

FINAL

**Total Maximum Daily Loads of Fecal Bacteria
for the Wills Creek Basin
in Allegany and Garrett Counties, Maryland**

FINAL



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

Submitted to:

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

August 2006

EPA Submittal Date: Sept. 7, 2007
EPA Approval Date: Nov. 6, 2007

Table of Contents

List of Figures..... i

List of Tables ii

List of Abbreviations iv

EXECUTIVE SUMMARY vi

1.0 INTRODUCTION..... 1

2.0 SETTING AND WATER QUALITY DESCRIPTION..... 3

2.1 General Setting 3

2.2 Water Quality Characterization..... 10

2.3 Water Quality Impairment 13

2.4 Source Assessment 18

3.0 TARGETED WATER QUALITY GOAL..... 30

4.0 TOTAL MAXIMUM DAILY LOADS AND SOURCE ALLOCATION 30

4.1 Overview..... 30

4.2 Analysis Framework 31

4.3 Estimating Baseline Loads 32

4.4 Critical Condition and Seasonality 38

4.5 Margin of Safety 41

4.6 TMDL Loading Caps 42

4.7 Scenario Descriptions 43

4.8 TMDL Allocations 47

4.9 Summary..... 50

5.0 ASSURANCE OF IMPLEMENTATION 51

REFERENCES 54

FINAL

Appendix A - Bacteria Data	A1
Appendix B - Flow Duration Curve Analysis to Define Strata.....	B1
Appendix C - Identifying Sources of Fecal Pollution in the Wills Creek Watershed, Maryland.....	C1
Appendix D - Estimating Human Allocation for Subwatershed WIL0000sub.....	D1

List of Figures

Figure 2.1.1: Location Map of the Wills Creek Basin.....	4
Figure 2.1.2: General Soil Series in the Wills Creek Basin.....	5
Figure 2.1.3: Land Use of the Wills Creek Watershed	8
Figure 2.1.4: Population Density in Wills Creek Basin.....	9
Figure 2.2.1: Monitoring Stations in the Wills Creek Basin.....	12
Figure 2.3.1: Conceptual Diagram of Flow Duration Zones	15
Figure 2.4.1: Location of Sanitary Sewer Overflows in MD’s Portion of the Wills Creek Watershed.....	20
Figure 2.4.2: Sanitary Sewer Service and Septics Areas in MD’s Portion of the Wills Creek Watershed.....	21
Figure 2.4.3: Permitted Point Sources Discharging Fecal Bacteria in the Wills Creek Watershed	23
Figure 2.4.4: Combined Sewer Overflows in the Wills Creek Watershed	26
Figure 4.2.1: Diagram of Non-tidal Bacteria TMDL Analysis Framework	32
Figure 4.3.1: Monitoring Stations and Subwatersheds in Wills Creek Basin.....	37
Figure A-1: <i>E. Coli</i> Concentration vs. Time for Wills Creek Monitoring Station BDK0000	A6
Figure A-2: <i>E. Coli</i> Concentration vs. Time for Wills Creek Monitoring Station JEN0036	A6
Figure A-3: <i>E. Coli</i> Concentration vs. Time for Wills Creek Monitoring Station NJE0014	A7
Figure A-4: <i>E. Coli</i> Concentration vs. Time for Wills Creek Monitoring Station WIL0000	A7
Figure A-5: <i>E. Coli</i> Concentration vs. Time for Wills Creek Monitoring Station WIL0013	A8
Figure A-6: <i>E. Coli</i> Concentration vs. Time for Wills Creek Monitoring Station WIL0067	A8
Figure B-1: Wills Creek Flow Duration Curves	B2
Figure B-2: <i>E. Coli</i> Concentration vs. Flow Duration for Wills Creek Monitoring Station BDK0000	B5
Figure B-3: <i>E. Coli</i> Concentration vs. Flow Duration for Wills Creek Monitoring Station JEN0036.....	B5
Figure B-4: <i>E. Coli</i> Concentration vs. Flow Duration for Wills Creek Monitoring Station NJE0014.....	B6
Figure B-5: <i>E. Coli</i> Concentration vs. Flow Duration for Wills Creek Monitoring Station WIL0000	B6
Figure B-6: <i>E. Coli</i> Concentration vs. Flow Duration for Wills Creek Monitoring Station WIL0013	B7
Figure B-7: <i>E. Coli</i> Concentration vs. Flow Duration for Wills Creek Monitoring Station WIL0067	B7
Figure C-1: Wills Creek Classification Model: Percent Correct versus Percent Unknown.	C9

List of Tables

Table 2.1.1: Wills Creek Watershed Contributing States	3
Table 2.1.2: Land Use Percentage Distribution for Wills Creek Basin	6
Table 2.1.3: Number of Dwellings Per Acre	7
Table 2.1.4: Total Population Per Subwatershed in Wills Creek Watershed	7
Table 2.2.1: Historical Monitoring Data in the Wills Creek Watershed	11
Table 2.2.2: Locations of DNR (CORE) Monitoring Station in the Wills Creek Watershed.....	11
Table 2.2.3: Locations of MDE Monitoring Stations in the Wills Creek Watershed	11
Table 2.2.4: Locations of USGS Gauging Stations in Wills Creek Watershed	11
Table 2.3.1: Bacteria Criteria Values (from Table 1 COMAR 26.08.02.03-3 Water Quality Criteria Specific to Designated Use).....	13
Table 2.3.2: Weighting Factors for Average Hydrology Year Used for Estimation of Geometric Means in the Wills Creek Watershed.....	16
Table 2.3.3: Wills Creek Annual Steady State Geometric Means by Stratum per Subwatersheds	17
Table 2.3.4: Wills Creek Seasonal (May 1 st -September 30 th) Period Steady-State Geometric Means by Stratum per Subwatersheds	18
Table 2.4.1: Septic Systems and Households Per Sub-Watershed in Wills Creek Watershed MD Only.....	19
Table 2.4.2: Municipal NPDES Permit Holders in the Wills Creek Watershed.....	22
Table 2.4.3: Locations of Combined Sewer Overflows in the Wills Creek Watershed	25
Table 2.4.4: Distribution of Fecal Bacteria Source Loads in the Wills Creek Basin for the Annual Period	28
Table 2.4.5: Distribution of Fecal Bacteria Source Loads in the Wills Creek Basin for the Seasonal Period (May 1 st – September 30 th).....	29
Table 4.3.1: Baseline Load Calculations	36
Table 4.4.1: Hydrological Conditions Used to Account for Critical Condition and Seasonality	39
Table 4.4.2: Required Reductions of Fecal Bacteria to Meet Water Quality Standards	40
Table 4.6.1: Wills Creek Watershed TMDL Summary.....	43
Table 4.7.1: Bacteria Source Distributions and Corresponding Baseline Loads Used in the TMDL Analysis	43
Table 4.7.2: Maximum Practicable Reduction Targets	44
Table 4.7.3: Practicable Reduction Scenario Results	45
Table 4.7.4: TMDL Scenario Results: Percent Reductions Based on Optimization Model Allowing Up to 98% Reduction*	47
Table 4.7.5: TMDL Scenario Results: Reduced Loads by Source Category.....	47
Table 4.8.1: Potential Source Contributions for TMDL Allocations	48
Table 4.9.1: Wills Creek Watershed TMDL.....	50
Table A-1: Measured Bacteria Concentration with Daily Flow Frequency	A1
Table B-1: USGS Gages in the Wills Creek Watershed	B1
Table B-2: Definition of Flow Regimes	B2
Table B-3: Weighting Factors for Estimation of Geometric Mean	B4
Table C-1: Antibiotics and concentrations used for ARA	C5

Table C-2a: Georges Creek. Category, total number, and number of unique patterns in the Georges Creek portion of the combined Georges-Wills known-source library.C7

Table C-2b: Wills Creek. Category, total number, and number of unique patterns in the Wills Creek portion of the combined Georges-Wills known-source library.....C7

Table C-3: Wills Creek. Number of isolates not classified, percent unknown, and percent correct for six (6) cutoff probabilities for Wills Creek known-source isolates using the combined Georges-Wills known-source library.C8

Table C-4: Wills Creek. Actual species categories versus predicted categories, with a 70% probability cutoff, with rates of correct classification (RCC) for each category.....C9

Table C-5: Potential host sources of Wills Creek Watershed water isolates by species category, number of isolates, percent isolates classified at a cutoff probability of 70%.....C10

Table C-6: Wills Creek. *Enterococcus* isolates obtained from water collected during the fall, winter, spring, and summer seasons for each of the six (6) monitoring stations.C10

Table C-7: Wills Creek: BST Analysis: Number of Isolates per Station per DateC11

Table C-8: BST Analysis: Percentage of Sources per Station per Date.C12

Table D-1: Estimating Non-CSO/“Background” Human Loading RateD1

List of Abbreviations

ARA	Antibiotic Resistance Analysis
ARCC	Average Rate of Correct Classification
BMP	Best Management Practice
BST	Bacteria Source Tracking
CAFO	Confined Animal Feeding Operations
cfs	Cubic Feet per Second
CFR	Code of Federal Regulations
CFU	Colony Forming Units
COMAR	Code of Maryland Regulations
CSO	Combined Sewer Overflow
CWA	Clean Water Act
CWP	Center for Watershed Protection
DNR	Maryland Department of Natural Resources
EPA	Environmental Protection Agency
GIS	Geographic Information System
LA	Load Allocation
LTCP	Long Term Control Plan
MACS	Maryland Agricultural Cost Share Program
MD	Maryland
MDE	Maryland Department of the Environment
MDP	Maryland Department of Planning
MGD	Million Gallons Per Day
ml	Milliliter(s)
MOS	Margin of Safety
MPN	Most Probable Number
MPR	Maximum Practicable Reduction
MRLC	Multi-Resolution Land Cover
MS4	Municipal Separate Storm Sewer System
MST	Microbial Source Tracking
NPDES	National Pollutant Discharge Elimination System
PA	Pennsylvania
PADEP	Pennsylvania Department of Environmental Protection
RCC	Rate of Correct Classification
RESAC	Regional Earth Science Application Center
SHA	State Highway Administration
SSO	Sanitary Sewer Overflows
STATSGO	State Soils Geographic Database
TARSA	Technical and Regulatory Services Administration
TMDL	Total Maximum Daily Load
USGS	United States Geological Survey

FINAL

WQIA	Water Quality Improvement Act
WLA	Wasteload Allocation
WQLS	Water Quality Limited Segment
WRAS	Watershed Restoration Action Strategy
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 Wills Creek (basin number 02-14-10-03). 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, the state is 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 Wills Creek and its tributaries, North Branch Jennings Run, Jennings Run, and Braddock Run, in the State of Maryland's 303(d) List as impaired by nutrients (1996), sediments (1996), toxics - cyanide (1996), low pH (1998), fecal bacteria (2002), and impacts to biological communities (2002). Wills Creek is a designated Use IV-P (Water Contact Recreation, Protection of Aquatic Life, Recreational Trout Waters and Public Water Supply) waterbody, and its tributaries are designated Use III-P (Water Contact Recreation, Protection of Aquatic Life, Non-tidal Cold Water and Public Water Supply) waterbodies. [See Code of Maryland Regulations \(COMAR\) 26.08.02.08R](#). This document proposes to establish a TMDL for fecal bacteria in Wills Creek that will allow for attainment of the beneficial use designation, primary contact recreation. The listings for nutrients, suspended sediments, impacts to biological communities, and toxics will be addressed separately at a future date. 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 Wills Creek watershed has been divided into six subwatersheds, including the tributaries North Branch Jennings Run, Jennings Run, and Braddock Run. The pollutant loads set forth in this document are for these six subwatersheds. To establish baseline and allowable pollutant loads for this TMDL, a flow duration curve approach was employed, using flow strata estimated from United States Geological Survey (USGS) daily flow monitoring data and bacteria monitoring data. The sources of fecal bacteria are estimated at six representative stations in the Wills 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 (agricultural related animals), and wildlife (mammals and waterfowl) source categories.

The allowable load is determined by estimating a baseline load from current monitoring data. The baseline load is estimated using a long-term geometric mean and weighting factors from the flow duration curve. The TMDL for fecal bacteria entering Wills Creek is established after considering four different hydrological conditions: high flow and low flow annual conditions; and high flow and low flow seasonal conditions (the period between May 1st and September 30th where water contact recreation is more prevalent). This allowable load 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 five of the six subwatersheds, it was estimated that water quality standards could not be attained with the MPRs. Thus, for these subwatersheds, the second scenario with higher maximum reductions was applied.

The fecal bacteria TMDL developed for the Wills Creek watershed, which includes the tributaries North Branch Jennings Run, Jennings Run, and Braddock Run is 1,509 billion MPN *E. coli*/day. The TMDL is distributed between load allocations (LA) for nonpoint sources and waste load allocations (WLA) for point sources, including National Pollutant Elimination System (NPDES) wastewater treatment plants (WWTPs), and NPDES Combined Sewer Overflows (CSOs). The margin of safety (MOS) for this TMDL has been incorporated using a conservative assumption by estimating the loading capacity of the stream based on a more stringent water quality endpoint concentration. The *E. coli* water quality endpoint concentration was reduced by 5%, from 126 MPN/100ml to 119.7 MPN/100ml. The TMDL has been allocated among the six subwatersheds of Wills Creek as follows:

Wills Creek Fecal Bacteria TMDL Allocations

Subwatershed	TMDL	LA	WLA CSOs
	Billion MPN <i>E. coli</i> /day		
Wills Creek upstream of Maryland/PA line (WIL0067)	629	629	N/A
North Branch Jennings Run (NJE0014)	62	62	N/A
Jennings Run upstream of the confluence with North Branch Jennings Run (JEN0036)	23	23	0
Braddock Run (BDK0000)	543	543	0
Wills Creek between Maryland/PA line and the confluence with Braddock Run (WIL0013sub)	61	61	N/A
Wills Creek between the confluence with Braddock Run and the confluence with the North Potomac River (WIL0000sub)	191	136	55
TOTAL	1,509	1,454	55

FINAL

Once the EPA has approved a TMDL, and it is known what measures must be taken to reduce pollution levels, implementation of best management practices (BMPs) is expected to take place. As previously stated, water quality standards cannot be met in all subwatersheds of Wills Creek using the MPR scenario. This may occur in subwatersheds where wildlife is a significant component, or in subwatersheds that require very high reductions of fecal bacteria loads to meet water quality standards. Therefore, MDE proposes a staged approach to implementation of the required reductions, beginning with the MPR scenario, as an iterative process that first addresses those sources making the largest impacts on water quality and creating 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 effectiveness of implementation efforts.

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 Wills Creek (basin number 02-14-10-03). 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.

The Wills Creek mainstem (basin number 02-14-10-03) has been designated a Use IV-P waterbody and its tributaries (North Branch Jennings Run, Jennings Run, and Braddock Run) as Use III-P waterbodies. [See Code of Maryland Regulations \(COMAR\) 26.08.02.08R](#). The Maryland Department of the Environment (MDE) has identified Wills Creek on the State's 303(d) List as impaired by the following: nutrients (1996), sediments (1996), toxics- cyanide (1996), low pH (1998), fecal bacteria (2002), and impacts to biological communities (2002). This document, upon approval by the EPA, establishes a TMDL of fecal bacteria in Wills Creek that will allow for attainment of its designated uses. All other impairments in Wills Creek will be addressed at a future date. A data solicitation for fecal bacteria was conducted by MDE in 2003, and all readily available data from the past five years were considered in the TMDL analysis.

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 (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*

FINAL

and enterococci can all be classified as fecal bacteria. The results of the EPA study (EPA, 1986) demonstrated that fecal coliform showed less correlation to swimming-associated gastroenteritis than did either *E. coli* or enterococci.

The Wills Creek watershed was listed on the Maryland 303(d) List using fecal coliform as the indicator organism. Based on EPA's guidance (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 Maryland's current bacteria water quality criteria, either *E. coli* or enterococci. The indicator organism used in the Wills Creek TMDL analysis was *E. coli*.

2.0 SETTING AND WATER QUALITY DESCRIPTION

2.1 General Setting

Location

The Wills Creek watershed is located in Allegany and Garrett Counties in Maryland (MD) and Bedford and Somerset Counties in Pennsylvania (PA). The total drainage area of Wills Creek is approximately 253.6 square miles (162,284 acres), with 60.5 sq. miles (38,722 acres) in MD and 193.1 sq. miles (123,562 acres) in PA. The headwaters of Wills Creek originate in the Big Savage Mountains in PA, flowing east toward the city of Hyndman where the creek turns and continues south, entering MD at the town of Eilerslie and eventually emptying into the North Branch Potomac at Cumberland, MD.

There are three major drainage areas comprising the Wills Creek watershed: Jennings Run, North Branch Jennings Run, and Braddock Run. These branches are free-flowing (non-tidal) streams, and flow into Wills Creek at Corriganville and Homewood, MD before discharging into the North Branch Potomac River at Cumberland, MD. Table 2.1.1 lists the percentages of contributing states.

Table 2.1.1: Wills Creek Watershed Contributing States

STATE	AREA (sq. miles)	AREA (acres)	% of Total
Maryland	60.5	38,722	24
Pennsylvania	193.1	123,562	76
Total	253.6	162,284	100

Geology/Soils

The majority of the study area is in the Valley and Ridge district of the Appalachian physiographic province. The highest elevation in the study area is approximately 1,300 feet. The Valley and Ridge district is divided into the Great Valley (Hagerstown Valley in MD) to the east and the Allegheny Ridge area to the west. The Appalachian region in the study area is underlain by thick layers of sedimentary rocks of limestones and shale.

The Wills Creek watershed lies predominantly in the Gilpin-Dekalb-Cookport and Welkert-Calvin-Lehew soil association series in MD. Soils in this series are gently sloping to very steep, well-to moderately well-drained, shaly to very stony soils over sandstone and shale (Allegheny County, Soil Conservation Service, 1977) (Figure 2.1.2).

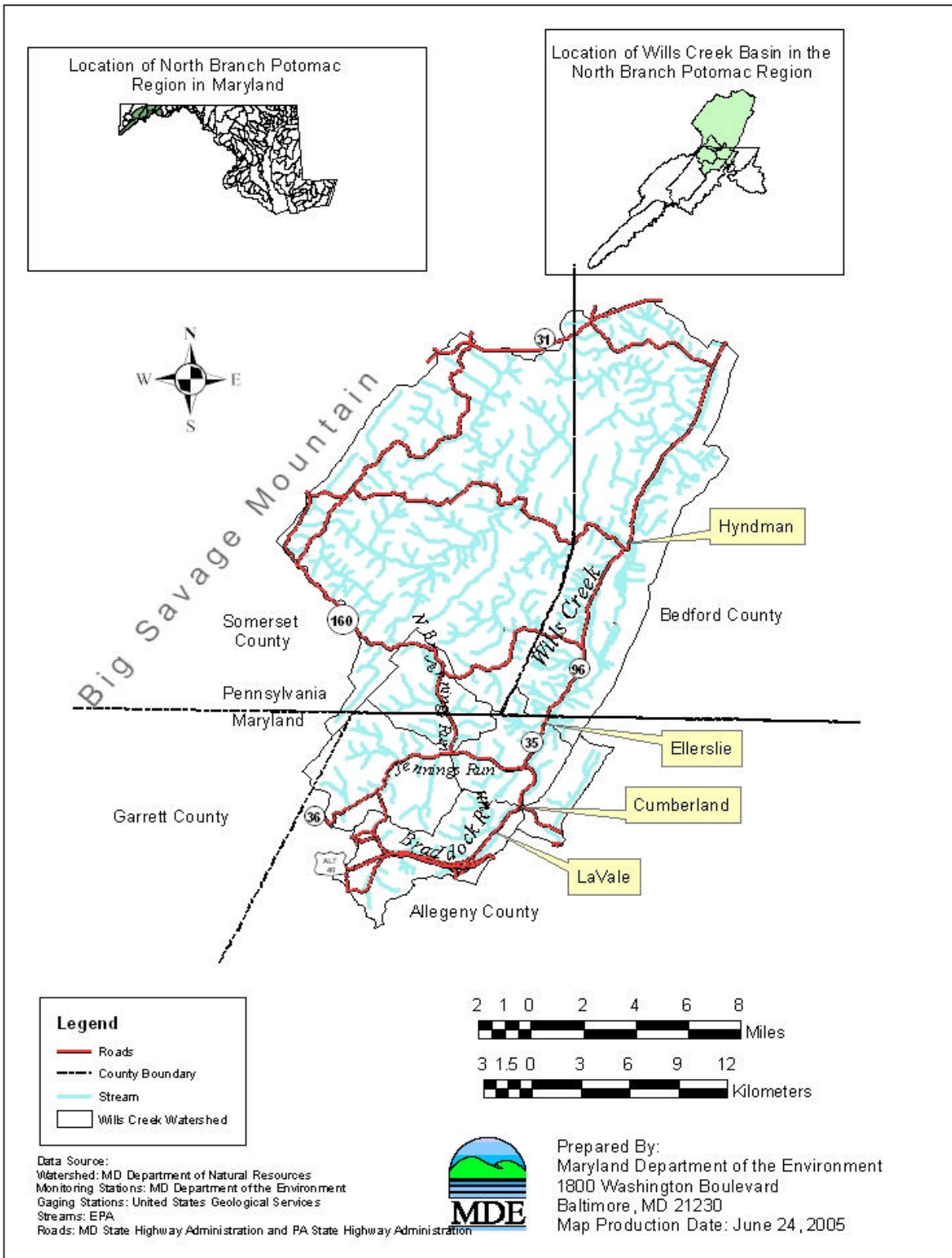


Figure 2.1.1: Location Map of the Wills Creek Basin

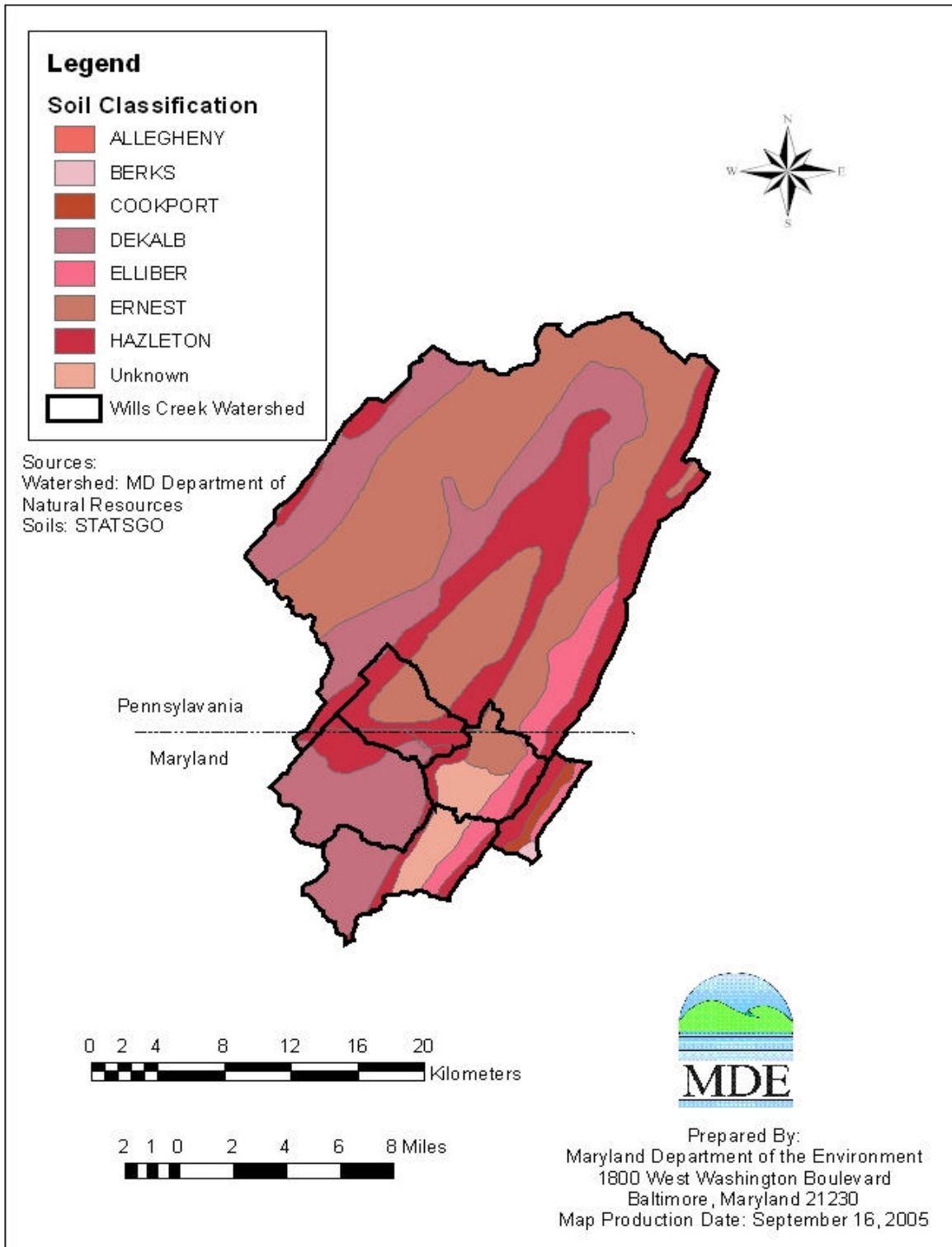


Figure 2.1.2: General Soil Series in the Wills Creek Basin

Land Use

The 2002 Maryland Department of Planning (MDP) land use/land cover data show that the watershed can be characterized as primarily forested for MD. Regional Earth Science Application Center (RESAC) land use/land cover was used to estimate the land use for the PA portion of Wills Creek. RESAC shows that the Wills Creek basin is also primarily forested in the PA portion of the basin. The headwaters of Wills Creek lie in the Big Savage Mountains in PA. West of Fairhope the area consists of public forestland. As the creek continues east toward Hyndman there are a few small rural communities and small farms of no significant impact.

The end of the watershed basin lies near West End in Bedford County at the headwaters of Little Wills Creek. The creek flows east along a relatively flat valley floor. Once it reaches Bard, the creek meets the steep slope of Buffalo Mountain and turns towards the south. Here it continues to flow along the boundary between the mountain and the valley floor. Eventually it meets Wills Creek just south of Hyndman. This region is primarily rural and sparsely developed. There seems to be minimal impact from wildlife sources and little, if any, impact from rural septic systems. Between Bard and Hyndman, there is row crop agriculture and forestland. As Wills Creek enters MD, it encounters a more urban environment. It receives water from Jennings Run and Braddock Run (MDE, 2002).

The land use percentage distribution for the Wills Creek Basin 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: Land Use Percentage Distribution for Wills Creek Basin

Land Type	Maryland Acreage	Maryland Percentage	Pennsylvania Acreage	Pennsylvania Percentage
Forest	28,885	74.6 %	103,981	84.2 %
Urban	6,495	16.8 %	2,922	2.4 %
Crops	1,905	4.9 %	14,010	11.3 %
Pasture	1,411	3.6 %	2,631	2.1 %
Water	26	0.1 %	18	0.0 %
Totals	38,722	100.0%	123,562	100.0%

Population

The total population in the Wills Creek watershed is estimated to be 32,017 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 Geographic Information Systems (GIS) 2000 U. S. Census Block and the MDP Land Use 2002 Cover and the RESAC coverage for PA that includes the Wills Creek watershed. Since the Wills Creek watershed is a sub-area of the Census Block, percentages of each land use within the watershed were used to extract the areas from the 2000 Census Block. Table 2.1.2 shows the number of dwellings per acre in the Wills Creek watershed. The number of dwellings per acre was derived from

information for residential density (low, medium, high) from the MDP land use cover and RESAC.

Table 2.1.3: Number of Dwellings Per Acre

Land use Code	Dwelling Per Acres
Low Density Residential	1
Medium Density Residential	5
High Density Residential	8

Based on the number of households from the Total Population from the Census Block and the number of dwellings per acre from the MDP Land Use Cover and RESAC, population per sub-watershed was estimated (see Table 2.1.3).

Table 2.1.4: Total Population Per Subwatershed in Wills Creek Watershed

Subwatershed	Station	Population
North Branch Jennings	NJE0014	615
Jennings Run	JEN0036	3,614
Braddock Run	BDK0000	7,612
Wills Creek	WIL0067	12,572
Wills Creek	WIL0013sub	2,284
Wills Creek	WIL0000sub	5,320
	TOTAL	32,017

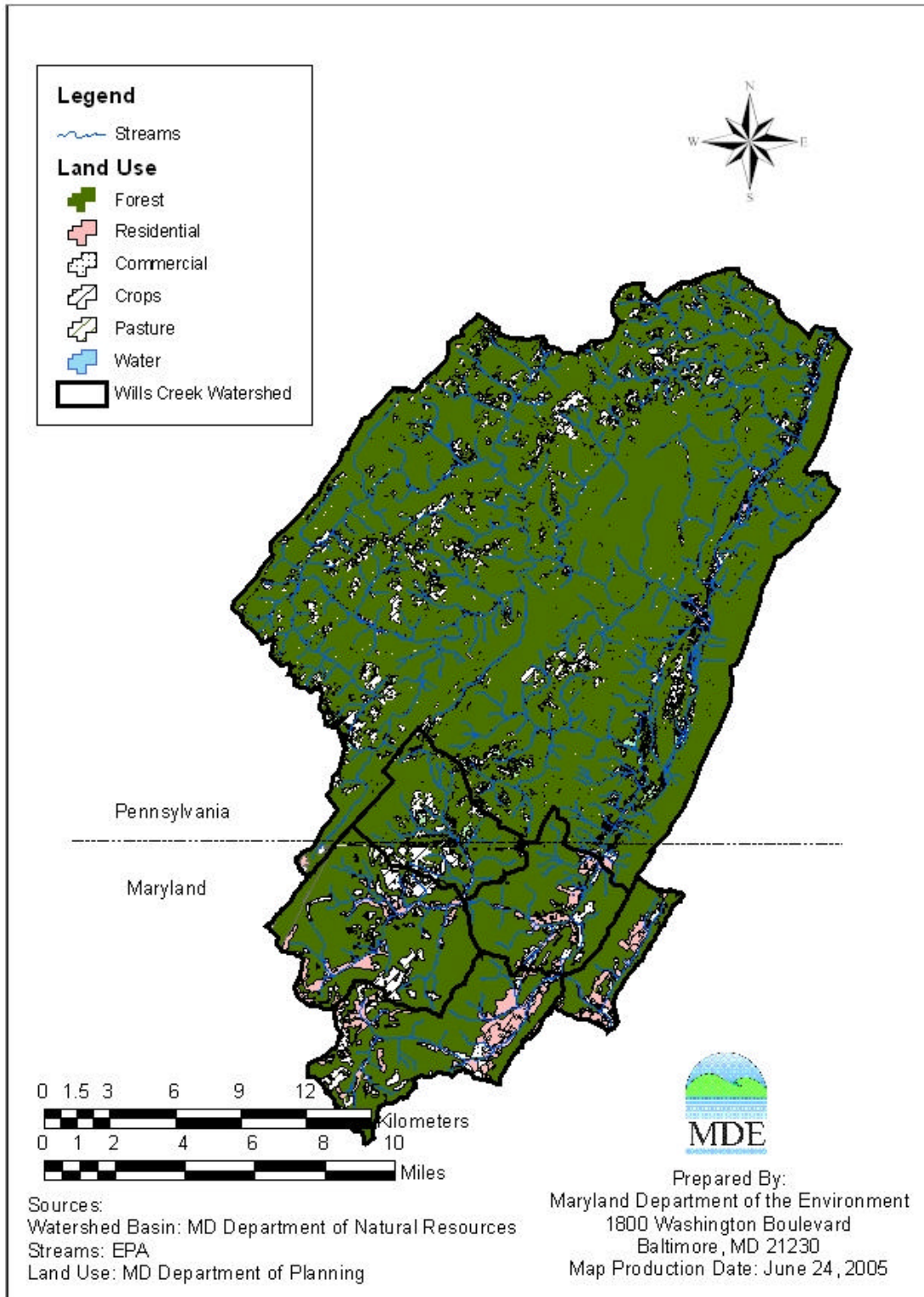


Figure 2.1.3: Land Use of the Wills Creek Watershed

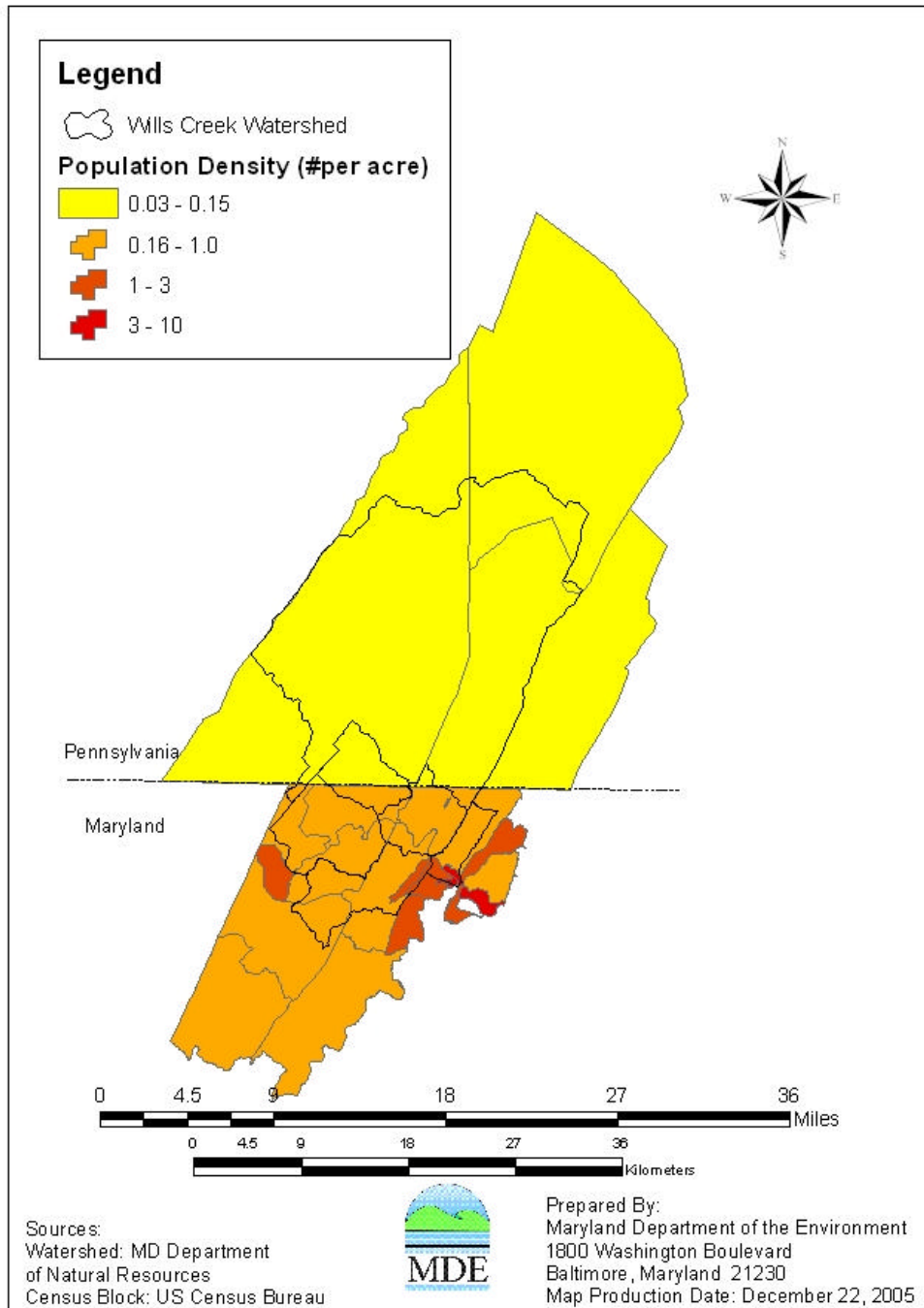


Figure 2.1.4: Population Density in Wills Creek Basin

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).

As per 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 (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.

Bacteria Monitoring

Table 2.2.1 lists the historical monitoring data for the Wills Creek watershed. MDE conducted monitoring sampling from October 2002 through October 2003. Monitoring Stations WIL0013 and BDK0000 (CORE) were used by the Maryland Department of Natural Resources (DNR) to identify the bacterial impairment. There are six MDE monitoring stations in the Wills Creek watershed. In addition to the bacteria monitoring stations, there are three United States Geological Survey (USGS) gauging stations used in deriving the surface flow in Wills Creek. The locations of these stations are shown in Tables 2.2.2 to Table 2.2.4 and in Figure 2.2.1. Observations recorded during the period 2002-2003 from the six MDE monitoring stations are shown in Appendix A. A table listing the monitoring results from the Wills Creek watershed appears in Appendix A.

Bacteria counts are highly variable and results are presented on a log scale for the six monitoring stations for data collected for September 2002 through November 2003. Bacteria counts ranged between 3 and 41,000 MPN/100 ml.

Table 2.2.1: Historical Monitoring Data in the Wills Creek Watershed

Sponsor	Location	Date	Design	Summary
Maryland Department of Natural Resources (DNR) CORE Monitoring	MD	1/8/97 – 4/1/98	Fecal Coliform*	BDK0000 and WIL0013 GM=317 MPN/100ml, n=15
MDE	MD	10/02 to 10/03	<i>E. coli</i>	6 stations 2 sample per month
MDE	MD	11/02 to 10/03	BST(ARA) (enterococci)*	6 stations 1 sample per month

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

Table 2.2.2: Locations of DNR (CORE) Monitoring Station in the Wills Creek Watershed

Tributary	Monitoring Station	Observation Period	Total Observations	LATITUDE Dec-Deg	LONGITUDE Dec-Deg
Braddock Run	BDK0000	1997 - 1998	15	39 40.224	78 47.511
Wills Creek	WIL0013	1997 - 1998	15	39 40.229	78 47.343

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

Tributary	Monitoring Station	Observation Period	Total Observations	LATITUDE Dec-Deg	LONGITUDE Dec-Deg
North Branch Jennings Run	NJE0014	2002 - 2003	23	39 42.800	78 50.378
Jennings Run	JEN0036	2002 - 2003	23	39 42.053	78 50.602
Braddock Run	BDK0000	2002 - 2003	23	39 40.224	78 47.511
Wills Creek	WIL0067	2002 - 2003	23	39 43.102	78 46.273
Wills Creek	WIL0013	2002 - 2003	23	39 40.229	78 47.343
Wills Creek	WIL0000	2002 - 2003	23	39 38.904	78 45.877

Table 2.2.4: Locations of USGS Gauging Stations in Wills Creek Watershed

Monitoring Station	Observation Period Used in TMDL Analysis	Total Observations	LATITUDE Dec-deg	LONGITUDE Dec-deg
01596500	1988 – 2003	5,477	39 34.203	79 06.116
01599000	1988 – 2003	5,477	39 29.634	79 02.681
01601500	1988 – 2003	5,477	39 40.146	78 47.281

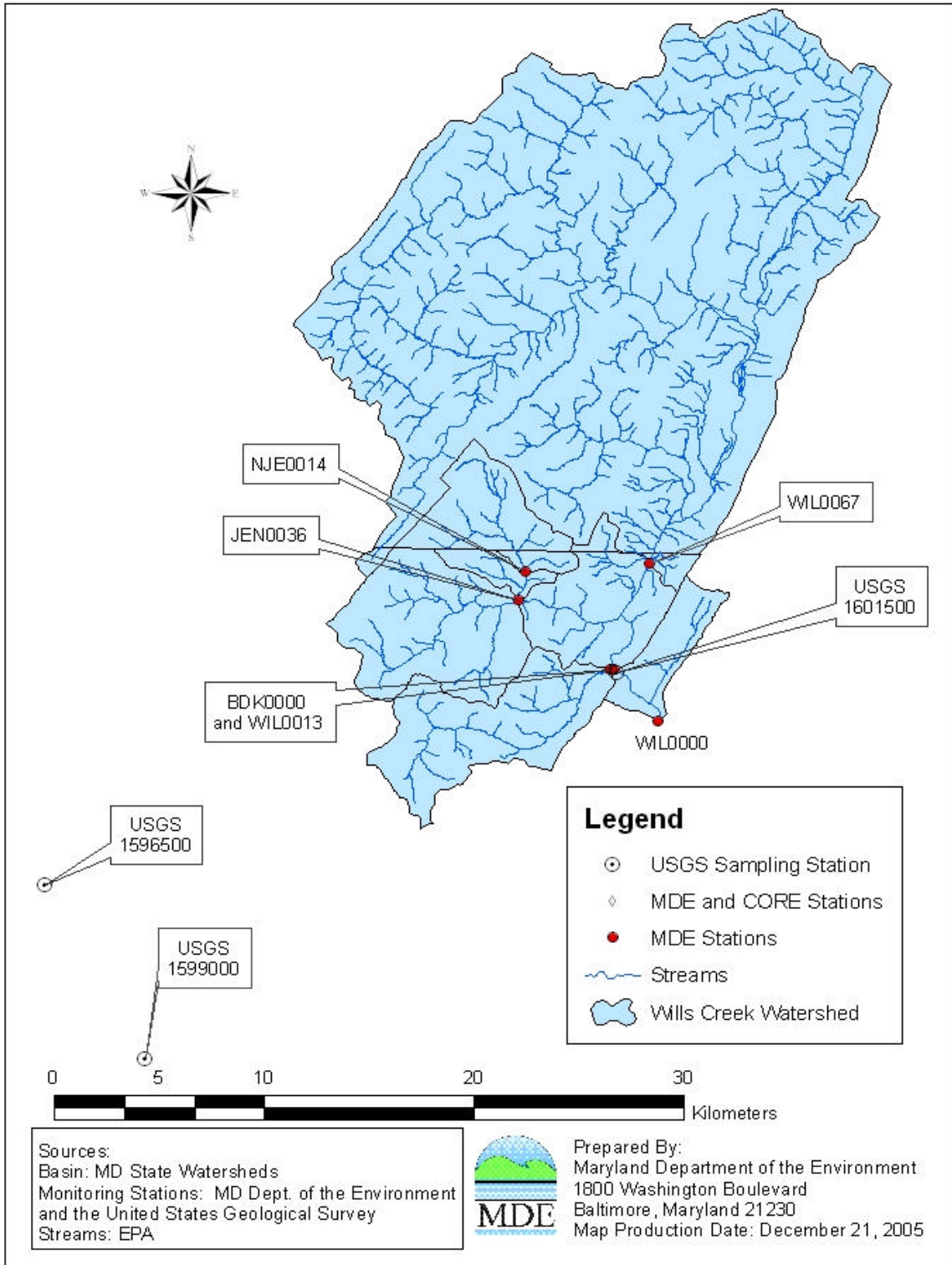


Figure 2.2.1: Monitoring Stations in the Wills Creek Basin

2.3 Water Quality Impairment

Designated Uses and Water Quality Standard

The Maryland water quality standards Surface Water Use Designations for Wills Creek is Use IV-P (Water Contact Recreation, Protection of Aquatic Life, Recreational Trout Waters and Public Water Supply), and its tributaries are designated Use III-P (Water Contact Recreation, Protection of Aquatic Life, Non-tidal Cold Water and Public Water Supply) (COMAR 26.08.02.08R(b)). The Wills Creek watershed was first listed in the State's 2002 303(d) List as impaired by fecal coliform bacteria, and has been included on the final 2004 Integrated 303(d) List.

Water Quality Criteria

The State water quality standard for bacteria (*E. coli*) used in this study is as follows (COMAR Section 26.08.02.03-3):

Table 2.3.1: Bacteria Criteria Values (from Table 1 COMAR 26.08.02.03-3 Water Quality Criteria Specific to Designated Use)

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

Interpretation of Bacteria Data for General Recreational Use

The listing methodology as per 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 cfu/100 ml *E. coli* 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. If the resulting steady-state geometric mean of the available data for each year is greater than 126 cfu/100 ml *E. coli* in freshwater, the waterbody or beach will be listed as impaired.

FINAL

The single sample maximum criterion applies only to beaches and is 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 Wills 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. Since warm temperatures can occur early in May and last until the end of September or early October, a longer seasonal period than the official beach season (Memorial Day to Labor Day) was used for the water quality assessment, as a conservative assumption in the analysis. 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 Wills 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.

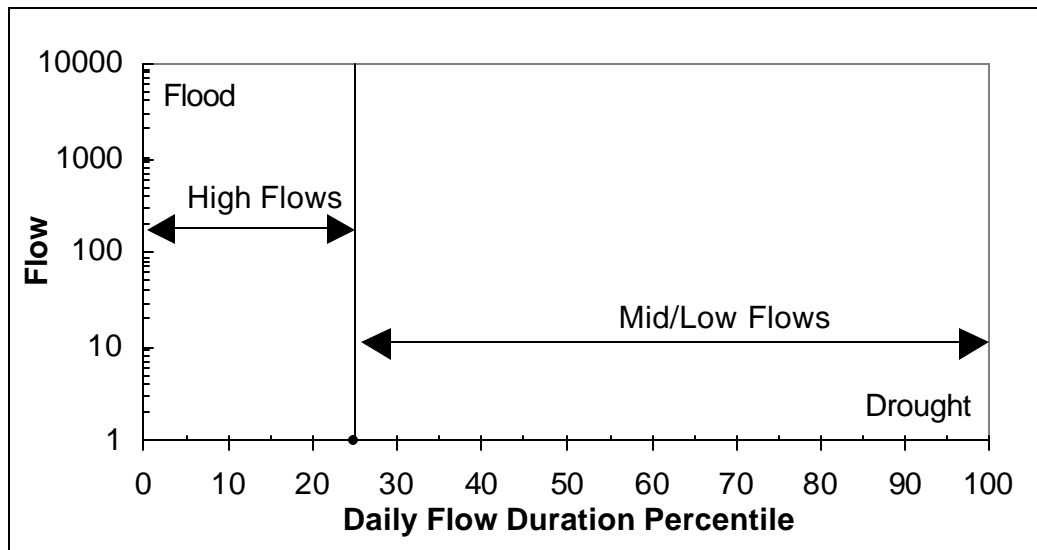


Figure 2.3.1: Conceptual Diagram of Flow Duration Zones

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 a flow analysis of several watersheds throughout Maryland, it was determined that flows within the 25th to 30th daily flow duration percentiles were representative of average daily flows. It is assumed for this analysis that flows above the 25th percentile represent high flows, and flows below the 25th percentile 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 Wills 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 Wills Creek Watershed

Flow Duration Zone	Duration Interval	Weighting Factor
High Flows	0 – 25%	0.25
Mid/Low Flows	25 – 100%	0.75

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

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

where

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

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:

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

C_{gm} = Steady-state geometric mean concentration

Table 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 Wills Creek subwatersheds for the annual and the seasonal (May 1st –September 30th) periods.

Table 2.3.3: Wills Creek Annual Steady State Geometric Means by Stratum per Subwatersheds

Tributary Station	Flow Stratum	# of Samples	<i>E. coli</i> Minimum Concentration (MPN/100ml)	<i>E. coli</i> Maximum Concentration (MPN/100ml)	Annual Steady-State Geometric Mean (MPN/100ml)	Annual Weighted Geometric Mean (MPN/100ml)
North Branch Jennings NJE0014	High	9	6.3	1,396	223	149
	Low	14	20	1,785	130	
Jennings Run JEN0036	High	9	97	399	201	270
	Low	14	35.4	5,794	298	
Braddock Run BDK0000	High	9	20	41,060	4,499	372
	Low	14	8.5	24,192	162	
Wills Creek WIL0067	High	10	3.1	384	88	76
	Low	13	10	1,421	73	
Wills Creek WIL0013	High	10	7.4	1,076	146	80
	Low	13	10	1,439	65	
Wills Creek WIL0000	High	10	11	4,884	626	218
	Low	13	20	24,192	154	

Table 2.3.4: Wills Creek Seasonal (May 1st-September 30th) Period Steady-State Geometric Means by Stratum per Subwatersheds

Tributary Station	Flow Stratum	# of Samples	<i>E. coli</i> Minimum Concentration (MPN/100ml)	<i>E. coli</i> Maximum Concentration (MPN/100ml)	Seasonal Steady-State Geometric Mean (MPN/100ml)	Seasonal Weighted Geometric Mean (MPN/100ml)
North Branch Jennings NJE0014	High	4	246	933	395	358
	Low	6	85	1,785	346	
Jennings Run JEN0036	High	4	132	399	284	668
	Low	6	226	5,794	887	
Braddock Run BDK0000	High	4	175	41,060	3,522	1,201
	Low	6	275	24,192	839	
Wills Creek WIL0067	High	4	85	384	178	160
	Low	6	51	1,421	154	
Wills Creek WIL0013	High	4	161	1,076	367	190
	Low	6	41	1,439	152	
Wills Creek WIL0000	High	4	479	4,884	1,228	635
	Low	6	84	24,192	510	

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. 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. As the runoff occurs during rain events, surface runoff transports water and fecal bacteria over the land surface and discharges to the stream system. 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 activities generally arise from failing septic systems and their associated drain fields or leaking infrastructure (*i.e.*, sewer systems). In summary, the transport of fecal bacteria from the land surface to the stream system is dictated by the rainfall, soil type, land use, and topography of the watershed.

Sewer Systems and 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 31 SSOs reported to MDE between September 2002 and November 2003 in Allegany County. Approximately 12,826,500 gallons of SSOs were discharged through various waterways (surface water, groundwater, sanitary sewers, *etc.*) in the Allegany County portion of the Wills Creek watershed. Pennsylvania Department of Environmental Protection (PADEP) was contacted for information of septics and sewers in the PA portion of Wills Creek. At this time, PADEP does not have sewer and SSO information available in the Wills Creek watershed (PADEP, January 9, 2006). Figure 2.4.1 depicts the locations where SSOs occurred in the Wills Creek watershed (MD) between September 2002 and November 2003.

Septic Systems

On-site disposal (septic) systems are located throughout the Wills Creek watershed. Table 2.4.1 presents the total households and the number of septic systems per subwatershed for MD only. PADEP does not have any available information other than the fact that there are some septics and sewers in the Wills Creek watershed (PADEP, January 9, 2006). Figure 2.4.2 depicts the areas that are serviced by sewers and septic systems for MD only.

Table 2.4.1: Septic Systems and Households Per Sub-Watershed in Wills Creek Watershed MD Only

Tributary	Station	Households per Subwatershed	Septic Systems (units)
North Branch Jennings	NJE0014	74	30
Jennings Run	JEN0036	3,146	472
Braddock Run	BDK0000	6,419	209
Wills Creek	WIL0067	190	19
Wills Creek	WIL0013sub	2,704	146
Wills Creek	WIL0000sub	2,809	76
	TOTAL	15,342	952

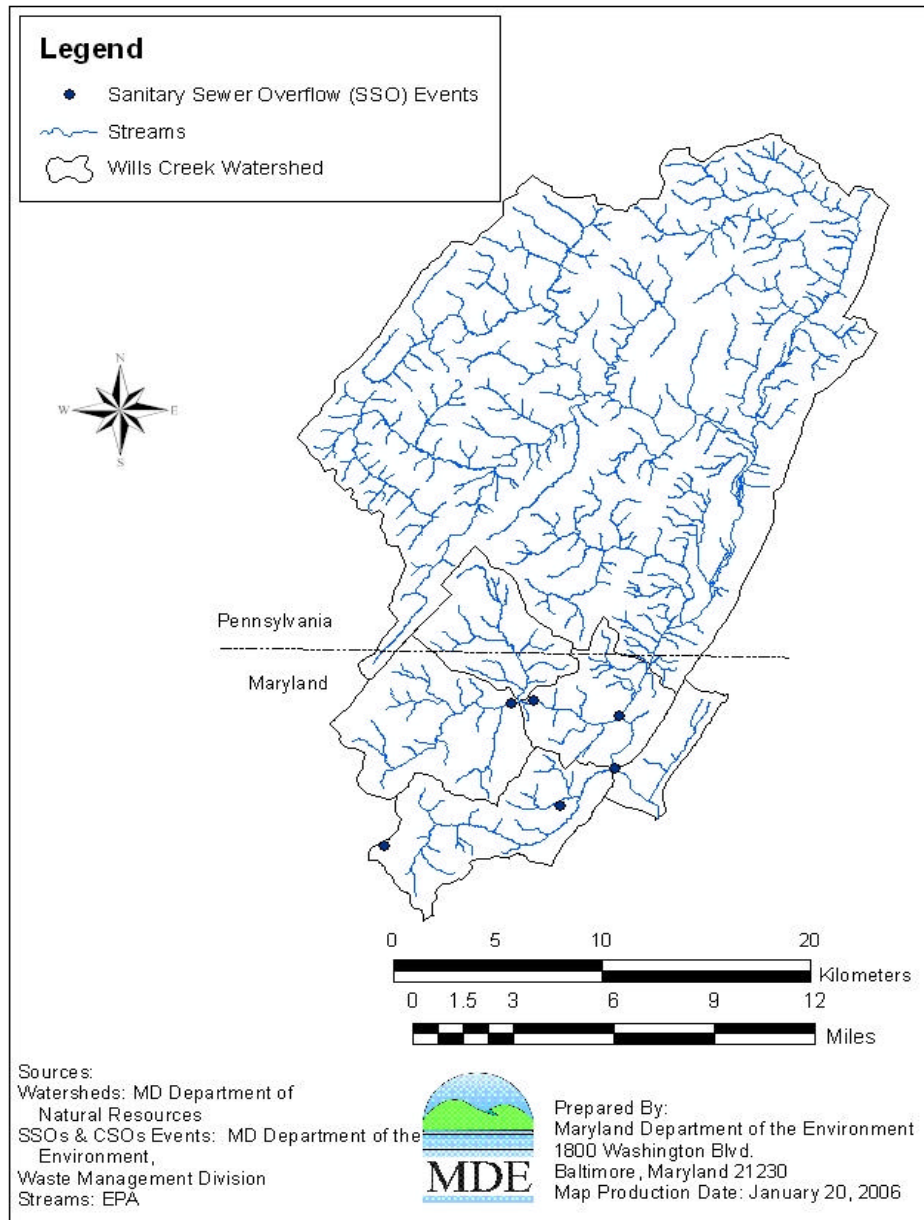


Figure 2.4.1: Location of Sanitary Sewer Overflows in MD's Portion of the Wills Creek Watershed

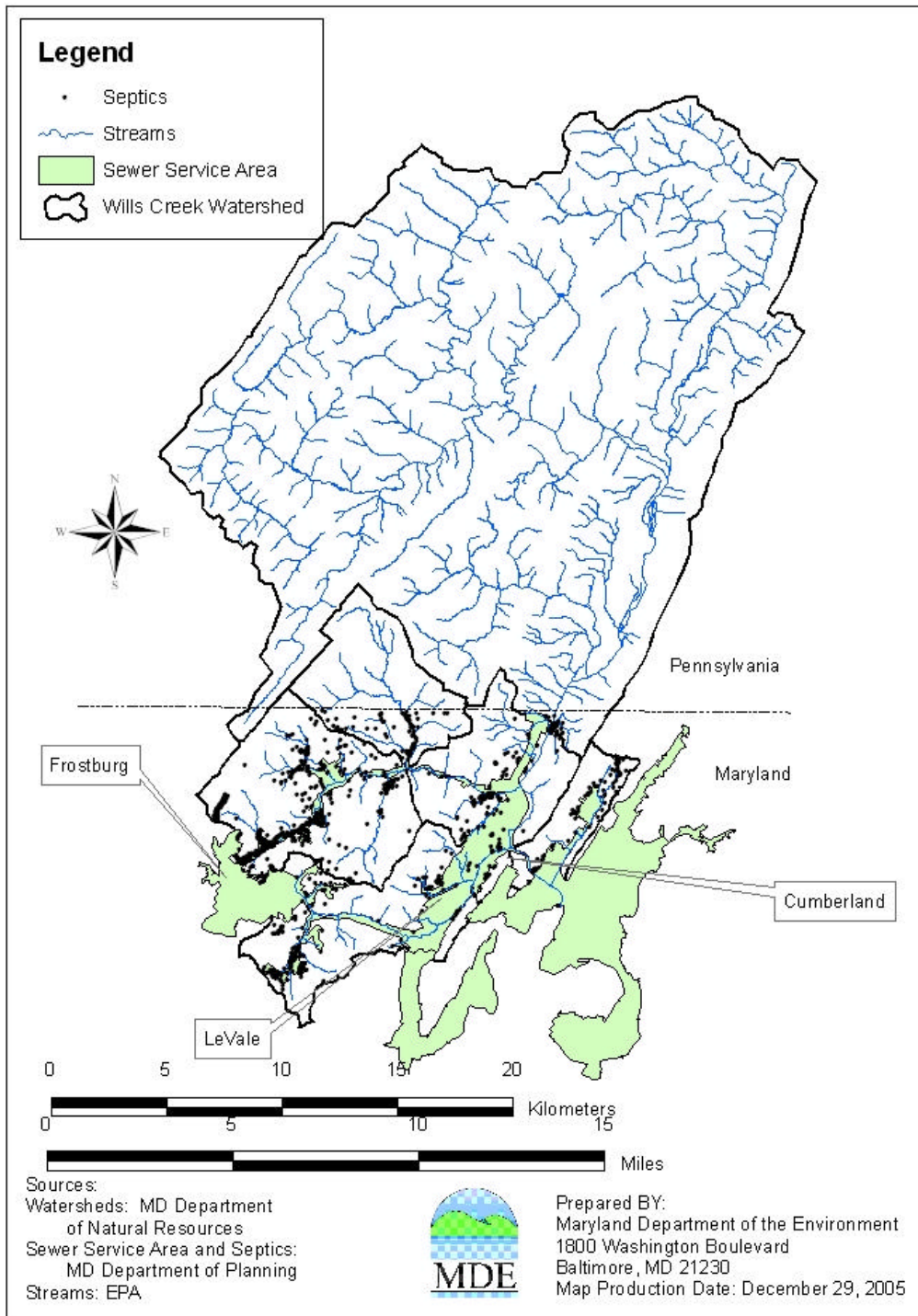


Figure 2.4.2: Sanitary Sewer Service and Septics Areas in MD's Portion of the Wills Creek Watershed

Point Source Assessment

Stormwater

The Wills Creek watershed is located in Allegany and Garrett Counties, MD, jurisdictions that are not required to obtain National Pollutant Discharge Elimination System (NPDES) Municipal Separate Storm Sewer System (MS4) permit coverage under the federal Clean Water Act. These jurisdictions have no NPDES MS4 permits to regulate stormwater discharges.

Municipal and Industrial WWTPs

Wastewater treatment plants are designed to treat wastewater before it can be 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 the point source permitting information, there is one NPDES permitted point source facility discharging fecal bacteria directly into the Wills Creek watershed (Table 2.4.2 and Figure 2.4.3). This WWTP uses an activated sludge process to treat approximately 200,000 gallons per day (0.2 MGD). The Hyndman Borough Municipal Authority WWTP is located in Bedford County, PA, outside of MD's jurisdiction. The TMDL and reductions that will be estimated at station WIL0067 will consider all point and nonpoint source loads located in PA and upstream of the monitoring station, including the Hyndman WWTP. The WWTP information presented below is only for informational purposes.

Table 2.4.2: Municipal NPDES Permit Holders in the Wills Creek Watershed

Permittee	NPDES Permit No.	County	Average Annual Flow (MGD)	Fecal Coliform Concentrations Annual AVG (MPN/100ml)	Fecal Coliform Load Per Day (Billion MPN/day)
Hyndman Borough Municipal	PA0020851	Bedford, PA	0.199	571.3	4.3

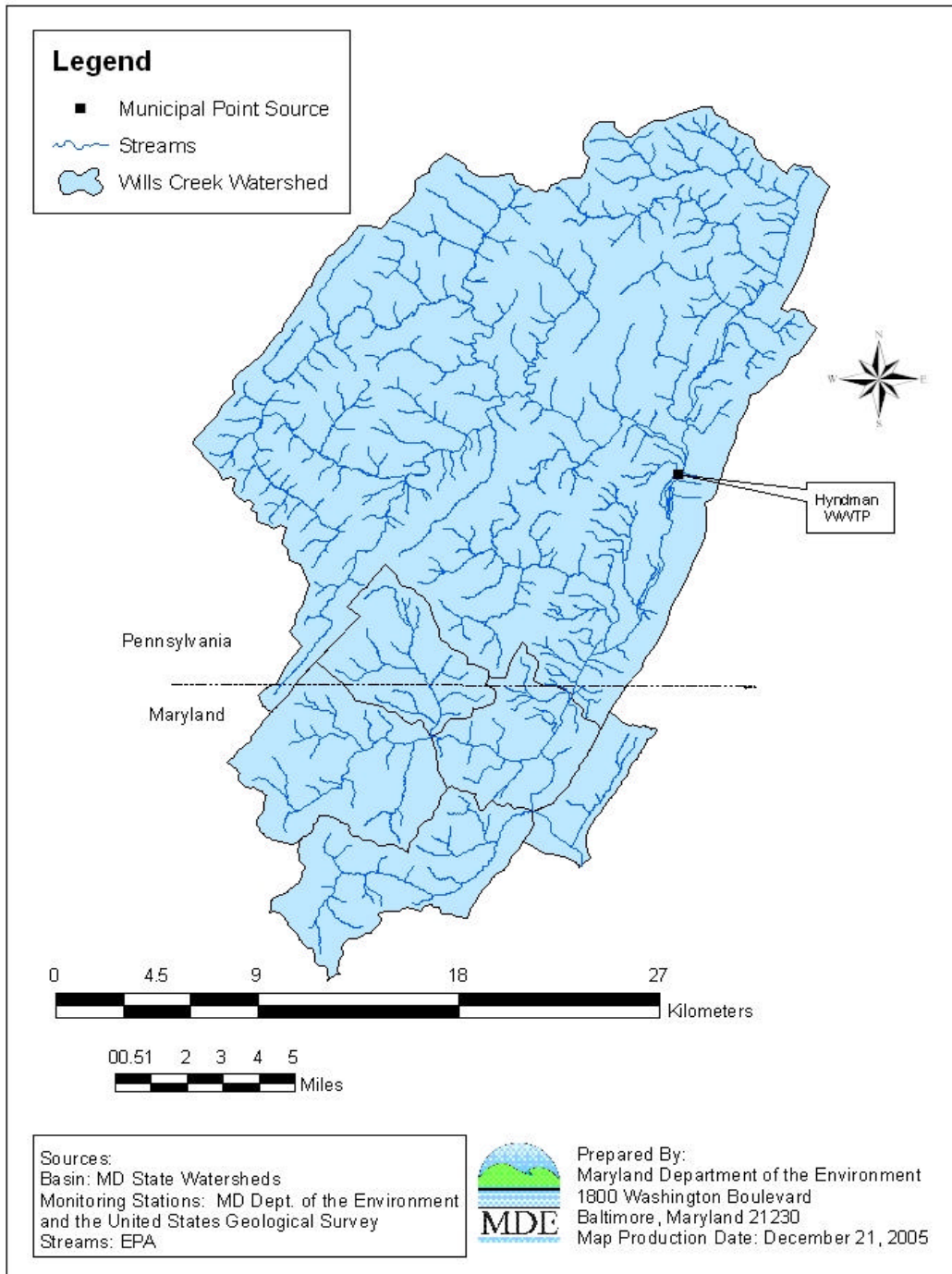


Figure 2.4.3: Permitted Point Sources Discharging Fecal Bacteria in the Wills Creek Watershed

Combined Sewer Overflow Systems

Wills Creek and its tributaries flow through several communities, large and small, with sewer systems that collect both sanitary sewage and stormwater runoff. These systems are referred to as combined sewer systems (CSSs). CSSs in the Wills Creek watershed transport wastewater to the sewage treatment plant in Cumberland, which discharges into the North Branch Potomac River. Therefore, the creek can receive untreated human and industrial waste.

Combined Sewer Overflows (CSOs) occur when the capacity of a separate or combined sanitary sewer is exceeded. Like SSOs, there are several factors that may contribute to CSOs from a sewerage system, including pipe capacity, operations and maintenance effectiveness, sewer design, age of system, pipe materials, geology and building codes. CSOs are designed to discharge, unlike SSOs, which are accidental releases, and are subject to NPDES permit requirements. The CSOs in Wills Creek are significant sources of bacteriological loading.

Braddock Run is a large source of acid mine drainage from numerous active and inactive area mines. The acid originated from mines in the Georges Creek basin. To mitigate the constant flooding in the mines, the Hoffman Tunnel was constructed between 1903 and 1906 to route excess water from the upper reaches of Georges Creek to the Wills Creek basin. The tunnel passes beneath the ridge of Dans Mountain and eventually forms the headwaters of Braddock Run. In roughly the first thirty percent of Braddock Run, bacteriological loading is almost nonexistent due to acidic waters (extremely low pH). Once the stream nears LaVale, the geochemistry raises the pH. At this point, the remaining seventy percent of Braddock Run has been known to receive wastewater from various CSOs. This same scenario has been seen in the Wills Creek tributary, Jennings Run (MDE, 2002).

The City of Cumberland owns and operates a WWTP and collection system that serves the City and the surrounding areas in the Wills Creek watershed. The WWTP discharges into the North Branch of the Potomac River.

Collection systems that are tributary to the Cumberland collection system are:

1. The Frostburg Combined Sewer System
2. The Allegany County Department of Public Works Sewer System
3. The Town of LaVale Sewer System
4. The Town of Ridgely, W. Virginia Sewer System (not part of Wills Creek Watershed).

The receiving waters of the Cumberland collection system include Wills Creek, Evitts Creek, Georges Creek, Braddock Run and the Potomac River. In the Wills Creek watershed there are four NPDES permitted CSSs: the City of Frostburg, the Town of LaVale, Allegany, and the City of Cumberland. The City of Frostburg owns and operates a wastewater collection system that delivers its sewage to the City of Cumberland through the Allegany County Sanitary Commission and the LaVale Sanitary Commission conveyance systems. The City of Frostburg (NPDES Permit # MD0067423 and State Permit No. 02-DP-3164) has an agreement with the Allegany County Sanitary Commission for the acceptance and conveyance of wastewater. The Allegany County Sanitary Commission has an agreement with the LaVale Sanitary Commission for the acceptance and

conveyance of wastewater. The LaVale Sanitary Commission (NPDES Permit # MD0067547 and State Permit No. 95-DP-3164) has an agreement with the City of Cumberland for the treatment and disposal of wastewater. The sewer collection system within the Wills Creek watershed is a Combined Sewer System (CSS), receiving stormwater as well as wastewater. The City of Cumberland is authorized to discharge from the CSO outfalls under NPDES Permit # MD0021598 and State Permit No. 01-DP-0567.

Long Term Control Plans (LTCP) have been developed for all Combined Sewer Systems (CSS) in Maryland under consent decrees between MDE and jurisdictions operating the CSSs to control or eliminate all CSOs. Implementation of LTCPs is required for all jurisdictions by October 1, 2023. For more detailed information on the consent decree and the jurisdictions' LTCPs, please refer to the Consent Decree Case Number 01-C-00-18342L and the LTCP documents in the References section. Figure 2.4.1 depicts the areas that are serviced by sewers and septic systems for MD only. Table 2.4.2 and Figure 2.4.3 depict the locations of the CSOs.

Table 2.4.3: Locations of Combined Sewer Overflows in the Wills Creek Watershed

CSS Permit System	NPDES #	Outfall	Location	Receiving Waters	Latitude	Longitude
Town of LaVale Combined Sewer System	MD0067547	001	Pumping Station	Braddock Run	39 40.200	78 47.583
		003	Arlington Avenue	Braddock Run	39 39.247	78 48.588
		006	Red Hill	Braddock Run	39 38.374	78 51.213
City of Frostburg Combined Sewer System	MD0067423	004	Bealls Lane	Jennings Run	39 39.633	78 55.167
		005	Fairview Street	Jennings Run	39 39.683	78 55.633
		006	N. Water Street	Jennings Run	39 39.533	78 55.917
		014	Rt. 40	Braddock Run	39 39.067	78 54.783
		015	Rt. 40	Braddock Run	39 39.083	78 54.733
City of Cumberland Combined Sewer System	MD0021598	008	Bedford Street	Wills Creek	39 39.144	78 45.833
		010	North Mechanic Street	Wills Creek	39 39.445	78 46.362
		011	Franklin Street	Wills Creek	39 39.472	78 46.410
		012	Valley Street	Wills Creek	39 39.359	78 46.204
		013	Market Street	Wills Creek	39 39.232	78 45.927
		014	Green Street	Wills Creek	39 38.987	78 45.861

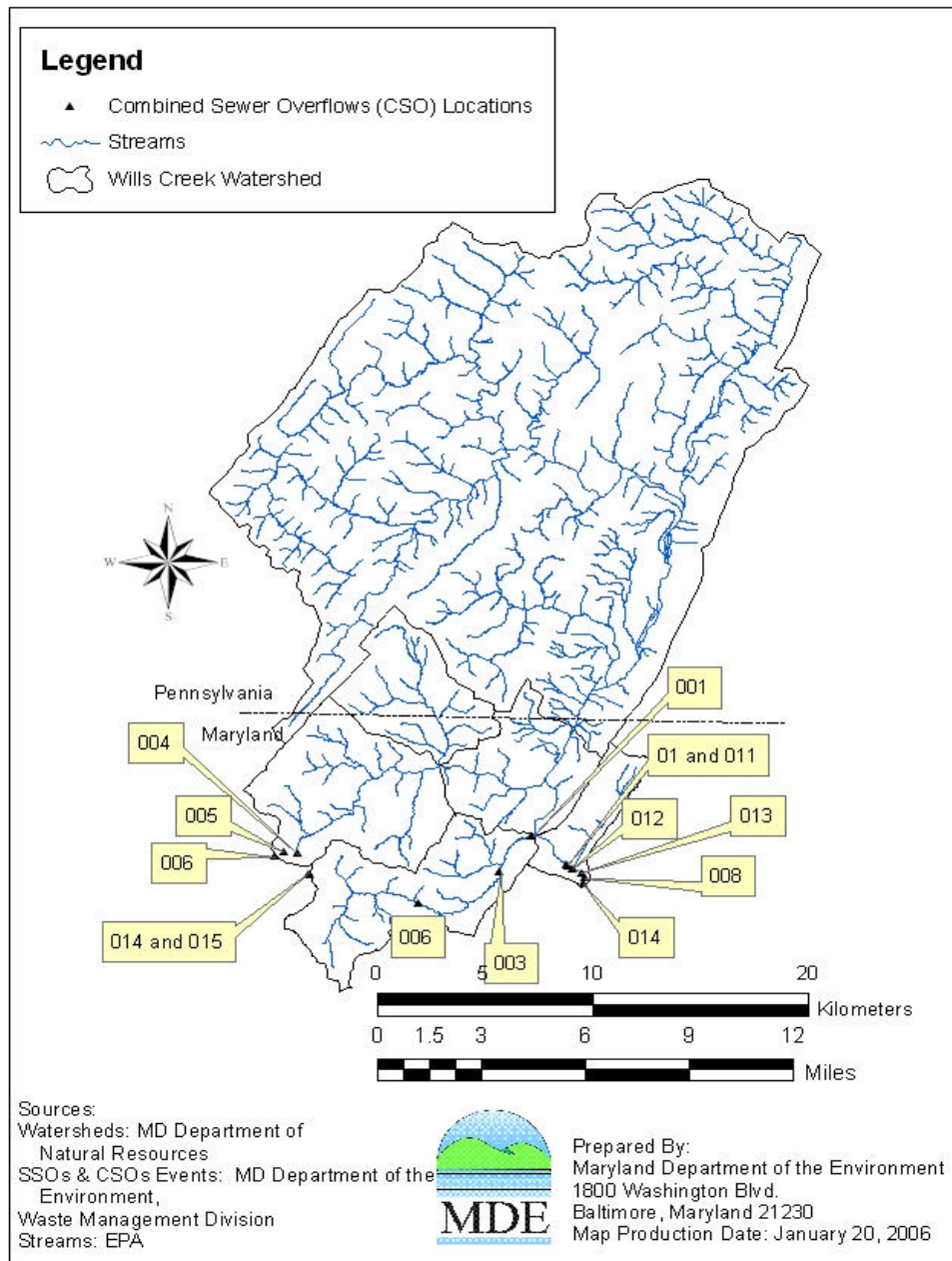


Figure 2.4.4: Combined Sewer Overflows in the Wills Creek Watershed

Bacteria Source Tracking

Bacteria source tracking (BST) was used to identify the relative contribution of bacteria to in-stream water samples. BST monitoring was conducted at six stations throughout the Wills Creek watershed where 12 samples (one per month) were collected 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 samples. Details of the BST methodology and data can be found in Appendix C.

An accurate representation of the expected average 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 the 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. The final weighted mean source percentage, for each source category, is based on the proportion of time in each flow duration zone (i.e. high flow=0.3, low flow=0.7).

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

$$MS_k = \sum_{i=1}^2 MS_{i,k} * W_i \quad (4)$$

where

$$MS_{i,k} = \frac{\sum_{j=1}^{n_i} \log_{10}(C_{i,j}) * S_{i,j,k}}{\sum_{j=1}^{n_i} \log_{10}(C_{i,j})} \quad (5)$$

$MS_{i,k}$ = Weighted mean proportion of isolates for source k in stratum I

MS_k = weighted mean proportion of isolates of source k

W_i = Proportion covered by stratum i

i = stratum

j = sample

k = Source category (1 = human, 2 = domestic, 3 = livestock, 4 = wildlife, 5 = unknown)

$C_{i,j}$ = 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.

Table 2.4.4: Distribution of Fecal Bacteria Source Loads in the Wills Creek Basin for the Annual Period

STATION	Flow Stratum	% Domestic Animals	% Human	% Livestock	% Wildlife	% Unknown
NJE0014	High Flow	11.1	41.3	2.0	16.6	29.0
	Low Flow	7.7	26.3	17.0	32.3	16.7
	Weighted	8.5	30.0	13.2	28.4	19.8
JEN0036	High Flow	8.7	57.4	2.3	9.4	22.2
	Low Flow	5.7	24.0	10.2	24.5	35.6
	Weighted	6.5	32.3	8.26	20.7	32.2
BDK0000	High Flow	10.1	73.4	0.0	7.5	9.0
	Low Flow	19.4	25.3	4.2	26.8	24.3
	Weighted	17.1	37.3	3.13	22.0	20.5
WIL0067	High Flow	8.3	31.4	0.0	30.6	29.7
	Low Flow	6.1	25.5	11.7	28.2	28.5
	Weighted	6.6	27.0	8.7	28.8	29.0
WIL0013	High Flow	5.4	33.3	6.1	27.6	27.7
	Low Flow	10.8	32.5	6.2	16.1	34.4
	Weighted	9.5	32.7	6.2	19.0	32.7
WIL0000	High Flow	9.3	69.1	0.5	9.6	11.5
	Low Flow	11.8	33.5	7.0	24.1	23.7
	Weighted	11.1	42.4	5.3	20.4	20.6

Table 2.4.5: Distribution of Fecal Bacteria Source Loads in the Wills Creek Basin for the Seasonal Period (May 1st – September 30th)

STATION	Flow Stratum	% Domestic Animals	% Human	% Livestock	% Wildlife	% Unknown
NJE0014	High Flow	8.3	45.8	0.0	20.9	25.0
	Low Flow	12.0	31.2	4.6	32.5	19.7
	Weighted	11.0	34.9	3.4	29.6	21.0
JEN0036	High Flow	30.4	8.7	4.3	21.7	34.8
	Low Flow	5.6	23.4	8.1	36.8	26.1
	Weighted	11.8	19.7	7.2	33.0	28.2
BDK0000	High Flow	20.8	62.5	0.0	12.5	4.2
	Low Flow	20.5	26.1	2.3	30.4	20.7
	Weighted	20.5	35.2	1.7	25.9	16.6
WIL0067	High Flow	16.7	4.2	0.0	66.6	12.5
	Low Flow	7.5	24.5	12.1	33.0	22.9
	Weighted	9.7	19.4	9.0	41.2	20.3
WIL0013	High Flow	0.0	21.7	0.0	60.9	17.4
	Low Flow	9.0	27.2	9.3	14.2	40.3
	Weighted	6.8	25.8	6.9	25.9	34.6
WIL0000	High Flow	8.3	75.0	0.0	8.3	8.3
	Low Flow	15.9	32.1	2.5	24.1	25.4
	Weighted	14.0	42.8	1.9	20.1	21.1

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 Wills Creek watershed area. 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. The 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 TMDL loading caps. 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 (WLA) for point sources, load allocations (LA) 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, and 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 (EPA, 1985) is a direct estimate of the bacteria colonies (Method 1600), and the second 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 be problematic due to the many assumptions required and the limited available data. For example, when considering septic systems, information is required on the spatial location of failing septic systems, consideration of transport to in-stream assessment location and estimation of the load from the septic system (degree of failure). Secondary sources, such as illicit discharges, also add to the uncertainty in a bacteria water quality model.

Estimating domestic animal sources requires information regarding the pet population in a watershed, how often the owners clean up after them, and the spatial location of the pet waste relative to the near stream (for upland transport). Livestock sources are limited by spatial resolution of Agricultural Census information (available at the county level), site-specific issues relating to animals' confinement, and confidentiality of data related to the development of Nutrient Management Plans. The most uncertain source category is wildlife. In an urban environment, this can result from the increased deer populations near streams to rat populations in storm sewers. In rural areas, estimation of wildlife populations and habitat locations in a watershed is required.

MDE appreciates the inherent uncertainty in developing traditional water quality models for the calculation of bacteria TMDLs. Traditional water quality modeling is very expensive and time-consuming and, as identified, contains many potential uncertainties. MDE believes it should be reserved for specific constituents and complex situations. In this TMDL, MDE applies an analytical method which, when combined with BST, appears to provide reasonable results (Cleland, 2003) and allows addressing more impaired streams in the same time period than if using the traditional water quality modeling methods.

4.2 Analysis Framework

This TMDL analysis uses flow duration curves to identify flow intervals that are used as indicator hydrological conditions (*i.e.*, annual average and critical conditions). As explained previously, this analytical method combined with water quality monitoring data and BST can provide a better description of water quality concerns while meeting TMDL requirements.

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

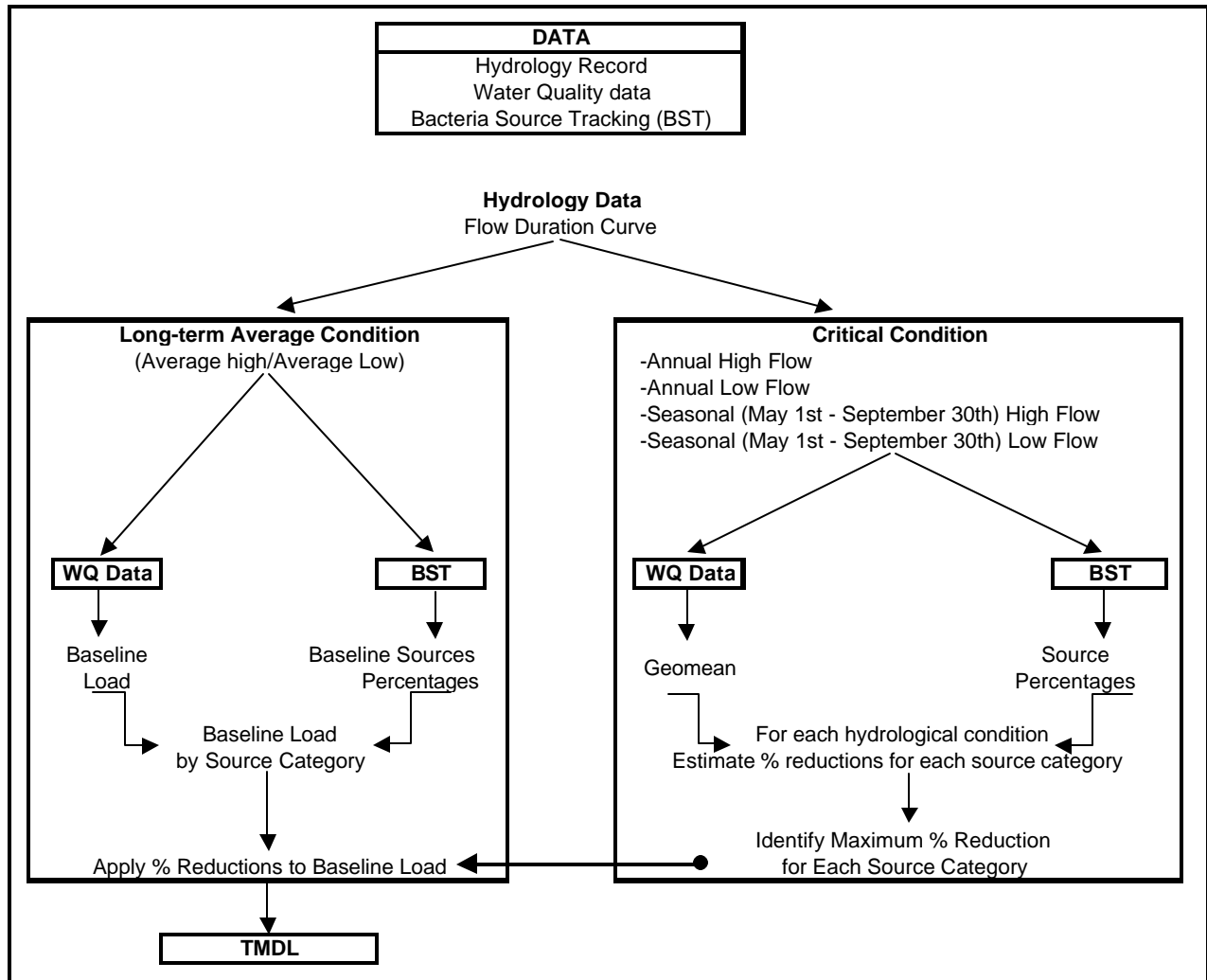


Figure 4.2.1: Diagram of Non-tidal Bacteria TMDL Analysis Framework

4.3 Estimating Baseline Loads

Baseline loads estimated in this TMDL analysis are reported in long-term average loads, using bacteria monitoring data and 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.

FINAL

To estimate baseline loads for each of the six subwatersheds of Wills Creek, bias correction factors, daily average flows and geometric mean concentrations for each stratum are first estimated.

The bias correction factor for each stratum is estimated as follows:

$$F1_i = A_i/C_i \quad (6)$$

where

$F1_i$ = 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:

$$L_i = Q_i * C_i * F_1 * F_2 \quad (7)$$

where

L_i = Daily average load (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_1 = Bias correction factor
 F_2 = Unit conversion factor (2.4466×10^7)

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

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

L = Daily average load at station (MPN/day)
 W_i = Proportion of stratum i

In the Wills Creek watershed, a weighting factor of 0.25 for high flow and 0.75 for low/mid flows were used to estimate the annual baseline load expressed as Billion MPN *E. coli*/day.

Estimating Subwatershed Loads

To treat each subwatershed as a separate entity, thus allowing separate load and reduction targets for watersheds that have one or more upstream monitored subwatersheds, they were subdivided into unique watershed segments. Wills Creek has six subwatersheds, two of them with upstream and downstream monitoring stations. These two subwatersheds are monitored at stations WIL0000 and WIL0013 (Figure 4.3.1). The subwatersheds were defined with the extension sub to the station name (WIL0013sub and WIL0000sub) and the total baseline loads from the upstream watersheds, estimated from the monitoring data, were multiplied by a transport factor derived from first order decay. The decay factor for *E. coli* used in the analysis was obtained from the study “Pathogen Decay in Urban Waters” by Easton *et al.* (2001), and was estimated by linear regression of counts of microorganisms versus time (die-off plots). The estimated transported loads were then subtracted from the downstream cumulative load to estimate the adjacent subwatershed load. The general equation for the flow mass balance is:

$$\sum Q_{us} + Q_{sub} = Q_{ds} \quad (9)$$

where

Q_{us} = Upstream flow
 Q_{sub} = Subwatershed flow
 Q_{ds} = Downstream flow

and the general equations for bacteria loading mass balance:

$$\sum (e^{-kt} Q_{us} C_{us}) + Q_{sub} C_{sub} = Q_{ds} C_{ds} \quad (10)$$

where

C_{us} = Upstream bacteria concentration
 k = Bacteria (*E. coli*) decay coefficient (1/day) = 0.762 day⁻¹
 t = travel time from upstream watershed to outlet
 C_{sub} = Subwatershed bacteria concentration
 C_{ds} = Downstream bacteria concentration

The concentrations in the subwatersheds were estimated by considering the ratio of high flow concentration to low flow concentrations in the upstream watersheds. If the total load and average flow were used to estimate the geometric mean concentration, this estimated concentration would be biased if there was a correlation with flow and concentration. For example, in two strata, the steady state geometric mean is estimated as follows:

$$L = Q_{high} W_{high} C_{high} + Q_{low} W_{low} C_{low} \quad (11)$$

L = Average Load
 Q_i = Average flow for stratum i

FINAL

W_i = Proportion of stratum i

C_i = Concentration for stratum i

n_i = number of samples in stratum i

Notice that the load in equation (10) is based on two concentrations and therefore, when using the mass balance approach and the total load, this results in two unknowns, C_{high} and C_{low} , with one equation. Thus a relationship between C_{high} and C_{low} , must be estimated to solve for the concentration in both strata. This relationship is estimated using the average of the ratios estimated from the monitoring data in the upstream watersheds. Using this relationship, the following two equations result:

$$C_{low} = \frac{L}{Q_{high}R + Q_{low}W_{low}} \quad (12)$$

where

$$R = \frac{C_{high}}{C_{low}} \quad (13)$$

and the final geometric mean concentration is estimated as follows:

$$GM = 10^{W_{high}\log_{10}(C_{high}) + W_{low}\log_{10}(C_{low})} \quad (14)$$

To estimate subwatershed WIL0000sub, the load measured at station WIL0013 and the transported load from BDK0000, estimated as explained above, will be subtracted from the load measured at station WIL0000. The difference is assigned to subwatershed WIL0000sub.

Several anthropogenic and non-anthropogenic factors such as soil, geology, the presence of septic systems or CSOs, can affect bacteria loadings into the streams. As explained in the CSOs point source assessment, a special scenario has been seen in Braddock Run and in Jennings Run, both tributaries of Wills Creek. In Jennings Run, the bacteria loadings upstream of station JEN0036 are significantly greater than the cumulative loads at the downstream station WIL0013. Bacteria loads are greater in the upper reaches of Jennings Run due to the existence of CSOs, which greatly elevate bacteria levels during storm events. As these bacteria loads are transported downstream, they come into contact with high concentrations of metals and acidity from acid mine drainage, in which bacteria cannot survive and quickly die off. For this reason, transported loads from station JEN0036 to station WIL0013 will not be considered in the estimation of loads from subwatershed WIL0013sub, and the load as measured at station WIL0013 together with the transported loads from stations WIL0067 and NJE0014 will be assigned to the subwatershed WIL0013sub.

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

FINAL

for WIL0000sub and WIL0013sub were assigned from the analysis for WIL0000 and WIL0013, respectively.

Results of the baseline load calculations are presented in Table 4.3.1.

Table 4.3.1: Baseline Load Calculations

Station	Area (sq. miles)	USGS Reference Gage	Unit flow (cfs/sq. mile)	Q (cfs)	<i>E. Coli</i> Concentration (MPN/100ml)	Unit flow (cfs/sq. mile)	Q (cfs)	<i>E. Coli</i> Concentration (MPN/100ml)	Baseline Load (Billion MPN/day)	Weighted Geometric Mean Conc. MPN/100ml
WIL0067	187.7	1601500	4.121	773	88	0.540	101.4	73	46,313	76
NJE0014	12.9	1596500	4.676	60	223	0.578	7.4	130	8,315	149
JEN0036	20.1	1599000	3.459	69	201	0.482	9.7	298	10,414	270
WIL0000	257.9	1601500	4.121	1063	626	0.540	139.3	154	582,997	218
WIL0000sub	6.1			37	4,946		4.4	2,506	214,206	2,971
BDK0000	18.5	1599000	3.459	64	4,499	0.482	8.9	162	293,247	372
WIL0013	233.4	1601500	4.121	962	146	0.540	126.1	65	87,898	80
WIL0013sub	12.8			59	1,119		7.6	766	45,187	842

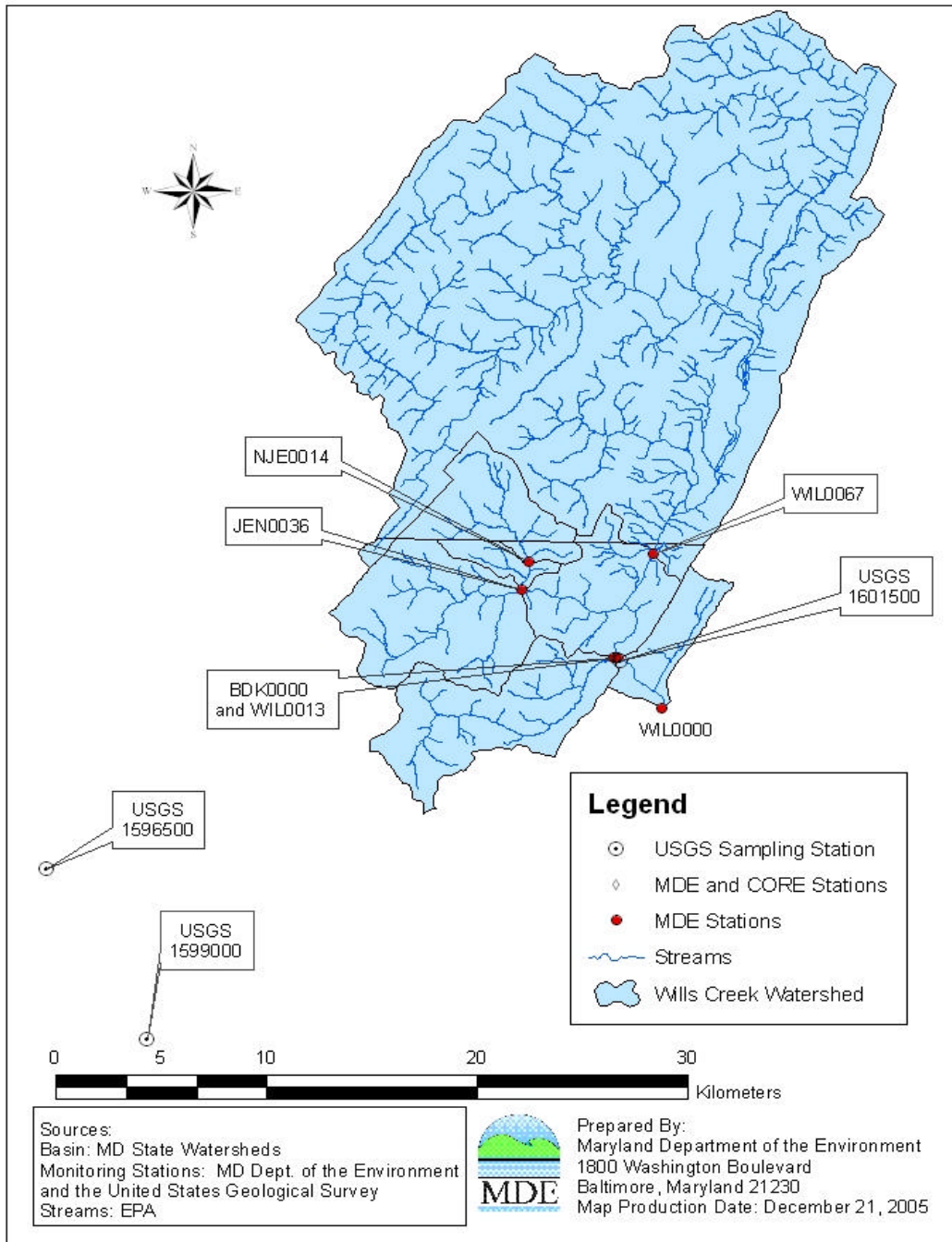


Figure 4.3.1: Monitoring Stations and Subwatersheds in Wills Creek Basin

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 wet and dry periods. Seasonality is captured by assessing the time period when water contact recreation is expected (May 1st - September 30th). The average hydrological condition over a 15-year period is approximately 25% high flow and 75% 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 25% and a low flow condition as occurring when the daily flow duration interval is greater than 25%, critical hydrological condition can be estimated by the percent of high or low flows during a specific period.

As stated above, Maryland's proposed fecal bacteria TMDL for Wills Creek has been determined by assessing various hydrological conditions to account for seasonal and annual averaging periods. The five 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	Subwatershed	Fraction High Flow	Fraction Low Flow	Period	
Annual	Average Condition	365 days	All	All	0.25	0.75	Long Term Average	
	High	365 days	All	WIL0067; WIL0013; WIL0013; WIL0000; WIL0000sub	0.54	0.46	April 22 nd , 2001– April 23 rd , 2002	
				NJE0014	0.57	0.43	Feb 22 st , 1990 – Feb 23 st , 1991	
				JEN0036; BDK0000	0.56	0.44	Jan 8 th , 1997 – Jan 7 th , 1998	
	Low	365 days	All	WIL0067; WIL0013; WIL00000	0.08	0.92	Dec 28 st , 1995 – Dec 28 th , 1996	
				NJE0014	0.14	0.86	Dec 28 st , 1995 – Dec 28 th , 1996	
				JEN0036; BDK0000	0.06	0.94	May 28 th , 1995 – May 27 th , 1996	
	Season	High	May 1 st – Sept 30 th	May 1 st – Sept 30 th	WIL0067; WIL0013; WIL0000	0.44	0.56	May 1 st – Sept 30 th , 1996
					NJE0014	0.51	0.49	May 1 st – Sept 30 th , 1996
JEN0036; BDK0000					0.46	0.54	May 1 st – Sept 30 th , 2003	
Low		May 1 st – Sept 30 th	May 1 st – Sept 30 th	WIL0067; WIL0013; WIL0000	0.0	1.0	May 1 st – Sept 30 th , 1991	
				NJE0014	0.0	1.0	May 1 st – Sept 30 th , 1991	
				JEN0036; BDK0000	0.0	1.0	May 1 st – Sept 30 th , 2002	

The critical condition is determined by the maximum reduction per source that satisfies all hydrological conditions, and that is required to meet the water quality standard while minimizing the risk to water contact recreation. It is assumed that the reduction that can be implemented to a bacteria source category will be constant through all conditions.

The monitoring data for all stations located in the Wills Creek watershed cover a sufficient temporal span (at least one year) to estimate annual and seasonal conditions.

Table 4.4.2 shows the reductions of fecal bacteria required in each subwatershed of Wills Creek to meet water quality standards for both Maryland and PA designated uses.

Table 4.4.2: Required Reductions of Fecal Bacteria to Meet Water Quality Standards

Subwatershed	Hydrological Condition		Domestic Animals %	Human %	Livestock %	Wildlife %
WIL0067	Annual	Average	0%	0%	0%	0%
		High Flow	0%	0%	0%	0%
		Low Flow	0%	0%	0%	0%
	Seasonal	High Flow	30%	95%	46%	0%
		Low Flow	0%	70%	0%	0%
Maximum Source Reduction			30%	95%	46%	0%
NJE0014	Annual	Average	53%	18%	43%	0%
		High Flow	0%	69%	0%	0%
		Low Flow	0%	41%	0%	0%
	Seasonal	High Flow	98%	98%	98%	9%
		Low Flow	98%	98%	98%	17.5%
Maximum Source Reduction			98%	98%	98%	17.5%
JEN0036	Annual	Average	73%	85%	72%	0%
		High Flow	0%	86%	0%	0%
		Low Flow	61%	98%	98%	0%
	Seasonal	High Flow	98%	98%	98%	49%
		Low Flow	98%	98%	98%	75%
Maximum Source Reduction			98%	98%	98%	75%
WIL0000sub	Annual	Average	98%	98%	91%	62%
		High Flow	98%	98%	98%	59%
		Low Flow	98%	98%	98%	59%
	Seasonal	High Flow	98%	98%	98%	92%
		Low Flow	98%	98%	98%	91%
Maximum Source Reduction			98%	98%	98%	92%

Subwatershed	Hydrological Condition		Domestic Animals %	Human %	Livestock %	Wildlife %
BDK0000	Annual	Average	%	%	%	%
		High Flow	98%	98%	98%	51%
		Low Flow	3%	98%	60%	0%
	Seasonal	High Flow	98%	98%	98%	78%
		Low Flow	98%	98%	98%	66%
	Maximum Source Reduction		98%	98%	98%	78%
WIL0013sub	Annual	Average	0 %	0 %	0 %	0 %
		High Flow	0%	0%	0%	0%
		Low Flow	0%	0%	0%	0%
	Seasonal	High Flow	98%	98%	98%	82%
		Low Flow	98%	98%	98%	65%
	Maximum Source Reduction		98%	98%	98%	82%

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, April 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. For this TMDL, the second approach was used 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 TMDL Loading Caps

The TMDL loading caps are estimates of the assimilative capacity of the monitored subwatersheds and are provided in MPN/day. These loadings are for the six subwatersheds located upstream of monitoring station WIL0000: WIL0067, NJE0014, JEN0036, WIL0013sub, BDK0000, and WIL0000sub.

The TMDLs are based on a long-term geometric mean of bacteria levels. Estimation of the TMDLs requires knowledge of how the bacteria concentrations vary with flow rate or flow duration interval. This concentration versus flow relationship is accounted for by using the strata defined on the flow duration curve.

The TMDL caps are estimated by first determining the baseline or current condition geometric mean bacteria concentration and the associated load from the available monitoring data. The baseline loads are estimated using the geometric mean concentrations and average daily flows for each flow stratum. The loads from these two strata are then weighted (same as the estimated concentration, see Table 4.3.1), based on the proportion of each stratum, to estimate the total long term loading rate.

Next, the percent reduction (based on the critical condition) required to meet the water quality criterion is estimated from the observed bacteria concentrations. It is assumed that 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.

$$TMDL = L_b * (1 - R) \quad (15)$$

where

L_b = Current or baseline load estimated from monitoring data

R = Reduction required from baseline to meet water quality criterion

The bacteria TMDLs for the subwatersheds upstream of monitoring station WIL0000 are shown in Table 4.6.1.

Table 4.6.1: Wills Creek Watershed TMDL Summary

Subwatershed ID	Baseline Load <i>E. Coli</i> (Billion MPN/day)	TMDL Load <i>E. Coli</i> (Billion MPN/day)	% Target Reduction
WIL0067	1,133	629	45%
NJE0014	203	62	69%
JEN0036	255	23	91%
WIL0013sub	1,106	61	94%
BDK0000	7,175	543	92%
WIL0000sub	5,241	191	96%
Total	15,113	1,509	

4.7 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” were removed and the known sources were then scaled up proportionally so that they totaled 100%. The source distribution and baseline loads used in the TMDL scenarios are presented in Table 4.7.1. As stated in Section 4.3, the source distributions for subwatersheds WIL0013sub and WIL0000sub, were based on the sources identified at stations WIL0013 and WIL0000, respectively.

Table 4.7.1: Bacteria Source Distributions and Corresponding Baseline Loads Used in the TMDL Analysis

Subwatershed	Domestic		Human		Livestock		Wildlife		Total Billion <i>E. coli</i> MPN/day
	%	Load Billion <i>E. coli</i> MPN/day	%	Load Billion <i>E. coli</i> MPN/day	%	Load Billion <i>E. coli</i> MPN/day	%	Load Billion <i>E. coli</i> MPN/day	
WIL0067	9.3%	105.2	38.0%	430.3	12.2%	138.6	40.5%	459.0	1,133
NJE0014	10.6%	21.6	37.5%	76.2	16.5%	33.5	35.5%	72.1	203
JEN0036	9.6%	24.4	47.6%	121.4	12.2%	31.2	30.5%	77.8	255
WIL0013sub	14.1%	156.1	48.6%	537.2	9.2%	101.8	28.1%	310.5	1,106
BDK0000	21.5%	1,543.2	47.0%	3,366.2	4.0%	279.8	27.7%	1,985.4	7,175
WIL0000sub	14.0%	734.5	53.5%	2,805.7	6.7%	350.7	25.8%	1,349.9	5,241

First Scenario: Fecal Bacteria Practicable Reduction Targets

The maximum practicable reduction (MPR) for each of the four source categories is listed in Table 4.7.2. 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 and CSOs are located in the upstream watershed, this is considered in the MPR so as to not violate the permitted loads. 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 4.7.2: Maximum Practicable Reduction Targets

Max Practicable Reduction per Source	Human	Domestic	Livestock	Wildlife
		95%	75%	75%
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

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

²Preliminary Data Summary of Urban Storm Water Best Management Practices. EPA-821-R-99-012. U.S. Environmental Protection Agency, Washington, DC. EPA. 1999.

³Agricultural BMP Descriptions as Defined for The Chesapeake Bay Program Watershed Model. Nutrient Subcommittee Agricultural Nutrient Reduction Workshop. EPA. 2004.

⁴Environmental Indicators and Shellfish Safety. 1994. Edited by Cameron, R., Mackeney and Merle D. Pierson, Chapman & Hall.

As previously stated, these practicable reduction targets are based on the available literature and best professional judgment. There is much uncertainty with estimated reductions from best management practices (BMP). The BMP efficiency for bacteria reduction ranged from -6% to +99% based on a total of 10 observations (EPA, 1999). The MPR to agricultural lands was based on sediment reductions identified by the EPA (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 4.7.2). The model was defined as follows:

FINAL

$$\text{Min } \sum_{i=1}^4 (P_h * 5 + P_d * 3 + P_l * 3 + P_w * 1) \quad i = \text{hydrological condition} \quad (16)$$

Subject to

$$\begin{aligned} C &= C_{cr} \\ 0 &\leq R_h \leq 95\% \\ 0 &\leq R_l \leq 75\% \\ 0 &\leq R_d \leq 75\% \\ R_w &= 0 \\ P_h, P_l, P_d, P_w &\geq 0\% \end{aligned}$$

Where

P_h = % human source in final allocation
 P_d = % domestic animal source in final allocation
 P_l = % livestock source in final allocation
 P_w = % wildlife source in final allocation
 C = In-stream concentration
 C_{cr} = Water quality criterion
 R_h = % Reduction applied to human sources
 R_l = % Reduction applied to livestock sources
 R_d = % Reduction applied to domestic animal sources
 R_w = % Reduction applied to wildlife sources

In five of the six 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 4.7.3.

Table 4.7.3: Practicable Reduction Scenario Results

Subwatershed	Applied Reductions				Achievable?
	Domestic %	Human %	Livestock %	Wildlife %	
WIL0067	75%	95%	75%	0%	Yes
NJE0014	75%	95%	75%	0%	No
JEN0036	75%	95%	75%	0%	No
WIL0013sub	75%	95%	75%	0%	No
BDK0000	75%	95%	75%	0%	No
WIL0000sub	75%	95%	75%	0%	No

Second Scenario: Fecal Bacteria Reductions Higher than Maximum Practicable Reductions

The TMDL must specify load allocations that will meet the water quality standards. In the practicable reduction targets scenario, five of the six subwatersheds of Wills Creek could not meet water quality standards based on MPRs.

The first scenario results showed that only one subwatershed (WIL0067) met water quality standards with MPRs. To further develop the TMDL, a second scenario was analyzed in which the constraints on the MPRs were relaxed in the five subwatersheds where water quality attainment was not achievable with MPRs. In these subwatersheds, the maximum allowable reduction was increased to 98% for all sources, including wildlife. A similar optimization procedure 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 as follows:

$$\text{Min } \sum_{i=1}^4 (P_h * 5 + P_d * 3 + P_l * 3 + P_w * 1) \quad i = \text{hydrological condition}$$

Subject to

$$\begin{aligned} C &= C_{cr} \\ 0 &\leq R_h \leq 98\% \\ 0 &\leq R_l \leq 98\% \\ 0 &\leq R_d \leq 98\% \\ 0 &\leq R_w \leq 98\% \\ P_h, P_l, P_d, P_w &\geq 0\% \end{aligned}$$

Where

$$\begin{aligned} P_h &= \% \text{ human source in final allocation} \\ P_d &= \% \text{ domestic animal source in final allocation} \\ P_l &= \% \text{ livestock source in final allocation} \\ P_w &= \% \text{ wildlife source in final allocation} \\ C &= \text{In-stream concentration} \\ C_{cr} &= \text{Water quality criterion} \\ R_h &= \% \text{ Reduction applied to human sources} \\ R_l &= \% \text{ Reduction applied to livestock sources} \\ R_d &= \% \text{ Reduction applied to domestic animal sources} \\ R_w &= \% \text{ Reduction applied to wildlife sources} \end{aligned}$$

The summary of the analysis is presented in Tables 4.7.4 and 4.7.5.

Table 4.7.4: TMDL Scenario Results: Percent Reductions Based on Optimization Model Allowing Up to 98% Reduction*

Station	Domestic %	Human %	Livestock %	Wildlife %	Target Reduction %
WIL0067	30%	95%	46%	0%	45%
NJE0014	98%	98%	98%	17.5%	69%
JEN0036	98%	98%	98%	75%	91%
WIL0013sub	98%	98%	98%	82%	94%
BDK0000	98%	98%	98%	78%	92%
WIL0000sub	98%	98%	98%	91%	96%

* For subwatersheds not meeting WQS with MPRs

Table 4.7.5: TMDL Scenario Results: Reduced Loads by Source Category

Station	Domestic	Human	Livestock	Wildlife	Total
	Billion MPN <i>E. coli</i> /day				
WIL0067	74.0	21.5	74.7	459.0	629.2
NJE0014	0.4	1.5	0.7	59.5	62.2
JEN0036	0.5	2.4	0.6	19.5	23.1
WIL0013sub	3.1	10.7	2.0	44.7	60.6
BDK0000	30.9	67.3	5.6	439.1	542.9
WIL0000sub	14.7	56.1	7.0	113.4	191.2

4.8 TMDL Allocations

The TMDL allocations include the load allocation (LA) for nonpoint sources and waste load allocations (WLA) for WWTPs (if WWTPs are present in the watershed), for stormwater (where MS4 permits are required), and for CSOs (in watersheds with permitted CSOs and LTCPs not expecting complete elimination of CSOs). The margin of safety is explicit and is expressed as a 5% reduction of the *E. coli* water quality criterion concentration, from 126 MPN/100ml to 119.7 MPN/100ml. The final loads are based on average hydrological conditions, with reductions estimated based on critical hydrological conditions. The load reduction scenario results in a load allocation 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.

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 or nonpoint sources and the WLA or point sources (WWTPs, MS4 permits and CSOs, if applicable). Only the final LA or WLA is reported in this TMDL.

Table 4.8.1: Potential Source Contributions for TMDL Allocations

Allocation Category	LA	WLA		
		CSOs	MS4s	WWTPs (N/A)
Human	X	X	N/A	N/A
Domestic	X			
Livestock	X			
Wildlife	X			

Load Allocation (LA)

All four bacteria source categories can contribute to nonpoint source loads (LA). For the human sources, the nonpoint source contribution (LA) in subwatersheds with WWTPs and CSOs is estimated by subtracting the WWTP (if applicable) and CSOs loads (if applicable) from the final human load. There are no NPDES WWTPs in the Maryland portion of the Wills Creek watershed. There is only one subwatershed in the Wills Creek watershed with assigned NPDES CSS WLA.

A domestic animals (pets) allocation is assigned to the LA if no MS4 permits exist for the watershed. The Wills Creek watershed is not covered by NPDES MS4 permits; therefore, bacteria loads from domestic animal sources are assigned to the LA in all six subwatersheds of Wills Creek. A domestic animal allocation would be assigned to the MS4 WLA if there were MS4 permit(s) covering the watershed.

Livestock loads are all assigned to the LA. Wildlife loads are distributed between the LA and WLA MS4 if the watershed is covered by NPDES permits. No NPDES MS4 permits exist in the Wills Creek watershed; therefore, all wildlife allocations are assigned to the LA.

Waste Load Allocation (WLA)

Stormwater

In Allegany and Garrett Counties, where the Wills Creek watershed is located, there are no NPDES Municipal Separate Storm Sewer (MS4) permits to regulate stormwater discharges.

Municipal and Industrial WWTP

As explained in the source assessment section above, there are no industrial WWTPs with permits regulating the discharge of bacteria into Wills Creek. There is one municipal WWTP

with a permit regulating the discharge of bacteria directly into the Wills Creek watershed: the Hyndman Borough Municipal Authority WWTP located in Hyndman, PA. The TMDL for subwatershed WIL0067 represents the total load allocated for the area upstream of station WIL0067, which is mainly in Pennsylvania. This load includes any bacteria sources from the Hyndman WWTP, and no explicit allocation is given to the WWTP.

Combined Sewer Systems

There are four jurisdictions with NPDES CSSs within the Wills Creek watershed (See section 2.4, Source Assessment, for more detailed information). Three of these four jurisdictions with CSOs permitted to discharge in Wills Creek have developed their Long Term Control Plans (LTCP). The LTCPs of three jurisdictions (Allegheny County, City of Frostburg and Town of La Vale) state that CSOs are to be eliminated by the dates noted in the LTCPs. Therefore, no allocation is assigned to CSOs in these jurisdictions, and the final human load in the corresponding subwatersheds is allocated to the LA. The fourth jurisdiction with a NPDES CSS permit in the watershed is the City of Cumberland. Cumberland's LTCP is not finalized at the time of the development of this TMDL, but the City has informed MDE that the LTCP will not propose the complete elimination of CSOs. Therefore, part of the final human load in the subwatershed where the City of Cumberland is located (WIL0000sub) will be assigned to the WLA-CSOs.

As reported in Section 4.7 (Tables 4.7.4 and 4.7.5), reductions needed to meet water quality standards in five of the six subwatersheds of Wills Creek are very high. For subwatershed WIL0000sub, the percent reductions for all bacteria source categories are the highest, and final loads are therefore very strict. CSOs contribute human bacteria loadings into a stream; therefore, CSO allocation to subwatershed WIL0000sub should be derived from the final human load allocation for that subwatershed. The sources of this final human load are both nonpoint sources and point sources (CSOs). A human-load-to-watershed-area ratio analysis was performed to estimate the percentage of this final human load that will be allocated to the LA (nonpoint sources) and to the WLA (City of Cumberland CSOs located in subwatershed WIL0000sub).

The CSO allocation analysis consisted of estimating a non-CSO or "background" human loading rate derived from subwatersheds in the Wills Creek, and in the nearby Georges Creek, that do not have human source load contributions from CSOs or SSOs. These "background" human loading rate will represent human contributions into the stream from human sources other than CSOs or SSOs (*i.e.*, septic failure). The resulting "background" loading rate is then applied to subwatershed WIL0000sub to estimate the non-CSO human load in the subwatershed. The difference between the total human load and the non-CSO human load will be allocated to the City of Cumberland CSOs located in the subwatershed. For details on this analysis please refer to Appendix D.

4.9 Summary

The TMDL for the Wills Creek watershed is presented below.

Table 4.9.1: Wills Creek Watershed TMDL

Subwatershed	TMDL	LA	WLA CSOs
	Billion MPN <i>E. coli</i> /day		
Wills Creek upstream of Maryland/PA line (WIL0067)	629	629	N/A
North Branch Jennings Run (NJE0014)	62	62	N/A
Jennings Run upstream of the confluence with North Branch Jennings Run (JEN0036)	23	23	0
Braddock Run (BDK0000)	543	543	0
Wills Creek between Maryland/PA line and the confluence with Braddock Run (WIL0013sub)	61	61	N/A
Wills Creek between the confluence with Braddock Run and the confluence with the North Potomac River (WIL0000sub)	191	136	55
TOTAL	1,509	1,454	55

In five out of six subwatersheds, based on the maximum practicable reduction rates specified, water quality standards cannot be achieved. This may occur in watersheds that require very high reductions to meet water quality standards. However, if there is no feasible TMDL scenario, then MPRs are increased to provide estimates of the reductions required to meet water quality standards. For these watersheds, it is noted that the reductions may be beyond practical limits. In these cases, it is expected that the first stage of implementation will be to implement 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 Wills Creek watershed, the TMDL analysis indicates that, for five of the six 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. Wills Creek and its tributaries North Jennings Run, Jennings Run and Braddock Run may not be able to attain water quality standards. The fecal bacteria load reductions required to meet water quality criteria in five of the six subwatersheds of the Wills Creek are not feasible by implementing effluent limitations and cost-effective, reasonable BMPs to nonpoint sources. Therefore, MDE proposes a staged approach to implementation of the required reductions beginning with the MPR scenario, with regularly scheduled follow-up monitoring to assess the effectiveness of the implementation plan.

The most significant planned implementation measures in the Wills Creek watershed involve the upgrade or separation of combined sewer systems in the City of Frostburg, the Town of LaVale, Allegany County, and the City of Cumberland. Each of these jurisdictions is obligated under a judicial consent decree and judgment to adopt and implement a long term control plan (“LTCP”) to eliminate dry weather overflows and minimize wet weather overflows. See *Maryland Department of the Environment v. Major and City Council of Frostburg, et al.*, Consent Decree and Judgment, Consolidated Case Number 01-C-00-18342L, (December 14, 2001). Frostburg, LaVale, and Allegany County have submitted and MDE has approved LTCPs that will separate their sanitary and stormwater sewers and/or eliminate all CSO outfalls. The City of Cumberland has not finalized its LTCP yet, but the City proposes to meet its legal obligations through the construction of a storage facility that will contain storm-related flows until the Cumberland Wastewater Treatment Plant can treat them. It is anticipated that the final LTCP will provide controls sufficient to meet water quality standards in Wills Creek. The judicial decree and judgment requires the jurisdictions to implement these LTCPs by 2023. Deadlines for LTCP implementation will be incorporated into NPDES permits and, if shorter than the court ordered deadline, permits will reflect what can be feasibly accomplished with consideration to the complexity of the engineering, the availability of resources, and the need for inter-jurisdictional coordination.

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.

In 2000, the Maryland DNR initiated the Watershed Restoration Action Strategy (WRAS) Program as one of several new approaches to implement water quality and habitat restoration and protection. The WRAS Program encourages local governments to focus on priority watersheds for restoration and protection. Since the program's inception, local governments have received grants and technical assistance from DNR for twenty WRAS projects in which local people identify local watershed priorities for restoration, protection and implementation. WRAS information provides a potential targeting tool to direct future efforts in implementation.

Additionally, MDE's "Managing Maryland for Results" document (MDE, 2005) states the following related to sewage overflows:

Objective 4.5: Reduce the quantity in gallons of sewage overflows [total for Combined Sewer System Overflows (CSO) and Separate Sewer System Overflows (SSO)] equivalent to a 50% reduction of 2001 amounts (50,821,102 gallons) by the year 2010 through implementation of EPA's minimum control strategies, long term control plans (LTCP), and collection system improvements in capacity, inflow and infiltration reduction, operation and maintenance.

Strategy 4.5.1: MDE will implement regulations adopted in FY 2004 to ensure that all jurisdictions are reporting all sewage overflows to the Department, notifying the public about significant overflows, and are taking appropriate steps to address the cause(s) of the overflows.

Strategy 4.5.2: MDE will inspect and take enforcement actions against those CSO jurisdictions that have not developed long-term control plans with schedules for completion and require that enforceable schedules are incorporated in consent decrees or judicial orders.

Strategy 4.5.3: MDE will take enforcement actions to require that jurisdictions experiencing significant or repeated SSOs take appropriate steps to eliminate overflows, and will fulfill the commitment in the EPA 106 grant for NPDES enforcement regarding the initiation of formal enforcement actions against 20% of jurisdictions in Maryland with CSOs and significant SSO problems annually.

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 not meet water quality standards. Neither Maryland nor EPA is proposing the elimination of wildlife to allow for the attainment of water quality standards, although 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 nonpoint sources may also reduce some wildlife inputs to the waters.

FINAL

REFERENCES

American Society of Agricultural Engineers (ASAE) (1998). *ASAE Standards, 45th edition: Standards, Engineering Practices, Data*. St. Joseph, MI.

Cleland, Bruce. 2003. TMDL Development from the “Bottom Up” – Part III: Duration Curves and Wet-weather Assessments. America’s Clean Water Foundation. Washington D.C.

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 06/24/05.

Code of Maryland Regulations, 26.08.02.03-3A(1), 26.08.02.08N. Website <http://www.dsd.state.md.us/comar>, last visited 06/24/05.

Code of Maryland Regulations, 26.08.10. Website <http://www.dsd.state.md.us/comar>, last visited 07/29/05.

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

Dillow, J.J.A., (1996). Technique for Estimating Magnitude and Frequency of Peak Flow in Maryland: U.S. Geological Survey Water –Resources Investigations Report 97-4279.

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.

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

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

Geldrenich, E. and E. A. Kenner. (1969). Concepts of Fecal Streptococci in Stream Pollution. *Journal of Water Pollution Federation*. 41:R336-R352.

Hirsch, R.M. 1982. “A Comparison of Four Record Extension Techniques”. *Water Resources Research*, V. 18, No. 4, p. 1082-1088.

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

*Wills Creek TMDL Fecal Bacteria
Document version: August 30, 2006*

FINAL

Maryland Department of the Environment, *2006 List of Impaired Surface Waters [303(d) List] and Integrated Assessment of Water Quality in Maryland.*

Maryland Department of the Environment, *2004 FINAL List of Impaired Surface Waters [303(d) List] and Integrated Assessment of Water Quality in Maryland.*

Maryland Department of the Environment, *2002 Bacteriological TMDL Survey of Wills Creek.*

Maryland Department of the Environment, 2006. Personal communication with Pennsylvania Department of Environmental Protection, Edward Muzic, Permits Engineer.

Maryland Department of Planning, 2000, 2000 Land Use, Land Cover Map Series.

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

Maryland Dept. of the Envir. v. Mayor and City Council of Frostburg, *et al.*, Consolidated Case Number: 01-C-00-18342L, Consent Decree and Judgment (entered Dec. 14, 2001).

Moglen, G.E., Thomas, W.O. and Miller, A.C. (2002). Evaluation of Alternative Statistical Method for Estimating Frequency of Peak Flows in Maryland: Maryland Department of Transportation State Highway Administration Final Report SP907CRB.

Pennsylvania DEP Watershed Notebook, website:

<http://www.dep.state.pa.us/DEP/DEPutate/Watermgmt/Wc/Subjects/WSNoteBks/ws13a.htm>

Pettyjohn, W.A., and Henning R. 1979. Preliminary Estimate of Ground-Water Recharge Rates, Related Streamflow and Water Quality in Ohio: Ohio State University Resources Center Project Completion Report Number 552. 323p.

Reiss, K.G. and Friesz, P.J., (2000). Methods for Estimating Low-Flow Statistics for Massachusetts Streams: U.S. Geological Survey Water –Resources Investigations Report 00-4135.

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.

Soil Conservation Service (SCS). *Soil Survey of AllegenyCounty, MD, 1977.*

Soil Conservation Service (SCS). *Soil Survey of Garret County, MD, 1967.*

FINAL

Swann, C, 1999. A survey of Residential Nutrient Behaviors in the Chesapeake Bay. Widener Burrows, Inc. Chesapeake Research Consortium. Center For Watershed Protection. Ellicott City, MD. 112 pp.

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

U.S. Department of Agriculture. (1995). State Soil Geographic (STATSGO) DataBase.

U.S. Department of Agriculture. (1997). Census of Agriculture: Maryland State and County Data. Washington, DC: National Agricultural Statistic Service.

U.S. Environmental Protection Agency. *Ambient Water Quality Criteria for Bacteria-1986*. EPA-440/5-84-002, 1986.

U.S. Environmental Protection Agency. Guidance for water quality-based decisions: The TMDL Process, EPA 440/4-91-001. 1991.

U.S. Environmental Protection Agency. *National Recommended Water Quality Criteria: 2002*. EPA-822-R-02-047. November 2002.

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

U.S. Environmental Protection Agency. Preliminary Data Summary of Urban Storm Water Best Management Practices. EPA-821-R-99-012. U.S. Environmental Protection Agency, Washington, DC. EPA. 1999.

U.S. Environmental Protection Agency. Agricultural BMP Descriptions as Defined for The Chesapeake Bay Program Watershed Model. Nutrient Subcommittee Agricultural Nutrient Reduction Workshop. 2004.

U.S. Environmental Protection Agency. *Implementation Guidance for Ambient Water Quality Criteria for Bacteria: Draft*, U.S. Environmental Protection Agency. Office of Water, Washington, D.C. EPA-823-B-02-003. 2003.

U.S. Environmental Protection Agency. Test Methods for Escherichia coli and Enterococci in Water by the Membrane Filter Procedure. EPA600/4-85-076. Washington, DC. NTIS PB86-158052. 1985.

U.S. Environmental Protection Agency. Office of Water (2000). Bacteria Indicator Tool User's Guide. EPA-823-B-01-003.

U.S. Environmental Protection Agency. . Protocol for developing Pathogen TMDLs, EPA 841-R-00-002, Office of Water (4503F), United States Environmental Protection Agency, Washington, DC. 134pp. 2001.

Wills Creek TMDL Fecal Bacteria
Document version: August 30, 2006

FINAL

U.S. Environmental Protection Agency. Stormwater Manager Resource Center. Website: <http://www.stormwatercenter.net/>.

U.S. Environmental Protection Agency. Chesapeake Bay Program. Chesapeake Bay Program: Watershed Model Application to Calculate Bay Nutrient Loadings: Final Findings and Recommendations, and Appendices, Annapolis, MD 1996.

USGS. 1996. HYSEP: A Computer Program for Streamflow Hydrograph Separation and Analysis. USGS Water-Resource Investigations Report 96-4040.

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

VA DEQ (2002) Fecal Coliform TMDL for Dodd Creek Watershed, Virginia, June 2002.

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

Wickham, J.D., Nash, M.S., Wade, T.G and Currey, D.L (2005). Statewide empirical modeling of bacterial contamination of surface waters. Journal of the American Water Resources Association (In Press).

Appendix A - Bacteria Data

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

SAMPLING STATION IDENTIFIER	Date	Daily flow frequency	E. Coli MPN/100ml
BDK0000	10/08/2002	97.3160	135.4
BDK0000	11/07/2002	28.4645	201.0
BDK0000	11/20/2002	27.2412	8.5
BDK0000	12/02/2002	57.5863	24.9
BDK0000	12/17/2002	38.5978	422.0
BDK0000	01/07/2003	16.1585	24192.0
BDK0000	01/21/2003	40.9896	52.0
BDK0000	02/03/2003	54.0807	10.0
BDK0000	03/18/2003	1.1320	17329.0
BDK0000	04/01/2003	18.7329	20.0
BDK0000	04/15/2003	9.4395	24192.0
BDK0000	04/21/2003	18.8607	24192.0
BDK0000	04/28/2003	30.5094	20.0
BDK0000	05/05/2003	33.5403	24192.0
BDK0000	05/19/2003	8.2344	26020.0
BDK0000	06/02/2003	6.2991	41060.0
BDK0000	06/16/2003	8.8917	175.0
BDK0000	07/07/2003	35.0009	448.0
BDK0000	07/21/2003	50.4108	448.0
BDK0000	08/04/2003	39.6567	275.0
BDK0000	08/18/2003	66.4050	583.0
BDK0000	09/08/2003	61.7674	448.0
BDK0000	09/22/2003	5.1305	823.0
JEN0036	10/08/2002	97.3160	41.9
JEN0036	11/07/2002	28.4645	278.0
JEN0036	11/20/2002	27.2412	35.4
JEN0036	12/02/2002	57.5863	547.5
JEN0036	12/17/2002	38.5978	299.0
JEN0036	01/07/2003	16.1585	122.0

SAMPLING STATION IDENTIFIER	Date	Daily flow frequency	E. Coli MPN/100ml
JEN0036	01/21/2003	40.9896	158.0
JEN0036	02/03/2003	54.0807	132.0
JEN0036	03/18/2003	1.1320	142.0
JEN0036	04/01/2003	18.7329	97.0
JEN0036	04/15/2003	9.4395	262.0
JEN0036	04/21/2003	18.8607	183.0
JEN0036	04/28/2003	30.5094	63.0
JEN0036	05/05/2003	33.5403	5794.0
JEN0036	05/19/2003	8.2344	399.0
JEN0036	06/02/2003	6.2991	132.0
JEN0036	06/16/2003	8.8917	328.0
JEN0036	07/07/2003	35.0009	354.0
JEN0036	07/21/2003	50.4108	990.0
JEN0036	08/04/2003	39.6567	3255.0
JEN0036	08/18/2003	66.4050	327.0
JEN0036	09/08/2003	61.7674	226.0
JEN0036	09/22/2003	5.1305	379.0
NJE0014	10/08/2002	95.9649	24.0
NJE0014	11/07/2002	16.4871	305.0
NJE0014	11/20/2002	18.4773	6.3
NJE0014	12/02/2002	49.0780	62.4
NJE0014	12/17/2002	37.5023	74.0
NJE0014	01/07/2003	20.4492	1396.0
NJE0014	01/21/2003	46.9418	20.0
NJE0014	02/03/2003	65.2547	216.0
NJE0014	03/18/2003	0.6756	52.0
NJE0014	04/01/2003	30.0347	52.0
NJE0014	04/15/2003	15.3186	399.0
NJE0014	04/21/2003	36.0599	98.0
NJE0014	04/28/2003	52.1088	98.0
NJE0014	05/05/2003	31.5684	1785.0

SAMPLING STATION IDENTIFIER	Date	Daily flow frequency	E. Coli MPN/100ml
NJE0014	05/19/2003	10.1150	275.0
NJE0014	06/02/2003	5.3314	388.0
NJE0014	06/16/2003	14.4057	246.0
NJE0014	07/07/2003	55.7970	413.0
NJE0014	07/21/2003	68.1212	85.0
NJE0014	08/04/2003	51.2142	670.0
NJE0014	08/18/2003	75.6984	422.0
NJE0014	09/08/2003	67.1170	97.0
NJE0014	09/22/2003	11.0462	933.0
WIL0000	10/08/2002	97.2430	108.1
WIL0000	11/07/2002	21.2890	282.0
WIL0000	11/20/2002	21.7272	11.0
WIL0000	12/02/2002	51.5246	36.8
WIL0000	12/17/2002	23.5348	246.0
WIL0000	01/07/2003	17.6191	1725.0
WIL0000	01/21/2003	50.7760	61.0
WIL0000	02/03/2003	58.1158	20.0
WIL0000	03/18/2003	1.4607	1658.0
WIL0000	04/01/2003	28.9757	20.0
WIL0000	04/15/2003	14.0040	1850.0
WIL0000	04/21/2003	30.4546	771.0
WIL0000	04/28/2003	42.9250	20.0
WIL0000	05/05/2003	35.4939	24192.0
WIL0000	05/19/2003	6.1347	1872.0
WIL0000	06/02/2003	8.9100	4884.0
WIL0000	06/16/2003	22.7679	520.0
WIL0000	07/07/2003	56.4543	135.0
WIL0000	07/21/2003	68.3221	238.0
WIL0000	08/04/2003	57.6410	1989.0
WIL0000	08/18/2003	76.7026	84.0
WIL0000	09/08/2003	70.4218	135.0

SAMPLING STATION IDENTIFIER	Date	Daily flow frequency	E. Coli MPN/100ml
WIL0000	09/22/2003	23.9365	479.0
WIL0013	10/08/2002	97.2430	41.9
WIL0013	11/07/2002	21.2890	335.0
WIL0013	11/20/2002	21.7272	7.4
WIL0013	12/02/2002	51.5246	71.7
WIL0013	12/17/2002	23.5348	218.0
WIL0013	01/07/2003	17.6191	364.0
WIL0013	01/21/2003	50.7760	31.0
WIL0013	02/03/2003	58.1158	20.0
WIL0013	03/18/2003	1.4607	122.0
WIL0013	04/01/2003	28.9757	41.0
WIL0013	04/15/2003	14.0040	10.0
WIL0013	04/21/2003	30.4546	41.0
WIL0013	04/28/2003	42.9250	10.0
WIL0013	05/05/2003	35.4939	233.0
WIL0013	05/19/2003	6.1347	309.0
WIL0013	06/02/2003	8.9100	161.0
WIL0013	06/16/2003	22.7679	1076.0
WIL0013	07/07/2003	56.4543	262.0
WIL0013	07/21/2003	68.3221	84.0
WIL0013	08/04/2003	57.6410	1439.0
WIL0013	08/18/2003	76.7026	41.0
WIL0013	09/08/2003	70.4218	41.0
WIL0013	09/22/2003	23.9365	341.0
WIL0067	10/08/2002	97.2430	21.1
WIL0067	11/07/2002	21.2890	218.0
WIL0067	11/20/2002	21.7272	3.1
WIL0067	12/02/2002	51.5246	36.4
WIL0067	12/17/2002	23.5348	336.0
WIL0067	01/07/2003	17.6191	243.0
WIL0067	01/21/2003	50.7760	52.0

SAMPLING STATION IDENTIFIER	Date	Daily flow frequency	E. Coli MPN/100ml
WIL0067	02/03/2003	58.1158	74.0
WIL0067	03/18/2003	1.4607	52.0
WIL0067	04/01/2003	28.9757	63.0
WIL0067	04/15/2003	14.0040	10.0
WIL0067	04/21/2003	30.4546	63.0
WIL0067	04/28/2003	42.9250	10.0
WIL0067	05/05/2003	35.4939	218.0
WIL0067	05/19/2003	6.1347	85.0
WIL0067	06/02/2003	8.9100	132.0
WIL0067	06/16/2003	22.7679	231.0
WIL0067	07/07/2003	56.4543	158.0
WIL0067	07/21/2003	68.3221	86.0
WIL0067	08/04/2003	57.6410	1421.0
WIL0067	08/18/2003	76.7026	51.0
WIL0067	09/08/2003	70.4218	63.0
WIL0067	09/22/2003	23.9365	384.0

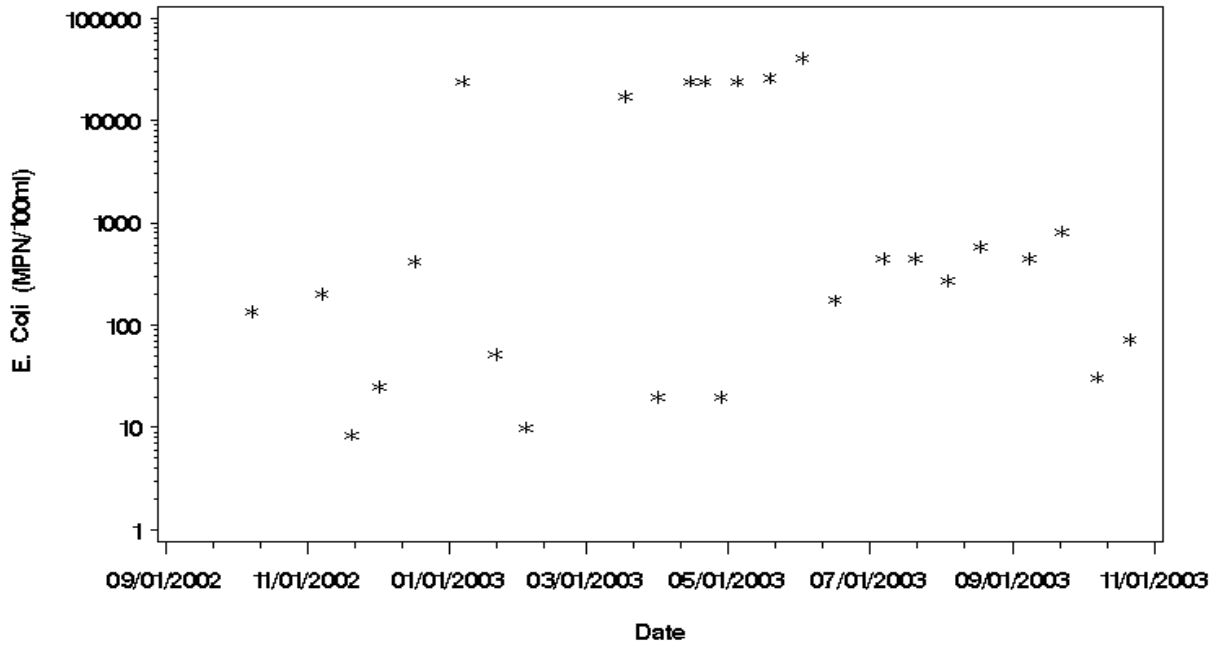


Figure A-1: E. Coli Concentration vs. Time for Wills Creek Monitoring Station BDK0000

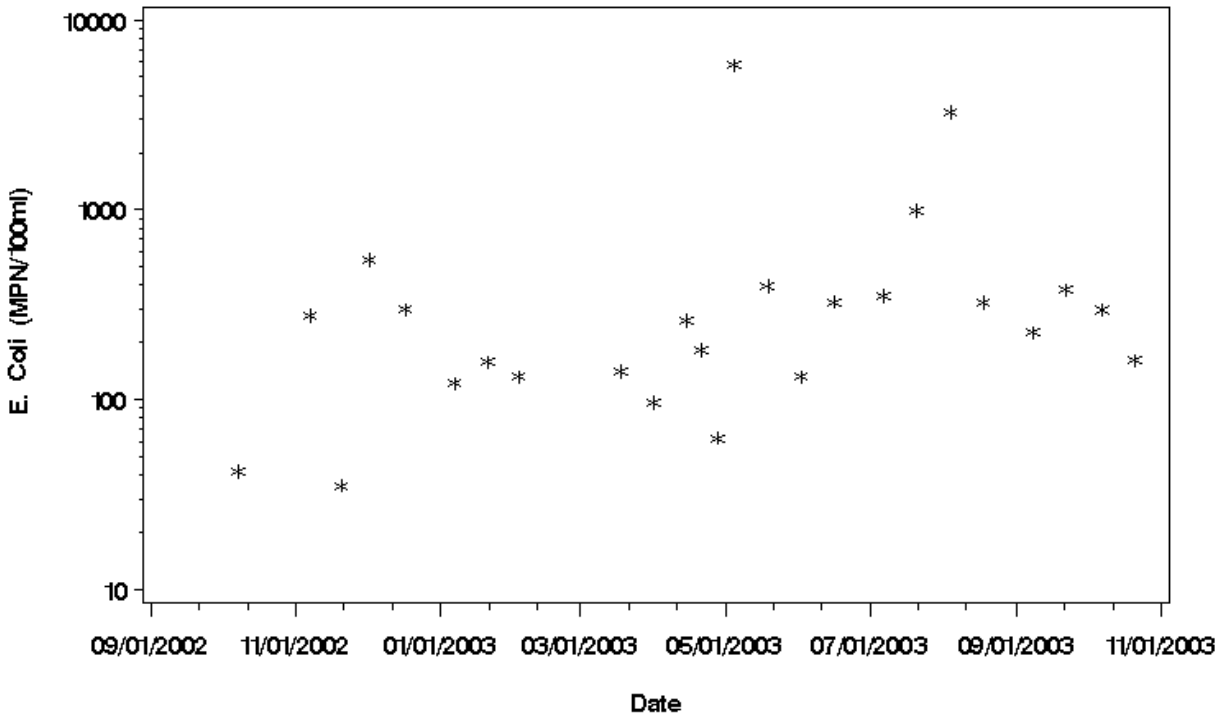


Figure A-2: E. Coli Concentration vs. Time for Wills Creek Monitoring Station JEN0036

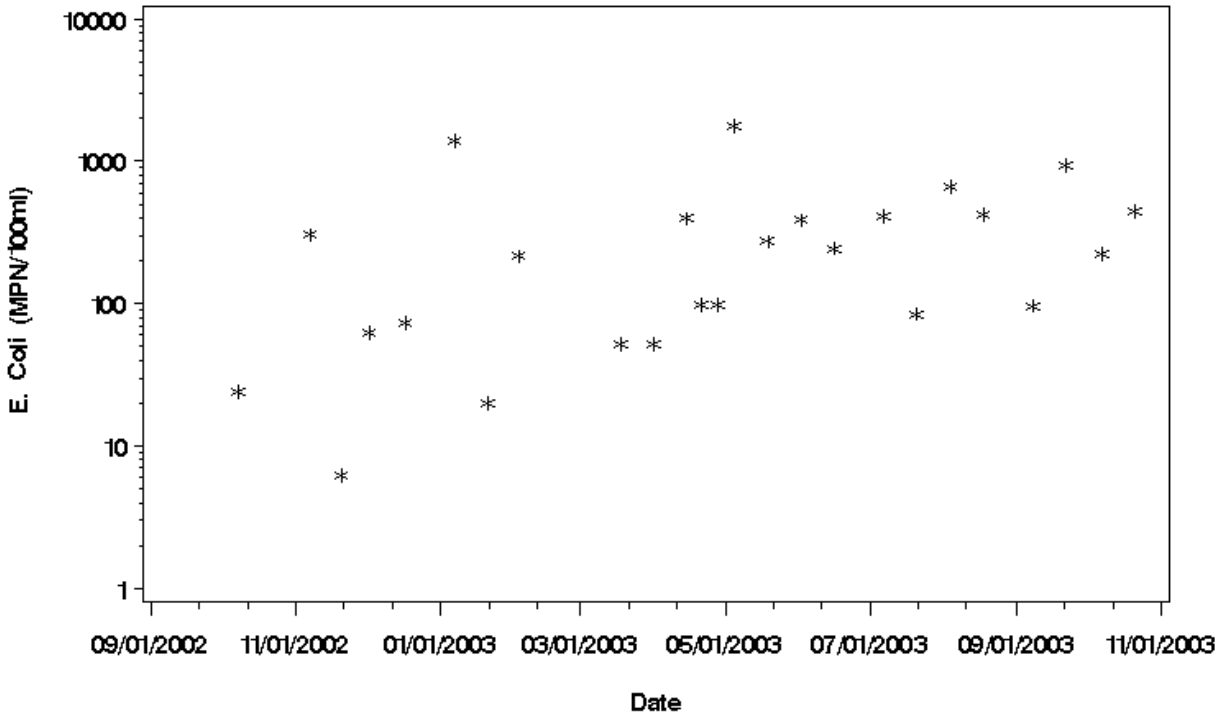


Figure A-3: *E. Coli* Concentration vs. Time for Wills Creek Monitoring Station NJE0014

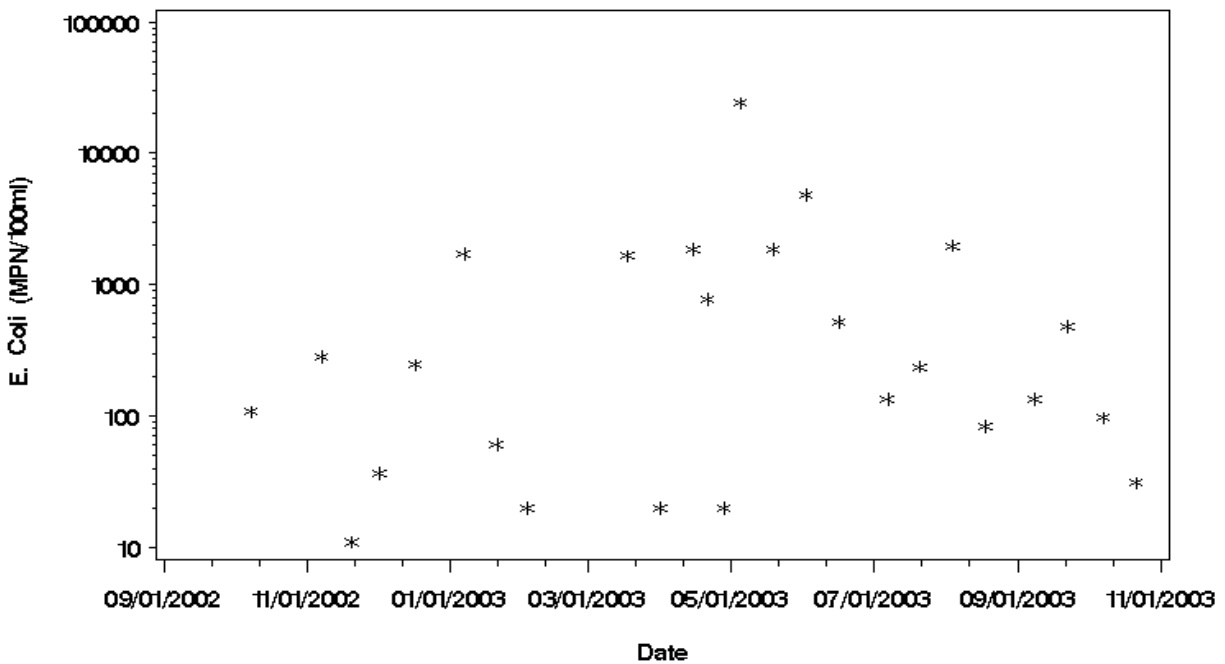


Figure A-4: *E. Coli* Concentration vs. Time for Wills Creek Monitoring Station WIL0000

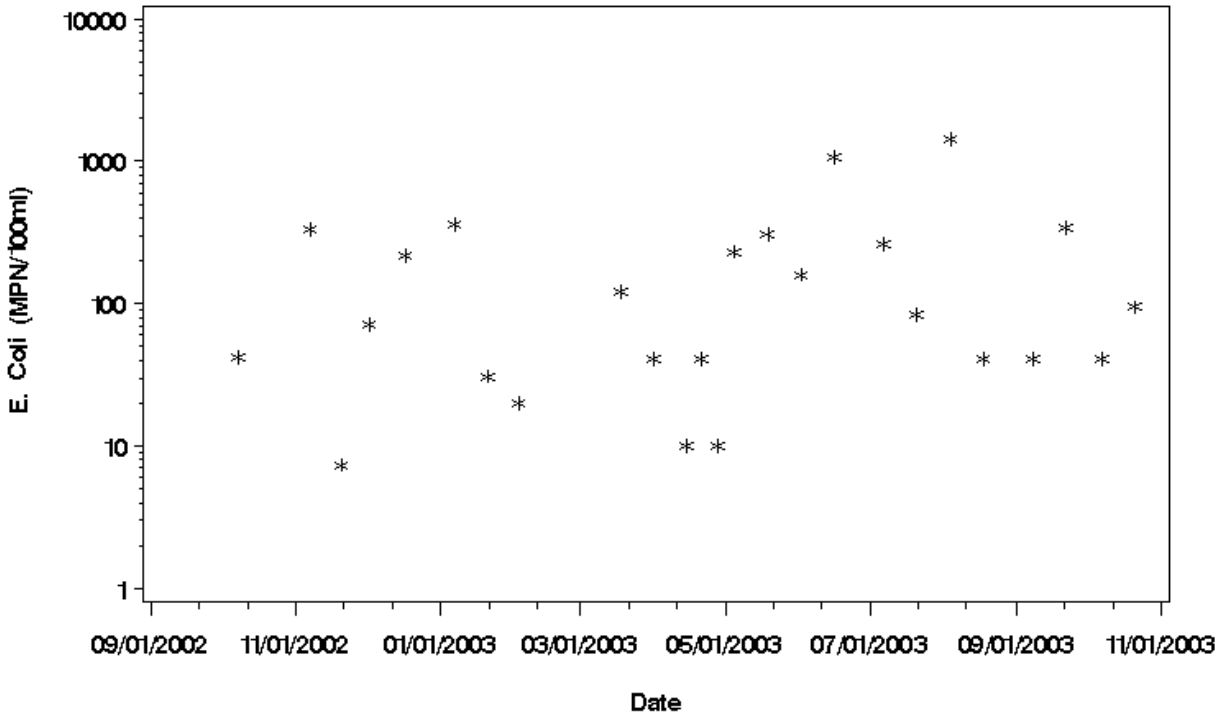


Figure A-5: *E. Coli* Concentration vs. Time for Wills Creek Monitoring Station WIL0013

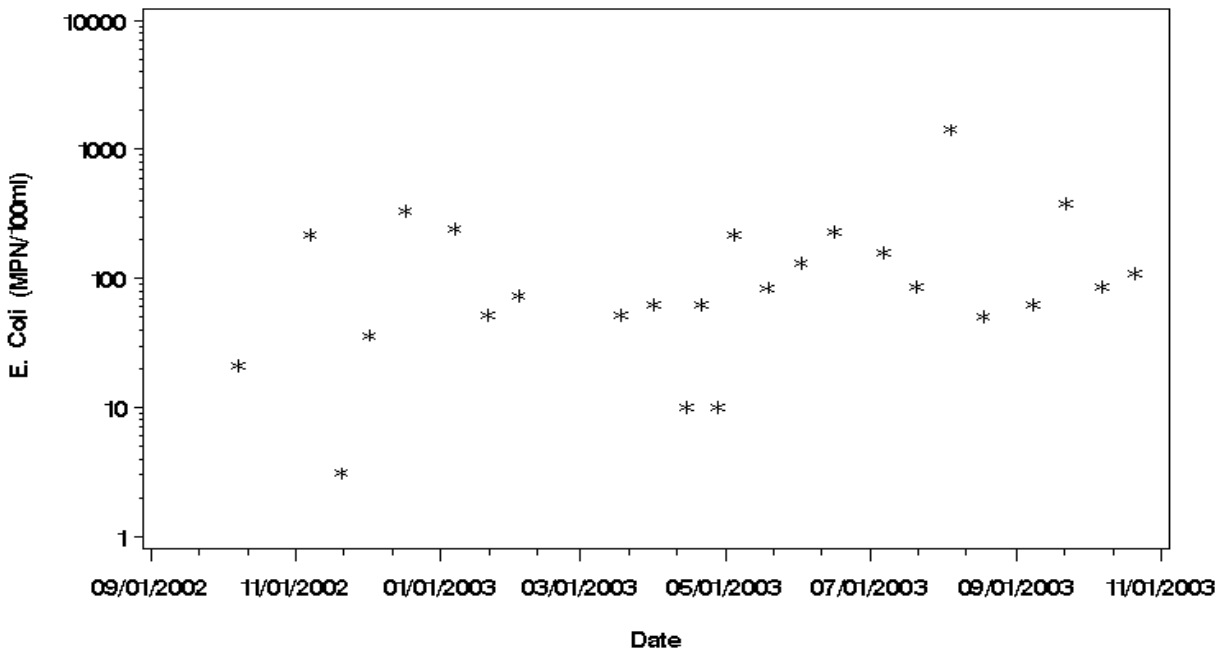


Figure A-6: *E. Coli* Concentration vs. Time for Wills Creek Monitoring Station WIL0067

Appendix B - Flow Duration Curve Analysis to Define Strata

The Wills 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 soil antecedent 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 Wills Creek Watershed has one active (01601500) USGS flow gage. Two additional active gages were used in this analysis. These are located outside the Wills Creek watershed; one is located in the Georges Creek watershed (01599000) and another in the Savage River watershed (01596500). The gages and dates of information used are as follows:

Table B-1: USGS Gages in the Wills Creek Watershed

USGS Gage #	Dates used	Description
01596500	October 1, 1988 to September 30, 2003	Savage River near the Town of Barton
01599000	October 1, 1988 to September 30, 2003	Georges Creek in Town of Westernport
01601500	October 1, 1988 to September 30, 2003	Wills Creek near Cumberland, MD

A flow duration curve for this gage is presented in Figure B-1.

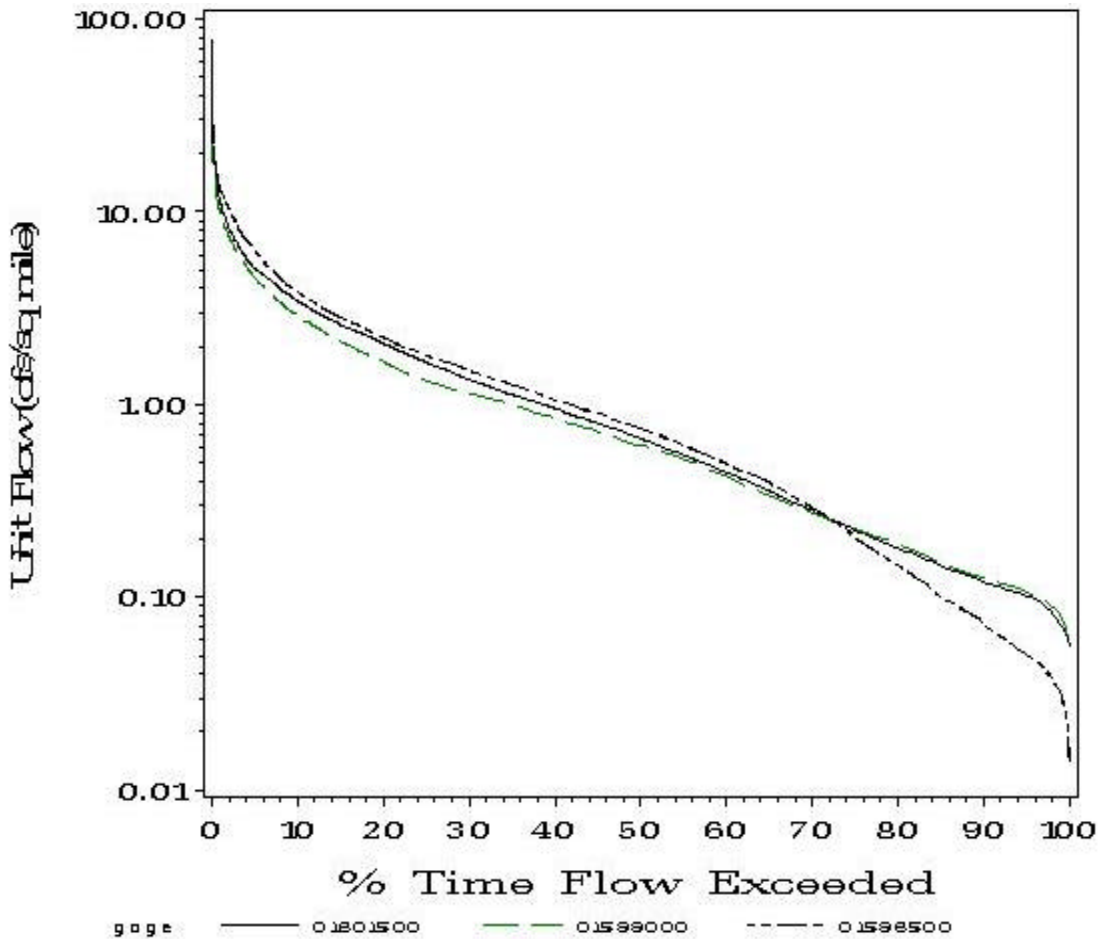


Figure B-1: Wills Creek Flow Duration Curves

Based on the long-term flow data for the Wills Creek watershed and other watersheds in the region (*i. e.*, Georges Creek), the long term average daily unit flow range between 1.2 to 1.6 cfs/sq. mile, which corresponds to a range of 20th to 28th flow frequency based on the flow duration curves of these watersheds. Using the definition of a high flow condition occurring when flows are higher than the long-term average flow and a low flow condition occurring when flows are lower than the long-term average flow, the 25th percentile threshold was selected to define the limits between high flows and low flows. Therefore, a high flow condition will be defined as occurring when the daily flow duration percentile is less than 25% and a low flow condition will be defined as occurring when the daily flow duration percentile is greater than 25%. Definitions of high and low range flows are presented in Table B-2.

Table B-2: 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 (stratum) based on the daily flow duration percentile of the date of sampling.

Figures B-2 to B-7 show the Wills Creek *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 the 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 fewer than five representative sampling events for an area being assessed are available, data from the previous two years will be evaluated. In Wills Creek, there are sufficient samples in the high flow strata to estimate the geometric mean. For the low flow strata only three samples exist, therefore the mid and low flow strata will be combined to calculate the geometric mean.

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-3. 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) High Flow Condition
- (5) Seasonal (May 1st – September 30th) Low 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-7.

Table B-3: Weighting Factors for Estimation of Geometric Mean

Hydrological Condition		Subwatershed	Weighting Factor High Flow	Weighting Factor Low Flow	
Annual	Average Condition	All Subwatersheds	0.30	0.70	
	High Flow	WIL0067; WIL0013; WIL0013; WIL0000; WIL0000sub	0.54	0.46	
		NJE0014	0.57	0.43	
		JEN0036; BDK0000	0.56	0.44	
	Low Flow	WIL0067; WIL0013; WIL00000	0.08	0.92	
		NJE0014	0.14	0.86	
		JEN0036; BDK0000	0.06	0.94	
	Season	High Flow	WIL0067; WIL0013; WIL0000	0.44	0.56
			NJE0014	0.51	0.49
JEN0036; BDK0000			0.46	0.54	
Low Flow		WIL0067; WIL0013; WIL0000	0.0	1.0	
		NJE0014	0.0	1.0	
		JEN0036; BDK0000	0.0	1.0	

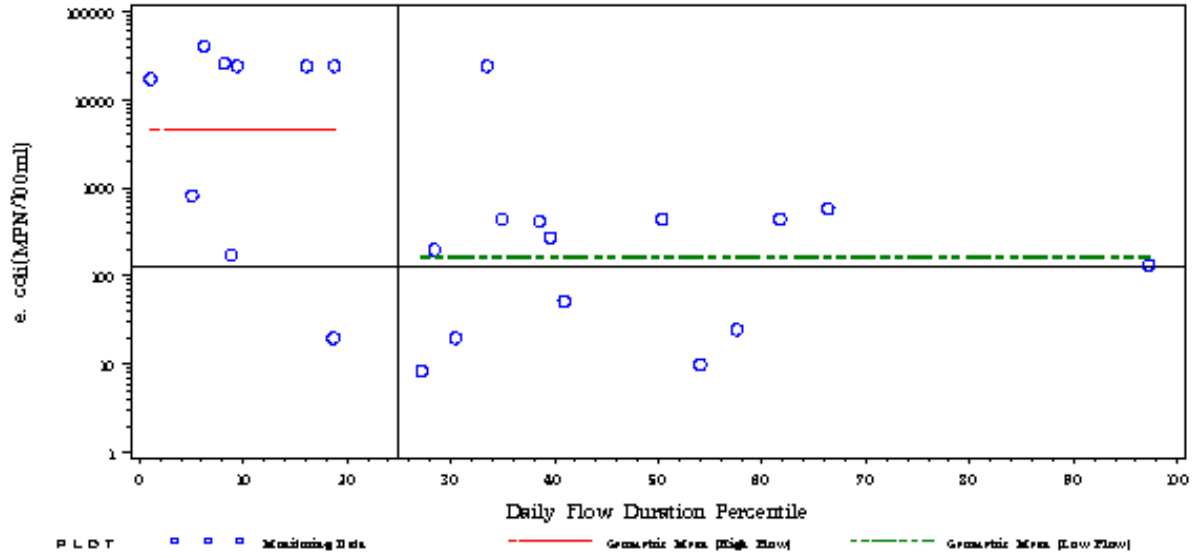


Figure B-2: *E. Coli* Concentration vs. Flow Duration for Wills Creek Monitoring Station BDK0000

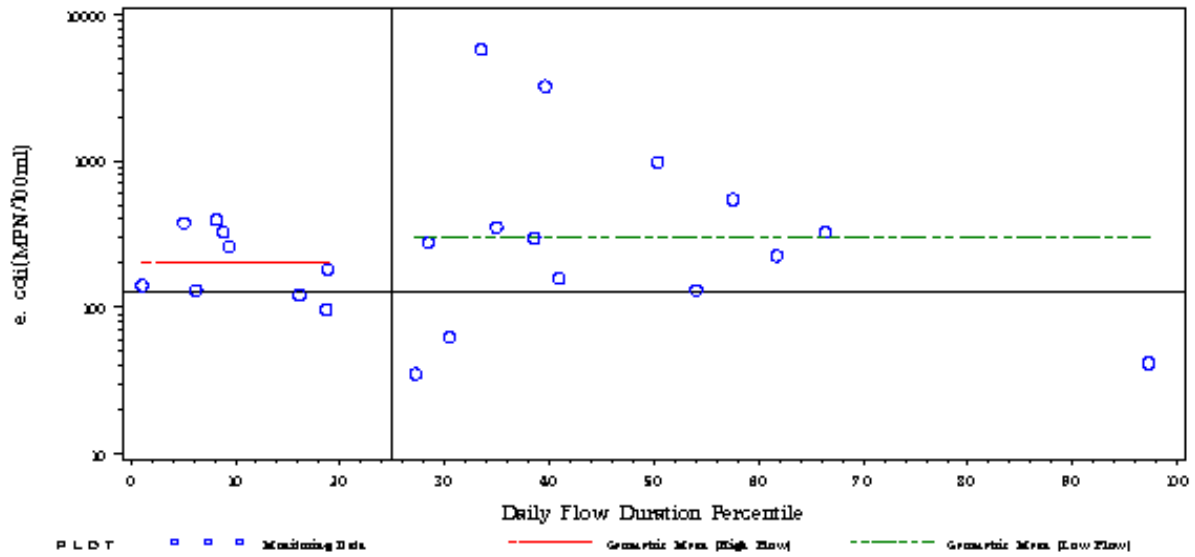


Figure B-3: *E. Coli* Concentration vs. Flow Duration for Wills Creek Monitoring Station JEN0036

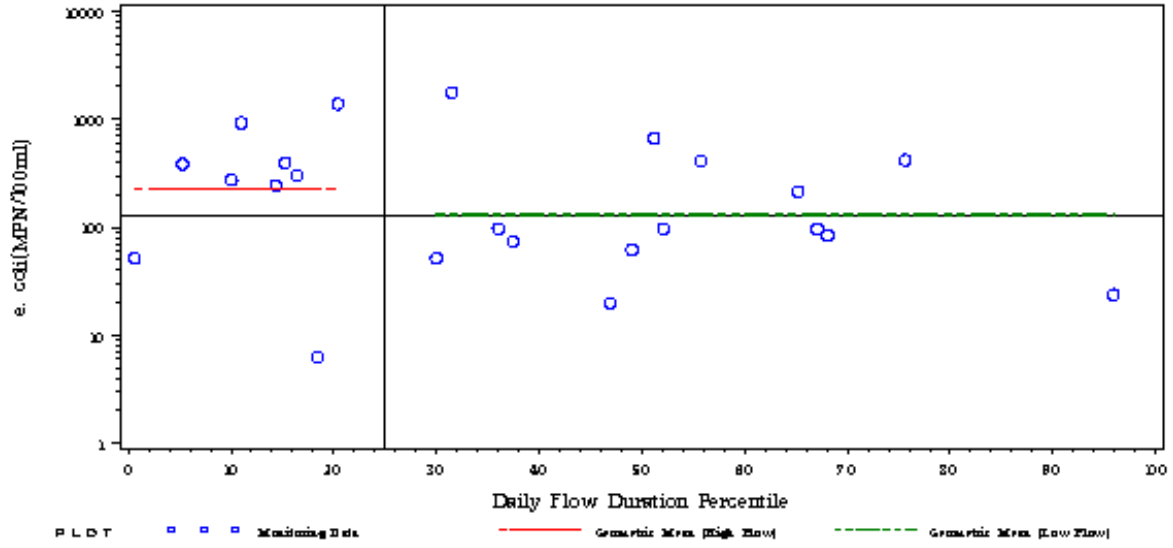


Figure B-4: *E. Coli* Concentration vs. Flow Duration for Wills Creek Monitoring Station NJE0014

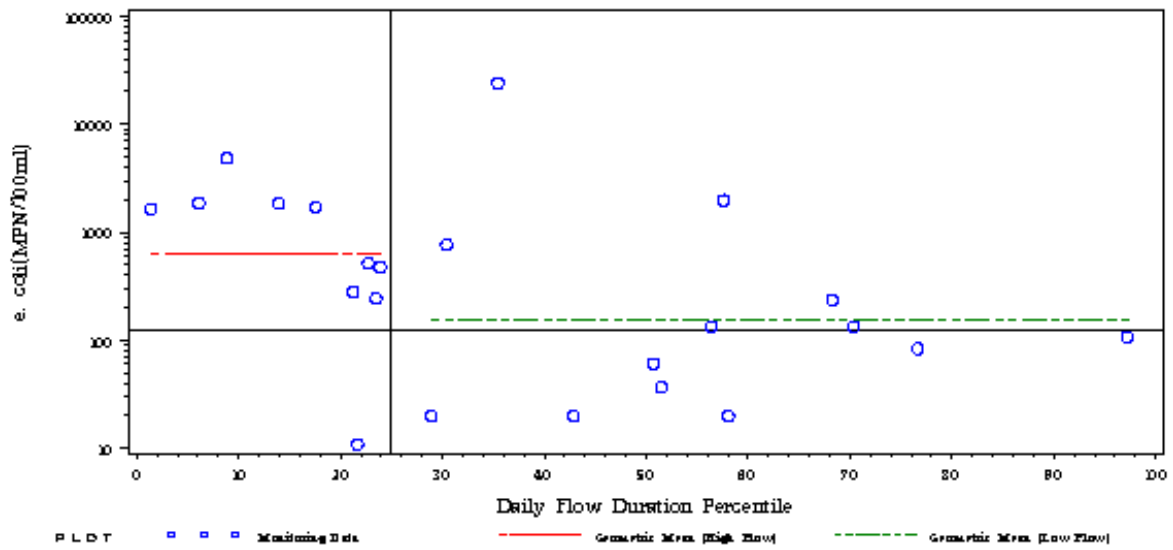


Figure B-5: *E. Coli* Concentration vs. Flow Duration for Wills Creek Monitoring Station WIL0000

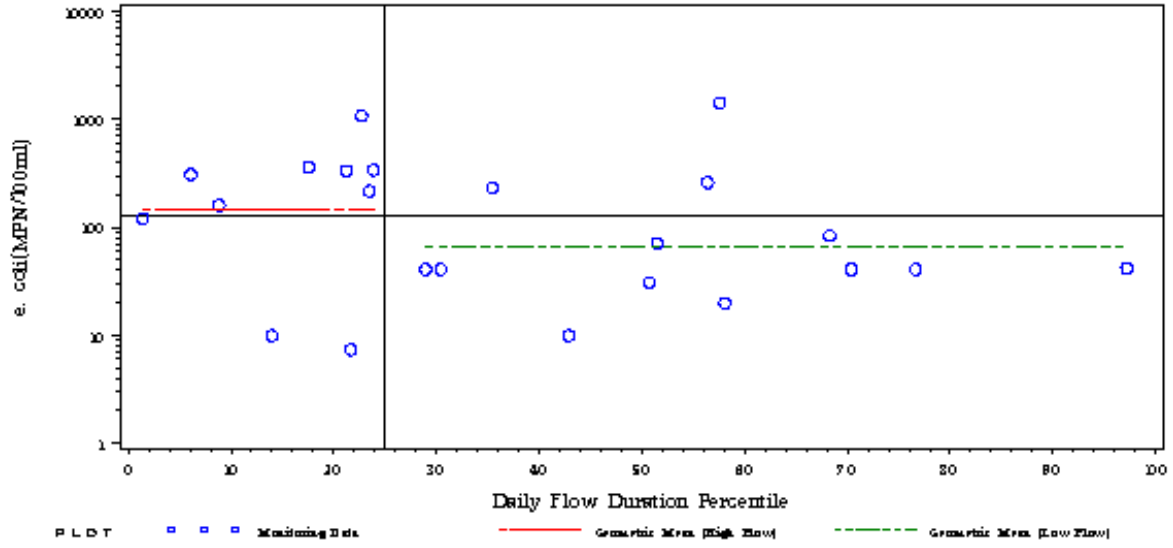


Figure B-6: *E. Coli* Concentration vs. Flow Duration for Wills Creek Monitoring Station WIL0013

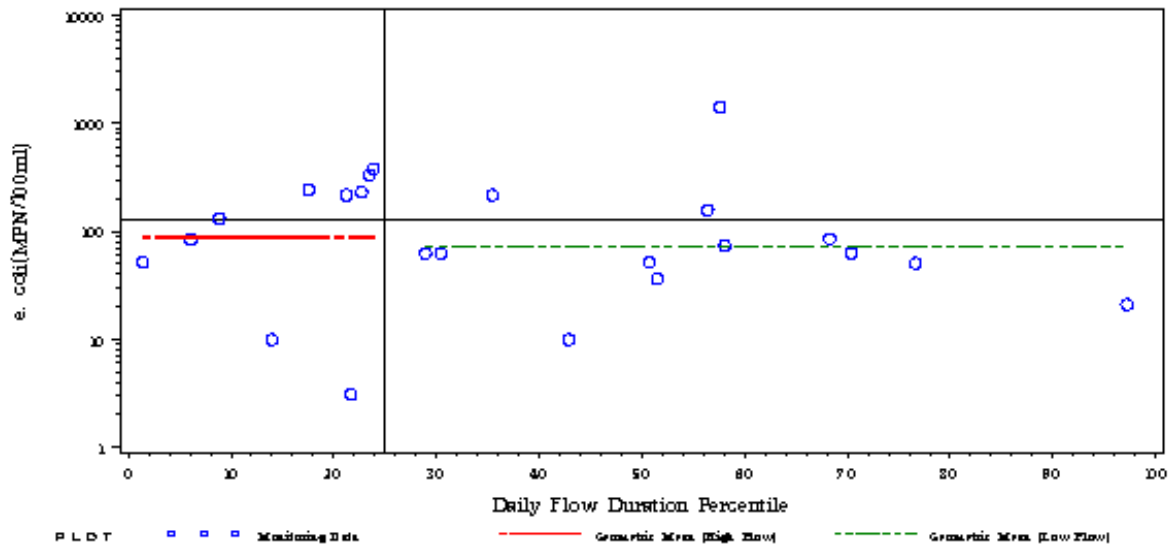


Figure B-7: *E. Coli* Concentration vs. Flow Duration for Wills Creek Monitoring Station WIL0067

FINAL

**Appendix C - Identifying Sources of Fecal Pollution in the Wills Creek Watershed,
Maryland**

November 1, 2003 – October 31, 2005

**Final Report
January 31, 2006**

Revised 02.03.2006

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

Table of Contents

Introduction	C3
Laboratory Methods	C4
Known-Source Library	C5
Statistical Analysis	C6
ARA Results	C7
1. Georges Creek Watershed	C7
2. Wills Creek Watershed	C7
References	C15
Acknowledgements	C15

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: Gwynns Falls, Jones Falls, Herring Run, Georges Creek, and Wills Creek. Also included in the study was the Patuxent 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 Patuxent 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

FINAL

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).

Pulsed-Field Gel Electrophoresis

In PFGE, the total microbial genome of the indicator organism is digested with a restriction enzyme specifically chosen to produce a limited number of DNA fragments (10 – 30). These fragments are too large (contain too many base pairs) to be separated by standard DNA gel electrophoresis. The PFGE apparatus is designed to move the DNA through the gel by changing the angle of DNA migration and the application of pulsed time and voltage over an extended run time (approximately 20 hours). The resulting DNA banding pattern from each isolate is digitized and the profile of that banding pattern entered into a fingerprint analysis program (BioNumerics®). The software program is then used to compare banding patterns from multiple isolates looking for similarities based upon isolate source. The Centers for Disease Control and Prevention use this method for their “National Molecular Subtyping Network for Foodborne Disease Surveillance” (<http://www.cdc.gov/pulsenet/>)

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 10 *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. *Enterococcus* 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

<u>Antibiotic</u>	<u>Concentration (µg/ml)</u>
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

Pulsed-Field Gel Electrophoresis: DNA characterization was performed using contour-clamped homogenous electric field (CHEF) PFGE. *Enterococcus* isolates were identified to species (*E. faecalis*, *E. faecium*, *E. casseliflavus*) using the Biolog, Inc. Microstation™ System and MicroLog™ software. Isolates were then prepared for analysis using CHEF Bacterial Genomic DNA Plug Kit (Bio-Rad Laboratories, Inc., Hercules, CA). The DNA in each plug was cut with *Sma*I restriction enzyme. DNA fragments were separated according to base pair size using the CHEF Mapper® XA Chiller System (Bio-Rad Laboratories, Inc., Hercules, CA.). Gel bands were stained with either ethidium bromide or SYBR® green and were photographed on a long-wave UV transilluminator and analyzed with Kodak Digital Science Electrophoresis Documentation and Analysis System (Eastman Kodak Co., Rochester, NY.). Banding patterns were analyzed using BioNumerics®, a product of Applied Maths, Inc., Austin, TX.

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, beaver, coyote, 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 Georges Creek and Wills Creek watersheds using patterns from scat obtained from both watersheds, and the water isolate patterns of each were compared to the combined library.

STATISTICAL ANALYSIS

We applied a tree classification method, ¹CART[®], 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 that is most populous among the library isolates in the node. Each water sample isolate (*i.e.*, an isolate with an unknown source), based 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

We imposed an additional requirement in our classification method for determining the sources of water sample isolates. We interpreted the proportion of the majority source among the library isolates in a *terminal node* as a probability. This proportion is an estimate of the probability that an isolate with unknown source, but with the same antibiotic resistance pattern as the library isolates in the *terminal node*, came from the source of the majority of the library isolates in the *terminal node*. If that probability was less than a specified *acceptable source identification probability*, we did not assign a source to the water sample isolates identified with that *terminal node*. Instead we assigned “Unknown” as the source for that node and “Unknown” for the source of all water sample isolates identified with that node. The *acceptable source identification probability* for the tree-classification model for an individual watershed is shown in the Results section for that watershed.

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

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.

ARA Results: Wills Creek Watershed**Known-Source Library**

An 827 known-source isolate library was constructed that included 436 isolates from sources in the Georges Creek Watershed combined with the 391 isolates from the adjacent Wills Creek Watershed. The known sources in the combined library were grouped into four categories: domestic (pets, specifically dogs), human, livestock (cow), and wildlife (deer, coyote, beaver, fox, rabbit) (Tables C-2a and C-2b). 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-2a: Georges Creek. Category, total number, and number of unique patterns in the Georges Creek portion of the combined Georges-Wills known-source library.

<u>Category</u>	<u>Potential Sources</u>	<u>Total Isolates</u>	<u>Unique Patterns</u>
Pet	dog	55	33
Human	human	135	93
Livestock	cow	54	8
Wildlife	rabbit, fox, deer	192	45
Total		436	179

Table C-2b: Wills Creek. Category, total number, and number of unique patterns in the Wills Creek portion of the combined Georges-Wills known-source library

<u>Category</u>	<u>Potential Sources</u>	<u>Total Isolates</u>	<u>Unique Patterns</u>
Human	human	84	54
Livestock	cow	69	32
Pet	dog	59	25
Wildlife	deer, coyote, beaver, fox, rabbit	179	45
Total		391	156

Table C-3: Wills Creek. Number of isolates not classified, percent unknown, and percent correct for six (6) cutoff probabilities for Wills Creek known-source isolates using the combined Georges-Wills known-source library.

Cutoff Probability	Number Not Classified	Percent Unknown	Percent Correct
.25	0	0%	83%
.375	0	0%	83%
.50	94	24%	84%
.60	114	29%	86%
.70	172	44%	92%
.80	228	58%	95%
.90	231	59%	98%

For Wills Creek Watershed, a cutoff probability of 0.70 (70%) was shown to yield a high ARCC of 92%. An increase to a 0.80 (80%) cutoff would only slightly increase the rate of correct classification (Figure 1-WI) and increase the percent unknowns significantly. Therefore, using a cutoff probability of 0.70 (70%), the 172 isolates that were not useful in the prediction of probable sources were removed; leaving 655 isolates remaining in the combined library. This library was then used in the statistical prediction of probable sources of bacteria in water samples collected from the Wills Creek Watershed. The rates of correction classification for the four categories of sources in the Wills Creek portion of the library, with a probability cutoff of 0.70 (70%), are shown in Table C-4 below.

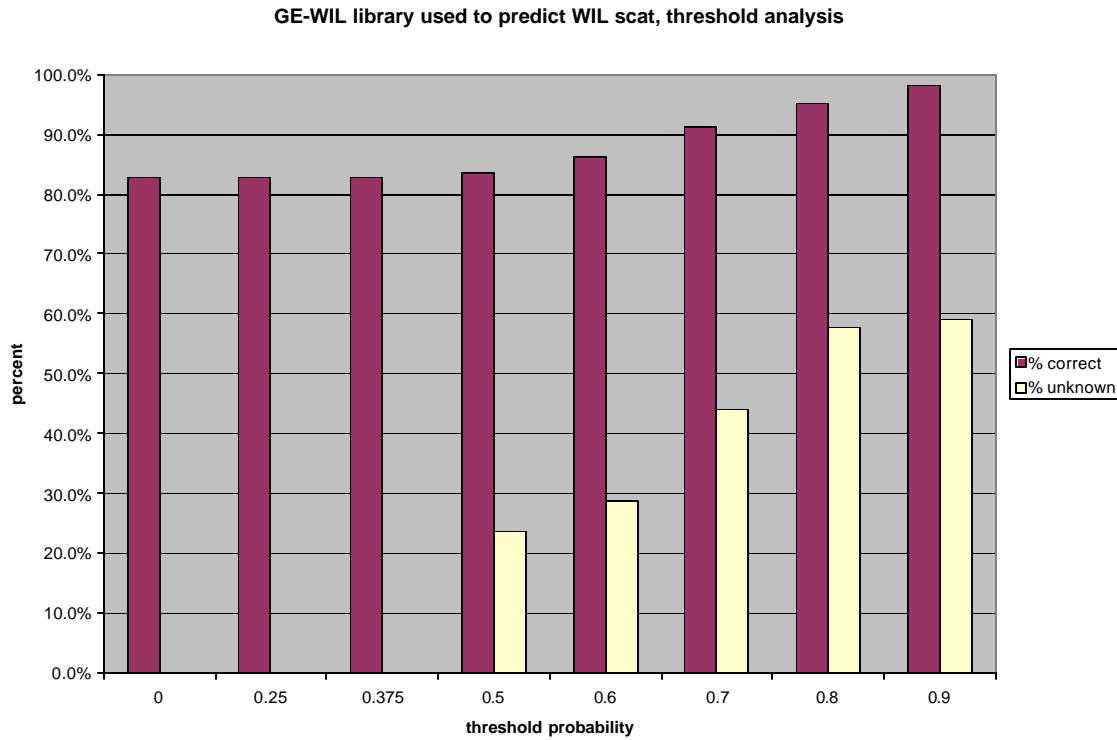


Figure C-1: Wills Creek Classification Model: Percent Correct versus Percent Unknown.

Table C-4: Wills Creek. Actual species categories versus predicted categories, with a 70% probability cutoff, with rates of correct classification (RCC) for each category

Actual ?	Predicted ?				TOTAL	RCC ¹
	HUMAN	LIVESTOCK	PET	WILDLIFE		
HUMAN	58	2	2	0	62	94%
LIVESTOCK	1	39	0	0	40	98%
PET	0	0	58	0	58	100%
WILDLIFE	0	11	3	45	59	76%
Total	59	52	63	45	219	91%

¹RCC = Actual number of predicted species category / Total number predicted. Example: One hundred sixty-three (163) domestic correctly predicted /175 total number predicted for domestic = 163/175 = 93%.

Wills Creek Water Samples. Monthly monitoring from six (6) stations on Wills Creek was the source of water samples. The maximum number of *Enterococcus* isolates per water sample was 24, although the number of isolates that actually grew was sometimes fewer

Wills Creek TMDL Fecal Bacteria
 Document version: August 30, 2006

than 24. A total of 1411 *Enterococcus* isolates were analyzed by statistical analysis. The BST results by species category, shown in Table C-5, indicates that 73% of the water isolates were classified after excluding unknowns when using a 0.70 (70%) probability cutoff.

Table C-5: Potential host sources of Wills Creek Watershed water isolates by species category, number of isolates, percent isolates classified at a cutoff probability of 70%.

Category	Number	% Isolates Classified 70% Prob.	% Isolates Classified (excluding unknowns)
DOMESTIC	132	9%	13%
HUMAN	487	35%	48%
LIVESTOCK	84	6%	8%
WILDLIFE	322	23%	31%
UNKNOWN	386	27%	
Missing Data	0		
Total	1411		
% Classified	73%		

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

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

Station	Spring	Summer	Fall	Winter	Total
Braddock	76	63	60	30	229
Ellerslie	85	71	50	34	240
Gage	65	71	56	39	231
Jennings	72	88	45	22	227
NB Jennings	86	85	37	34	242
Outlet	74	101	38	29	242
Total	458	479	286	188	1411

Tables C-7 and C-8 below show the number and percent of probable sources of *Enterococcus* contamination in the watershed.

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

Site	Date	human	livestock	pet	wildlife	unknown	Total
Braddock	11/20/02	3	4	4	3	8	22
Ellerslie	11/20/02	7	0	2	4	8	21
Gage	11/20/02	7	5	0	0	8	20
Jennings	11/20/02	4	1	3	1	14	23
Jennings	11/20/02	2	1	1	4	5	13
Outlet	11/20/02	5	1	1	7	6	20
Braddock	12/02/02	4	0	2	3	9	18
Ellerslie	12/02/02	1	2	0	6	13	22
Gage	12/02/02	6	0	0	8	7	21
Jennings	12/02/02	5	0	1	0	9	15
NJennings	12/02/02	2	0	0	16	6	24
Outlet	12/02/02	3	1	0	2	3	9
Braddock	01/07/03	16	0	0	0	4	20
Ellerslie	01/07/03	12	0	1	2	9	24
Gage	01/07/03	11	1	1	3	8	24
Jennings	01/07/03	8	0	0	2	7	17
NJennings	01/07/03	8	0	2	2	12	24
Outlet	01/07/03	21	0	0	1	2	24
Braddock	02/03/03	3	2	2	1	2	10
Ellerslie	02/03/03	6	1	1	0	2	10
Gage	02/03/03	15	0	0	0	0	15
Jennings	02/03/03	1	2	0	0	2	5
NJennings	02/03/03	0	9	0	1	0	10
Outlet	02/03/03	4	0	0	0	1	5
Outlet	04/01/03	0	1	0	1	0	2
Braddock	04/01/03	0	0	2	2	1	5
Ellerslie	04/01/03	2	2	0	5	6	15
Gage	04/01/03	1	0	3	1	2	7
Jennings	04/01/03	2	0	0	0	0	2
NJennings	04/01/03	8	2	0	4	2	16
Braddock	04/15/03	23	0	0	0	0	23
Ellerslie	04/15/03	10	0	0	3	10	23
Gage	04/15/03	3	1	3	2	3	12
Jennings	04/15/03	17	1	1	1	3	23
NJennings	04/15/03	12	1	4	4	1	22
Outlet	04/15/03	14	0	5	2	3	24
Braddock	05/05/03	9	0	10	2	3	24
Ellerslie	05/05/03	7	2	4	5	5	23

Table C-7: Wills Creek: BST Analysis: Number of Isolates per Station per Date (Continued)

FINAL

Gage	05/05/03	1	3	2	3	14	23
Jennings	05/05/03	2	5	1	7	9	24
NJennings	05/05/03	4	1	5	6	8	24
Outlet	05/05/03	8	1	5	6	4	24
Braddock	06/02/03	15	0	5	3	1	24
Ellerslie	06/02/03	1	0	4	16	3	24
Gage	06/02/03	5	0	0	14	4	23
Jennings	06/02/03	2	1	7	5	8	23
NJennings	06/02/03	11	0	2	5	6	24
Outlet	06/02/03	18	0	2	2	2	24
Braddock	07/07/03	9	1	1	9	4	24
Ellerslie	07/07/03	2	4	0	11	7	24
Gage	07/07/03	8	4	1	6	5	24
Jennings	07/07/03	4	0	0	15	5	24
NJennings	07/07/03	7	1	0	14	2	24
Outlet	07/07/03	7	0	4	8	5	24
Braddock	08/04/03	5	0	2	11	5	23
Ellerslie	08/04/03	4	2	2	8	5	21
Gage	08/04/03	6	0	3	2	8	19
Jennings	08/04/03	10	1	2	5	6	24
NJennings	08/04/03	8	2	4	5	4	23
Outlet	08/04/03	7	0	3	5	8	23
Braddock	09/08/03	0	1	2	7	6	16
Ellerslie	09/08/03	12	4	0	6	4	26
Gage	09/08/03	12	3	1	2	10	28
Jennings	09/08/03	11	1	4	18	6	40
NJennings	09/08/03	20	0	2	10	6	38
Outlet	09/08/03	19	3	5	9	18	54
Braddock	10/07/03	6	0	4	1	9	20
Ellerslie	10/07/03	0	0	1	3	3	7
Gage	10/07/03	0	5	3	1	6	15
Jennings	10/07/03	1	0	2	1	3	7
Outlet	10/07/03	0	1	0	0	8	9
Total		487	84	132	322	386	1411

Table C-8: BST Analysis: Percenta

ge of Sources per Station per Date.

Site	Date	human	livestock	pet	wildlife	unknown
Braddock	11/20/02	14%	18%	18%	14%	36%
Ellerslie	11/20/02	33%	0%	10%	19%	38%
Gage	11/20/02	35%	25%	0%	0%	40%
Jennings	11/20/02	17%	4%	13%	4%	61%
NJennings	11/20/02	15%	8%	8%	31%	38%
Outlet	11/20/02	25%	5%	5%	35%	30%
Braddock	12/02/02	22%	0%	11%	17%	50%
Ellerslie	12/02/02	5%	9%	0%	27%	59%

FINAL

Gage	12/02/02	29%	0%	0%	38%	33%
Jennings	12/02/02	33%	0%	7%	0%	60%
NJennings	12/02/02	8%	0%	0%	67%	25%
Outlet	12/02/02	33%	11%	0%	22%	33%
Braddock	01/07/03	80%	0%	0%	0%	20%
Ellerslie	01/07/03	50%	0%	4%	8%	38%
Gage	01/07/03	46%	4%	4%	13%	33%
Jennings	01/07/03	47%	0%	0%	12%	41%
NJennings	01/07/03	33%	0%	8%	8%	50%
Outlet	01/07/03	88%	0%	0%	4%	8%
Braddock	02/03/03	30%	20%	20%	10%	20%
Ellerslie	02/03/03	60%	10%	10%	0%	20%
Gage	02/03/03	100%	0%	0%	0%	0%
Jennings	02/03/03	20%	40%	0%	0%	40%
NJennings	02/03/03	0%	90%	0%	10%	0%
Outlet	02/03/03	80%	0%	0%	0%	20%
Outlet	04/01/03	0%	50%	0%	50%	0%
Braddock	04/01/03	0%	0%	40%	40%	20%
Ellerslie	04/01/03	13%	13%	0%	33%	40%
Gage	04/01/03	14%	0%	43%	14%	29%
Jennings	04/01/03	100%	0%	0%	0%	0%
NJennings	04/01/03	50%	13%	0%	25%	13%
Braddock	04/15/03	100%	0%	0%	0%	0%
Ellerslie	04/15/03	43%	0%	0%	13%	43%
Gage	04/15/03	25%	8%	25%	17%	25%
Jennings	04/15/03	74%	4%	4%	4%	13%
NJennings	04/15/03	55%	5%	18%	18%	5%
Outlet	04/15/03	58%	0%	21%	8%	13%
Braddock	05/05/03	38%	0%	42%	8%	13%
Ellerslie	05/05/03	30%	9%	17%	22%	22%
Gage	05/05/03	4%	13%	9%	13%	61%
Jennings	05/05/03	8%	21%	4%	29%	38%

Table C-8: BST Analysis: Percentage of Sources per Station per Date (Continued)

NJennings	05/05/03	17%	4%	21%	25%	33%
Outlet	05/05/03	33%	4%	21%	25%	17%
Braddock	06/02/03	63%	0%	21%	13%	4%
Ellerslie	06/02/03	4%	0%	17%	67%	13%
Gage	06/02/03	22%	0%	0%	61%	17%
Jennings	06/02/03	9%	4%	30%	22%	35%
NJennings	06/02/03	46%	0%	8%	21%	25%
Outlet	06/02/03	75%	0%	8%	8%	8%
Braddock	07/07/03	38%	4%	4%	38%	17%
Ellerslie	07/07/03	8%	17%	0%	46%	29%
Gage	07/07/03	33%	17%	4%	25%	21%
Jennings	07/07/03	17%	0%	0%	63%	21%
NJennings	07/07/03	29%	4%	0%	58%	8%
Outlet	07/07/03	29%	0%	17%	33%	21%
Braddock	08/04/03	22%	0%	9%	48%	22%
Ellerslie	08/04/03	19%	10%	10%	38%	24%
Gage	08/04/03	32%	0%	16%	11%	42%
Jennings	08/04/03	42%	4%	8%	21%	25%
NJennings	08/04/03	35%	9%	17%	22%	17%
Outlet	08/04/03	30%	0%	13%	22%	35%
Braddock	09/08/03	0%	6%	13%	44%	38%
Ellerslie	09/08/03	46%	15%	0%	23%	15%
Gage	09/08/03	43%	11%	4%	7%	36%
Jennings	09/08/03	28%	3%	10%	45%	15%
NJennings	09/08/03	53%	0%	5%	26%	16%
Outlet	09/08/03	35%	6%	9%	17%	33%
Braddock	10/07/03	30%	0%	20%	5%	45%
Ellerslie	10/07/03	0%	0%	14%	43%	43%
Gage	10/07/03	0%	33%	20%	7%	40%
Jennings	10/07/03	14%	0%	29%	14%	43%
Outlet	10/07/03	0%	11%	0%	0%	89%
	Total	35%	6%	9%	23%	27%

Wills Creek Summary

The use of ARA was successful for identification of bacterial sources in the Wills Creek Watershed as evidenced by the high ARCC (91%) for the library. The lower RCC for wildlife of 76% is still acceptable, especially given that no remedial action will be taken for wildlife sources. When water isolates were compared to the library and potential sources predicted, 73% of the isolates were classified by statistical analysis. The largest category of potential sources in the watershed as a whole was human (48%), followed by wildlife, domestic, and livestock (31%, 13%, and 8% of the classified isolates, respectively).

REFERENCES FOR APPENDIX C

- Bell, J.B., Elliott, G.E. & Smith, D.W. (1983). Influence of Sewage Treatment and Urbanization on Selection of Multiple Resistance in Fecal Coliform Populations. *Appl. Environ. Microbiol.* 46, 227-32
- 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. We also want to acknowledge Dr. Bertram Price of Price Associates, Inc., for his contribution to the statistical analysis in this project.

Appendix D - Estimating Human Allocation for Subwatershed WIL0000sub

Four subwatersheds with unknown CSO or SSO load contributions were used for the analysis, two from the Wills Creek watershed and two from the Georges Creek subwatershed. The non-CSO or “background” human loading rate for each of these four subwatersheds was estimated as follows:

$$BLR_i = Lh_i/A_i$$

Where

BLR_i = Background human loading rate for subwatershed I (billion MPN *E. coli*/day/sq. mile)

Lh_i = Final human load for subwatershed i (billion MPN *E. coli*/day)

A_i = Area of subwatershed i (square miles)

The “background” human loading rate to be applied to subwatershed WIL0000sub is estimated as the average of the four loading rates. A summary of the analysis is presented in Table D-1.

Table D-1: Estimating Non-CSO/“Background” Human Loading Rate

Subwatersheds with no CSO or SSO bacteria load contribution	Final Human Load (bill MPN <i>E. coli</i> /day/sq. miles)	Area (sq. miles)	Non-CSO “Background” Human Loading Rate (bill MPN <i>E. coli</i> /day/sq. miles)
WIL0067 (Wills Creek)	21.5	189.34	0.11
NJE0014 (Wills Creek)	1.5	10.45	0.15
GEO0011sub (Georges Creek)	1.2	19.9	0.06
GEO0065sub (Georges Creek)	3.8	18.2	0.21
Average Non-CSO Human Loading Rate =>			0.13

The WLA for the City of Cumberland CSOs located in subwatershed WIL0000sub is estimated as follows:

From Table 4.7.5 of the TMDL main document, the WIL0000sub final human load is 56.1 billion MPN *E. coli*/day. The area of subwatershed WIL0000sub is 6.1 sq. miles. The background human loading rate as estimated above is 0.13 billion MPN *E. coli*/day. The background (non-CSO) human load for subwatershed WIL0000sub is 0.81 billion MPN *E. coli*/day. This is summarized in the equation below:

$$WLA - CSO \text{ for subwatershed WIL0000sub} = 56.1 - 0.81 = 55.3 \text{ billion MPN } E. coli/\text{day}$$