Practices. EPA-821-R-99-012.

————. 2002a. Establishing Total Maximum Daily Load (TMDL) Wasteload Allocations (WLAs) for Storm Water Sources and NPDES Permit Requirements Based on Those WLAs. November 22, 2002 U.S. EPA Memorandum.

————. 2002b. National Recommended Water Quality Criteria: 2002. EPA-822-R-02-047.

————. 2003. Implementation Guidance for Ambient Water Quality Criteria for Bacteria: Draft. Office of Water, Washington, D.C. EPA-823-B-02-003.

————. 2004. Agricultural BMP Descriptions as Defined for The Chesapeake Bay Program Watershed Model. Nutrient Subcommittee Agricultural Nutrient Reduction Workshop.

————. 2006a. Establishing TMDL "Daily" Loads in Light of the Decision by the U.S. Court of Appeals for the D.C. Circuit in *Friends of the Earth, Inc. v. EPA, et al.*, No. 05-5015, (April 25, 2006) and Implications for NPDES Permits. Memorandum from Benjamin Grumbles, Assistant Administrator. Office of Water, Washington, D.C. November 15, 2006.

————. 2006b. Approaches For Developing a Daily Load Expression for TMDLs Computed for Longer Term Averages. Draft Guidance Document. Office of Water. Washington, D.C. October 2006.

————. 2007. An Approach for Using Load Duration Curves in the Development of TMDLs. EPA 841-B-07-006.

U.S. Geological Survey. 1996. HYSEP: A Computer Program for Streamflow Hydrograph Separation and Analysis. USGS Water-Resource Investigations Report 96-4040. Website http://pa.water.usgs.gov/reports/wrir96-4040.pdf, last visited 08/16/07.

University of Maryland, Mid-Atlantic Regional Earth Science Applications Center, version 1.05, 2000.

Versar. 2004. Development of Regional Flow Duration Curves in Maryland. Prepared for Maryland Department of the Environment.

Appendix A – Bacteria Data

Table A-1: Measured Bacteria Concentration with Daily Flow Frequency

Station	Date	Daily flow frequency	*E. coli* Concentration (MPN/100ml)
ANT0002	10/09/2002	98.4450	170
	10/23/2002	87.6588	50
	11/12/2002	61.4214	140
	11/26/2002	47.6347	120
	12/04/2002	62.8778	10
	12/18/2002	21.9996	70
	01/08/2003	13.8305	150
	01/23/2003	36.6404	30
	02/05/2003	36.3885	110
	03/05/2003	13.5129	90
	03/19/2003	5.4205	100
	04/23/2003	12.8778	160
	05/07/2003	24.2554	150
	05/21/2003	4.4569	496
	06/04/2003	3.8874	24190
	06/18/2003	4.0188	990
	06/25/2003	6.9864	380
	07/09/2003	20.3132	360
	07/23/2003	27.2449	860
	08/06/2003	39.0495	230
	08/20/2003	39.9036	290
	08/27/2003	31.5922	15530
	09/10/2003	25.3176	460
	09/24/2003	3.7122	15530
	10/08/2003	21.0578	110
	10/22/2003	26.4893	250

Station	**Date**	**Daily flow frequency**	***E. coli*** **Concentration (MPN/100ml)**
ANT0044	10/09/2002	98.4450	190
	10/23/2002	87.6588	70
	11/12/2002	61.4214	160
	11/26/2002	47.6347	60
	12/04/2002	62.8778	100
	12/18/2002	21.9996	270
	01/08/2003	13.8305	120
	01/23/2003	36.6404	20
	02/05/2003	36.3885	90
	03/05/2003	13.5129	140
	03/19/2003	5.4205	130
	04/23/2003	12.8778	380
	05/07/2003	24.2554	240
	05/21/2003	4.4569	1120
	06/04/2003	3.8874	24190
	06/18/2003	4.0188	1080
	06/25/2003	6.9864	350
	07/09/2003	20.3132	350
	07/23/2003	27.2449	1730
	08/06/2003	39.0495	170
	08/20/2003	39.9036	360
	08/27/2003	31.5922	8160
	09/10/2003	25.3176	450
	09/24/2003	3.7122	8170
	10/08/2003	21.0578	150
	10/22/2003	26.4893	210

Station	Date	Daily flow frequency	*E. coli* Concentration (MPN/100ml)
ANT0132	10/09/2002	98.4450	120
	10/23/2002	87.6588	110
	11/12/2002	61.4214	3650
	11/26/2002	47.6347	30
	12/04/2002	62.8778	80
	12/18/2002	21.9996	210
	01/08/2003	13.8305	130
	01/23/2003	36.6404	30
	02/05/2003	36.3885	80
	03/05/2003	13.5129	90
	03/19/2003	5.4205	30
	04/23/2003	12.8778	620
	05/07/2003	24.2554	380
	05/21/2003	4.4569	410
	06/04/2003	3.8874	24190
	06/18/2003	4.0188	2100
	06/25/2003	6.9864	430
	07/09/2003	20.3132	290
	07/23/2003	27.2449	5480
	08/06/2003	39.0495	160
	08/20/2003	39.9036	470
	08/27/2003	31.5922	8660
	09/10/2003	25.3176	710
	09/24/2003	3.7122	13000
	10/08/2003	21.0578	170
	10/22/2003	26.4893	120

Station	Date	Daily flow frequency	*E. coli* Concentration (MPN/100ml)
ANT0223	10/09/2002	98.4450	170
	10/23/2002	87.6588	250
	11/12/2002	61.4214	300
	11/26/2002	47.6347	14
	12/04/2002	62.8778	10
	12/18/2002	21.9996	220
	01/08/2003	13.8305	200
	01/23/2003	36.6404	60
	02/05/2003	36.3885	320
	03/05/2003	13.5129	130
	03/19/2003	5.4205	250
	04/23/2003	12.8778	1330
	05/07/2003	24.2554	540
	05/21/2003	4.4569	700
	06/04/2003	3.8874	24190
	06/18/2003	4.0188	2910
	06/25/2003	6.9864	750
	07/09/2003	20.3132	450
	07/23/2003	27.2449	7700
	08/06/2003	39.0495	1080
	08/20/2003	39.9036	640
	08/27/2003	31.5922	2760
	09/10/2003	25.3176	2360
	09/24/2003	3.7122	7270
	10/08/2003	21.0578	170
	10/22/2003	26.4893	210

Station	Date	Daily flow frequency	*E. coli* Concentration (MPN/100ml)
ANT0277	10/23/2002	87.6588	370
	11/12/2002	61.4214	2610
	11/26/2002	47.6347	23
	12/04/2002	62.8778	70
	12/18/2002	21.9996	310
	01/08/2003	13.8305	300
	01/23/2003	36.6404	70
	02/05/2003	36.3885	310
	03/19/2003	5.4205	200
	04/23/2003	12.8778	750
	05/07/2003	24.2554	400
	05/21/2003	4.4569	860
	06/04/2003	3.8874	24190
	06/18/2003	4.0188	4110
	06/25/2003	6.9864	410
	07/09/2003	20.3132	390
	07/23/2003	27.2449	3260
	08/06/2003	39.0495	910
	08/20/2003	39.9036	800
	08/27/2003	31.5922	9800
	09/10/2003	25.3176	1090
	09/24/2003	3.7122	1730
	10/08/2003	21.0578	360
	10/22/2003	26.4893	350

Station	**Date**	**Daily flow frequency**	***E. coli* Concentration (MPN/100ml)**
ANT0366	10/09/2002	98.4450	1400
	10/23/2002	87.6588	750
	11/12/2002	61.4214	2600
	11/26/2002	47.6347	17
	12/04/2002	62.8778	310
	12/18/2002	21.9996	460
	01/08/2003	13.8305	300
	01/23/2003	36.6404	470
	02/05/2003	36.3885	320
	03/05/2003	13.5129	610
	03/19/2003	5.4205	190
	04/23/2003	12.8778	670
	05/07/2003	24.2554	780
	05/21/2003	4.4569	5170
	06/04/2003	3.8874	14140
	06/18/2003	4.0188	5790
	06/25/2003	6.9864	830
	07/09/2003	20.3132	640
	07/23/2003	27.2449	5790
	08/06/2003	39.0495	4570
	08/20/2003	39.9036	1010
	08/27/2003	31.5922	5480
	09/10/2003	25.3176	770
	09/24/2003	3.7122	1040
	10/08/2003	21.0578	530
	10/22/2003	26.4893	490

Station	**Date**	**Daily flow frequency**	***E. coli*** **Concentration (MPN/100ml)**
BEC0001	10/09/2002	98.4450	390
	10/23/2002	87.6588	260
	11/12/2002	61.4214	670
	11/26/2002	47.6347	230
	12/04/2002	62.8778	110
	12/18/2002	21.9996	80
	01/08/2003	13.8305	230
	01/23/2003	36.6404	60
	02/05/2003	36.3885	150
	03/05/2003	13.5129	100
	03/19/2003	5.4205	100
	04/23/2003	12.8778	310
	05/07/2003	24.2554	400
	05/21/2003	4.4569	1400
	06/04/2003	3.8874	10460
	06/18/2003	4.0188	1210
	06/25/2003	6.9864	570
	07/09/2003	20.3132	400
	07/23/2003	27.2449	760
	08/06/2003	39.0495	1530
	08/20/2003	39.9036	1550
	08/27/2003	31.5922	5170
	09/10/2003	25.3176	790
	09/24/2003	3.7122	1180
	10/08/2003	21.0578	350
	10/22/2003	26.4893	410

Station	**Date**	**Daily flow frequency**	***E. coli*** **Concentration (MPN/100ml)**
LAS0004	10/09/2002	98.4450	320
	10/23/2002	87.6588	510
	11/12/2002	61.4214	350
	11/26/2002	47.6347	110
	12/04/2002	62.8778	130
	12/18/2002	21.9996	130
	01/08/2003	13.8305	200
	01/23/2003	36.6404	20
	02/05/2003	36.3885	70
	03/05/2003	13.5129	130
	03/19/2003	5.4205	110
	04/23/2003	12.8778	450
	05/07/2003	24.2554	500
	05/21/2003	4.4569	830
	06/04/2003	3.8874	12030
	06/18/2003	4.0188	990
	06/25/2003	6.9864	520
	07/09/2003	20.3132	2310
	07/23/2003	27.2449	7270
	08/06/2003	39.0495	410
	08/20/2003	39.9036	860
	08/27/2003	31.5922	9800
	09/10/2003	25.3176	360
	09/24/2003	3.7122	870
	10/08/2003	21.0578	200
	10/22/2003	26.4893	750

Station	Date	Daily flow frequency	*E. coli* Concentration (MPN/100ml)
MRS0000	10/09/2002	98.4450	680
	10/23/2002	87.6588	740
	11/12/2002	61.4214	1660
	11/26/2002	47.6347	7
	12/04/2002	62.8778	110
	12/18/2002	21.9996	90
	01/08/2003	13.8305	170
	01/23/2003	36.6404	80
	02/05/2003	36.3885	500
	03/05/2003	13.5129	400
	03/19/2003	5.4205	110
	04/23/2003	12.8778	320
	05/07/2003	24.2554	800
	05/21/2003	4.4569	710
	06/04/2003	3.8874	24190
	06/18/2003	4.0188	2280
	06/25/2003	6.9864	680
	07/09/2003	20.3132	660
	07/23/2003	27.2449	4610
	08/06/2003	39.0495	1670
	08/20/2003	39.9036	1790
	08/27/2003	31.5922	24190
	09/10/2003	25.3176	1610
	09/24/2003	3.7122	3080
	10/08/2003	21.0578	880
	10/22/2003	26.4893	490

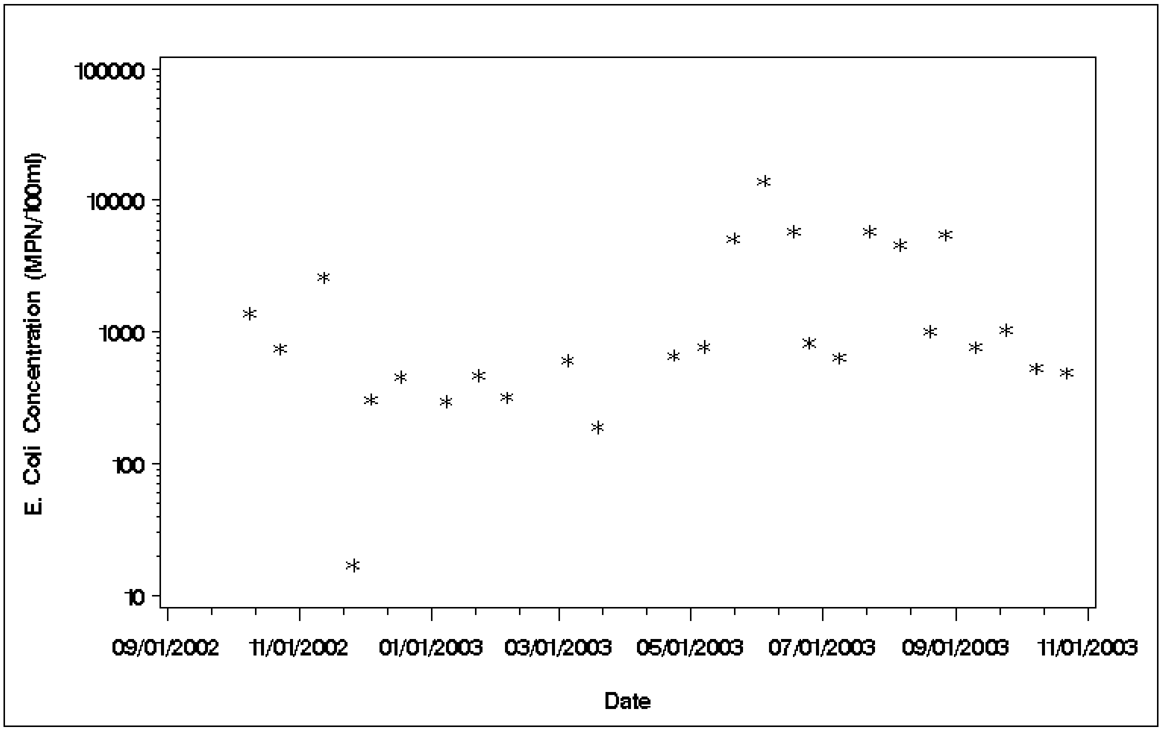


Figure A-1: ***E. coli*** **Concentration vs. Time for MDE Monitoring Station ANT0366**

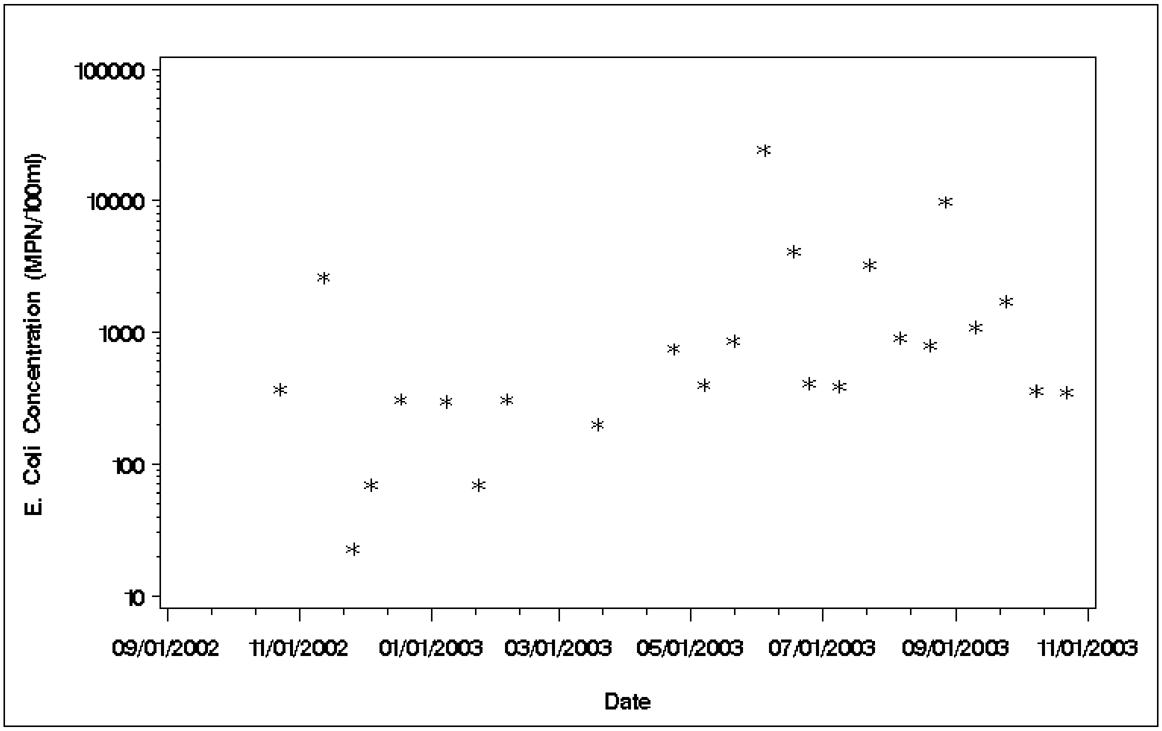


Figure A-2: ***E. coli*** **Concentration vs. Time for MDE Monitoring Station ANT0277**

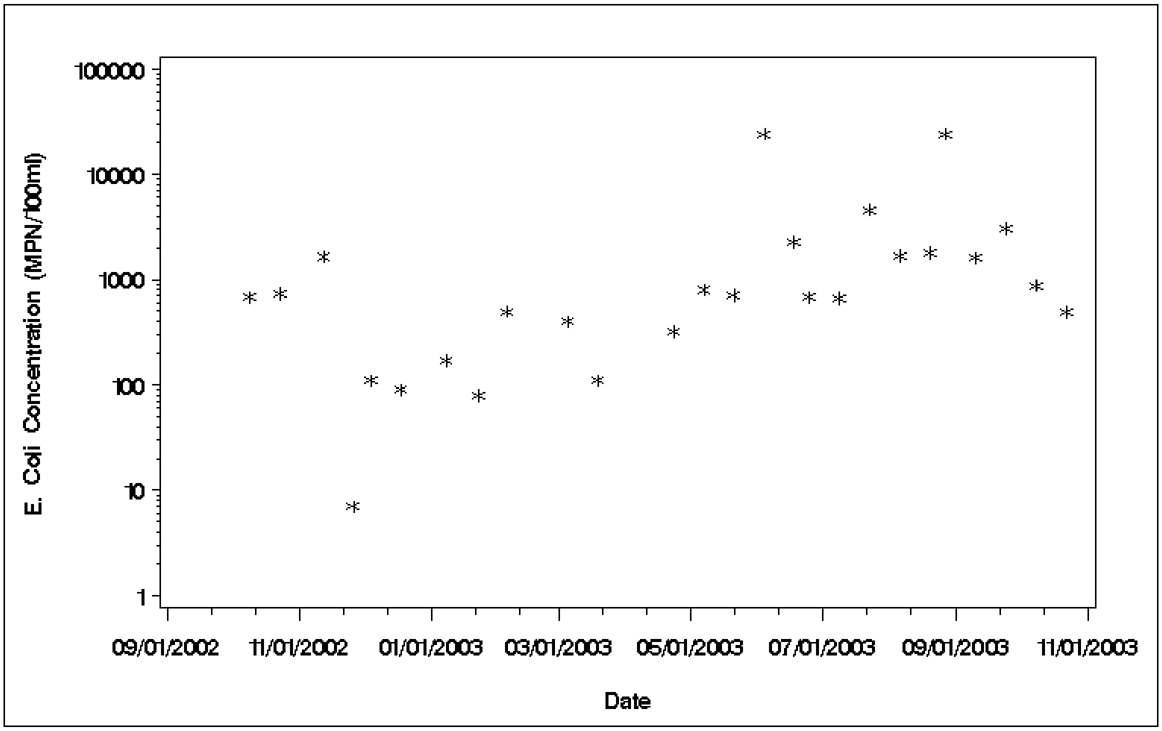


Figure A-3: ***E. coli*** **Concentration vs. Time for MDE Monitoring Station MRS0000**

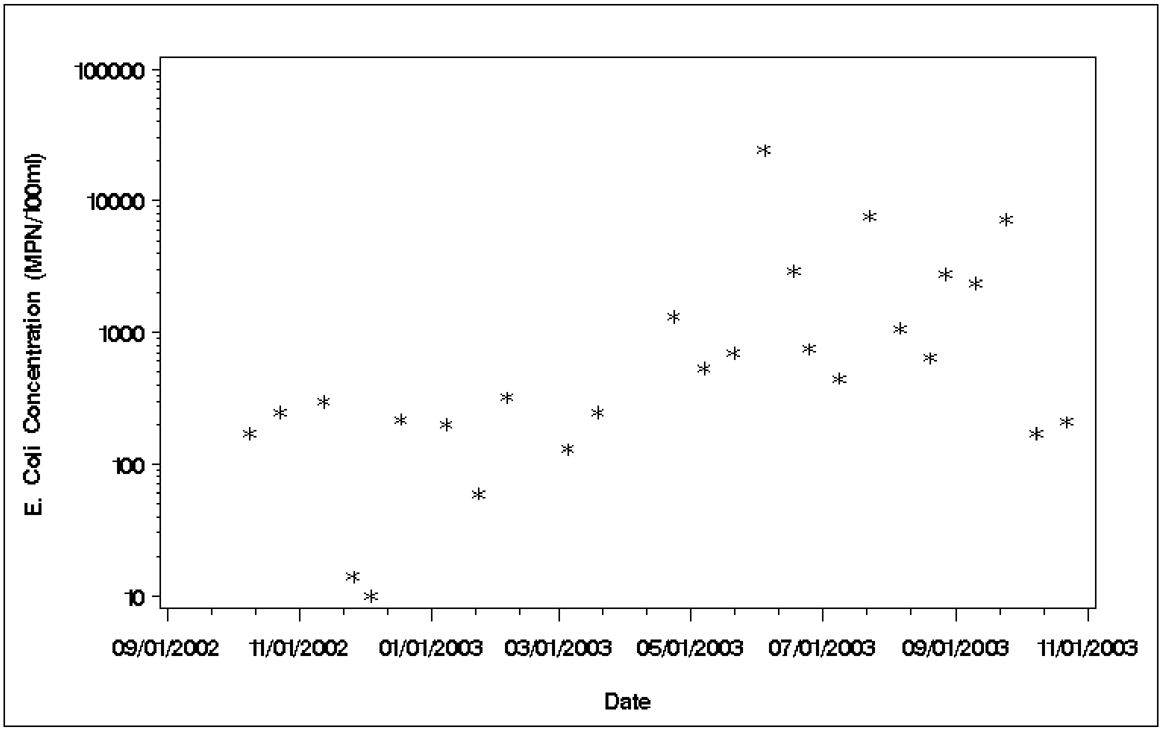


Figure A-4: ***E. coli*** **Concentration vs. Time for MDE Monitoring Station ANT0223**

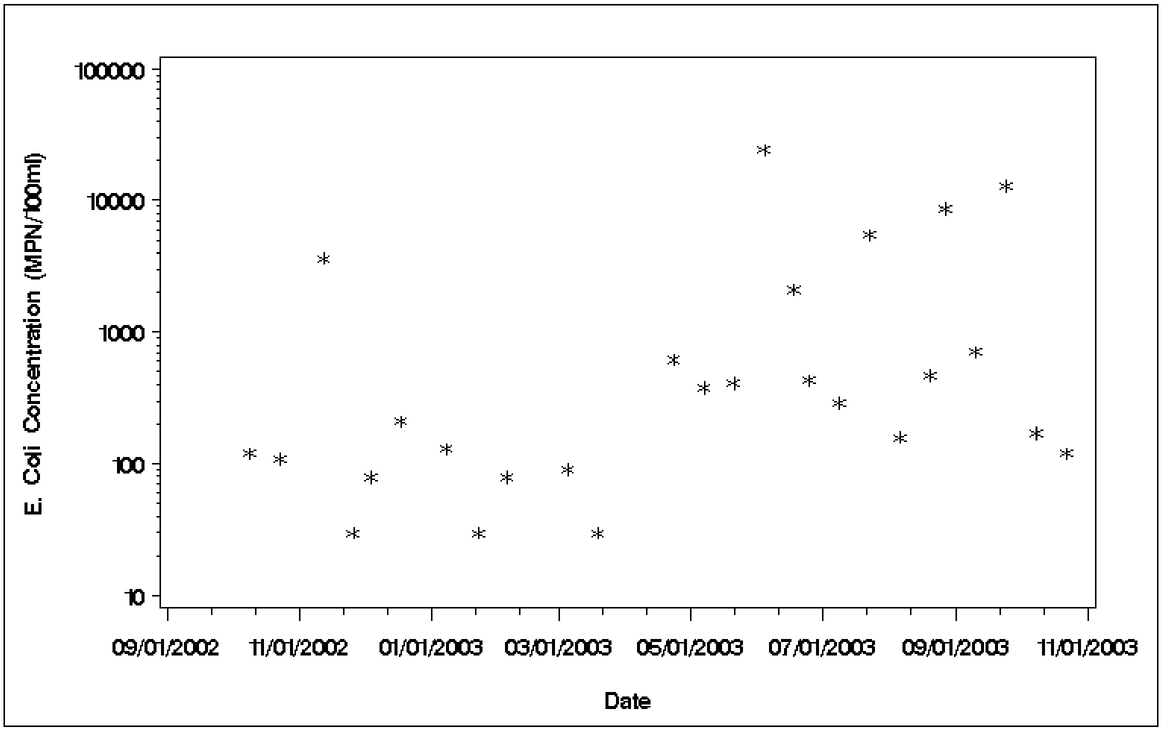


Figure A-5: *E. coli* Concentration vs. Time for MDE Monitoring Station ANT0132

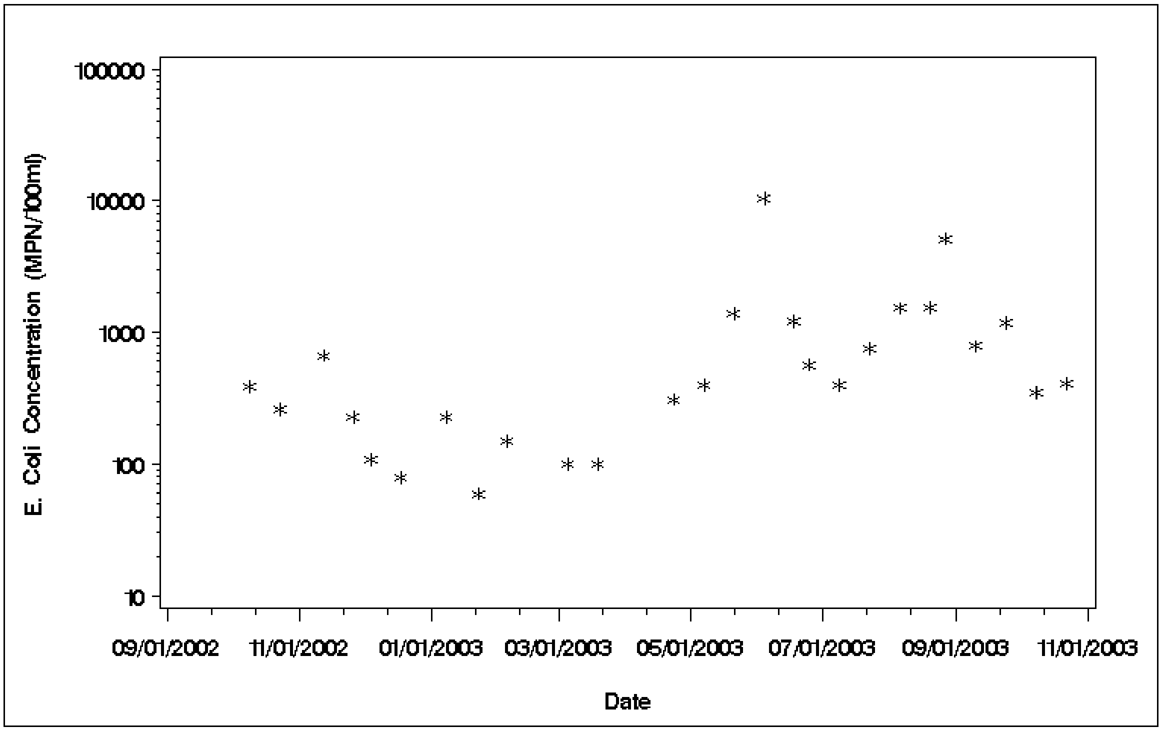


Figure A-6: *E. coli* Concentration vs. Time for MDE Monitoring Station BEC0001

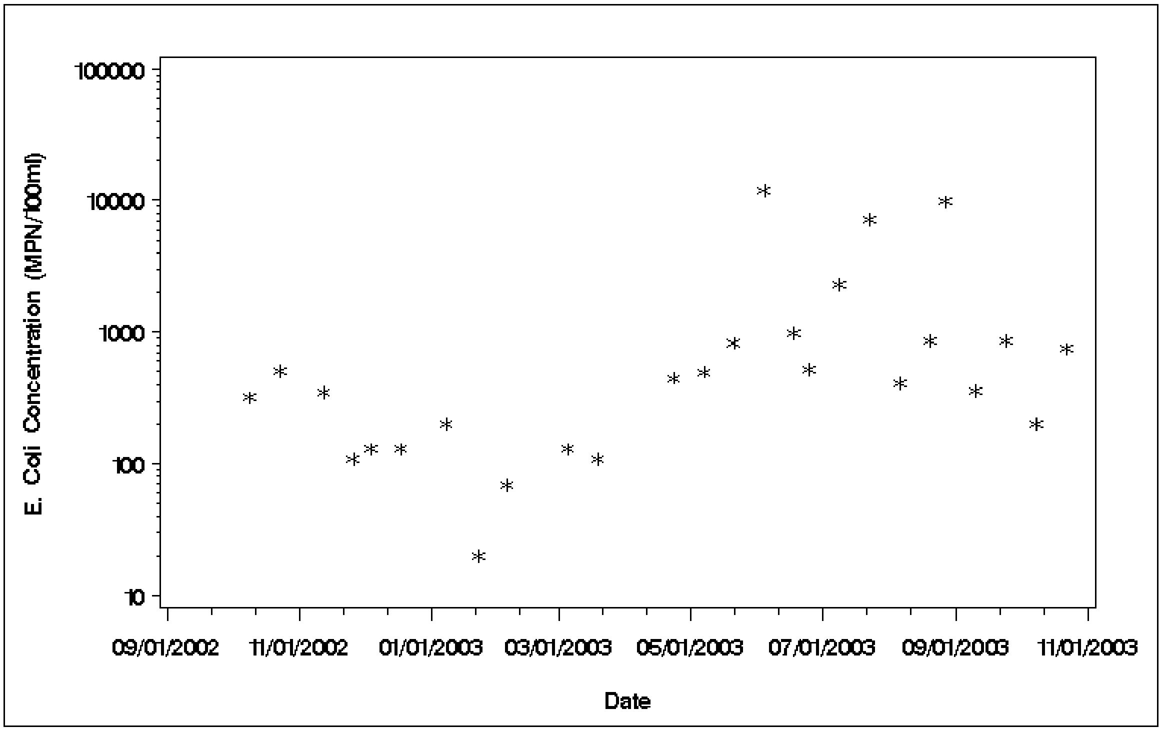


Figure A-7: ***E. coli*** **Concentration vs. Time for MDE Monitoring Station LAS0004**

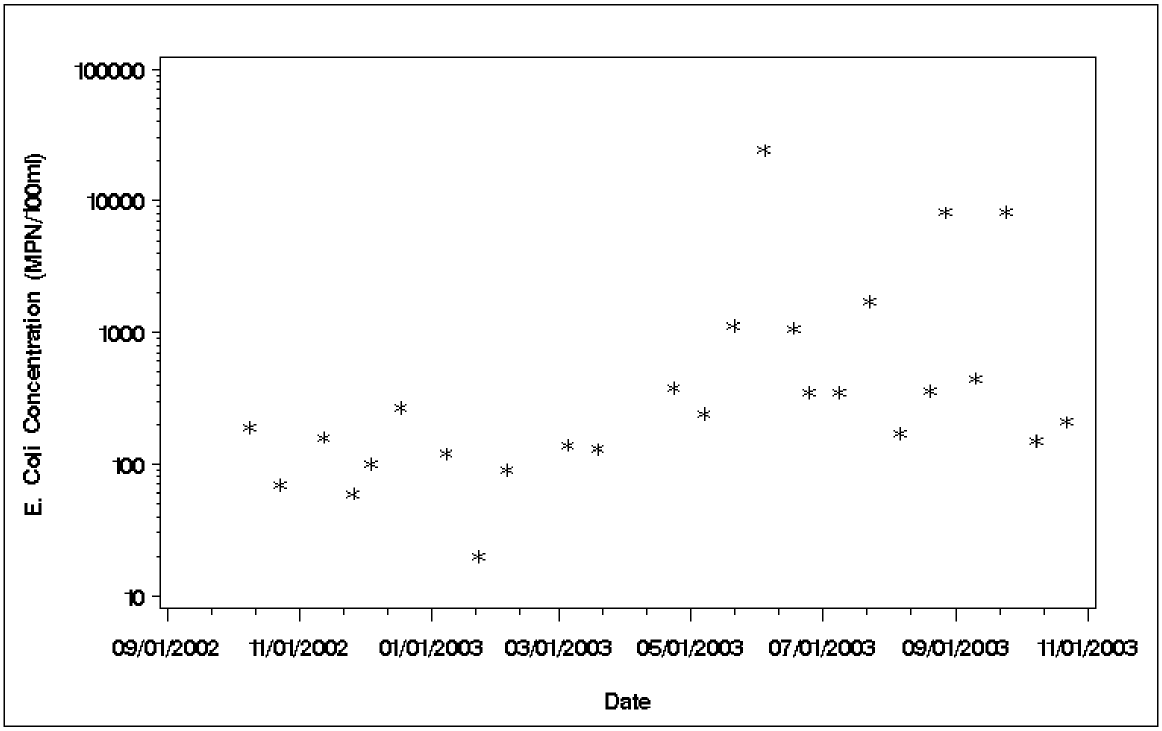


Figure A-8: ***E. coli*** **Concentration vs. Time for MDE Monitoring Station ANT0044**

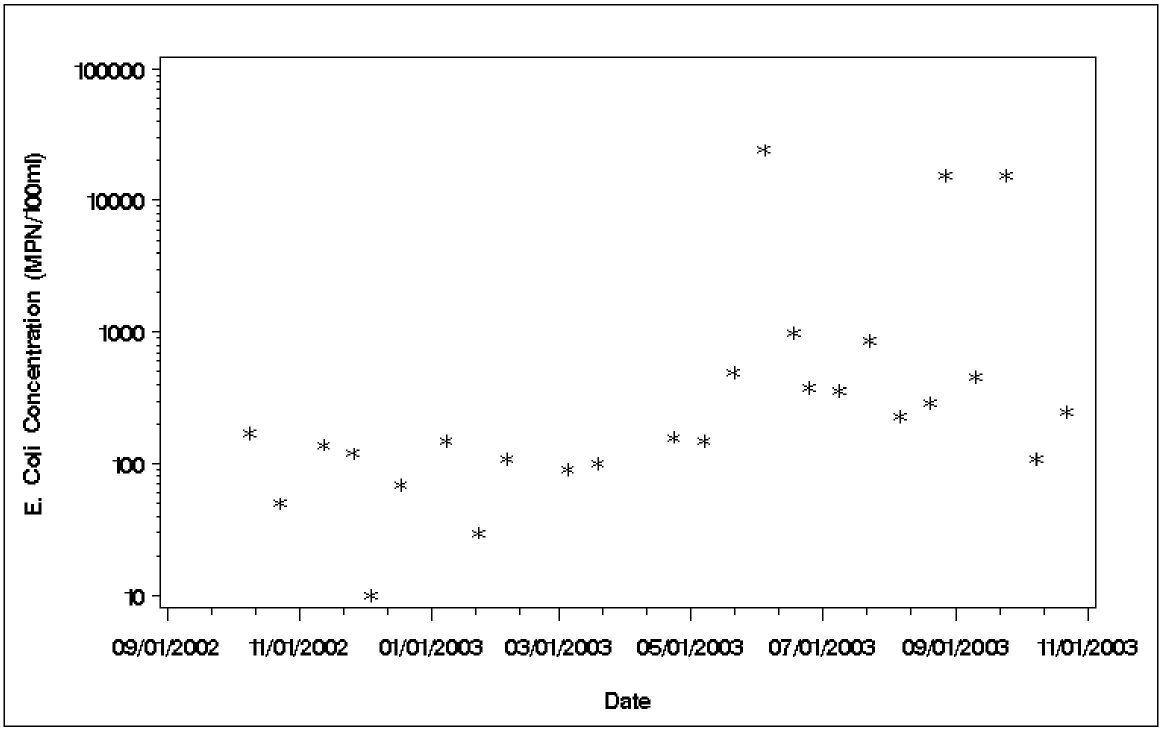


Figure A-9: ***E. coli*** **Concentration vs. Time for MDE Monitoring Station ANT0002**

Appendix B - Flow Duration Curve Analysis to Define Strata

The Antietam Creek watershed was assessed to determine hydrologically significant strata. The purpose of these strata is to apply weights to monitoring data and thus (1) reduce bias associated with the monitoring design and (2) approximate a critical condition for TMDL development. The strata group hydrologically similar water quality samples and provide a better estimate of the mean concentration at the monitoring station.

The flow duration curve for a watershed is a plot of all possible daily flows, ranked from highest to lowest, versus their probability of exceedance. In general, the higher flows will tend to be dominated by excess runoff from rain events and the lower flows will result from drought type conditions. The mid-range flows are a combination of high base flow with limited runoff and lower base flow with excess runoff. The range of these mid-level flows will vary with antecedent soil moisture conditions. The purpose of the following analysis is to identify hydrologically significant groups, based on the previously described flow regimes, within the flow duration curve.

Flow Analysis

The one active USGS gage station in the Antietam Creek watershed, station #01619500 Antietam Creek near Sharpsburg, MD, was used for the analysis. The dates of information used were from October 1, 1981 to September 30, 2006. A flow duration curve for this gage station is presented in Figure B-1.

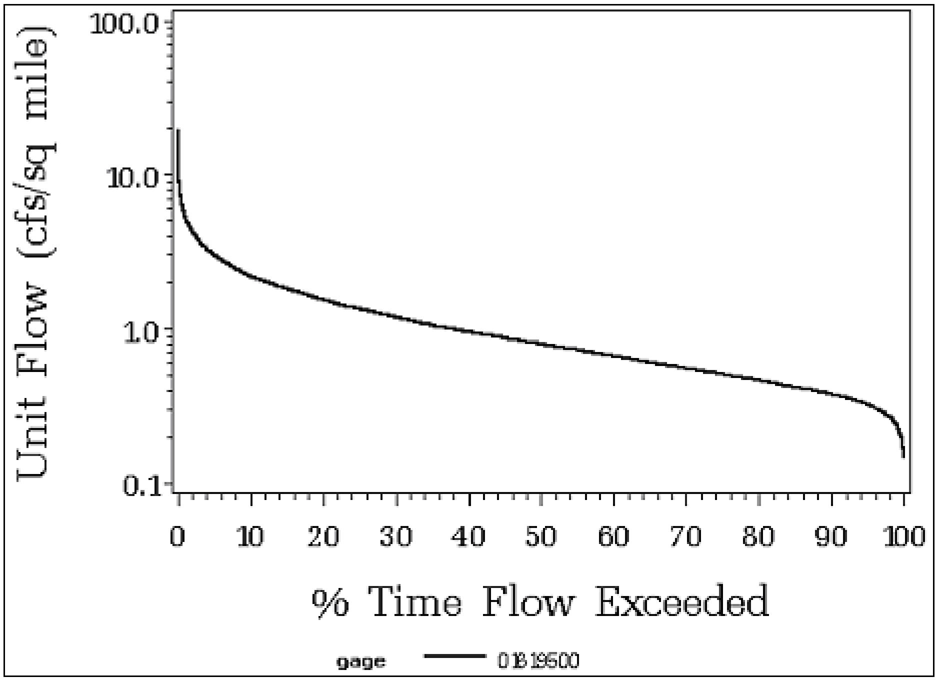


Figure B-1: Flow Duration Curve for USGS Gage 016195000

Based on the flow data from the Antietam Creek gage station the long-term average daily unit flow is 1.13 cfs/sq. mile, which corresponds to a flow frequency of 32.2%. Using the definition of a high flow condition as occurring when flows are higher than the long-term average flow and a low flow condition as occurring when flows are lower than the long-term average flow, the 32.2 percentile threshold was selected to define the limits between high flows and low flows in this watershed. Therefore, a high flow condition will be defined as occurring when the daily flow duration percentile is less than 32.2% and a low flow condition will be defined as occurring when the daily flow duration percentile is greater than 32.2%. Definitions of high and low range flows are presented in Table B-1.

Table B-1: Definition of Flow Regimes

High Flow	Represents conditions where stream flow tends to be dominated by surface runoff.
Low Flow	Represents conditions where stream flow tends to be more dominated by groundwater flow.

Flow Data Analysis

The final analysis to define the daily flow duration intervals (flow regions, strata) includes the bacteria monitoring data. Bacteria (*E. coli*) monitoring data are “placed” within the regions (strata) based on the daily flow duration percentile of the date of sampling. Figures B-2 to B-19 show the Antietam Creek watershed *E. coli* monitoring data with corresponding flow frequency for the average annual and the seasonal conditions.

Maryland’s water quality standards for bacteria state that, when available, the geometric mean indicator should be based on at least five samples taken representatively over 30 days. Therefore, in situations in which fewer than five samples “fall” within a particular flow regime interval, the interval and the adjacent interval will be joined. In the Antietam Creek watershed, for the annual average flow condition, there are sufficient samples in both the high flow and low flow strata to estimate the geometric means. However, in the seasonal (May 1st – September 30th) flow condition, there are only two samples within the low flow strata; therefore, for this condition an average seasonal geometric mean will be calculated.

Weighting factors for estimating a weighted geometric mean are based on the frequency of each flow stratum during the averaging period. The weighting factors for the averaging periods and hydrological conditions are presented in Table B-2. Averaging periods are defined in this report as:

(1) Average Annual Hydrological Condition
(2) Annual High Flow Condition
(3) Annual Low Flow Condition
(4) Seasonal (May 1st – September 30th) Average Flow Condition

Weighted geometric means for the average annual and the seasonal conditions are plotted with the monitoring data on Figures B-2 to B-19.

Table B-2: Weighting Factors for Estimation of Geometric Mean

Hydrological Condition		Averaging Period	Water Quality Data Used	Fraction High Flow	Fraction Low Flow	Condition Period
Annual	Average	365 days	All	0.322	0.678	Long-Term Average
	Wet (High Flow)	365 days	All	1.000	0	Jan. 1996 – Jan. 1997
	Dry (Low Flow)	365 days	All	0	1.000	Apr. 2001 – Apr. 2002
Seasonal	Average	May 1st – Sept. 30th	May 1st – Sept. 30th	1.000		N/A

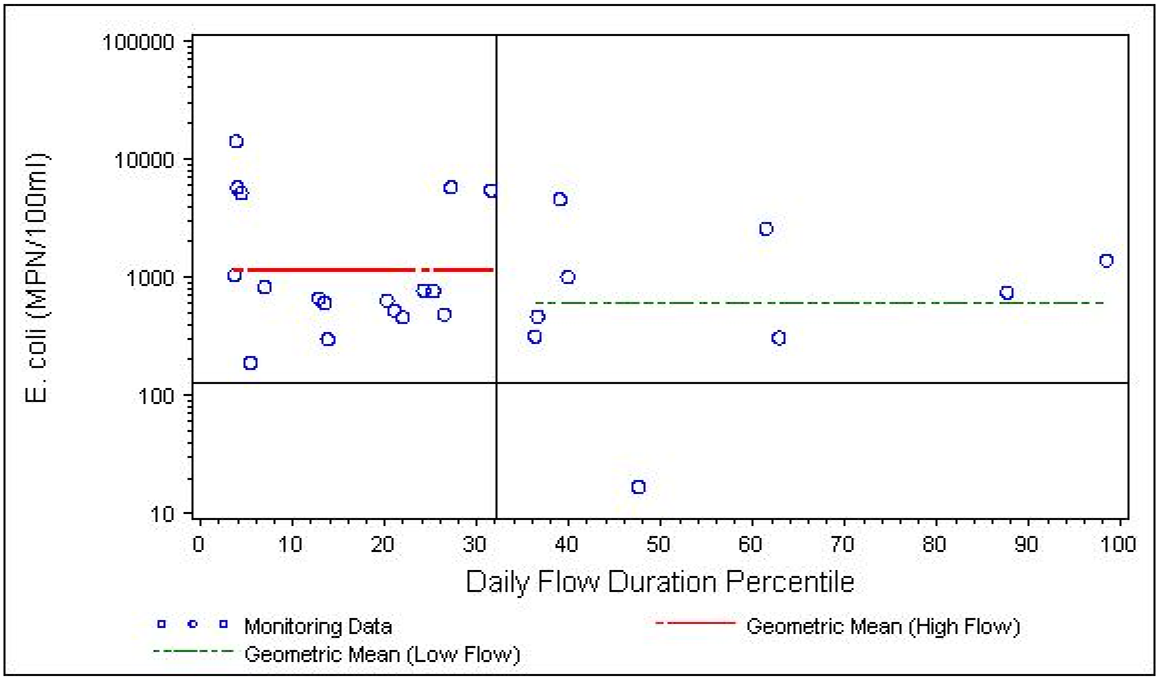


Figure B-2: *E. coli* Concentration vs. Flow Duration for Monitoring Station ANT0366 (Annual Condition)

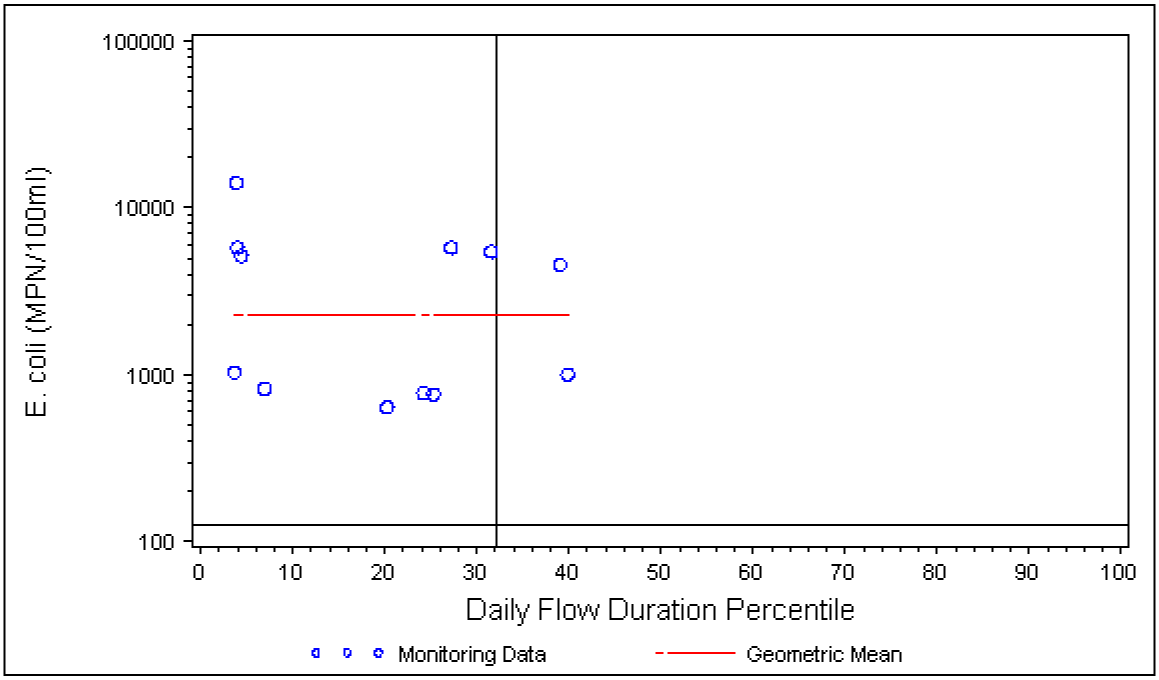


Figure B-3: *E. coli* Concentration vs. Flow Duration for Monitoring Station ANT0366 (Seasonal Condition)

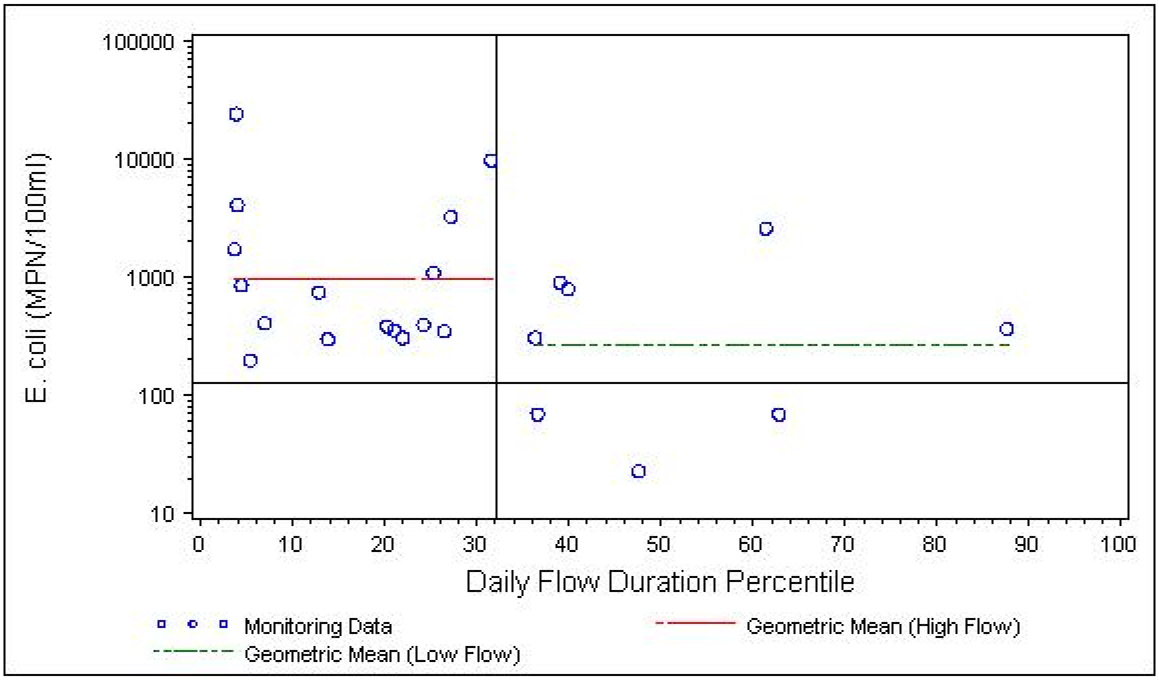


Figure B-4: ***E. coli*** **Concentration vs. Flow Duration for Monitoring Station ANT0277 (Annual Condition)**

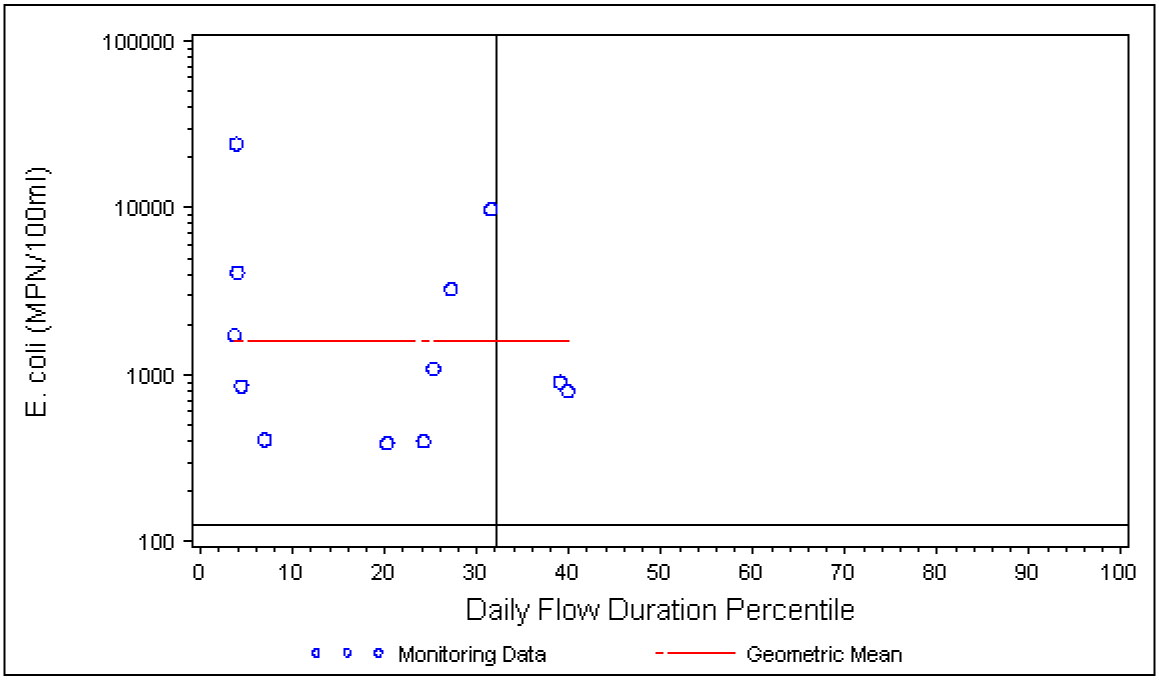


Figure B-5: ***E. coli*** **Concentration vs. Flow Duration for Monitoring Station ANT0277 (Seasonal Condition)**

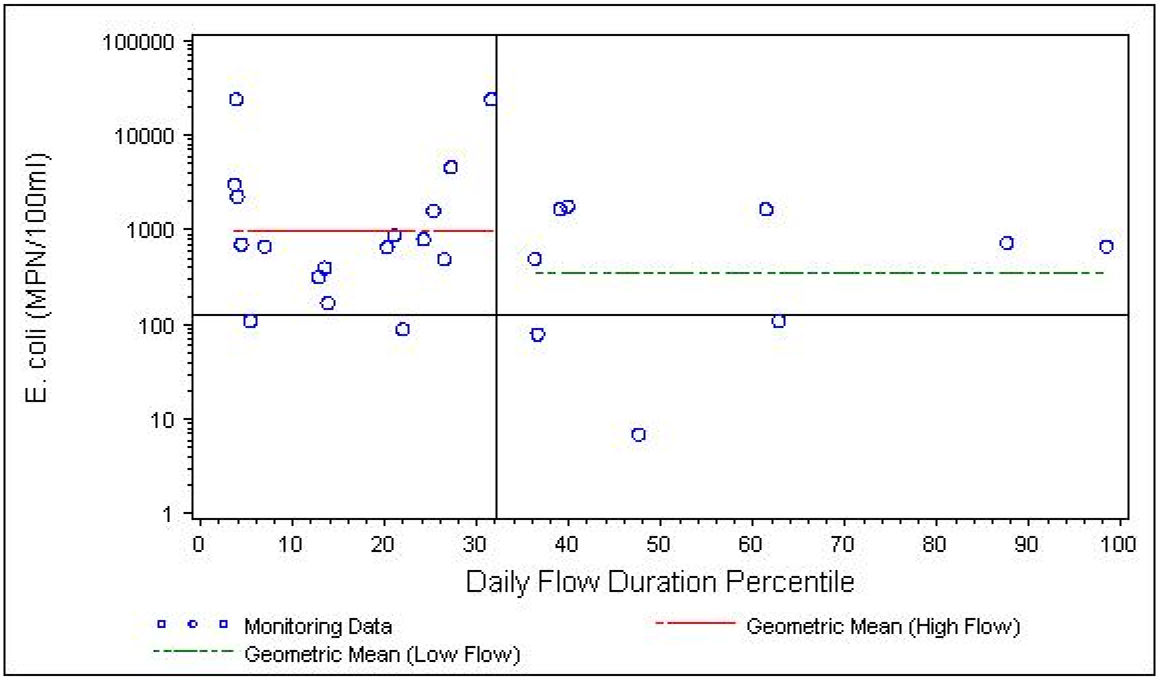


Figure B-6: *E. coli* Concentration vs. Flow Duration for Monitoring Station MRS0000 (Annual Condition)

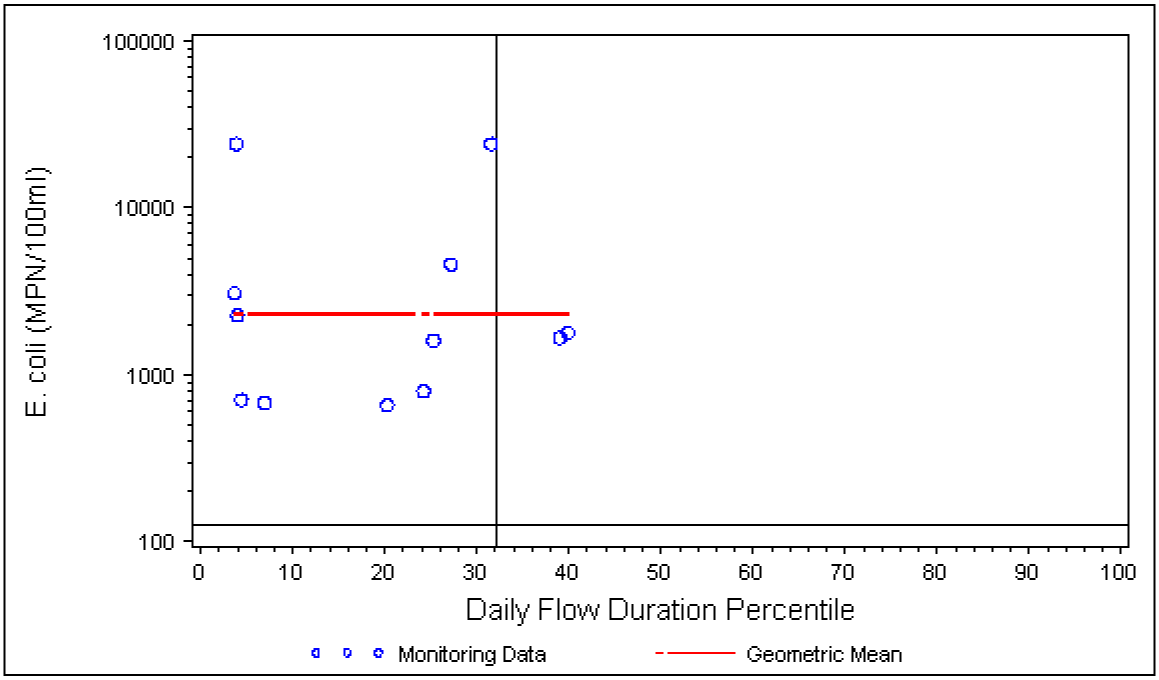


Figure B-7: *E. coli* Concentration vs. Flow Duration for Monitoring Station MRS0000 (Seasonal Condition)

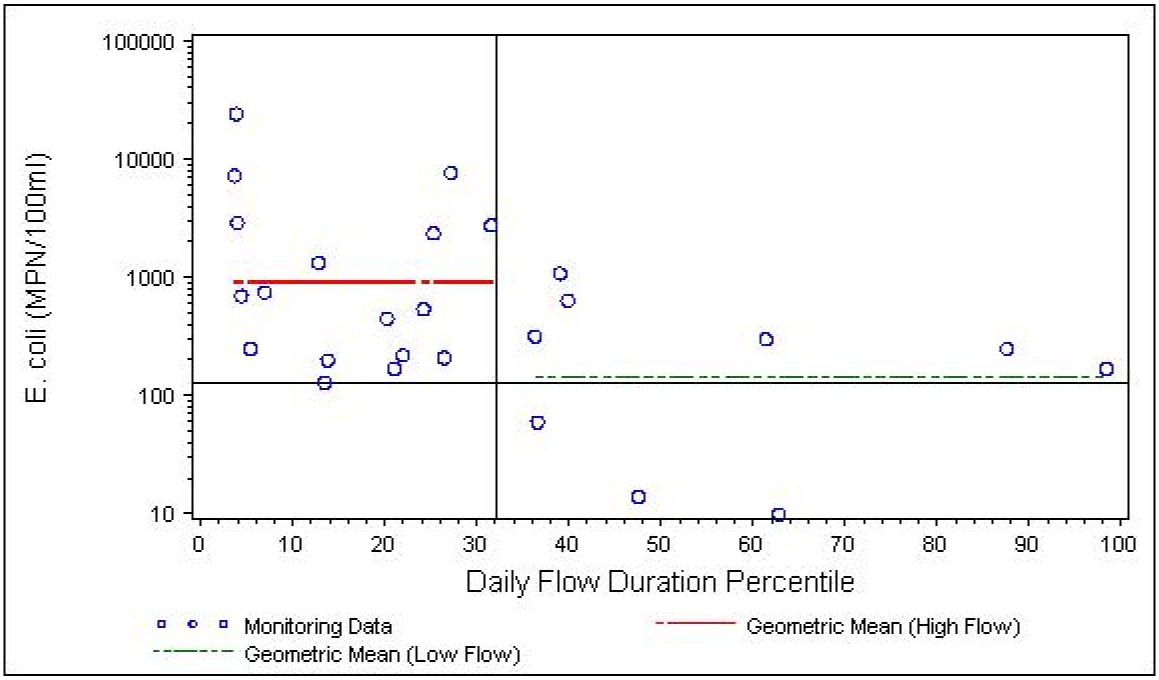


Figure B-8: *E. coli* Concentration vs. Flow Duration for Monitoring Station ANT0223 (Annual Condition)

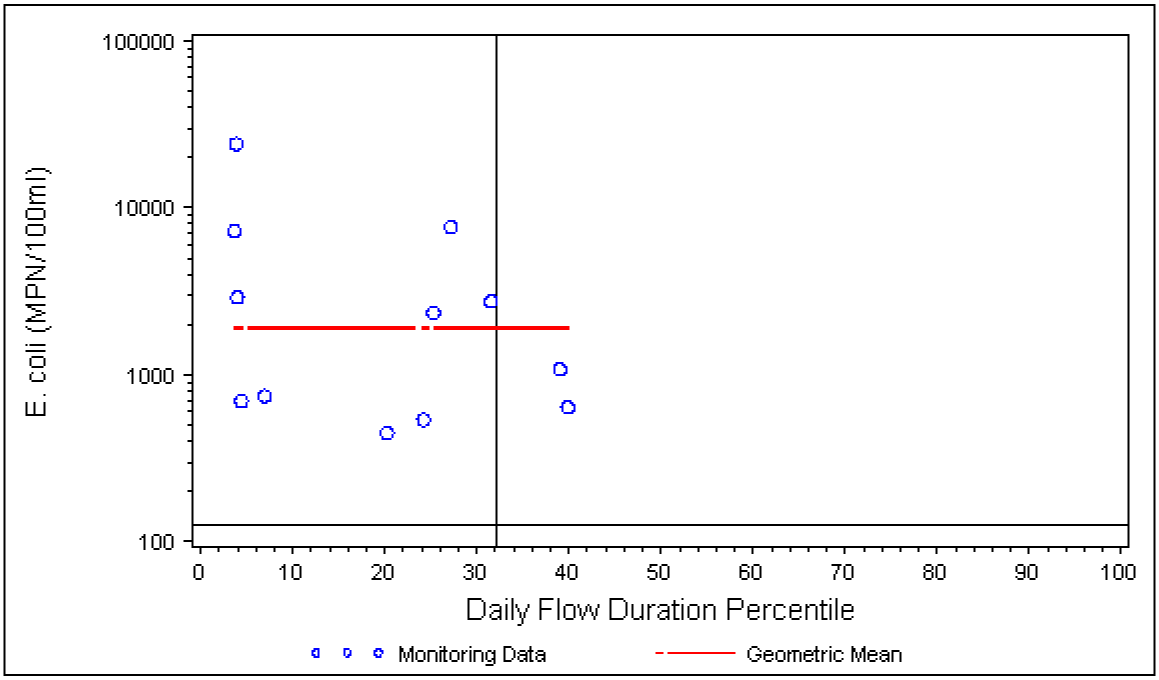


Figure B-9: *E. coli* Concentration vs. Flow Duration for Monitoring Station ANT0223 (Seasonal Condition)

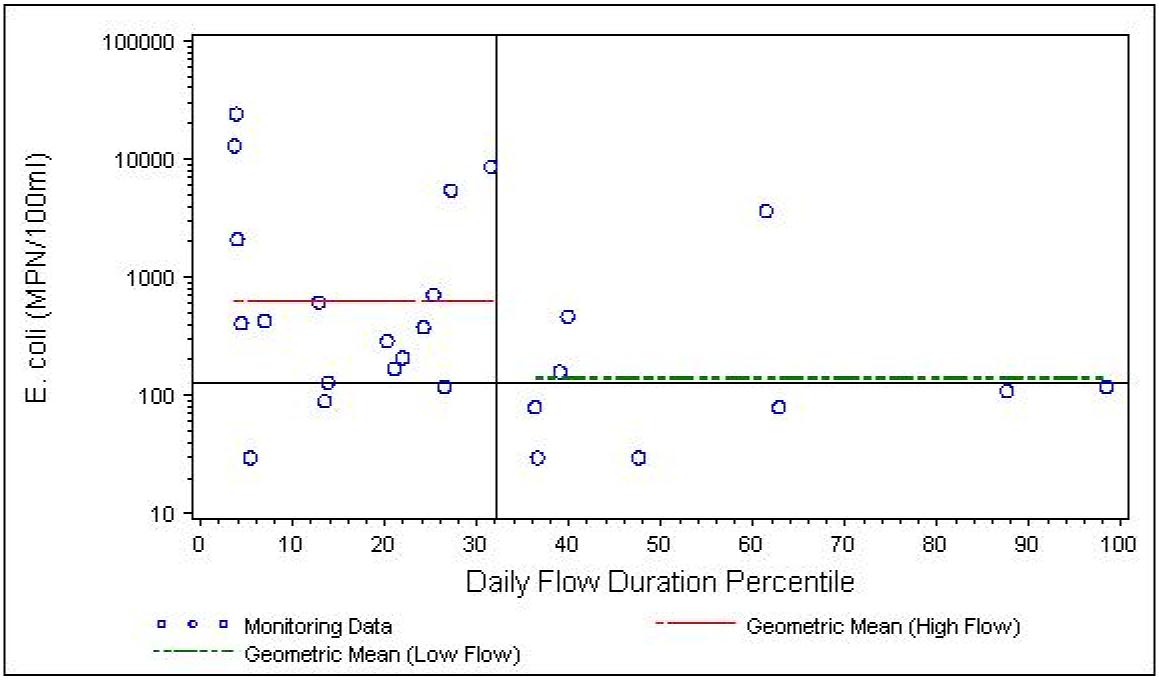


Figure B-10: *E. coli* Concentration vs. Flow Duration for Monitoring Station ANT0132 (Annual Condition)

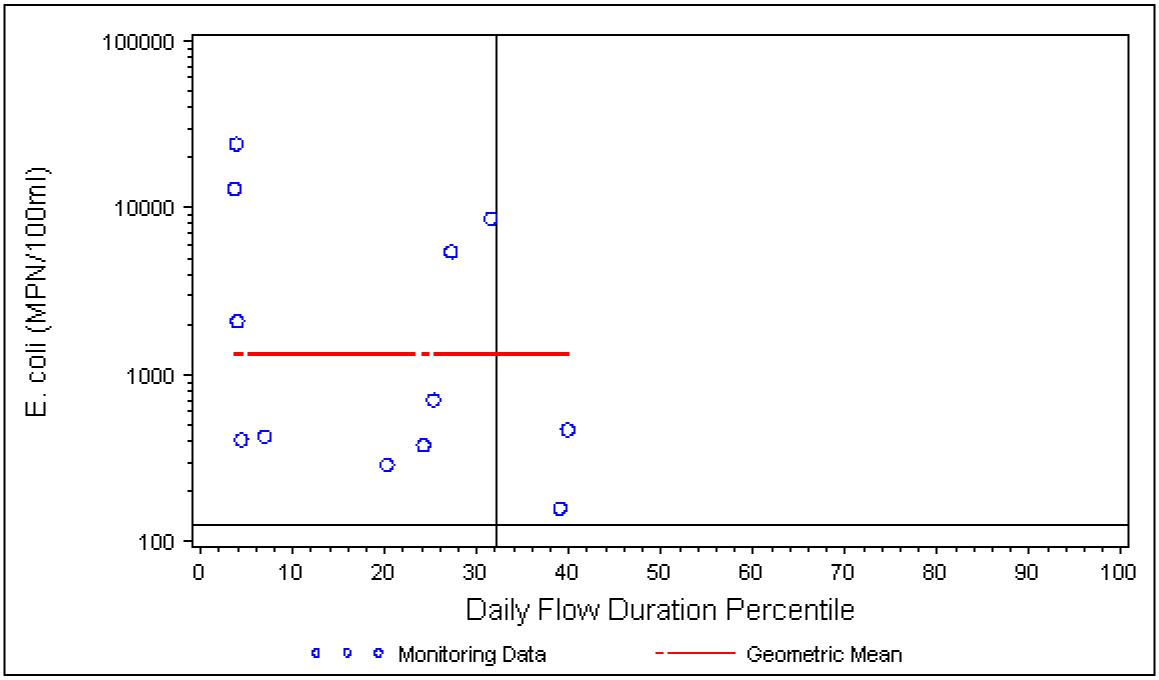


Figure B-11: *E. coli* Concentration vs. Flow Duration for Monitoring Station ANT0132 (Seasonal Condition)

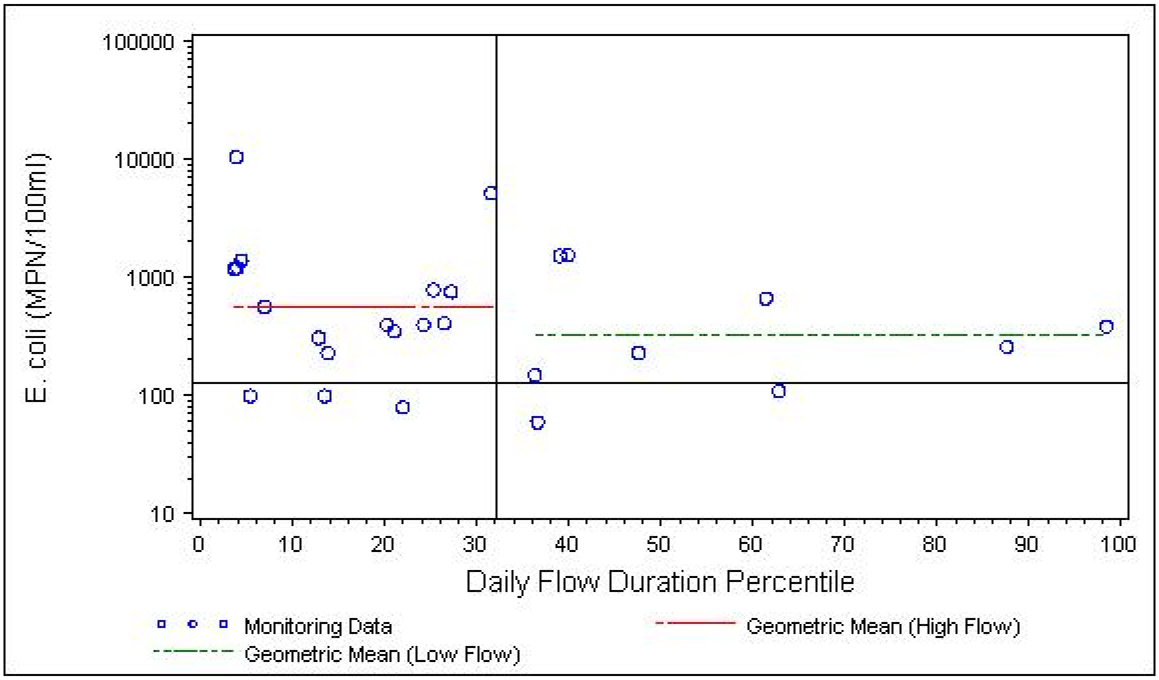


Figure B-12: *E. coli* Concentration vs. Flow Duration for Monitoring Station BEC0001 (Annual Condition)

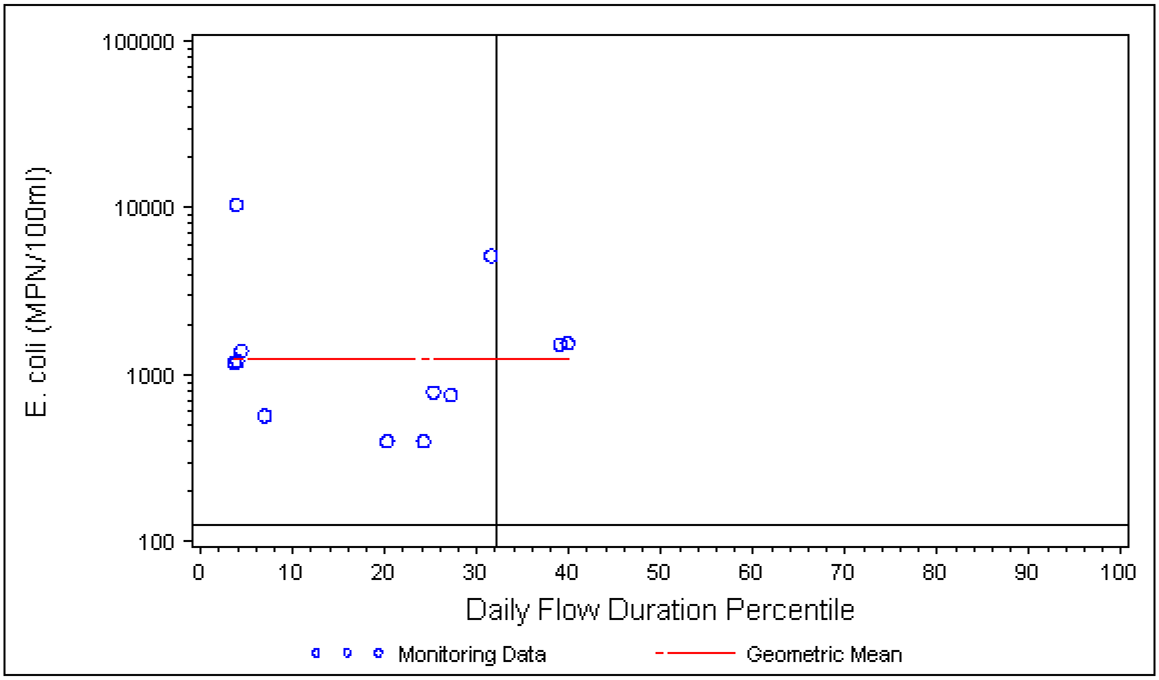


Figure B-13: *E. coli* Concentration vs. Flow Duration for Monitoring Station BEC0001 (Seasonal Condition)

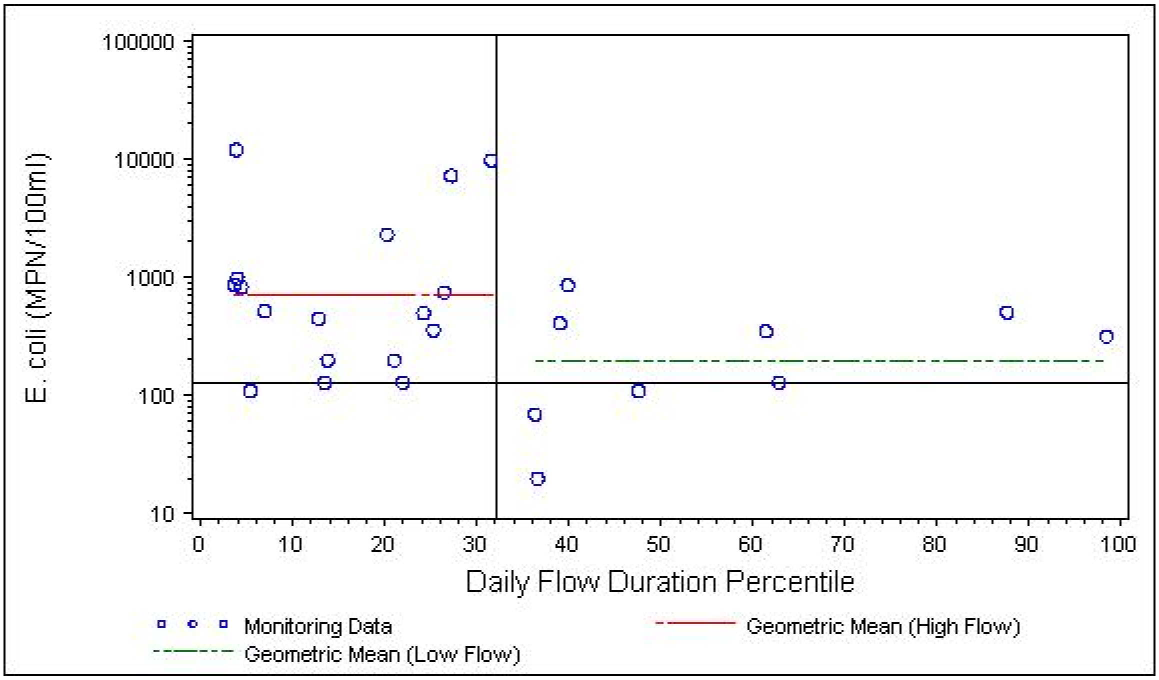


Figure B-14: *E. coli* Concentration vs. Flow Duration for Monitoring Station LAS0004 (Annual Condition)

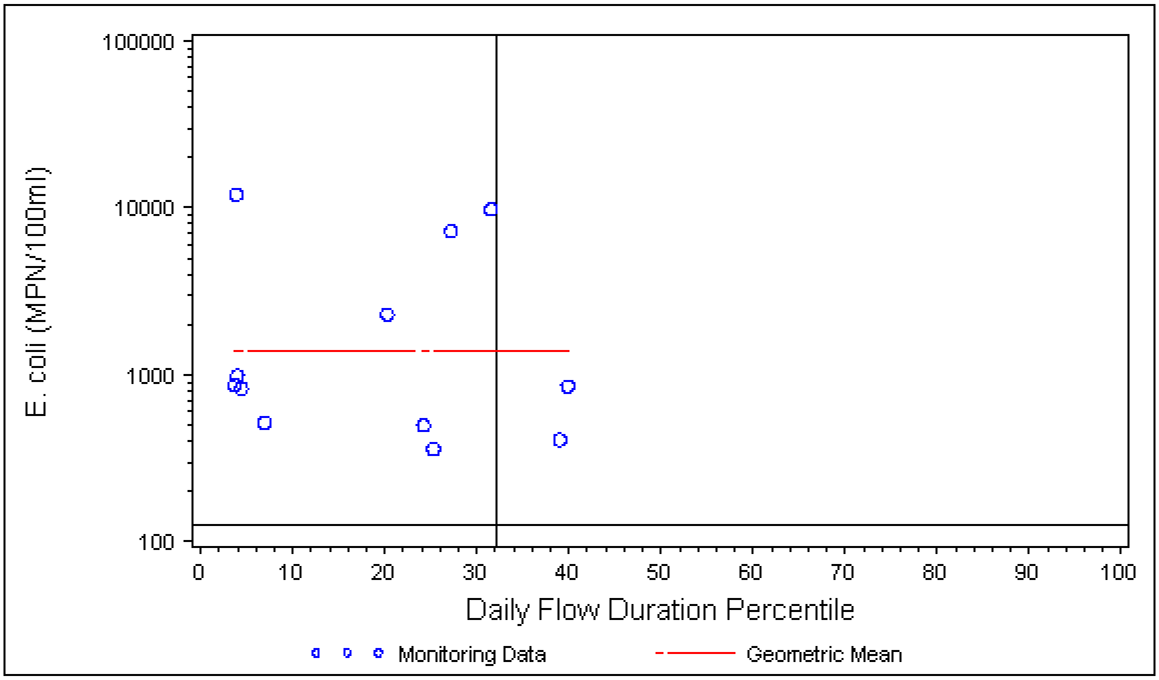


Figure B-15: *E. coli* Concentration vs. Flow Duration for Monitoring Station LAS0004 (Seasonal Condition)

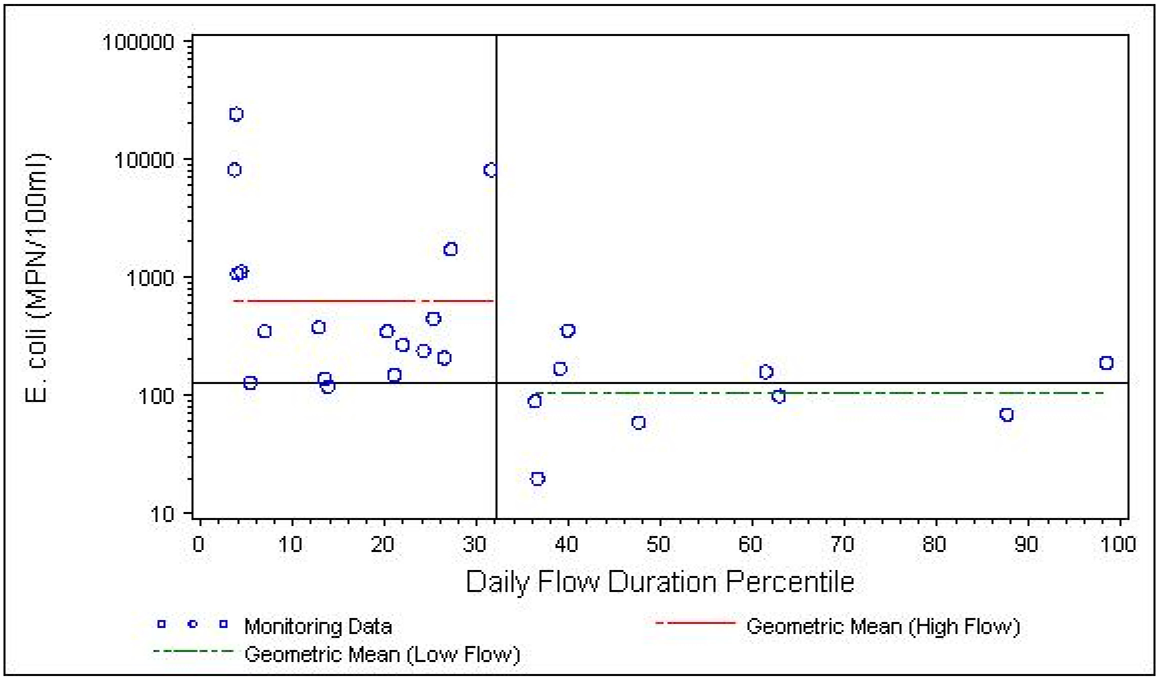


Figure B-16: *E. coli* Concentration vs. Flow Duration for Monitoring Station ANT0044 (Annual Condition)

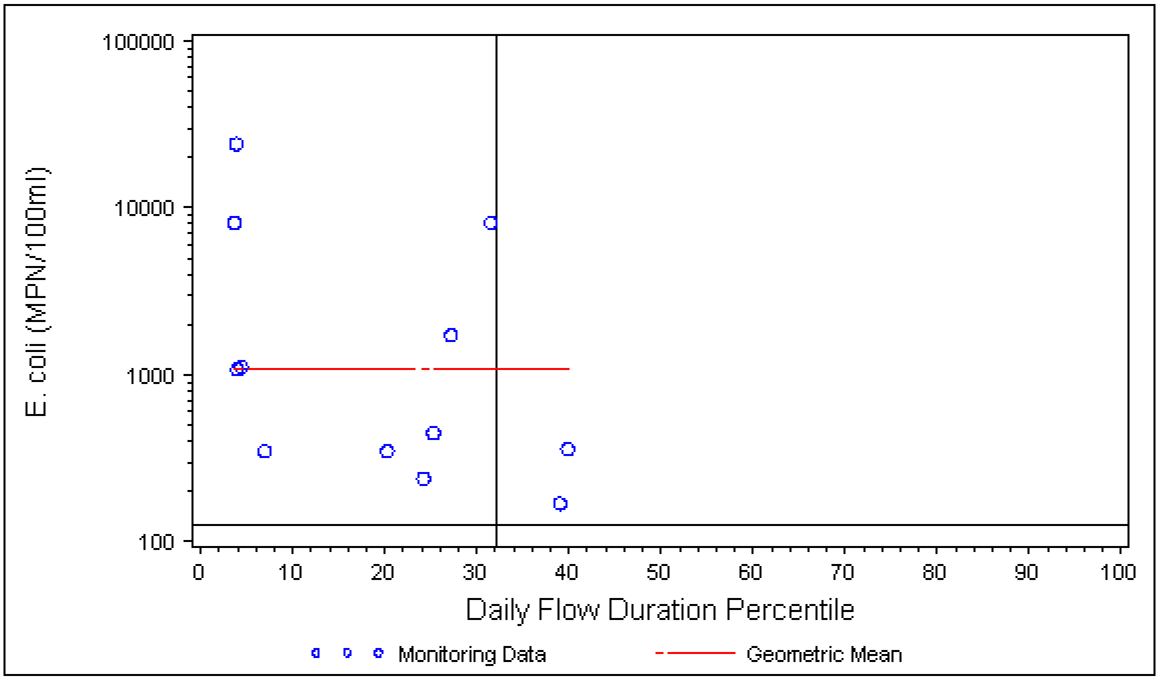


Figure B-17: *E. coli* Concentration vs. Flow Duration for Monitoring Station ANT0044 (Seasonal Condition)

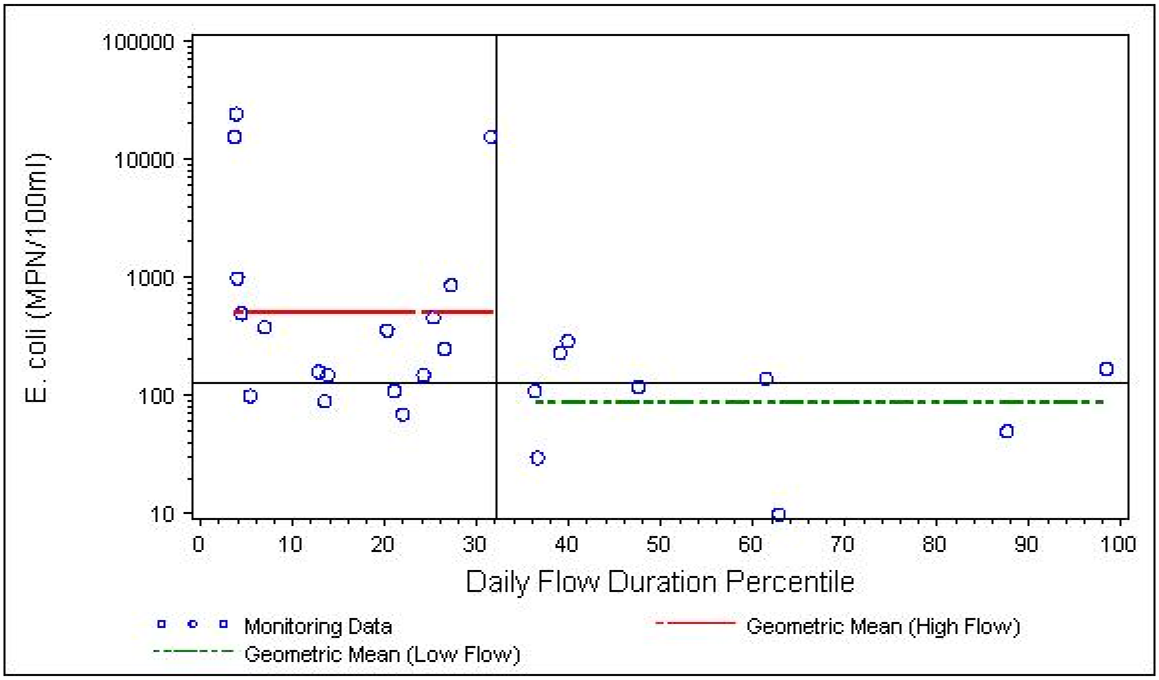


Figure B-18: ***E. coli*** **Concentration vs. Flow Duration for Monitoring Station ANT0002 (Annual Condition)**

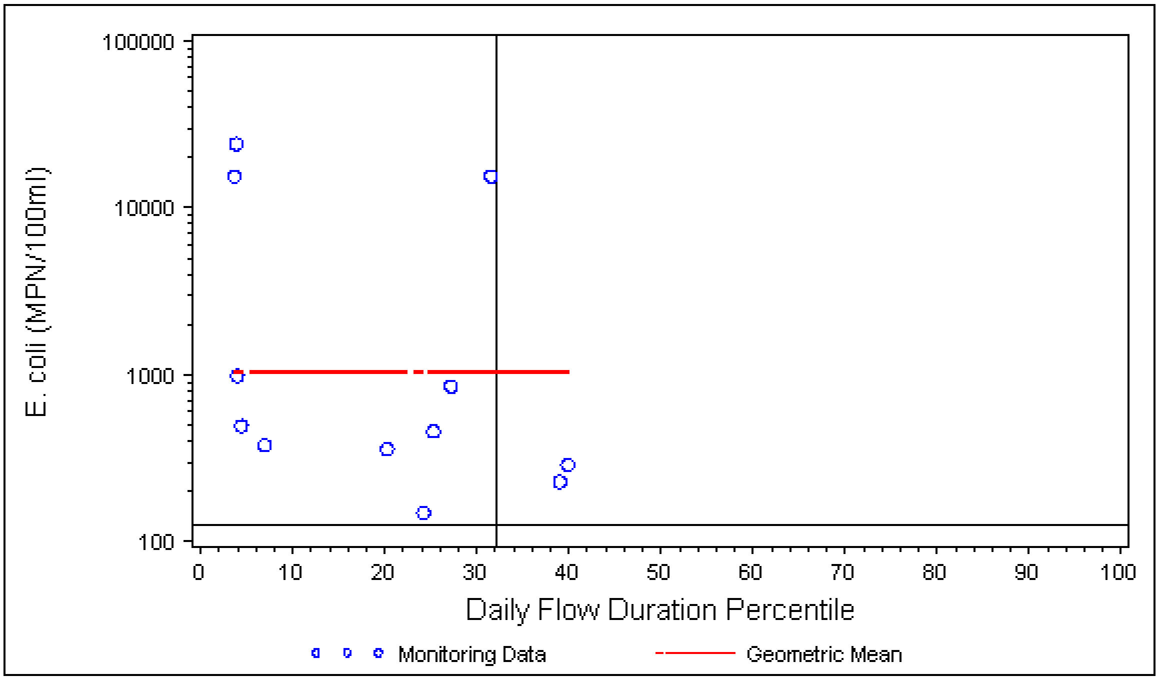


Figure B-19: ***E. coli*** **Concentration vs. Flow Duration for Monitoring Station ANT0002 (Seasonal Condition)**

Appendix C – BST Report

Maryland Department of the Environment

Identifying Sources of Fecal Pollution in Shellfish and Nontidal Waters in Maryland Watersheds

June 2004 – October 2006

Mark F. Frana, Ph.D. and Elichia A. Venso, Ph.D.
Department of Biological Sciences and Environmental Health Science
Salisbury University, Salisbury, MD

Final Report
October 31, 2006

Table of Contents

INTRODUCTION

Microbial Source Tracking. Microbial Source Tracking (MST) is a relatively recent scientific and technological innovation designed to distinguish the origins of enteric microorganisms found in environmental waters. Several different methods and a variety of different indicator organisms (both bacteria and viruses) have successfully been used for MST, as described in recent reviews (Scott et al., 2002; Simpson et al., 2002). When the indicator organism is bacteria, the term Bacterial Source Tracking (BST) is often used. Some common bacterial indicators for BST analysis include: *E. coli, Enterococcus* spp., *Bacteroides-Prevotella,* and *Bifidobacterium* spp.

Techniques for MST can be grouped into one of the following three categories: molecular (genotypic) methods, biochemical (phenotypic) methods, or chemical methods. Ribotyping, Pulsed-Field Gel Electrophoresis (PFGE), and Randomly-Amplified Polymorphic DNA (RAPD) are examples of molecular techniques. Biochemical methods include Antibiotic Resistance Analysis (ARA), F-specific coliphage typing, and Carbon Source Utilization (CSU) analysis. Chemical techniques detect chemical compounds associated with human activities, but do not provide any information regarding nonhuman sources. Examples of this type of technology include detection of optical brighteners from laundry detergents or caffeine (Simpson et al., 2002).

Many of the molecular and biochemical methods of MST are “library-based,” requiring the collection of a database of fingerprints or patterns obtained from indicator organisms isolated from known sources. Statistical analysis determines fingerprints/patterns of known sources species or categories of species (i.e., human, livestock, pets, wildlife). Indicator isolates collected from water samples are analyzed using the same MST method to obtain their fingerprints or patterns, which are then statistically compared to those in the library. Based upon this comparison, the final results are expressed in terms of the “statistical probability” that the water isolates came from a given source (Simpson et al. 2002).

In this BST project, we studied the following Maryland nontidal watersheds: Antietam Creek, Concoheague Creek, Double Pipe Creek, Lower Monocacy River, and Upper Monocacy River. Also included in the study was the Potomac River Watershed shellfish harvesting area. The methodology used was the ARA with *Enterococcus* spp. as the indicator organism. Previous BST publications have demonstrated the predictive value of using this particular technique and indicator organism (Hagedorn, 1999; Wiggins, 1999). A pilot study using PFGE, a genotypic BST method, was used on a subset of known-source isolates collected from the Potomac River Watershed.

Antibiotic Resistance Analysis. A variety of different host species can potentially contribute to the fecal contamination found in natural waters. Many years ago, scientists speculated on the possibility of using resistance to antibiotics as a way of determining the sources of this fecal contamination (Bell et al., 1983; Krumperman, 1983). In ARA, the premise is that bacteria isolated from different hosts can be discriminated based upon differences in the selective pressure of microbial populations found in the gastrointestinal tract of those hosts (humans, livestock, pets, wildlife) (Wiggins, 1996). Microorganisms isolated from the fecal material of wildlife would be expected to have a much lower level of resistance to antibiotics than isolates collected from the fecal material of humans, livestock and pets. In addition, depending upon the specific antibiotics used in the analysis, isolates from humans, livestock and pets could be differentiated from each other.

In ARA, isolates from known sources are tested for resistance or sensitivity against a panel of antibiotics and antibiotic concentrations. This information is then used to construct a library of antibiotic resistance patterns from known-source bacterial isolates. Microbial isolates collected from water samples are then tested and their resistance results are recorded. Based upon a comparison of resistance patterns of water and library isolates, a statistical analysis can predict the likely host source of the water isolates. (Hagedorn 1999; Wiggins 1999).

LABORATORY METHODS

Isolation of *Enterococcus* from Known-Source Samples. Fecal samples, identified to source, were delivered to the Salisbury University (SU) BST lab by Maryland Department of the Environment (MDE) personnel. Fecal material suspended in phosphate buffered saline was plated onto selective m-Enterococcus agar. After incubation at 37° C, up to eight (8) *Enterococcus* isolates were randomly selected from each fecal sample for ARA testing.

Isolation of Enterococcus from Water Samples. Water samples were collected by MDE staff and shipped overnight to MapTech Inc, Blacksburg, Va. Bacterial isolates were collected by membrane filtration. Up to 24 randomly selected *Enterococcus* isolates were collected from each water sample and all isolates were then shipped to the SU BST lab.

Antibiotic Resistance Analysis. Each bacterial isolate from both water and scat were grown in Enterococcosel® broth (Becton Dickinson, Sparks, MD) prior to ARA testing. *Enterococci* are capable of hydrolyzing esculin, turning this broth black. Only esculin-positive isolates were tested for antibiotic resistance.

Bacterial isolates were plated onto tryptic soy agar plates, each containing a different concentration of a given antibiotic. Plates were incubated overnight at 37° C and isolates then scored for growth (resistance) or no growth (sensitivity). Data consisting of a “1” for resistance or “0” for sensitivity for each isolate at each concentration of each antibiotic was then entered into a spread-sheet for statistical analysis.

The following table includes the antibiotics and concentrations used for isolates in analyses for all the study watersheds.

Table C-1. Antibiotics and concentrations used for ARA.

Antibiotic	Concentration (μg/ml)
Amoxicillin	0.625
Cephalothin	10, 15, 30, 50
Chloramphenicol	10
Chlortetracycline	60, 80, 100
Erythromycin	10
Gentamycin	5, 10, 15
Neomycin	40, 60, 80
Oxytetracycline	20, 40, 60, 80, 100
Salinomycin	10
Streptomycin	40, 60, 80, 100
Tetracycline	10, 30, 50, 100
Vancomycin	2.5

KNOWN-SOURCE LIBRARY

Construction and Use. Fecal samples (scat) from known sources in each watershed were collected during the study period by MDE personnel and delivered to the BST Laboratory at SU. *Enterococcus* isolates were obtained from known sources (e.g., human, dog, cow, horse, deer, fox, rabbit, and goose). For each watershed, a library of patterns of *Enterococcus* isolate responses to the panel of antibiotics was analyzed using the statistical software CART® (Salford Systems, San Diego, CA). *Enterococcus* isolate response patterns were also obtained from bacteria in water samples collected at the monitoring stations in each basin. Using statistical techniques, these patterns were then compared to those in the appropriate library to identify the probable source of each water isolate. A combined library of known sources was used for Antietam Creek and Concocheaque Creek Watersheds using patterns from scat obtained from both watersheds, and the water isolate patterns of each were compared to the combined library. A combined known-source library was also used for Double Pipe Creek, Lower Monocacy River, and Upper Monocacy River, with water isolate patterns of each compared to this combined library.

STATISTICAL ANALYSIS

We applied a tree classification method, [1]CART®, to build a model that classifies isolates into source categories based on ARA data. CART® builds a classification tree by recursively splitting the library of isolates into two nodes. Each split is determined by the antibiotic variables (antibiotic resistance measured for a collection of antibiotics at varying concentrations). The first step in the tree-building process splits the library into two nodes by considering every binary split associated with every variable. The split is chosen that maximizes a specified index of homogeneity for isolate sources within each of the nodes. In subsequent steps, the same process is applied to each resulting node until a *stopping* criterion is satisfied. Nodes where an additional split would lead to only an insignificant increase in the *homogeneity index* relative to the *stopping* criterion are referred to as *terminal* nodes.[2] The collection of *terminal* nodes defines the classification model. Each *terminal* node is associated with one source, the source isolate with an unknown source), based that is most populous among the library isolates in the node. Each water sample isolate (i.e., an on its antibiotic resistance pattern, is identified with one specific *terminal* node and is assigned the source of the majority of library isolates in that *terminal* node.[3]

---

[1] The Elements of Statistical Learning: Data Mining, Inference, and Prediction. Hastie T, Tibshirani R, and Friedman J. Springer 2001.

[2] An ideal split, i.e., a split that achieves the theoretical maximum for homogeneity, would produce two nodes each containing library isolates from only one source.

[3] The CART® tree-classification method we employed includes various features to ensure the development of an optimal classification model. For brevity in exposition, we have chosen not to present details of those features, but suggest the following sources: Breiman L, et al. *Classification and Regression Trees.* Pacific Grove: Wadsworth, 1984; and Steinberg D and Colla P. *CART—Classification and Regression Trees.* San Diego, CA: Salford Systems, 1997.

Antietam Creek Watershed ARA Results

Known-Source Library. A 1,120 known-source isolate library was constructed that included 567 isolates from sources in the Antietam Creek Watershed, combined with the 553 isolates from the Conococheague Creek Watershed. The known sources in the combined library were grouped into four categories: pets (dogs), human, livestock (cow, horse), and wildlife (deer, fox, goose, rabbit) (Table C-2). The library was analyzed for its ability to take a subset of the library isolates and correctly predict the identity of their host sources when they were treated as unknowns. Average rates of correct classification (ARCC) for the library were found by repeating this analysis using several probability cutoff points, as described above. The number-not-classified for each probability was determined. From these results, the percent unknown and percent correct classification (RCCs) was calculated (Table C-3).

Table C-2: Category, total number, and number of unique patterns in the Antietam Creek known-source library and the combined Antietam-Conococheague library.

Category	Potential Sources	Total Isolates	Unique Patterns
Antietam Creek Library:			
Human	Human	85	50
Livestock	Cow, horse	178	64
Pet	Dog	66	29
Wildlife	Deer, rabbit, goose	238	40
Total		567	183
Combined ANT-CON Library:			
Human	Human	175	106
Livestock	Cow, horse	355	121
Pet	Dog	131	63
Wildlife	Deer, rabbit, goose	459	101
Total		1120	391

For Antietam Creek Watershed, a cutoff probability of 0.50 (50%) was shown to yield an ARCC of 67% (Table C-3). The resulting rates of correction classification (RCCs) for the four categories of sources in the Antietam Creek portion of the library are shown in Table C-4.

Table C-3: Number of isolates not classified, percent unknown, and percent correct for eight (8) cutoff probabilities for Antietam Creek known- source isolates using the combined Antietam-Conococheague known-source library.

Threshold	0	0.25	0.375	0.5	0.6	0.7	0.8	0.9
% Correct	60.5%	60.5%	61.0%	67.0%	71.8%	78.9%	87.2%	96.8%
% Unknown	0.0%	0.0%	0.9%	52.4%	65.6%	74.1%	83.4%	89.1%
# Not Classified	0	0	5	297	372	420	473	505

Figure C-1. Antietam Creek Classification Model: Percent Correct versus Percent Unknown using a combined Antietam-Conococheague library .

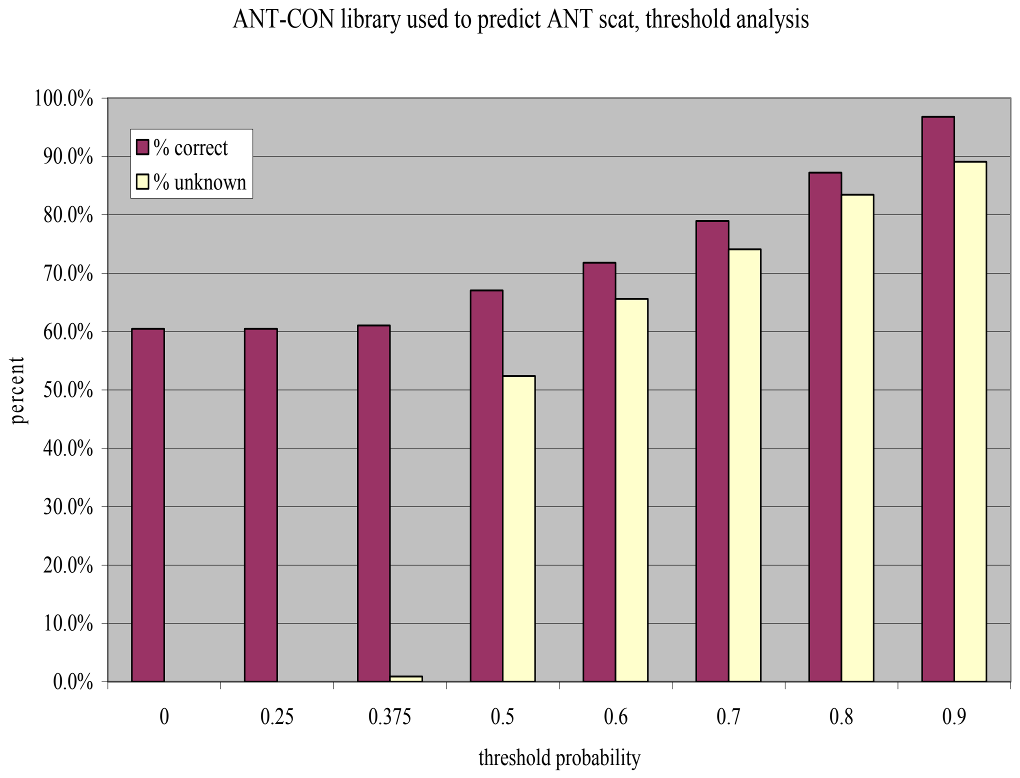


Table C-4: Antietam Creek. Actual species categories versus predicted categories, at 50% probability cutoff, with rates of correct classification (RCC) for each category.

	Predicted						
Actual	**Human**	**Livestock**	**Pet**	**Wildlife**	**Unknown**	**Total**	**RCC***
Human	54	0	7	1	23	85	87.1%
Livestock	19	26	9	29	95	178	31.3%
Pet	1	1	50	3	11	66	90.9%
Wildlife	4	3	12	51	168	238	72.9%
Total	78	30	78	84	297	567	

*RCC = Actual number of predicted species category / Total number predicted. Example: 163 pet correctly predicted / 175 total number predicted for pet = 163/175 = 93%.

Antietam Creek Water Samples. Monthly monitoring from nine (9) monitoring stations on Antietam Creek was the source of water samples (Figure C-2). The maximum number of *Enterococcus i*solates per water sample was 24, although the number of isolates that actually grew was sometimes less than 24. A total of 2,501 *Enterococcus* isolates were analyzed by statistical analysis. The BST results by species category, shown in Table C-5, indicate that 81% of the water isolates were able to be classified to a probable host source when using a 0.50 (50%) probability threshold.

Table C-5: Probable host sources of water isolates by species category, number of isolates, and percent isolates classified at a cutoff probability of 50%.

Category	**Number**	**% Assigned to category at 50% Probability**	**% Assigned to category (excluding unknowns)**
Human	530	21%	26%
Livestock	367	15%	18%
Pet	711	28%	35%
Wildlife	428	17%	21%
Unknown	465	19%	
Missing Data	0	-	0
Total	2,501	100%	100%
% Classified	**81%**		

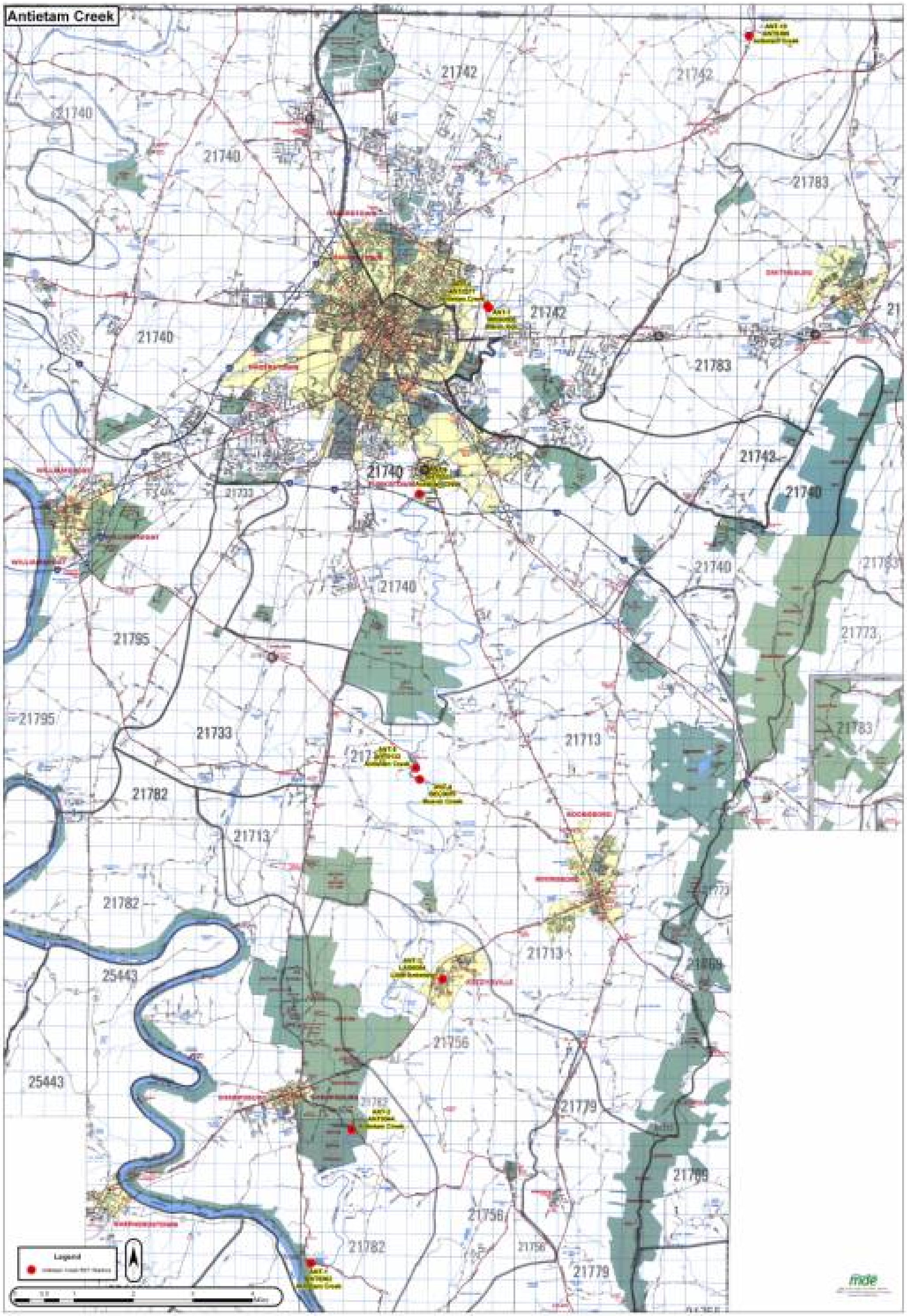
*Percentages may not add up to 100% due to rounding.

The seasonal distribution of water isolates from samples collected at each sampling station is shown below in Table C-6.

Table C-6: *Enterococcus* isolates obtained from water collected during the spring, summer, fall, and winter seasons for each of the monitoring stations.

Station	Spring	Summer	Fall	Winter	Total
ANT0002	71	68	90	62	291
ANT0044	71	65	92	64	292
ANT0132	72	64	90	61	287
ANT0223	72	70	67	61	270
ANT0277	72	89	63	48	272
ANT0366	72	69	66	71	278
BEC0001	69	70	57	63	259
LAS0004	72	68	85	48	273
MRS0000	72	62	72	63	279
Total	**643**	**635**	**682**	**541**	**2,501**

Figure C-2. Map of Antietam River Watershed.
Dots indicate water monitoring sites.



Tables C-7 and C-8 on the following pages show the number and percent of the probable sources for each monitoring station by month.

Table C-7: BST Analysis: Number of Isolates per Station per Date.

		Predicted Source					
Station	**Date**	**Human**	**Livestock**	**Pet**	**Wildlife**	**Unknown**	**Total**
ANT0002	11/12/02	1	1	12	4	2	20
ANT0002	12/04/02	4	6	0	6	8	24
ANT0002	01/08/03	8	6	10	0	0	24
ANT0002	02/05/03	16	0	4	3	1	24
ANT0002	03/05/03	7	2	3	0	2	14
ANT0002	04/23/03	9	5	7	0	3	24
ANT0002	05/07/03	1	5	2	7	8	23
ANT0002	06/04/03	10	3	9	1	1	24
ANT0002	07/09/03	3	2	3	11	5	24
ANT0002	08/06/03	1	4	8	7	2	22
ANT0002	09/10/03	1	2	2	10	7	22
ANT0002	09/24/03	5	0	6	5	6	22
ANT0002	10/08/03	3	4	4	0	13	24
ANT0044	11/12/02	6	0	11	2	4	23
ANT0044	12/04/02	3	2	6	5	6	22
ANT0044	01/08/03	6	1	12	3	1	23
ANT0044	02/05/03	19	2	2	0	1	24
ANT0044	03/05/03	5	0	5	3	4	17
ANT0044	04/23/03	3	9	4	7	1	24
ANT0044	05/07/03	1	6	1	7	8	23
ANT0044	06/04/03	14	2	4	2	2	24
ANT0044	07/09/03	0	3	2	8	11	24
ANT0044	08/06/03	1	1	14	6	1	23
ANT0044	09/10/03	0	5	4	7	2	18
ANT0044	09/24/03	6	4	8	0	5	23
ANT0044	10/08/03	0	0	3	0	21	24
ANT0132	11/12/02	6	4	2	4	8	24
ANT0132	12/04/02	2	6	1	5	4	18
ANT0132	01/08/03	10	4	5	2	3	24
ANT0132	02/05/03	10	3	6	2	2	23
ANT0132	03/05/03	5	0	1	0	8	14
ANT0132	04/23/03	8	7	1	7	1	24
ANT0132	06/04/03	9	0	8	1	6	24
ANT0132	07/09/03	0	0	2	10	10	22
ANT0132	08/06/03	1	1	8	6	8	24
ANT0132	09/10/03	0	2	4	4	8	18
ANT0132	09/24/03	6	2	12	0	4	24
ANT0132	10/08/03	1	1	4	7	11	24
ANT0223	11/12/02	5	6	5	6	2	24

Table C-7: BST Analysis: Number of Isolates per Station per Date (continued).

		Predicted Source					
Station	**Date**	**Human**	**Livestock**	**Pet**	**Wildlife**	**Unknown**	**Total**
ANT0223	12/04/02	4	3	10	1	6	24
ANT0223	01/08/03	11	1	6	3	3	24
ANT0223	02/05/03	10	6	6	0	2	24
ANT0223	03/05/03	7	1	0	3	2	13
ANT0223	04/23/03	5	6	6	7	0	24
ANT0223	05/07/03	1	11	9	1	2	24
ANT0223	06/04/03	12	3	5	1	3	24
ANT0223	07/09/03	5	0	13	5	1	24
ANT0223	08/06/03	4	1	10	8	1	24
ANT0223	09/10/03	4	2	2	3	11	22
ANT0223	10/08/03	6	1	3	4	5	19
ANT0277	11/12/02	10	1	2	0	2	15
ANT0277	12/04/02	7	4	9	1	3	24
ANT0277	01/08/03	12	2	8	0	2	24
ANT0277	02/05/03	13	4	6	0	1	24
ANT0277	04/23/03	3	4	5	9	3	24
ANT0277	05/07/03	1	4	9	8	2	24
ANT0277	06/04/03	5	1	8	3	7	24
ANT0277	06/25/03	3	2	11	2	5	23
ANT0277	07/09/03	0	3	10	11	0	24
ANT0277	08/06/03	4	1	8	6	2	21
ANT0277	09/10/03	1	2	12	2	4	21
ANT0277	10/08/03	3	2	11	1	7	24
ANT0366	11/12/02	5	3	8	2	4	22
ANT0366	12/04/02	5	5	9	4	1	24
ANT0366	01/08/03	12	3	7	0	2	24
ANT0366	02/05/03	8	3	5	3	5	24
ANT0366	03/05/03	5	5	5	6	2	23
ANT0366	04/23/03	10	2	5	6	1	24
ANT0366	05/07/03	4	11	3	4	2	24
ANT0366	06/04/03	7	0	11	3	3	24
ANT0366	07/09/03	3	0	10	10	1	24
ANT0366	08/06/03	4	2	12	2	4	24
ANT0366	09/10/03	2	5	8	2	4	21
ANT0366	10/08/03	3	4	7	3	3	20
BEC0001	11/12/02	4	2	1	3	7	17
BEC0001	01/08/03	6	3	8	5	2	24
BEC0001	02/05/03	3	11	6	0	4	24
BEC0001	03/05/03	4	2	2	0	7	15
BEC0001	04/23/03	6	3	3	7	5	24
BEC0001	05/07/03	7	2	2	6	7	24

Table C-7: BST Analysis: Number of Isolates per Station per Date (continued).

		Predicted Source					
Station	**Date**	**Human**	**Livestock**	**Pet**	**Wildlife**	**Unknown**	**Total**
BEC0001	06/04/03	9	1	3	3	5	21
BEC0001	07/09/03	4	3	7	4	6	24
BEC0001	08/06/03	2	0	5	14	1	22
BEC0001	09/10/03	1	3	10	8	2	24
BEC0001	10/08/03	3	3	2	4	8	20
LAS0004	11/12/02	3	2	6	1	4	16
LAS0004	12/04/02	3	0	17	4	0	24
LAS0004	01/08/03	1	8	11	0	4	24
LAS0004	02/05/03	7	6	5	3	3	24
LAS0004	04/23/03	5	7	5	2	5	24
LAS0004	05/07/03	3	0	7	8	6	24
LAS0004	06/04/03	5	7	5	6	1	24
LAS0004	07/09/03	1	2	14	7	0	24
LAS0004	08/06/03	4	0	12	7	1	24
LAS0004	09/10/03	0	3	2	5	10	20
LAS0004	09/24/03	3	0	12	0	7	22
LAS0004	10/08/03	2	5	1	0	15	23
MRS0000	11/12/02	4	1	4	8	7	24
MRS0000	12/04/02	5	8	6	2	3	24
MRS0000	01/08/03	13	6	4	1	0	24
MRS0000	02/05/03	1	9	3	4	7	24
MRS0000	03/05/03	2	5	0	4	4	15
MRS0000	04/23/03	3	10	4	2	5	24
MRS0000	05/07/03	3	8	3	7	3	24
MRS0000	06/04/03	3	3	12	1	5	24
MRS0000	07/09/03	5	2	11	3	3	24
MRS0000	08/06/03	0	2	18	4	0	24
MRS0000	09/10/03	3	0	19	0	2	24
MRS0000	10/08/03	5	0	13	3	3	24
Total		530	367	711	428	465	2501

Table C-8: BST Analysis: Percent of Isolates per Station per Date.

		Predicted Source					
Station	**Date**	**Human**	**Livestock**	**Pet**	**Wildlife**	**Unknown**	**Total**
ANT0002	11/12/02	5.0%	5.0%	60.0%	20.0%	10.0%	100%
ANT0002	12/04/02	16.7%	25.0%	0.0%	25.0%	33.3%	100%
ANT0002	01/08/03	33%	25%	42%	0%	0%	100%
ANT0002	02/05/03	66.7%	0.0%	16.7%	12.5%	4.2%	100%
ANT0002	03/05/03	50.0%	14.3%	21.4%	0.0%	14.3%	100%
ANT0002	04/23/03	37.5%	20.8%	29.2%	0.0%	12.5%	100%
ANT0002	05/07/03	4.3%	21.7%	8.7%	30.4%	34.8%	100%
ANT0002	06/04/03	41.7%	12.5%	37.5%	4.2%	4.2%	100%
ANT0002	07/09/03	12.5%	8.3%	12.5%	45.8%	20.8%	100%
ANT0002	08/06/03	4.5%	18.2%	36.4%	31.8%	9.1%	100%
ANT0002	09/10/03	4.5%	9.1%	9.1%	45.5%	31.8%	100%
ANT0002	09/24/03	22.7%	0.0%	27.3%	22.7%	27.3%	100%
ANT0002	10/08/03	12.5%	16.7%	16.7%	0.0%	54.2%	100%
ANT0044	11/12/02	26.1%	0.0%	47.8%	8.7%	17.4%	100%
ANT0044	12/04/02	13.6%	9.1%	27.3%	22.7%	27.3%	100%
ANT0044	01/08/03	26.1%	4.3%	52.2%	13.0%	4.3%	100%
ANT0044	02/05/03	79.2%	8.3%	8.3%	0.0%	4.2%	100%
ANT0044	03/05/03	29.4%	0.0%	29.4%	17.6%	23.5%	100%
ANT0044	04/23/03	12.5%	37.5%	16.7%	29.2%	4.2%	100%
ANT0044	05/07/03	4.3%	26.1%	4.3%	30.4%	34.8%	100%
ANT0044	06/04/03	58.3%	8.3%	16.7%	8.3%	8.3%	100%
ANT0044	07/09/03	0.0%	12.5%	8.3%	33.3%	45.8%	100%
ANT0044	08/06/03	4.3%	4.3%	60.9%	26.1%	4.3%	100%
ANT0044	09/10/03	0.0%	27.8%	22.2%	38.9%	11.1%	100%
ANT0044	09/24/03	26.1%	17.4%	34.8%	0.0%	21.7%	100%
ANT0044	10/08/03	0.0%	0.0%	12.5%	0.0%	87.5%	100%
ANT0132	11/12/02	25.0%	16.7%	8.3%	16.7%	33.3%	100%
ANT0132	12/04/02	11.1%	33.3%	5.6%	27.8%	22.2%	100%
ANT0132	01/08/03	41.7%	16.7%	20.8%	8.3%	12.5%	100%
ANT0132	02/05/03	43.5%	13.0%	26.1%	8.7%	8.7%	100%
ANT0132	03/05/03	35.7%	0.0%	7.1%	0.0%	57.1%	100%
ANT0132	04/23/03	33.3%	29.2%	4.2%	29.2%	4.2%	100%
ANT0132	05/07/03	0.0%	62.5%	0.0%	16.7%	20.8%	100%
ANT0132	06/04/03	37.5%	0.0%	33.3%	4.2%	25.0%	100%
ANT0132	07/09/03	0.0%	0.0%	9.1%	45.5%	45.5%	100%
ANT0132	08/06/03	4.2%	4.2%	33.3%	25.0%	33.3%	100%
ANT0132	09/10/03	0.0%	11.1%	22.2%	22.2%	44.4%	100%
ANT0132	09/24/03	25.0%	8.3%	50.0%	0.0%	16.7%	100%
ANT0132	10/08/03	4.2%	4.2%	16.7%	29.2%	45.8%	100%
ANT0223	12/04/02	16.7%	12.5%	41.7%	4.2%	25.0%	100%
ANT0223	01/08/03	45.8%	4.2%	25.0%	12.5%	12.5%	100%
ANT0223	02/05/03	41.7%	25.0%	25.0%	0.0%	8.3%	100%
ANT0223	03/05/03	53.8%	7.7%	0.0%	23.1%	15.4%	100%

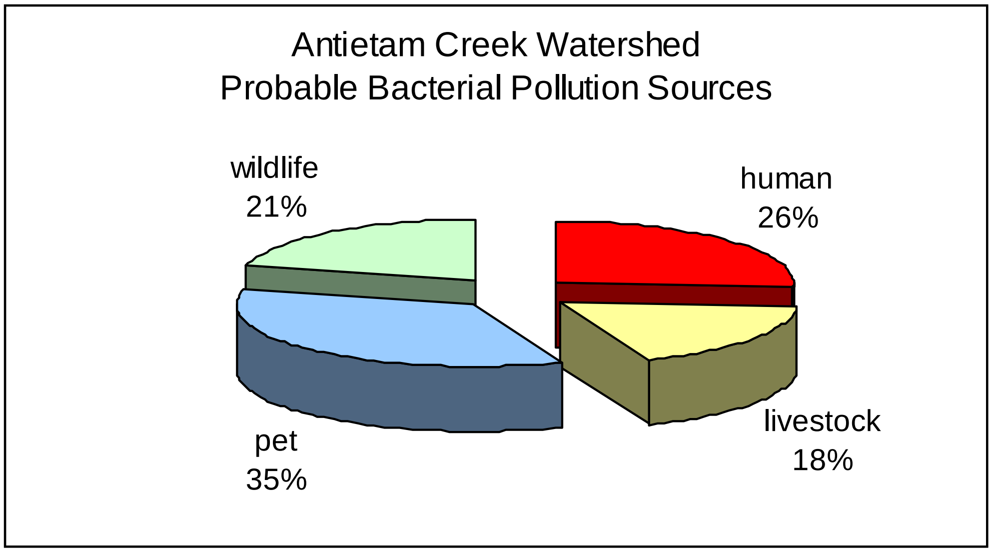
Table C-8: BST Analysis: Percent of Isolates per Station per Date (continued).

		Predicted Source					
Station	**Date**	**Human**	**Livestock**	**Pet**	**Wildlife**	**Unknown**	**Total**
ANT0223	04/23/03	20.8%	25.0%	25.0%	29.2%	0.0%	100%
ANT0223	05/07/03	4.2%	45.8%	37.5%	4.2%	8.3%	100%
ANT0223	06/04/03	50.0%	12.5%	20.8%	4.2%	12.5%	100%
ANT0223	07/09/03	20.8%	0.0%	54.2%	20.8%	4.2%	100%
ANT0223	08/06/03	16.7%	4.2%	41.7%	33.3%	4.2%	100%
ANT0223	09/10/03	18.2%	9.1%	9.1%	13.6%	50.0%	100%
ANT0223	10/08/03	31.6%	5.3%	15.8%	21.1%	26.3%	100%
ANT0277	11/12/02	66.7%	6.7%	13.3%	0.0%	13.3%	100%
ANT0277	12/04/02	29.2%	16.7%	37.5%	4.2%	12.5%	100%
ANT0277	01/08/03	50.0%	8.3%	33.3%	0.0%	8.3%	100%
ANT0277	02/05/03	54.2%	16.7%	25.0%	0.0%	4.2%	100%
ANT0277	04/23/03	12.5%	16.7%	20.8%	37.5%	12.5%	100%
ANT0277	05/07/03	4.2%	16.7%	37.5%	33.3%	8.3%	100%
ANT0277	06/04/03	20.8%	4.2%	33.3%	12.5%	29.2%	100%
ANT0277	06/25/03	13.0%	8.7%	47.8%	8.7%	21.7%	100%
ANT0277	07/09/03	0.0%	12.5%	41.7%	45.8%	0.0%	100%
ANT0277	08/06/03	19.0%	4.8%	38.1%	28.6%	9.5%	100%
ANT0277	09/10/03	4.8%	9.5%	57.1%	9.5%	19.0%	100%
ANT0277	10/08/03	12.5%	8.3%	45.8%	4.2%	29.2%	100%
ANT0366	11/12/02	22.7%	13.6%	36.4%	9.1%	18.2%	100%
ANT0366	12/04/02	20.8%	20.8%	37.5%	16.7%	4.2%	100%
ANT0366	01/08/03	50.0%	12.5%	29.2%	0.0%	8.3%	100%
ANT0366	02/05/03	33.3%	12.5%	20.8%	12.5%	20.8%	100%
ANT0366	03/05/03	21.7%	21.7%	21.7%	26.1%	8.7%	100%
ANT0366	04/23/03	41.7%	8.3%	20.8%	25.0%	4.2%	100%
ANT0366	05/07/03	16.7%	45.8%	12.5%	16.7%	8.3%	100%
ANT0366	06/04/03	29.2%	0.0%	45.8%	12.5%	12.5%	100%
ANT0366	07/09/03	12.5%	0.0%	41.7%	41.7%	4.2%	100%
ANT0366	08/06/03	16.7%	8.3%	50.0%	8.3%	16.7%	100%
ANT0366	09/10/03	9.5%	23.8%	38.1%	9.5%	19.0%	100%
ANT0366	10/08/03	15.0%	20.0%	35.0%	15.0%	15.0%	100%
BEC0001	11/12/02	23.5%	11.8%	5.9%	17.6%	41.2%	100%
BEC0001	12/04/02	10.0%	30.0%	20.0%	30.0%	10.0%	100%
BEC0001	01/08/03	25.0%	12.5%	33.3%	20.8%	8.3%	100%
BEC0001	02/05/03	12.5%	45.8%	25.0%	0.0%	16.7%	100%
BEC0001	03/05/03	26.7%	13.3%	13.3%	0.0%	46.7%	100%
BEC0001	04/23/03	25.0%	12.5%	12.5%	29.2%	20.8%	100%
BEC0001	05/07/03	29.2%	8.3%	8.3%	25.0%	29.2%	100%
BEC0001	06/04/03	42.9%	4.8%	14.3%	14.3%	23.8%	100%
BEC0001	07/09/03	16.7%	12.5%	29.2%	16.7%	25.0%	100%

Table C-8: BST Analysis: Percent of Isolates per Station per Date (continued).

		Predicted Source					
Station	**Date**	**Human**	**Livestock**	**Pet**	**Wildlife**	**Unknown**	**Total**
BEC0001	08/06/03	9.1%	0.0%	22.7%	63.6%	4.5%	100%
BEC0001	09/10/03	4.2%	12.5%	41.7%	33.3%	8.3%	100%
BEC0001	10/08/03	15.0%	15.0%	10.0%	20.0%	40.0%	100%
LAS0004	11/12/02	18.8%	12.5%	37.5%	6.3%	25.0%	100%
LAS0004	12/04/02	12.5%	0.0%	70.8%	16.7%	0.0%	100%
LAS0004	02/05/03	29.2%	25.0%	20.8%	12.5%	12.5%	100%
LAS0004	04/23/03	20.8%	29.2%	20.8%	8.3%	20.8%	100%
LAS0004	05/07/03	12.5%	0.0%	29.2%	33.3%	25.0%	100%
LAS0004	06/04/03	20.8%	29.2%	20.8%	25.0%	4.2%	100%
LAS0004	07/09/03	4.2%	8.3%	58.3%	29.2%	0.0%	100%
LAS0004	08/06/03	16.7%	0.0%	50.0%	29.2%	4.2%	100%
LAS0004	09/10/03	0.0%	15.0%	10.0%	25.0%	50.0%	100%
LAS0004	09/24/03	13.6%	0.0%	54.5%	0.0%	31.8%	100%
MRS0000	11/12/02	16.7%	4.2%	16.7%	33.3%	29.2%	100%
MRS0000	12/04/02	20.8%	33.3%	25.0%	8.3%	12.5%	100%
MRS0000	01/08/03	54.2%	25.0%	16.7%	4.2%	0.0%	100%
MRS0000	02/05/03	4.2%	37.5%	12.5%	16.7%	29.2%	100%
MRS0000	03/05/03	13.3%	33.3%	0.0%	26.7%	26.7%	100%
MRS0000	04/23/03	12.5%	41.7%	16.7%	8.3%	20.8%	100%
MRS0000	05/07/03	12.5%	33.3%	12.5%	29.2%	12.5%	100%
MRS0000	06/04/03	12.5%	12.5%	50.0%	4.2%	20.8%	100%
MRS0000	07/09/03	20.8%	8.3%	45.8%	12.5%	12.5%	100%
MRS0000	08/06/03	0.0%	8.3%	75.0%	16.7%	0.0%	100%
MRS0000	09/10/03	12.5%	0.0%	79.2%	0.0%	8.3%	100%
MRS0000	10/08/03	20.8%	0.0%	54.2%	12.5%	12.5%	100%

Figure C-3: Antietam Creek Watershed relative contributions by probable sources of *Enterococcus* contamination.



Antietam Creek Summary

The use of ARA was successful for identification of probable bacterial sources in the Antietam Creek Watershed. When water isolates were compared to the library and potential sources predicted, 81% of the isolates were classified as to category by statistical analysis. The highest RCC for the library was 91% (for pet), while human and wildlife had RCCs of 87 % and 73 %, respectively. The livestock category, as is often seen in BST, had a low RCC of 31 %, which is thought to be due to differing treatments (or no treatment) by livestock owners.

The largest category of potential sources in the watershed as a whole was pet (35% of classified water isolates), followed by human and wildlife (26% and 21% of classified isolates, respectively) (Fig. 3-ANT). The potential contribution by livestock was similar to that of wildlife and made up 18 % of classified isolates.

REFERENCES

Bell, J.B., Elliott, G.E. & Smith, D.W. 1983. Influence of Sewage Treatment an Urbanization on Selection of Multiple Resistance in Fecal Coliform Populations. *Appl. Environ. Microbiol.* 46, 227-32.

Department of Health and Human Services. Centers for Disease Control and Prevention. Pulsenet. 2006. "National Molecular Subtyping Network for Foodborne Disease Surveillance" http://www.cdc.gov/pulsenet [Available 01.26.06].

Hagedorn, C., Robinson, S.L., Filtz, J.R., Grubbs, S.M., Angier, T.A. & Beneau, R.B. 1999. Determining Sources of Fecal Pollution in a Rural Virginia Watershed with Antibiotic Resistance Patterns in Fecal Streptococci. *Appl. Environ. Microbiol.* 65, 5522-5531.

Krumperman, P.H. 1983. Multiple Antibiotic Resistance Indexing of *Escherichia coli* to Identify High-Risk Sources of Fecal Contamination of Foods. *Appl. Environ. Microbiol.* 46, 165-70.

Scott, T.M., Rose, J.B., Jenkins, T.M., Farrah, S.R. & Lukasik, J. 2002 Microbial Source Tracking: Current Methodology and Future Directions. *Appl. Environ. Microbiol.* 68(12), 3373-3385.

Simpson, J.M., Santo Domingo, J.W. & Reasoner, D.J. 2002 Microbial Source Tracking: State of the Science. *Environ. Sci. Technol.* 36(24), 5279-5288.

Wiggins, B.A. 1996. Discriminant Analysis of Antibiotic Resistance Patterns in Fecal Streptococci, a Method to Differentiate Human and Animal Sources of Fecal Pollution in Natural Waters. *Appl. Environ. Microbiol.* 62, 3997-4002.

Wiggins, B.A., Andrews, R.W., Conway, R.A., Corr, C.L., Dobratz, E. J., Dougherty, D.P., Eppard, J.R., Knupp, S.R., Limjoco, M.C., Mettenburg, J.M., Rinehardt, J.M., Sonsino, J., Torrijos, R.L. & Zimmerman, M.E. 1999. Use of Antibiotic Resistance Analysis to Identify Nonpoint Sources of Fecal Pollution. *Appl. Environ. Microbiol.* 65, 3483-3486.

ACKNOWLEDGEMENTS

We wish to thank the Richard A. Henson School of Science and Technology of Salisbury University, Salisbury, MD for its support. We also want to acknowledge Dr. Bertram Price and Joshua Greenberg of Price Associates, Inc., for their contributions to the statistical analysis in this project.

Adjustment of BST Results

As explained in the BST Summary for the Antietam Creek, the percent of correct classification (RCC) for bacteria sources, especially for the livestock category can introduce a potential misclassification of the more probable sources in the watershed. This is seen in Table C-4, which shows results of the analysis of samples from known sources. For example, out of 567, 85 isolates were known to be of human source but only 54 were classified by the analysis as being of human source. Of those 85, one isolate was classified as wildlife, 7 as pet and 23 as unknown. Similarly, of the other three categories, 19 isolates known to be livestock, one isolate knows to be from pets, and 4 wildlife isolates were classified as human, resulting in a total of 78 of all 567 isolates classified as human of which only 54 were known to be of human source.

The results provided by the BST methodology can be adjusted based on the known source percent of correct classification results provided in Table C-4.

Example:

The current BST methodology provides the following source percentages for station ANT0366 during high flow conditions:

Source Category	Original Percentage
Pets	31.44 %
Human	24.41 %
Livestock	15.75 %
Wildlife	18.20 %
Unknown	10.20 %

To get the correct human source percentage we redistributed the above percentages based on the % of correct classification as follows.

From Table C-4:

Source Category	Isolates known to be from Human Source	Total Isolates Predicted for Each category	Percentage
Pets	7	78	8.9 %
Human	54	78	69.2 %
Livestock	0	30	0 %
Wildlife	1	84	1.2 %
Unknown	23	297	7.7 %
Total	85	567	15 %

Applying those percentages to the original estimated source distribution presented above will result in the adjusted percentage for human sources:

= (8.9 x 31.44) + (69.2 x 24.41) + (0 x 15.75) + (1.2 x 18.20) + (7.7 x 10.20) = 20.73 %

Thus the correct human source percentage, the value used in the TMDL analysis, is 20.73% and not 24.41%. Corrected percentages are also calculated as above for domestic animal, livestock and wildlife sources. The classification of unknown is eliminated in the process as all known isolates are of known source. For station ANT0366 during high flow condition the corrected source percentages are as follows:

Source Category	**Adjusted Percentage**
Pets	22.0 %
Human	20.7 %
Livestock	32.8 %
Wildlife	24.5 %

Appendix D – Estimating Maximum Daily Loads

This appendix documents the technical approach used to define maximum daily loads of fecal bacteria consistent with the annual average TMDL which, when met, are protective of water quality standards in the Antietam Creek watershed. The approach builds upon the TMDL analysis that was conducted to ensure that compliance with the annual average target will result in compliance with the applicable water quality standards. The annual average loading target was converted into allowable *daily* values by using the loadings developed from the TMDL analysis. The approach is consistent with available EPA guidance on generating daily loads for TMDLs.

The available guidance for developing daily loads does not specify a single allowable approach; it contains a range of options. Selection of a specific method for translating a time-series of allowable loads into expression of a TMDL requires decisions regarding both the level of resolution (e.g., single daily load for all conditions vs. loads that vary with environmental conditions) and level of probability associated with the TMDL.

Level of Resolution

The level of resolution pertains to the amount of detail used in specifying the maximum daily load. The draft EPA guidance on daily loads provides three categories of options for level of resolution.

1. **Representative daily load:** In this option, a single daily load (or multiple representative daily loads) is specified that covers all time periods and environmental conditions.
2. **Flow-variable daily load:** This option allows the maximum daily load to vary based upon the observed flow condition.
3. **Temporally-variable daily load:** This option allows the maximum daily load to vary based upon seasons or times of varying source or water body behavior.

Probability Level

Essentially all TMDLs have some probability of being exceeded, with the specific probability being either explicitly specified or implicitly assumed. This level of probability reflects, directly or indirectly, two separate phenomena:

1. Water quality criteria consist of components describing acceptable magnitude, duration, and frequency. The frequency component addresses how often conditions can allowably surpass the combined magnitude and duration components.
2. Pollutant loads, especially from wet weather sources, typically exhibit a large degree of variability over time. It is rarely practical to specify a “never to be exceeded value” for a daily load, as essentially any loading value has some finite probability of being exceeded.

The draft daily load guidance states that the probability component of the maximum daily load should be “based on a representative statistical measure” that is dependent upon the specific TMDL and best professional judgment of the developers. This statistical measure represents how often the maximum daily load is expected/allowed to be exceeded. The primary options for selecting this level of protection would be:

1. **The maximum daily load reflects some central tendency:** In this option, the maximum daily load is based upon the mean or median value of the range of loads expected to occur. The variability in the actual loads is not addressed.
2. **The maximum daily load reflects a level of protection implicitly provided by the selection of some "critical" period**: In this option, the maximum daily load is based upon the allowable load that is predicted to occur during some critical period examined during the analysis. The developer does not explicitly specify the probability of occurrence.
3. **The maximum daily load is a value that will be exceeded with a pre-defined probability:** In this option, a "reasonable" upper bound percentile is selected for the maximum daily load based upon a characterization of the variability of daily loads. For example, selection of the 95th percentile value would result in a maximum daily load that would be exceeded 5% of the time.

Selected Approach for Defining Maximum Daily Loads for Nonpoint Sources and MS4

To calculate the Antietam Creek watershed MDL for non-point sources and MS4s, a "representative daily load" option was selected as the level of resolution, and a value "that will be exceeded with a pre-defined probability" was selected as the level of protection. In these options, the maximum daily load is one single daily load that covers the two flow strata, with an upper bound percentile that accounts for the variability of daily loads. The upper bound percentile and the maximum daily loads were estimated following EPA's "Technical Support Document for Water Quality-Based Toxics Control" (1991 TSD) (EPA 1991); and "Approaches For Developing a Daily Load Expression for TMDLs Computed for Longer Term Averages" (EPA 2006).

The 1991 TSD illustrates a way to identify a target maximum daily concentration from a long-term average concentration (LTA) based on a coefficient of variation (CV) and the assumption of a log-normal distribution of the data. The equations for determining both the upper boundary percentile and corresponding maximum daily load described in the TSD are as follows:

$$MDLC = LTA * e^{[Z\sigma - 0.5\sigma^2]} \qquad \text{(D1)}$$

and,

$$\text{MDL} = \text{MDLC*Q*F} \qquad \text{(D2)}$$

where,

MDLC = maximum daily load concentration (MPN/100ml)
LTAC = long-term average TMDL concentration (MPN/100ml)
MDL = Maximum Daily Load (MPN/day)
Z = z-score associated with upper bound percentile (unitless)

σ^2 = $\ln(CV^2 + 1)$
CV = coefficient of variation
Q = flow (cfs)
F = conversion factor

The first step is to use the bacteria monitoring data to estimate the upper bound percentile as the percentile of the highest observed bacteria concentration in each of the nine monitoring stations of the Antietam Creek watershed. Using the maximum value of *E. coli* observed in each monitoring station, and solving for the z-score using the above formula, the value of "z" and its corresponding percentile is found as shown below. The percentile associated with the particular value of z can be found in tables in statistics books or using the function NORMSINV(%) in EXCEL©.

$$Z = [\log_{10}(MOC) - \log(AM) + 0.5\sigma^2]/\sigma \qquad \text{(D3)}$$

where,

Z = z-score associated with upper bound percentile
MOC = maximum observed bacteria concentration (MPN/100ml)
AM = arithmetic mean observed bacteria concentrations (MPN/100ml)
σ^2 = $\ln(CV^2 + 1)$
CV = coefficient of variation (arithmetic)

Note that these equations use arithmetic parameters, not geometric parameters as used in the calculations of the long-term annual average TMDL. Therefore, bias correction factors are not necessary to estimate the loads as will be explained below.

The highest percentile of all the stations analyzed by stratum will define the upper bound percentile to be used in estimating the maximum daily limits. In the case of the Antietam Creek watershed, a value measured during high-flow conditions at the ANT0277 station resulted in the highest percentile of both strata of the nine stations. This value translates to the 98.9th percentile, which is the upper boundary percentile to be used in the computation of the maximum daily limits (MDLs) throughout this analysis. Results of the analysis to estimate the recurrence or upper boundary percentile are shown in Table D-1.

Table D-1: Percentiles of Maximum Observed Bacteria Concentrations

Subwatershed	Flow Stratum	Maximum Observed *E. coli* Concentration (MPN/100ml)	Percentile (%)
ANT0366[1]	High	14,140	97.8
	Low	4,570	89.6
ANT0277sub[1]	High	24,190	98.9
	Low	2,610	92.5
MRS0000[1]	High	24,190	97.6
	Low	1,790	81.1
ANT0223sub	High	24,190	98.2
	Low	1,080	89.4
ANT0132sub	High	24,190	97.2
	Low	3,650	98.6
BEC0001	High	10,460	98.8
	Low	1,550	91.8
LAS0004	High	12,030	97.1
	Low	860	89.7
ANT0044sub	High	24,190	98.8
	Low	360	93.3
ANT0002sub	High	24,190	98.1
	Low	290	86.5

[1] Subwatersheds partially located in Pennsylvania

The 98.9th percentile value results in a maximum daily load that would not be exceeded 98.9% of the time, as, in a similar manner, a TMDL that represents the long term average condition would be expected to be exceeded half the time even after all required controls were implemented.

The MDLCs are estimated based on a statistical methodology referred to as "Statistical Theory of Rollback (STR)". This method predicts concentrations of a pollutant after its sources have been controlled (post-control concentrations), in this case after annual average TMDL implementation. Using STR, the daily TMDLs are calculated as presented below.

First, the long-term average TMDL concentrations (C_{LTA}) by stratum are estimated by applying the required percent reduction to the baseline (monitoring data) concentrations (C_b) by stratum as follows:

From Section 4.3, equations (8) and (9):

$$L_b = L_{b\text{-}H} + L_{b\text{-}L}$$
$$L_b = Q_H*C_{bH}*F_{1H}*W_H + Q_L*C_{bL}*F_{1L}*W_L$$

And from equation (10):

$$Annual\ Average\ TMDL = L_b * (1 - R)$$

Therefore,

$$L_b*(1\text{-}R) = Q_H*C_H*F_{1H}*W_H *(1\text{-}R) + Q_L*C_L*F_{1L}*W_L*(1\text{-}R) \qquad \text{(D4)}$$

As explained before, a reduction in concentration is proportional to a reduction in load, thus the bacteria concentrations expected after reductions are applied are equal to the baseline concentrations multiplied by one minus the required reduction:

$$C_{LTA\text{-}H} = C_{b\text{-}H}*(1\text{-}R_H) \qquad \text{(D5)}$$

$$C_{LTA\text{-}L} = C_{b\text{-}L}*(1\text{-}R_L) \qquad \text{(D6)}$$

The TMDL concentrations estimated as explained above are shown in Table D-2.

Table D-2: Long-term Annual Average (LTA) TMDL Bacteria Concentrations

Subwatershed	Flow Stratum	LTA Geometric Mean *E. coli* Concentration (MPN/100ml)	LTA Arithmetic Mean* *E. coli* Concentration (MPN/100ml)
ANT0366[1]	High	59	128
	Low	30	113
ANT0277sub[1]	High	58	154
	Low	16	56
MRS0000[1]	High	57	208
	Low	20	112
ANT0223sub	High	59	199
	Low	9	34
ANT0132sub	High	56	343
	Low	12	37
BEC0001	High	52	122
	Low	30	57
LAS0004	High	55	165
	Low	15	30
ANT0044sub	High	62	228
	Low	10	15
ANT0002sub	High	50	289
	Low	9	16

*Only arithmetic parameters are used in the daily loads analysis.

[1] Subwatersheds partially located in Pennsylvania

The next step is to calculate the 98.9th percentile (the MDL concentrations) of these expected concentrations (LTA concentrations) using the coefficient of variation of the baseline concentrations. Based on a general rule for coefficient of variations, the coefficient of variation of the distribution of pollutant concentrations does not change after these concentrations have been reduced or controlled by a fixed proportion (Ott 1995). Therefore, the coefficient of variation estimated using the monitoring data concentrations does not change, and it can be used to estimate the 98.9th percentile of the long-term average TMDL concentrations (LTAC) using equation (D1). These values are shown in Table D-3.

Table D-3: Maximum Daily Load (MDL) Concentrations

Subwatershed	Flow Stratum	Coefficient of Variation	MDL *E. coli* Concentration (MPN/100ml)
ANT0366[1]	High	1.93	1,048
	Low	3.55	1,269
ANT0277sub[1]	High	2.45	1,452
	Low	3.38	620
MRS0000[1]	High	3.55	2,348
	Low	5.48	1,446
ANT0223sub	High	3.22	2,147
	Low	3.60	388
ANT0132sub	High	6.02	4,517
	Low	2.83	380
BEC0001	High	2.11	1,057
	Low	1.60	409
LAS0004	High	2.83	1,684
	Low	1.72	224
ANT0044sub	High	3.52	2,562
	Low	1.00	71
ANT0002sub	High	5.66	3,756
	Low	1.49	105

[1] Subwatersheds partially located in Pennsylvania

With the 98.9th percentiles of LTA TMDL bacteria concentrations estimated for both high flow and low flow strata as explained above, the maximum daily load for MS4 and non-point sources for each subwatershed can be now estimated as:

$$\begin{aligned} \textit{Daily TMDL (MPN/day)} &= Q_H*(98.9^{th}C_{LTA\text{-}H})*F_{1H}*W_H \\ &\quad + Q_L*(98.9^{th}C_{LTA\text{-}L})*F_{1L}*W_L \end{aligned} \qquad \text{(D7)}$$

Selected Approach for Defining Maximum Daily Loads for Other Point Sources

The TMDL also considers contributions from other point sources (i.e., municipal and industrial WWTP) in watersheds that have NPDES permits with fecal bacteria limits. The TMDL analysis that defined the average annual TMDL held each of these sources constant at their existing

NPDES permit limit (daily or monthly) for the entire year. The approach used to determine maximum daily loads was dependent upon whether a maximum daily load was specified within the permit. If a maximum daily load was specified within the permit, then the maximum design flow is multiplied by the maximum daily limit to obtain a maximum daily load. If a maximum daily limit was not specified in the permit, then the maximum daily loads are calculated from guidance in the TSD for Water Quality-based Toxics Control (EPA 1991). The long-term average annual TMDL was converted to maximum daily limits using Table 5-2 of the TSD assuming a coefficient of variation of 0.6 and a 99th percentile probability. This results in a dimensionless multiplication factor of 3.11. The average annual bacteria loads for WWTPs are reported in billion MPN/year. In the Antietam Creek watershed, to estimate the maximum daily loads for WWTPs, the annual average loads are multiplied by the multiplication factor as follows:

$$WWTP\text{-}WLA\ MDL_{\text{(billion MPN/day)}} = [WWTP\text{-}WLA_{\text{(billion MPN/year)}}]*(3.11/365) \quad \text{(D8)}$$

The Maximum Daily Loads for the Antietam Creek subwatersheds, including those partially located in PA, are presented in Table D-4 below.

Table D-4: Maximum Daily Loads Summary

Subwatershed	Flow Stratum	Maximum Daily Load (Billion *E. coli* MPN/day) by Stratum	Maximum Daily Load (Billion *E. coli* MPN/day) Weighted by Stratum
ANT0366[1]	High Low	5,265 1,834	2,939
ANT0277sub[1]	High Low	3,264 401	1,323
MRS0000[1]	High Low	3,971 703	1,755
ANT0223sub	High Low	1,724 90	616
ANT0132sub	High Low	4,293 104	1,453
BEC0001	High Low	1,899 211	755
LAS0004	High Low	2,545 97	886
ANT0044sub	High Low	2,349 19	769
ANT0002sub	High Low	2,126 17	696

[1] Subwatersheds partially located in Pennsylvania

Maximum Daily Loads Allocations

Using the MDLs estimated as explained above, loads are allocated following the same methodology as the annual average TMDL (See section 4.8). The maximum daily load allocations for the Antietam Creek watershed are presented in Table D-5.

Table D-5: Antietam Creek Watershed Maximum Daily Loads

Subwatershed	Total Allocation	LA	SW-WLA	WWTP-WLA
	(Billion MPN *E. coli* /day)			
ANT0366[1]	248	185	54	9
ANT0277sub[1]	1,304	969	330	5
MRS0000[1]	722	593	129	0.2
ANT0223sub	616	98	399	119
ANT0132sub	1,453	1,048	378	27
BEC0001	755	588	143	24
LAS0004	886	703	175	8
ANT0044sub	769	651	118	0
ANT0002sub	696	546	148	2
Total	**7,449**	**5,381**	**1,874**	**194**

[1] MD portion of the subwatershed only.

REFERENCES

Limno-Tech, Inc. 2007. Draft Memorandum: Technical Approach for Four Alternative Options to Define Maximum Daily Loads for the Anacostia TMDL. Washington, DC. January 23, 2007.

EPA (U.S. Environmental Protection Agency). 2006. Approaches For Developing a Daily Load Expression for TMDLs Computed for Longer Term Averages. Draft guidance document. Washington, DC. October 2006.

EPA (U.S. Environmental Protection Agency). 1991. Technical Support Document for Water Quality-Based Toxics Control (1991 TSD). Washington, DC.

Ott, Wayne R. Environmental Statistics and Data Analysis. 1995. CRC Press. Pages 276 – 283.