Total Maximum Daily Loads of Fecal Bacteria
for the Anacostia River Basin
in Montgomery and Prince George's Counties, Maryland

FINAL

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MARYLAND

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List of Abbreviations

ARA	Antibiotic Resistance Analysis
ARCC	Average Rates of Correct Classification
BARC	Beltsville Agricultural Research Center
BMP	Best Management Practice
BPA	Blue Plains Advance
BST	Bacteria Source Tracking
CAFO	Confined Animal Feeding Operations
cfs	Cubic Feet per Second
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
COMAR	Code of Maryland Regulations
CSO	Combined Sewer Overflow
CSU	Carbon Source Utilization
CWA	Clean Water Act
CWP	Center for Watershed Protection
DCWASA	District of Columbia Waste and Sewer Authority
DNA	Deoxyribonucleic Acid
DNR	Maryland Department of Natural Resources
EPA	Environmental Protection Agency
FA	Future Allocation
GIS	Geographic Information System
LA	Load Allocation
LMM	Long-term Moving Median
MACS	Maryland Agricultural Cost Share Program
MASS	Maryland Agricultural Statistic Service
MDE	Maryland Department of the Environment
MDP	Maryland Department of Planning
Ml	Milliliter(s)
MOS	Margin of Safety
MPN	Most Probable Number
MPR	Maximum Practicable Reduction
MS4	Municipal Separate Storm Sewer System
MRLC	Multi-Resolution Land Cover
MST	Microbial Source Tracking
NPDES	National Pollutant Discharge Elimination System
PFGE	Pulsed Field Gel Electrophoresis
RAPD	Randomly-Amplified Polymorphic DNA
RCC	Rate of Correct Classification
SSO	Sanitary Sewer Overflows
STATSGO	State Soils Geographic Database

SU	Salisbury University
TARSA	Technical and Regulatory Services Administration
TMDL	Total Maximum Daily Load
USDA	United States Department of Agriculture
USGS	United States Geological Survey
WSSC	Washington Suburban Sanitary Commission
WQIA	Water Quality Improvement Act
WLA	Wasteload Allocation
WQLS	Water Quality Limited Segment
WRAS	Watershed Restoration Action Strategy
WWTP	Wastewater Treatment Plant

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EXECUTIVE SUMMARY

This document, upon approval by the U.S. Environmental Protection Agency (EPA), establishes a Total Maximum Daily Load (TMDL) for fecal bacteria in the Anacostia River (basin number 02-14-02-05). 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 the Anacostia River, a Use I-P, II, III and IV waterbody [Code of Maryland Regulations (COMAR) 26.08.02.08 O], on the State's 303(d) list as impaired by nutrients (1996), sediments (1996), fecal bacteria – non-tidal waters (2002), impacts to biological communities (2002), toxics – poly-chlorinated biphenyls (PCBs) (2002), toxics – heptachlor epoxide (2002), and fecal bacteria – tidal waters (2004). This document proposes to establish TMDLs of fecal bacteria in the tidal and non-tidal portions of the Anacostia River watershed within the State of Maryland that will allow for the attainment of the designated use of primary water contact recreation. The listings for other impairments in tidal and non-tidal waters 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.

The District of Columbia (DC) has developed a fecal coliform TMDL for the tidal Anacostia River, which has been approved by EPA. DC's TMDL assigns an allocation to Maryland's portion of the Anacostia River. This allocation is a summed load for both tidal and non-tidal segments of the Anacostia River drainage in Maryland. The pathogen indicator organism used in DC's TMDL analysis was fecal coliform, whereas Maryland, which recently adopted EPA's recommended bacteria indicator organisms (*E. coli* and enterococci), used enterococci for its bacteria TMDL analysis. MDE performed a correlation analysis between these two fecal bacteria indicators to convert DC's fecal coliform TMDL allocation into Maryland's enterococci TMDL. Based on the geometric mean resulting from this analysis, a ratio of 0.34 enterococci concentration to fecal coliform concentration converts DC's fecal coliform TMDL allocation for MD into an enterococci TMDL. This represents accurately the bacteria per acre per year loading rates of both DC and Maryland TMDLs. Although generated using a different pathogen indicator organism, Maryland's TMDL was based on the allocation stated in DC's TMDL. Therefore, Maryland's proposed TMDLs will meet both Maryland's and DC's water quality standards, and will be protective of downstream designated uses under all hydrological conditions.

Two drainage areas define the Anacostia River watershed within the State of Maryland: the non-tidal area located upstream of the confluence of Northwest Branch (NWB) and Northeast Branch (NEB) (hereafter referred as "the area upstream of the confluence"); and the area between the confluence and the Maryland/DC line (hereafter referred as "the area downstream of the

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confluence"), which includes tidal and non-tidal reaches of the Anacostia River. To establish baseline and allowable pollutant loads for the area located upstream of the confluence, a flow duration curve approach was used, incorporating flow strata estimated from United States Geological Survey (USGS) daily flow monitoring data and bacteria monitoring data. The sources of fecal bacteria for this area were estimated at six stations where samples were collected for a one-year duration. A multiple antibiotic resistance analysis (ARA) methodology was used to determine the relative proportion of source categories: domestic (pets and human associated animals); human (human waste); livestock (agricultural related animals); and wildlife (mammals and waterfowl). Allowable loads for the watershed area located downstream of the confluence were derived from DC's TMDL. The fecal bacteria sources for this area were obtained from DC's bacteria source tracking study. DC's bacteria source tracking (BST) methodology analysis includes ARA coupled with Pulsed Field Gel Electrophoresis (PFGE).
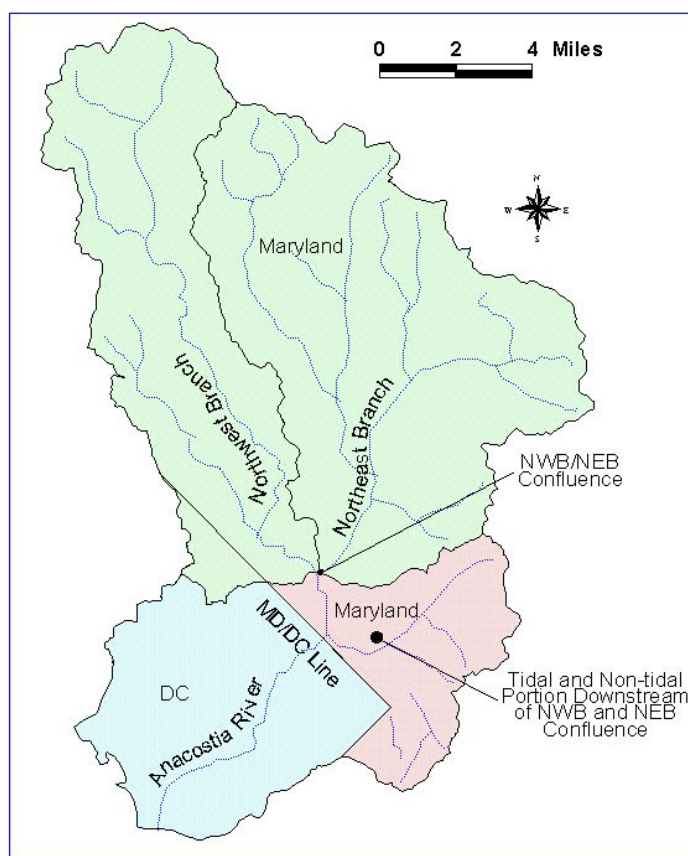
Anacostia River Watershed

The allowable load within the non-tidal watershed located upstream of the confluence is determined by first estimating a baseline load from current monitoring data. This baseline load is estimated using a long-term geometric mean and weighting factors from the flow duration curve. The TMDL for fecal bacteria entering the non-tidal Anacostia River located upstream of the confluence is established after considering six different hydrological conditions: high flow and low flow annual conditions; high flow and low flow seasonal conditions (the period between May 1st and September 30th when water contact recreation is more prevalent); and 30-day high flow and 30-day low flow conditions to be protective of DC waters designated uses (DC's

TMDL was based on a 30-day moving geometric mean). The TMDL for the Anacostia River area located downstream of the confluence was estimated by subtracting the upstream non-tidal area allowable load from the total allowable load derived from DC's TMDL. 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 and not a literal daily limit.

For the non-tidal watershed TMDL located upstream of the confluence, two scenarios were developed; the first assessing if attainment of current water quality standards could be achieved with maximum practicable reductions (MPRs) applied, and the second requiring higher maximum reductions. 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 source categories. In all non-tidal and tidal subwatersheds, it was estimated that water quality standards could not be attained with the MPRs. Thus, for these subwatersheds, a second scenario with higher maximum reductions was applied.

The fecal bacteria TMDL for the Anacostia River watersheds located within MD boundaries are 357 billion MPN enterococci/day. The TMDL is distributed between load allocation (LA) for nonpoint sources and waste load allocations (WLA) for point sources, including National Pollutant Discharge Elimination System (NPDES) wastewater treatment plants (WWTPs) and NPDES municipal separate storm sewer systems (MS4). The total LA is 146 billion MPN/day. The WWTPs WLA and MS4 WLA are 1.0 billion MPN/day and 210 billion MPN/day, respectively. The margin of safety (MOS) for the watershed area upstream of the confluence is explicit and has been incorporated by estimating the loading capacity of the stream based on a more stringent water quality endpoint concentration. The enterococci water quality criterion concentration was reduced by 5%, from 33 MPN/100ml to 31.35 MPN/100ml. The TMDL has been allocated among the non-tidal watersheds located upstream of the confluence and the watersheds located downstream of the confluence, as follows:

Anacostia River Fecal Bacteria TMDL Allocations

Subwatershed	TMDL	LA	WLA-MS4	WLA-WWTP
	Billion MPN Enterococci/day			
Upstream of Confluence of Northwest and Northeast Branches	310	130	179	1
Downstream of Confluence of Northwest and Northeast Branches and Upstream of MD/DC line*	47	16	31	0
TOTAL	357	146	210	1

*Derived by subtracting from DC's TMDL

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 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.

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1.0   INTRODUCTION

This document, upon approval by the U.S. Environmental Protection Agency (EPA), establishes a Total Maximum Daily Load (TMDL) for fecal bacteria in the Anacostia River (basin number 02-14-02-05). 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 water body 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 Anacostia River (basin number 02-14-02-05) has been designated a Use I-P, II, III, and IV waterbody [Code of Maryland Regulations (COMAR) 26.08.02.08 O].   The Maryland Department of the Environment (MDE) has identified the Anacostia River on the State's 303(d) list as impaired by the following: nutrients (1996); sediments (1996); fecal bacteria (non-tidal waters in 2002, tidal waters in 2004); impacts to biological communities (2002); and toxics (poly-chlorinated biphenyls and heptachlor epoxide) in 2002. The District of Columbia (DC) has established a fecal bacteria TMDL for the portion of the Anacostia River within DC's boundaries. DC's fecal bacteria allocation for Maryland's portion of the Anacostia River is 348,000 Billion Most Probable Number (MPN) fecal coliform/day. This document, upon approval by the U.S. EPA, establishes TMDLs of fecal bacteria in the tidal and non-tidal portions of the Anacostia River that will allow for attainment of its designated uses. All other impairments in the tidal and non-tidal portions of the Anacostia River 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 coliforms 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" whereby three indicator organisms were assessed to determine their correlation with swimming-associated illnesses. Fecal coliform, *E. coli* and enterococci were the indicators used in the analysis. Fecal coliform bacteria are a subgroup of total coliform bacteria and *E. coli* bacteria are a subgroup of fecal

coliform bacteria. Most *E. coli* are harmless and are found in great quantities in the intestines of people and warm-blooded animals. However, certain pathogenic strains may cause illness. Enterococci are a subgroup of bacteria in the fecal streptococcus group. Fecal coliform, *E. coli* and enterococci can all be classified as fecal bacteria. The results of the EPA study (EPA, 1986) demonstrated that fecal coliform showed less correlation to swimming-associated gastroenteritis than either *E. coli* or enterococci.

The Anacostia River watershed was listed on the Maryland 303(d) list using fecal coliform as the indicator organism. Based on EPA's guidance (USEPA, 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 Anacostia River TMDL analysis was enterococci.

2.0   SETTING AND WATER QUALITY DESCRIPTION

2.1   General Setting

Location

There are three major drainage areas comprising the Anacostia River watershed: the Northwest Branch, the Northeast Branch, and the tidal drainage. The Northwest and Northeast Branches are free-flowing (non-tidal) streams, and their confluence forms the tidal Anacostia River in the vicinity of Bladensburg, Maryland.

The Anacostia River proper begins at Bladensburg, Maryland, where the Northwest and Northeast Branches meet (Figure 2.1.1). The many smaller tributaries of the branches form a broad, fan-shaped drainage basin of 17 square miles. Just below Bladensburg, the Anacostia River drops to near sea level and changes from a free-flowing river into a tidal freshwater embayment of the Potomac estuary.

The tidal drainage area consists of the tidal river and its floodplain, as well as small Coastal Plain streams that flow directly to the tidal river. Most of these streams are enclosed in storm sewer systems. The tidal reach of the Anacostia River is 8.4 miles (13.5 kilometers) in length from the confluence of the Northwest and Northeast branches downstream to the Potomac River. The river joins the Potomac approximately 108 miles (174 kilometers) upstream of the Chesapeake Bay.

The vast majority of the tidal section of the Anacostia River is located in Washington DC, while the free-flowing segments are primarily within the State of Maryland, specifically Prince George's and Montgomery Counties. The regions this document will address are the free-flowing (non-tidal) sections of the Anacostia River watershed covering a surface area of approximately 80,000 acres, and the region between the confluence of the Northwest Branch and Northeast Branch and the MD/DC line, with an area of approximately 12,726 acres. The head of tide is just upstream of the Alternate Route 1 Bridge crossing in Bladensburg. Immediately upstream of the head of tide, the river splits into two segments, the Northeast and Northwest Branches.

The analysis presented in this report includes the non-tidal zone upstream of the Northwest Branch and Northeast Branch confluence and the zone between this confluence and the MD/DC line. Descriptions of these zones are described below.

Northwest Branch

The Northwest Branch subwatershed is approximately 32,000 acres, with the land use being almost exclusively heavily developed urban and suburban areas. The topography of the area is gently rolling hills. Eighty percent of the drainage area is within the eastern portion of Montgomery County, with the remaining lower reaches in Prince George's County. This sub-watershed has been divided into three segments: lower, middle, and headwater reaches.

Northeast Branch

The Northeast Branch watershed is approximately 48,000 acres, with land use ranging from urban to Federal Agricultural Research areas. The Federal Agricultural Research Centers are large tracts of land containing animal grazing fields, feed lots, barns, forested areas, clusters of institutional buildings, and two wastewater treatment facilities. The topography of the area is gently rolling hills. The majority of the watershed is located within Prince George's County. Only a small portion of the headwater regions is located in Montgomery County, and a couple of minor headwater areas are located in DC. This watershed has been divided into three segments: lower, middle, and headwater reaches.

Zone Downstream of the Northwest Branch and Northeast Branch Confluence

This region includes the tidal portion of the watershed and is approximately 35,890 acres, with the land use being almost exclusively heavily developed urban and suburban areas. The topography of the area is gently rolling hills. Approximately 12,726 acres are located in Maryland and another 23,163 acres are located in Washington DC.

Geology/Soils

The Anacostia River watershed is one of the most densely populated watersheds within the Chesapeake Bay drainage basin. The watershed extends into two physiographic provinces. Roughly mirroring the boundary between Montgomery and Prince George's Counties, Maryland, the fall line delineates the surface contact between the Piedmont, where the bedrock consists of metamorphic rocks of Paleozoic age, and Coastal Plain provinces. The Piedmont province is characterized by relatively narrow and steep-sloped valleys of moderately thin soils, as compared to the undulating Coastal Plain, which contains deeper sedimentary soil complexes and supports broader meandering streams. (Anacostia Watershed Network: www.anacostia.net)

The Northwest Branch of the Anacostia River lies predominantly in the Manor-Glenelg-Chester soil series. Soils in this series are fine-loamy, mixed, mesic Typic Hapludults and are very deep and well drained soils (Montgomery County, Maryland Soil Conservation Service, 1995). The Northeast Branch lies mostly in the Sunnyside-Christiana-Muirkirk soil series. The Sunnyside soils are mostly red, deep, and well drained. The Christiana-Muirkirk are also red and deep soils but are less permeable than the Sunnyside soils (Prince George's County, Maryland Soil Conservation Service, 1967) (Figure 2.1.2).

The tidal portion lies mainly in the Sunnyside-Christiana-Muirkirk soil series, and the Beltsville-Croom-Sasafras soil series (STATSGO). These soils are gently sloping to steep and dominantly gravelly soils. (Prince George's County, Maryland Soil Conservation Service, 1967) (Figure 2.1.2).
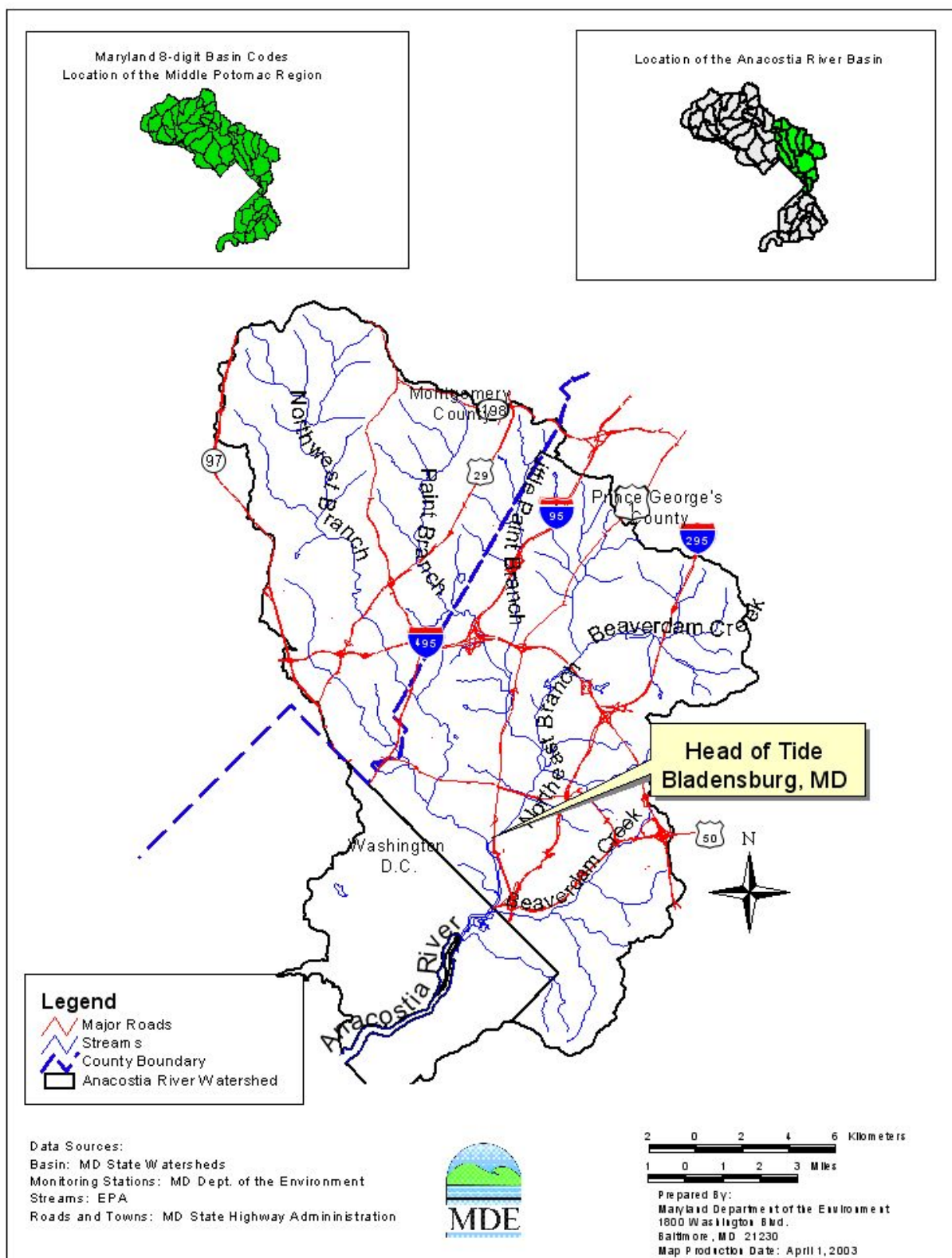
Figure 2.1.1: Location Map of the Anacostia River Basin
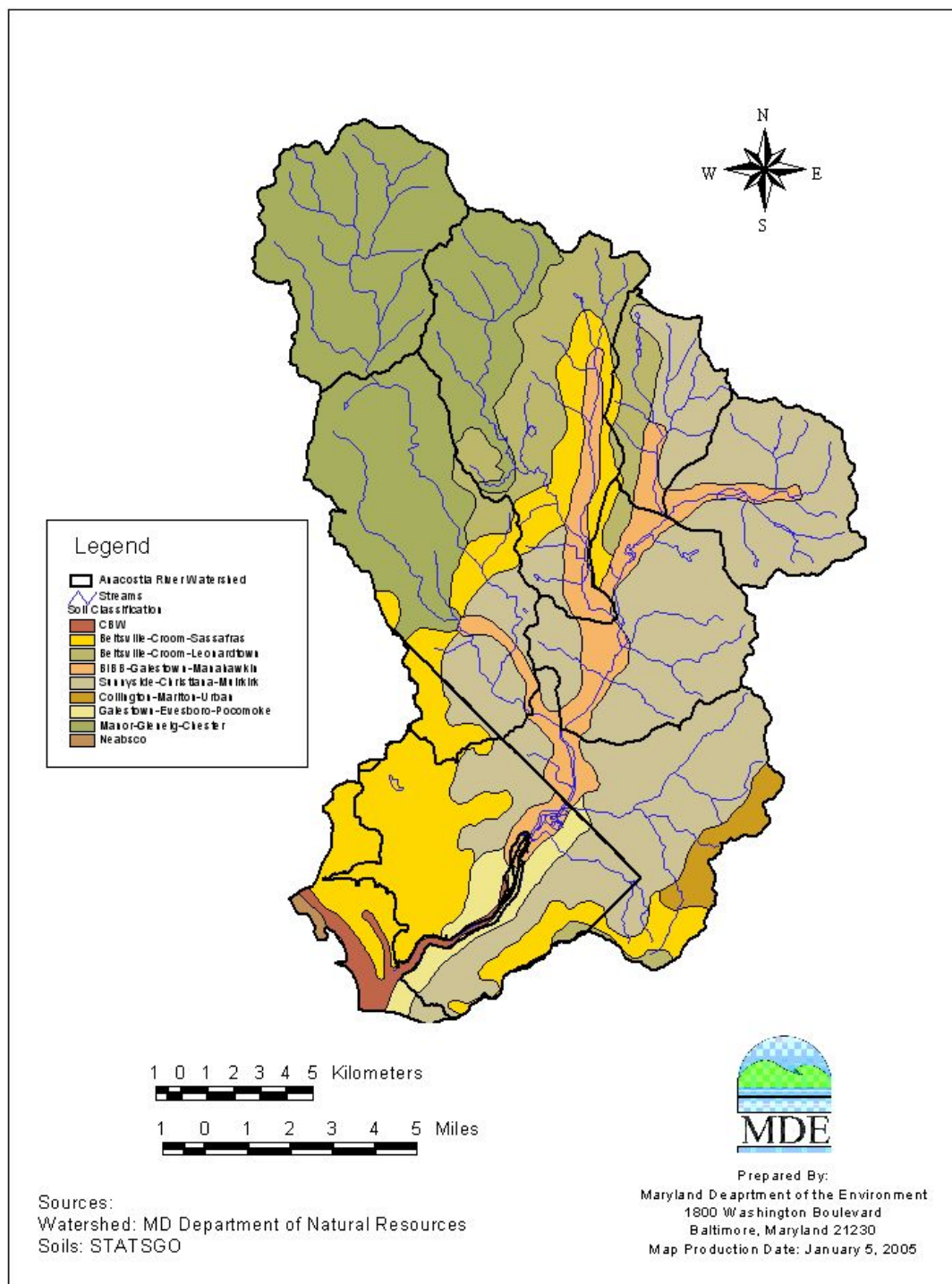
Figure 2.1.2: General Soil Series in the Anacostia River Basin

Land Use

The 2000 Maryland Department of Planning (MDP) land use/land cover data show that the watershed can be characterized as primarily residential and forested. Park and forest lands cover 24% of the watershed and are evenly dispersed throughout the watershed, such as the National Park Service facilities, the National Arboretum, Greenbelt Park, and Beltsville Agricultural Research Center. The industrial and manufacturing land use is largely confined to the tidal area of the basin such as Hickey Run, Lower Beaverdam Creek, and Indian Creek. These sub-watersheds contain as high as 80% impervious cover. The land use percentage distribution for Anacostia River Basin is shown in Table 2.1.1, and spatial distributions for each land use are shown in Figure 2.1.3.

Table 2.1.1: Land Use Percentage Distribution for Anacostia River Basin within the State of Maryland

Land Type	Acreage	Percentage
Urban	65,094	70.2
Forest	22,229	24.0
Agriculture	5,031	5.4
Wetlands	48	0.1
Water	324	0.3
Totals	**92,726**	**100.0**

Population

The total population in the Anacostia River watershed is estimated to be 539,389 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 Census Block and the MDP Land Use 2002 Cover that includes the Anacostia River watershed. Since the Anacostia River watershed is a subarea 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 Anacostia River watershed. The number of dwellings per acre was derived from information for residential density (low, medium, high) from the MDP land use cover.

Table 2.1.2: Number of Dwellings Per Acre

Land use Code	Dwelling Per Acres
11 Low Density Residential	1
12 Medium Density Residential	5
13 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, population per sub-watershed was estimated (see Table 2.1.3.)

Table 2.1.3: Total Population Per Subwatershed in Anacostia River Watershed within the State of Maryland

Subwatershed	Station	Population
Beaverdam Creek	BED0001	8,567
Indian Creek	INC0030	7,664
Northeast Branch	NEB0002	97,603
Northwest Branch	NWA0002	190,110
Northwest Branch	NWA0135	46,038
Paint Branch	PNT0001	92,364
Lower Beaverdam Creek		97,042
	TOTAL	**539,389**
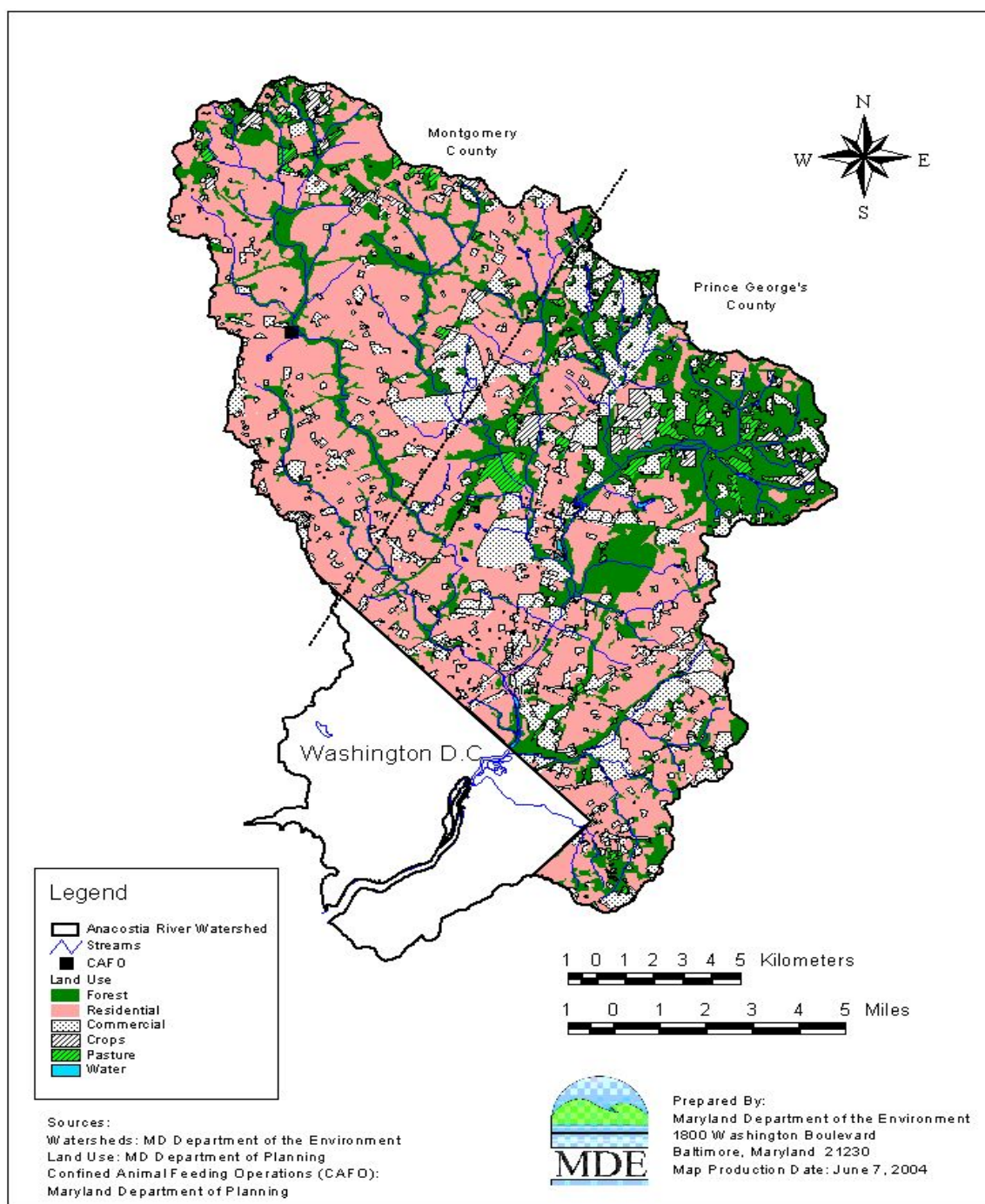
Figure 2.1.3: Land Use of the Anacostia River Watershed
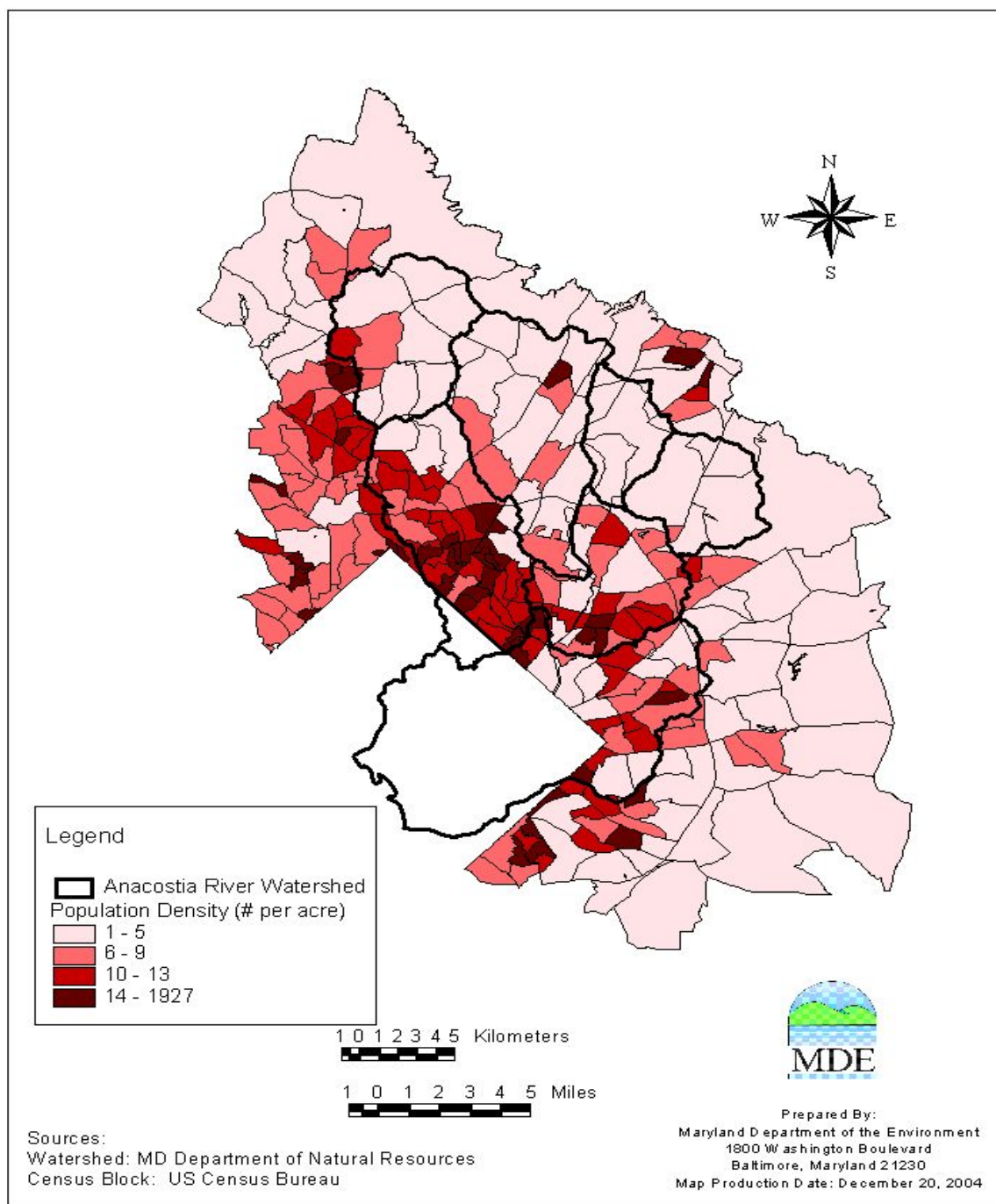
Figure 2.1.4: Population Density in Anacostia River Basin

2.2 Water Quality Characterization

From EPA's guidance document Ambient Water Quality Criteria for Bacteria (1986), 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), leading EPA to propose that states use *E. coli* or enterococci as pathogen indicators.

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 assessment was based on a geometric mean of the monitoring data, where the result could not exceed a geometric mean of 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 assure that risk levels are acceptable.

Bacteria Monitoring

Table 2.2.1 lists the historical monitoring data for the Anacostia River watershed. MDE conducted monitoring sampling from October 2002 through October 2003. Monitoring Station ANA0082 (CORE) was used by the Maryland Department of Natural Resources (DNR) to identify the bacterial impairment. There are five MDE bacteria monitoring stations in the Anacostia River basin, all five located upstream of the NWB and NEB confluence. In addition to the bacteria monitoring stations, there are three United States Geological Survey (USGS) gauging stations used in deriving the surface flow in the Anacostia River. The locations of these stations are shown in Tables 2.2.2 to Table 2.2.4 and Figure 2.2.1. Observations recorded during the period 2002-2003 from the five MDE monitoring stations are shown in Appendix A. In general, based on statewide monitoring data, fecal bacteria concentrations are higher in the headwaters. This is also consistent with findings from Wickham, *et al*. (2005), regarding pathogens in Maryland where the likelihood of an impairment decreases with watershed size. A table listing the monitoring results from the Anacostia River 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. Ranges were typically between 10 and 10,000 Most Probable Number (MPN)/100 ml.

Table 2.2.1: Historical Monitoring Data in the Anacostia River Watershed

Sponsor	Location	Date	Design	Summary
Maryland Department of Natural Resources (DNR) Core Monitoring	MD	1/8/97 – 4/1/98	Fecal Coliform	ANA0082 downstream of NE/NW Branch confluence GM=1509 MPN/100ml, n=15
District of Columbia Combined Sewer Overflow (DC CSO) Monitoring	DC		Fecal Coliform	6 stations located in Anacostia River and Rock Creek.
Anacostia Watershed Society	DC	8/20/02 and 8/29/02	Bacteria Source Tracking (BST)	DNA and Antibiotic Resistance Analysis (ARA) on two samples located near Bladensburg Road Bridge
Metropolitan Washington Council of Government (MWCOG)	DC	2002 – 2003	Microbial Source Tracking (MST)	DNA and ARA in DC Waters.
MDE	MD	11/02 to 10/03	Enterococci	6 stations 2x per month
MDE	MD	11/02 to 10/03	BST(ARA) (enterococci)	6 stations 1x per month
MWCOG / MDE	MD	2004	Optical brightener study	Ongoing.
USGS/Prince George's County/MDE	MD	1/2004 – 2005	*E. coli* and enterococci	Stations located at USGS gages on NE/NW Branch. Enumeration 1x per month.

Table 2.2.2: Locations of DNR (CORE) Monitoring Station in the Anacostia River Watershed

Watershed	Monitoring Station	Observation Period	Total Observations	LATITUDE Dec-Deg	LONGITUDE Dec-Deg
Tidal Anacostia River	ANA0082	1997 – 1998	15	38 56.32	76 56.62

Table 2.2.3: Locations of MDE Monitoring Stations in the Anacostia River Watershed

Subwatershed	Monitoring Station	Observation Period	Total Observations	LATITUDE Dec-Deg	LONGITUDE Dec-Deg
Beaverdam Creek	BED0001	2002 - 2003	26	39 0.975	76 53.84
Indian Creek	INC0030	2002 - 2003	25	39 0.97	76 53.88
Northeast Branch	NEB0002	2002 - 2003	26	38 56.74	76 56.45
Northwest Branch	NWA0002	2002 - 2003	26	38 56.76	76 57.82
Northwest Branch	NWA0135	2002 - 2003	26	39 03.89	77 01.68
Paint Branch	PNT0001	2002 - 2003	25	38 58.90	76 55.23

Table 2.2.4: Locations of USGS Gauging Stations in Anacostia River Watershed

Monitoring Station	Observation Period Used in TMDL Analysis	Total Observations	LATITUDE Dec-deg	LONGITUDE Dec-deg
01649500	1988 – 2003	5,509	38 57.60	76 55.50
01651000	1988 – 2003	5,577	38 57.00	76 58.56
01650500	1997 – 2003	2,099	39 03.60	77 01.80
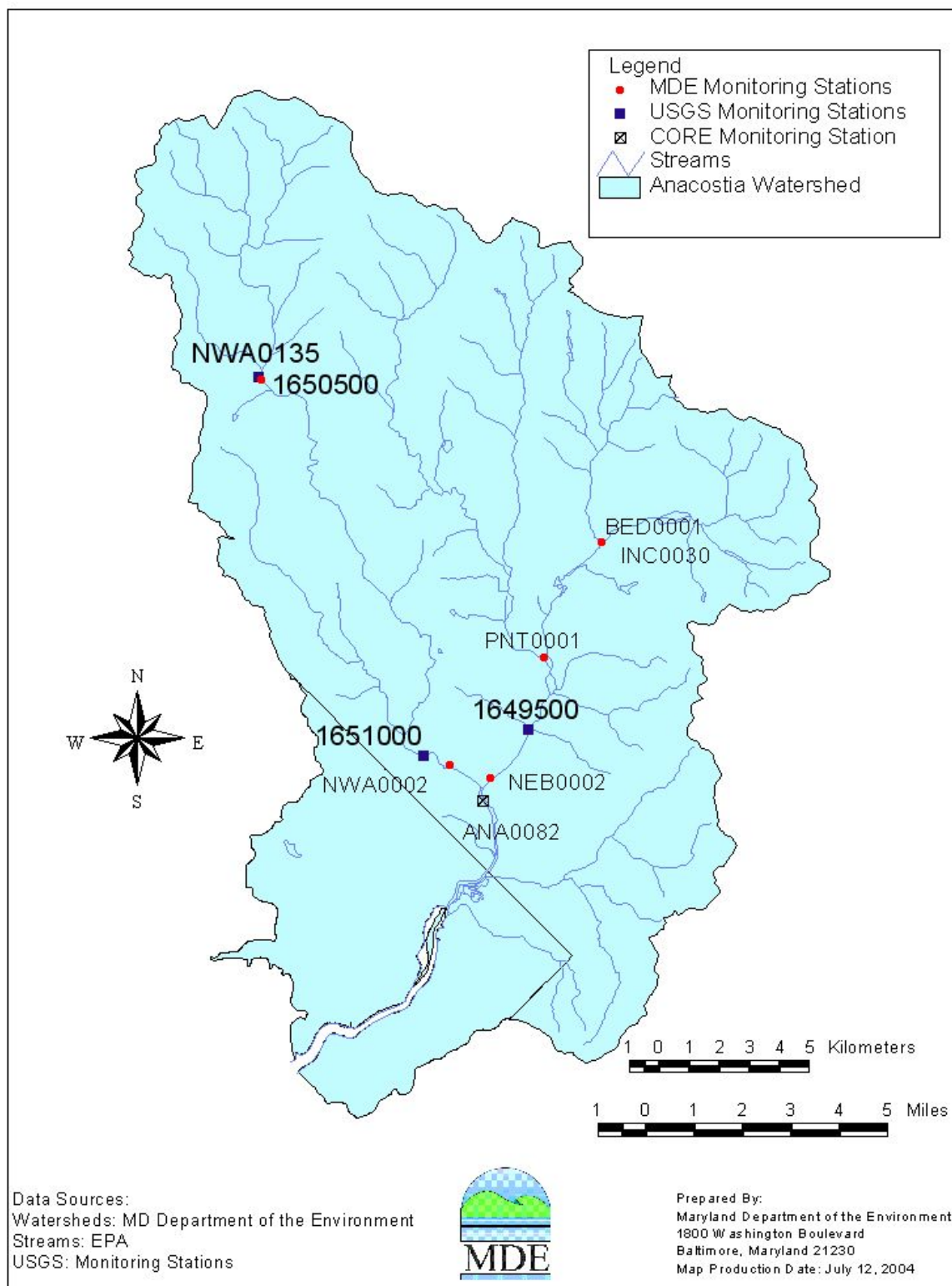
Figure 2.2.1: Monitoring Stations in the Anacostia River Basin

2.3 Water Quality Impairment

State of Maryland Designated Uses and Bacteriological Water Quality Standard

The Maryland water quality standards Surface Water Use Designations for this watershed area are Use I-P – Water Contact Recreation, Protection of Aquatic Life, and Public Drinking Supply; Use II – Support of Estuarine and Marine Aquatic Life and Shellfish Harvesting; Use III – Natural Trout Waters; and Use IV – Recreational Trout Waters (COMAR 26.08.02.08 O). The Maryland portion of the Anacostia River has been included on the final 2004 Integrated 303(d) List as impaired by fecal bacteria in both the non-tidal waters (2002) and the tidal waters (2004).

Water Quality Criteria

The State water quality standards for bacteria used for ALL Use waters are as follow (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 Uses.

Indicator	Steady State Geometric Mean Indicator Density
Freshwater	
E. coli	126 MPN/100ml
Enterococci*	33 MPN/100ml
Marine Water	
Enterococci	35 MPN/100ml

* Used in the Anacostia River analysis

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 5 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 35 coliform units (cfu)/100 ml enterococci in marine/estuarine waters, 33 cfu/100 ml enterococci in freshwater or 126 cfu/100 ml *E. coli* in freshwater, the water body will be listed as impaired. If fewer than 5 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 35 cfu/100 ml enterococci in marine/estuarine waters, 33 cfu/100 ml enterococci in freshwater or 126 cfu/100 ml *E. coli* in freshwater, the water body or beach will be listed as impaired.

The listing methodology for all general recreational use also applies to beaches. If the steady state geometric mean exceeds 35 cfu/100 ml enterococci in marine/estuarine waters, 33 cfu/100 ml enterococci in freshwater or 126 cfu/100 ml *E. coli* in freshwater, the beach area segment, as defined by the endpoint latitudes and longitudes, will be listed as impaired. The single sample maximum criteria applies only to beaches and is to be used for closure and advisory decisions based on short term exceedences of the geometric mean portion of the standard.

Washington, District of Columbia Designated Uses and Water Quality Standards

The following tables present the categories of uses; the Anacostia River designated uses and the bacteriological water quality standards in Washington, DC:

Table 2.3.2: Categories of Uses in Washington, D.C.

Surface Water of the District	Current Uses	Designated Uses
Anacostia River	B, C, D, E	A, B, C, D, E
Anacostia River Tributaries (except as listed below)	B, C, D	A, B, C, D
Hickey Run	B, C, D	B, C, D
Watts Branch	B, C, D	B, C, D

Table 2.3.3: Designated Uses of the Anacostia River in DC

Categories of Uses That Determine Water Quality Standards	Classes of Water
Primary contact recreation	A
Secondary contact recreation and aesthetic enjoyment	B
Protection & propagation of fish, shellfish and wildlife	C
Protection of human health related to consumption of fish & shellfish	D
Navigation	E

Table 2.3.4: DC's Bacteriological Water Quality Standards

Constituent	A	B
Bacteriological (No./100 mL)		
Fecal coliform (Maximum 30 day geometric mean for 5 samples)	200	1,000

Water Quality Assessment

A water quality impairment was assessed by comparing both the annual and the seasonal (May 1st – September 30th) steady state geometric means of enterococci 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. 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.

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 Anacostia River watershed. To estimate the steady state geometric mean, the monitoring data was 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.
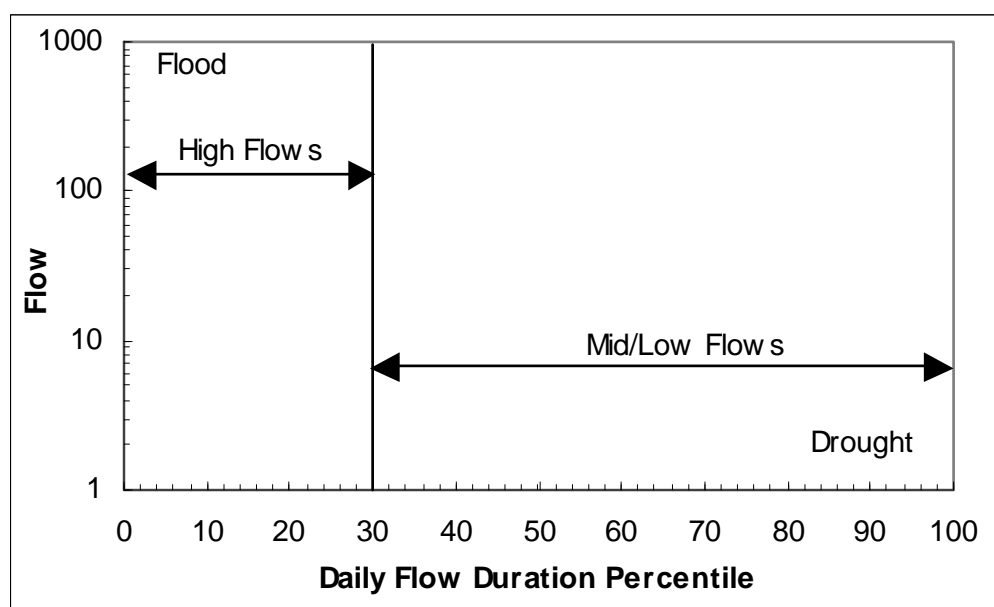
Figure 2.3.1: Conceptual Diagram of Flow Duration Zones

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 period (mid flows) between the high and low flow durations that is 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. The daily flow duration intervals that define these regions and supporting details of how these zones were developed are 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 Anacostia River 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 Anacostia River Watershed

Flow Duration Zone	Duration Interval	Weighting Factor

High Flows	0 – 30%	0.30
Low Flows	30 – 100%	0.70

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

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

where

$$M = \sum_{i=1}^{2} M_i * W_i \tag{2}$$

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

Finally, using the log mean concentration M, the steady state geometric mean C_{gm} is calculated using the following equation:

$$C_{gm} = 10^M \tag{3}$$

Table 2.3.3 and 2.3.4 present the geometric means by stratum and the overall steady state geometric mean for the Anacostia River subwatersheds for the annual and the seasonal (May 1st – September 30th) periods.

Table 2.3.3: Anacostia River Annual Steady State Geometric Mean by Stratum per Subwatersheds

Subwatershed	Station	Stratum	Geometric Mean	Steady State Geometric Mean
Beaverdam Creek	BED0001	High Flow	325	346
		Low Flow	355	
Indian Creek	INC0030	High Flow	215	126
		Low Flow	100	
Northeast Branch	NEB0002	High Flow	296	91
		Low Flow	55	
Northwest Branch	NWA0002	High Flow	386	133
		Low Flow	85	
Northwest Branch	NWA0135	High Flow	284	128
		Low Flow	91	
Paint Branch	PNT0001	High Flow	206	51
		Low Flow	28	

Table 2.3.4: Anacostia River Seasonal (May 1st- September 30th) Period Steady State Geometric Mean by Stratum per Subwatersheds

Subwatershed	Station	Stratum	Geometric Mean	Steady State Geometric Mean
Beaverdam Creek	BED0001	High Flow	154	250
		Low Flow	307	
Indian Creek	INC0030	High Flow	175	186
		Low Flow	190	
Northeast Branch	NEB0002	High Flow	148	67
		Low Flow	48	
Northwest Branch	NWA0002	High Flow	306	165
		Low Flow	127	
Northwest Branch	NWA0135	High Flow	212	258
		Low Flow	281	
Paint Branch	PNT0001	High Flow	126	54
		Low Flow	38	

Summary of Water Quality Data

The water quality impairment was assessed by comparing the annual and seasonal (May 1st – September 30th) steady state geometric mean concentrations at each monitoring station with the water quality criterion. Graphs illustrating these results can be found in Appendix B. Steady state geometric means of the monitoring data for both periods assessed and the water quality criterion are shown in Tables 2.3.5 and 2.3.6.

Table 2.3.5: Anacostia River Monitoring Data and Steady State Geometric Mean per Subwatershed for Average Annual Period

Watershed	Subwatershed	Station	# Samples	Enterococci Minimum MPN/100ml	Enterococci Maximum MPN/100ml	Enterococci Steady State Geometric Mean MPN/100ml	Enterococci Criterion MPN/100ml
02140205	Beaverdam Creek	BED0001	26	20	8,660	346	33
02140205	Indian Creek	INC0030	25	10	7,700	126	33
02140205	Northeast Branch	NEB0002	26	10	7,700	91	33
02140205	Northwest Branch	NWA0002	26	10	4,110	133	33
02140205	Northwest Branch	NWA0135	26	10	19,860	128	33
02140205	Paint Branch	PNT0001	25	10	4,350	51	33

Table 2.3.6: Anacostia River Monitoring Data and Steady State Geometric Mean per Subwatershed for the Seasonal Period (May 1st – September 30th)

Watershed	Subwatershed	Station	# Samples	Enterococci Minimum MPN/100ml	Enterococci Maximum MPN/100ml	Enterococci Steady State Geometric Mean MPN/100ml	Enterococci Criterion MPN/100ml
02140205	Beaverdam Creek	BED0001	12	50	470	250	33
02140205	Indian Creek	INC0030	12	60	7,700	186	33
02140205	Northeast Branch	NEB0002	12	10	500	67	33
02140205	Northwest Branch	NWA0002	12	20	4,110	165	33
02140205	Northwest Branch	NWA0135	12	10	19,860	258	33
02140205	Paint Branch	PNT0001	12	10	560	54	33

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 surface runoff occurs during rain events, it 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 and Septic Systems

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.

The majority of the sanitary sewer mains in the Anacostia River watershed flow to the Blue Plains Advanced (BPA) WWTP. The BPA WWTP is located downstream of, and outside, the Anacostia River watershed in Washington DC. The BPA WWTP serves the District of Columbia, portions of Maryland, and portions of Virginia, encompassing two to three million people. The BPA WWTP is part of the District of Columbia Waste and Sewer Authority (DCWASA). Washington Suburban Sanitary Commission (WSSC) provides safe drinking water and sewer services to Montgomery and Prince George's Counties and therefore shares the cost of maintaining the BPA WWTP with DCWASA.

There are also on-site disposal (septic) systems in the northern part of the Anacostia River watershed, specifically in the Northwest Branch around Sandy Spring and Spencerville and in the northern part of Northeast Branch around Beltsville and north of Beltsville. Table 2.4.1 presents the number of septic systems and total households per subwatershed. Figure 2.4.1 depicts the areas that are serviced by sewers and septic systems.

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 facility's permit, and must be reported to MDE's Water Management Administration, in accordance to COMAR 26.08.10, to be addressed under the State's enforcement program.

There were a total of 78 SSOs reported to MDE between September 2002 and November 2003 in Montgomery and Prince George's County. Approximately 5,662,960 gallons of SSO discharge was released through various waterways (surface water, groundwater, sanitary sewers, etc.) in the Prince George's County portion of Anacostia River watershed. In the Montgomery County portion of the Anacostia River watershed, there were approximately 31 SSOs reported between

September 2002 and November 2003, approximately 51,161 gallons of SSO discharge was released into various waterways (surface water and groundwater, sanitary sewers, etc.) (MDE, Water Management Agency). Figure 2.4.2 depicts the SSOs in the Anacostia River watershed.

Table 2.4.1: Septic Systems and Households Per Sub-Watershed in Anacostia River Watershed

Subwatershed	Station	Septic Systems (units)	Households per Subwatershed
Beaverdam Creek	BED0001	103	4,702
Indian Creek	INC0030	76	4,066
Northeast Branch	NEB0002	54	36,552
Northwest Branch	NWA0002	2	41,613
Northwest Branch	NWA0135	3,785	25,061
Paint Branch	PNT0001	2,927	41,172
Lower Beaverdam Creek		58	36,956
	TOTAL	**7,005**	**190,122**
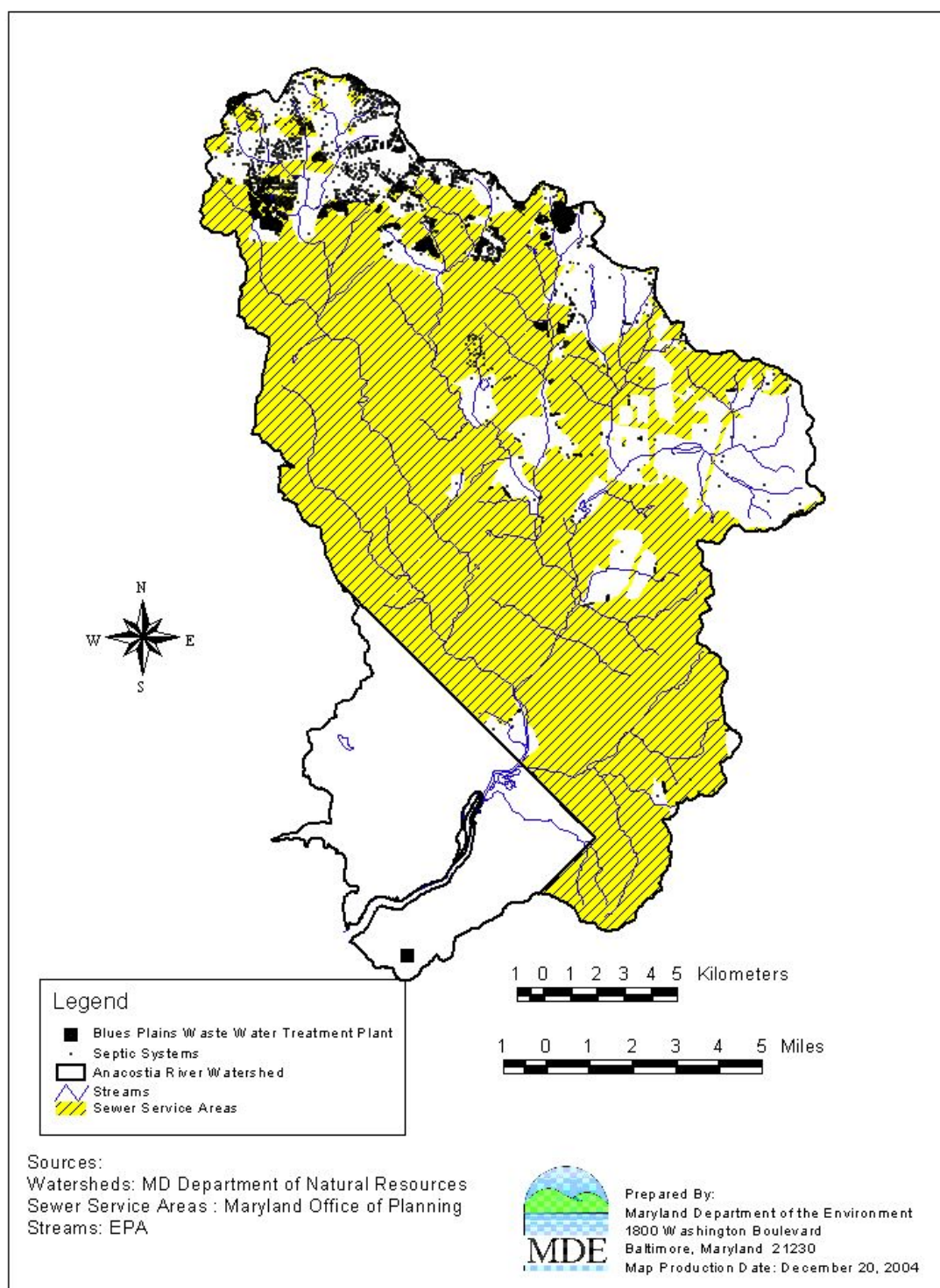
Figure 2.4.1: Sanitary Sewer Service and Septics Areas in the Anacostia River Watershed
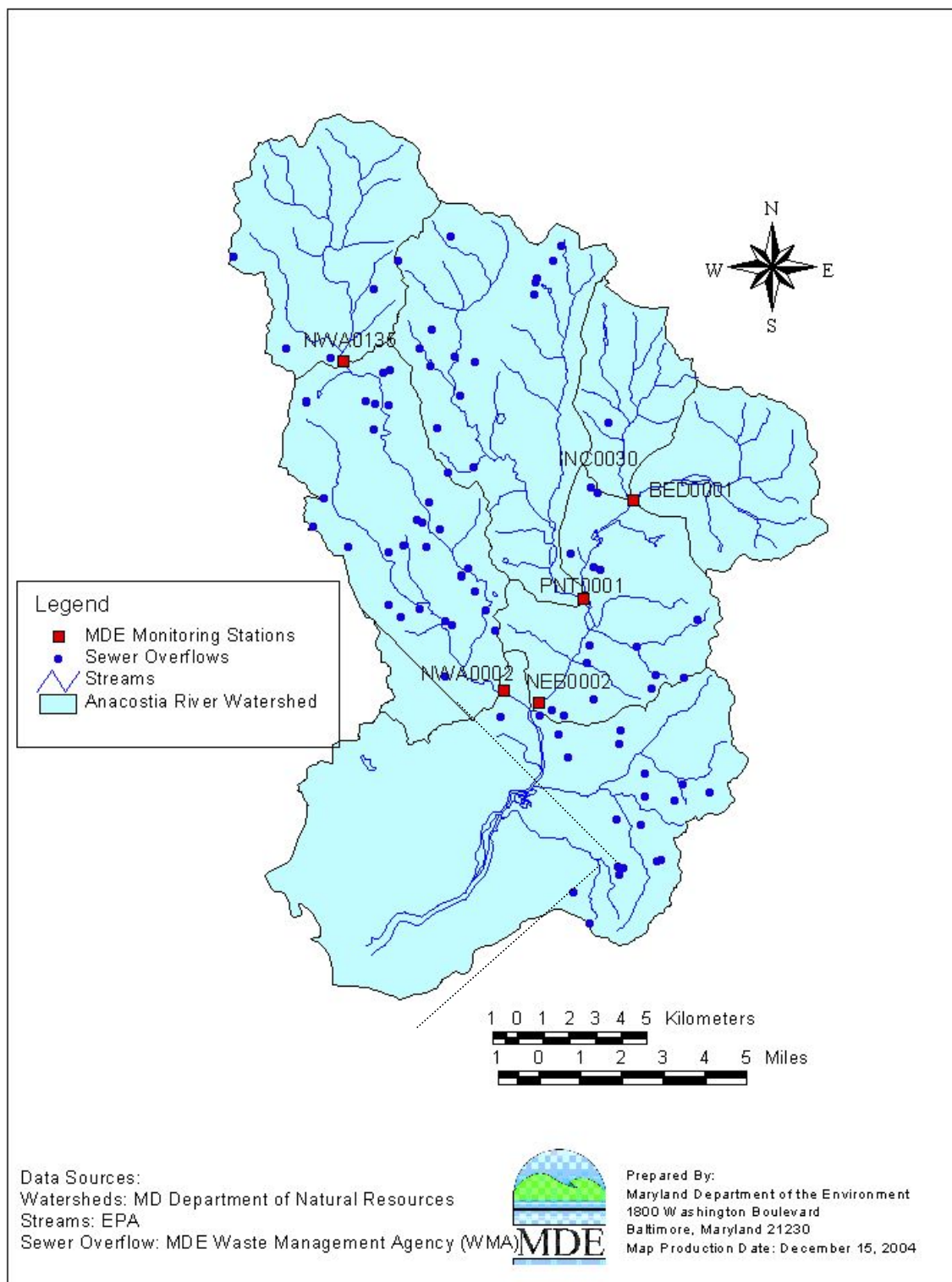
Figure 2.4.2: Sanitary Sewer Overflows in the Anacostia River Watershed

Point Source Assessment

Stormwater

The Anacostia River watershed is located in Montgomery and Prince George's Counties, which are both Phase I National Pollutant Discharge Elimination System (NPDES) Municipal Separate Storm Sewer System (MS-4) permit jurisdictions.   The MS-4 permits cover stormwater discharges from the municipal separate stormwater sewer systems in the Counties.

Municipal and Industrial WWTPs

Based on the point source permitting information, there are two NPDES permitted point source facilities regulated to discharge fecal bacteria directly into the Anacostia River watershed (Table 2.4.2 and Figure 2.4.3).   Based on personal communication with the Beltsville Agricultural Research Center and the USDA treatment plants operator (MDE, 2005), it is believed that approximately 30% of the fecal bacteria livestock contribution to the plant's inflow comes from livestock.

Table 2.4.2: Municipal NPDES Permit Holders in the Anacostia River Watershed (02-14-02-05)

Permittee	NPDES Permit No.	County	Average Annual Flow (MGD)	Fecal Coliform Concentrations Annual AVG (MPN/100ml)	Fecal Coliform Load Per Day (Billion MPN/day)
BARC East Side	MD0020842	Prince George's	0.198	2.09	0.25
Beltsville USDA West	MD0020851	Prince George's	0.124	4.03	0.15
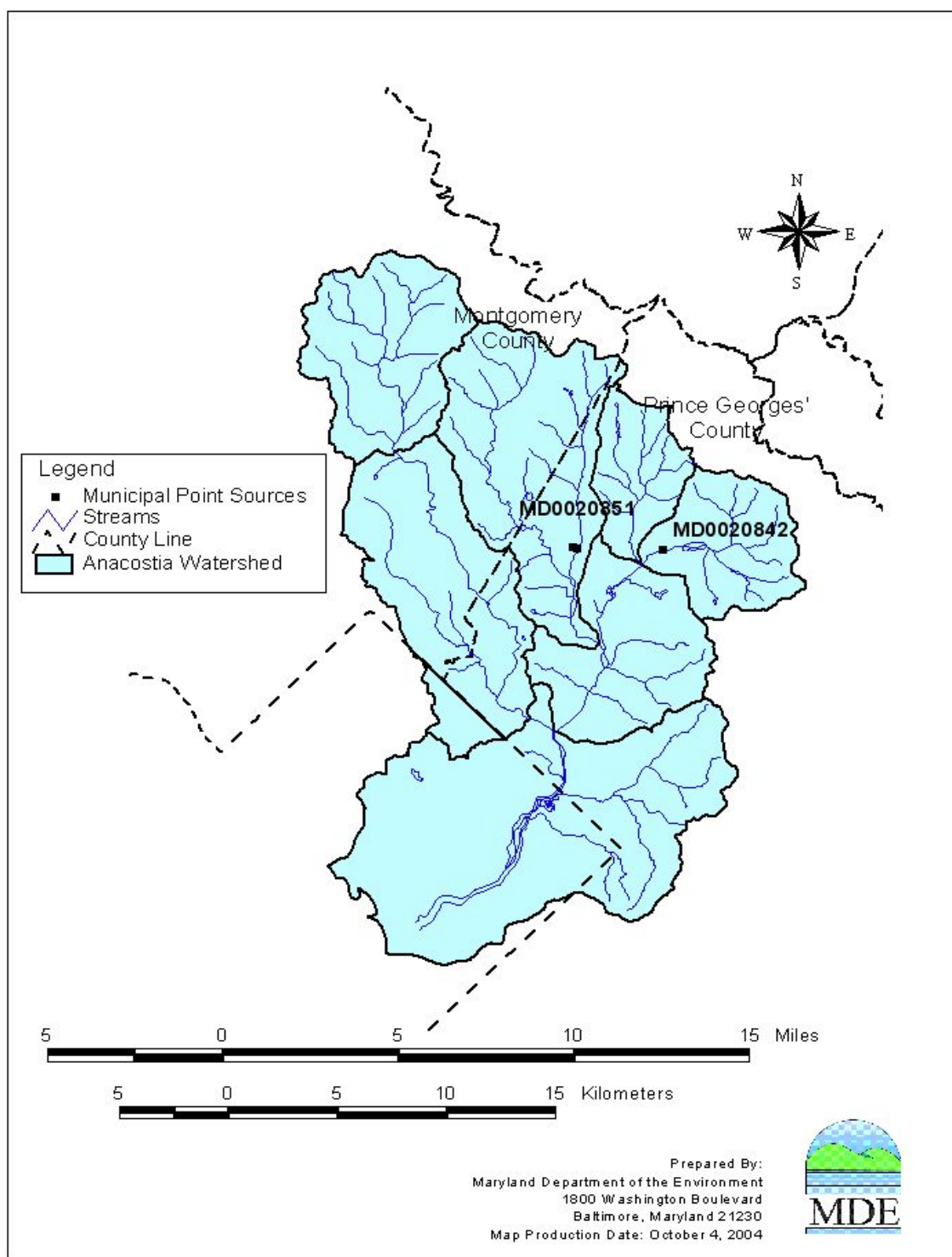
Figure 2.4.3: Permitted Point Sources Regulated to Discharge Fecal Bacteria in the Anacostia River Watershed

Bacteria Source Tracking

Bacteria source tracking (BST) was used to identify the relative contribution of bacteria to in-stream water samples. MDE conducted BST monitoring at six stations throughout the Anacostia River watershed area located upstream of the confluence, where 12 samples (one per month) were collected for a one-year duration. Bacteria 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 Maryland's BST methodology and data can be found in Appendix C. For the watershed area located downstream of the confluence, the fecal bacteria sources were obtained at a monitoring station, located at the Maryland/DC line, used in DC's bacteria source tracking study. DC's BST methodology analysis includes ARA coupled with Pulsed Field Gel Electrophoresis (PFGE). For information regarding DC's BST methodology, please refer to the document entitled "Identification of Fecal Bacteria Sources in District of Columbia Waterways Using Bacterial Source Tracking", August 2003.

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 both Maryland and DC's water quality standards in the Anacostia River watershed area. Maryland and DC 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 fecal bacteria TMDL development, with a discussion of the many complexities involved in the estimation of bacteria concentrations, loads and sources. The second section presents the TMDL analysis for the non-tidal area located upstream of the confluence, including 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 using DC's TMDL allocation to Maryland to derive the TMDL in the area downstream of the confluence. In section four, the TMDL for the entire Anacostia River watershed area within Maryland 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 non-point 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 accurate estimation of source inputs are difficult to develop. Finally, limited data are available to characterize the effectiveness of any program or practice in 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 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 and the TMDL previously developed by DC, appears to provide reasonable results (Cleland, 2003). Using this approach, Maryland can address more impaired streams in the same time period than by using the traditional water quality modeling methods.

4.2 TMDL Analysis for the Anacostia River Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence

Section 4.2 presents the TMDL analysis for the non-tidal Anacostia River watershed area located upstream of the NWB and NEB confluence. The first section presents the analysis framework and how the hydrological, water quality and BST data are linked together in the TMDL process. The second 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 specific to a free flowing stream system. The third section addresses the critical condition and seasonality. The fourth section presents the margin of safety. The fifth section discusses TMDL loading caps. The sixth section presents TMDL scenario descriptions. The seventh section presents the load allocations. Finally, in section eight, the TMDL equation for this area is summarized.

4.2.1 Analysis Framework for the Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence

The TMDL analysis for the non-tidal Anacostia River watershed area located upstream of the NWB and NEB confluence uses flow duration curves to identify flow intervals that are used as indicators of hydrological conditions (*i.e.,* annual average, 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 4.2.1.1 illustrates how the hydrological (flow duration curve), water quality and BST data are linked together for the TMDL development.
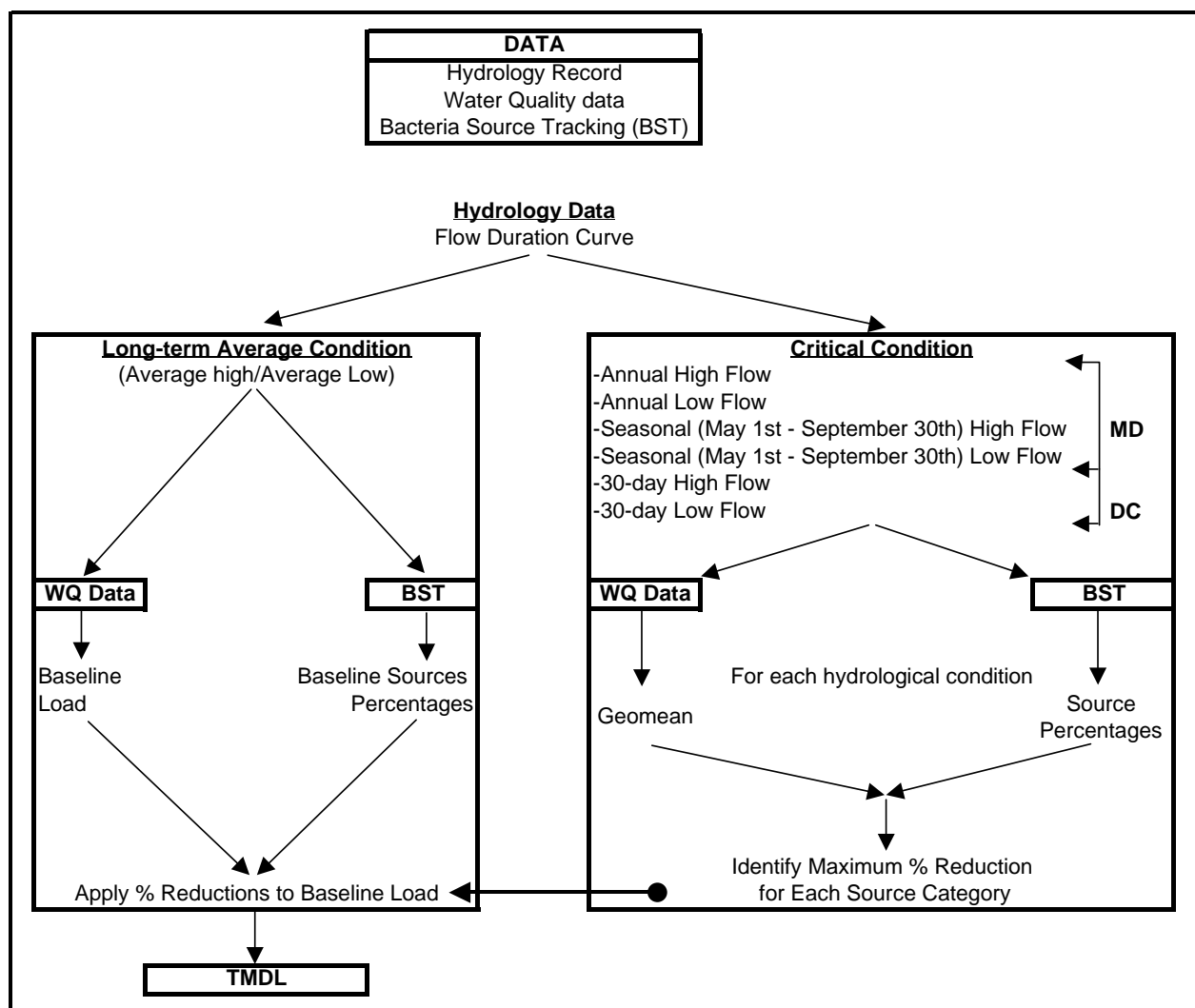
Figure 4.2.1.1: Diagram of the Bacteria TMDL Analysis Framework for the Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence

4.2.2 Estimating Baseline Loads in the Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence

Baseline loads estimated in this TMDL analysis for the non-tidal Anacostia River watershed area located upstream of the confluence are reported in long-term average loads.

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 smearing 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.

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:

$$L_i = Q_i * C_i * F_1 * F_2 \hspace{4cm} (6)$$

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 = Unit conversion factor (2.4466×10^9)
F_2 = Bias correction factor

To total baseline load is estimated as follows

$$L_t = \sum_{i=1}^{2} L_i * W_i \hspace{4cm} (7)$$

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

In the area located upstream of the confluence, a weighting factor of 0.3 for high flow and 0.7 for low flow were used to estimate the annual baseline load expressed as Billion MPN enterococci/day. Results are as follows:

Table 4.2.2.1: Baseline Load Calculations for the Subwatersheds Located Upstream of the Northwest Branch and Northeast Branch Confluence

Station	Area (sq. miles)	USGS Reference Gauge	Unit flow (cfs/sq. mile)	Q (cfs)	Enterococci Concentration (MPN/100ml)	Unit flow (cfs/sq. mile)	Q (cfs)	Enterococci Concentration (MPN/100ml)	Baseline Load (Billion MPN/day)	Weighted Geometric Mean Conc. MPN/100ml
BED0001	14.5	1650500	3.079	44.6	325	0.419	6.1	355	472.8	346
INC0030	9.8	1650500	3.079	30.3	215	0.419	4.1	100	162.6	126
PNT0001	32.2	1649500	3.381	109.0	206	0.473	15.2	28	544.6	51
NEB0002	76.8	1649500	3.381	259.8	296	0.473	36.4	55	1,839.7	91
NEB0002sub	20.3			75.9	140		10.9	37	258.9	55
NWA0135	21.8	1650500	3.079	67.1	284	0.419	9.1	90	478.5	127
NWA0002	52.2	1651000	2.818	147.1	386	0.431	22.5	85	1,354.8	134
NWA0002sub	30.4	N/A		80.0	163		13.4	42	318.0	63

FINAL

To treat each subwatershed as a separate entity, thus allowing separate load and reduction targets for watersheds that have one or more upstream monitored sub-watersheds, they were subdivided into unique watershed segments. The Northeast and Northwest Branches have upstream monitoring stations located upstream of NEB0002 and NWA0002 (Figure 4.2.2.1). The subwatersheds were defined with the extension sub to the station name (*e.g.*, NEB0002sub) 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. These 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:

$$\sum Q_{us} + Q_{sub} = Q_{ds} \qquad\qquad (8)$$

where

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

and the general equations for bacteria loading mass balance:

$$\sum (e^{kt} Q_{us} C_{us}) + Q_{sub} C_{sub} = Q_{ds} C_{ds} \qquad\qquad (9)$$

where

C_{us} = Upstream bacteria concentration
k = Bacteria decay coefficient (1/day)
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 concentrations 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 were a correlation with flow and concentration. For example, in two strata, the steady state geometric mean is estimated as follows:

$$L = Q_{high} W_{high} C_{high} + Q_{low} W_{low} C_{low} \qquad\qquad (10)$$

L = Average Load
Q_i = Average flow for stratum i
W_i= Proportion of stratum i
C_i = Concentration for stratum i
n_i = number of samples in stratum i

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:

$$C_{low} = \frac{L}{Q_{high}R*W_{high} + Q_{low}W_{low}} \qquad (11)$$

where

$$R = \frac{C_{high}}{C_{low}} \qquad (12)$$

and the final geometric mean concentration is estimated as follows:

$$GM = 10^{W_{high}\log_{10}(C_{high})+W_{low}\log_{10}(C_{low})} \qquad (13)$$

Source estimates from the bacteria source tracking analysis are completed for each station and are based on the contribution from the upstream watershed. Given the uncertainty of in-stream bacteria processes and the complexity involved in back-calculating an accurate source transport factor, the sources for NEB0002sub and NWA0002sub were assigned from the analysis for NEB0002 and NWA0002, respectively.
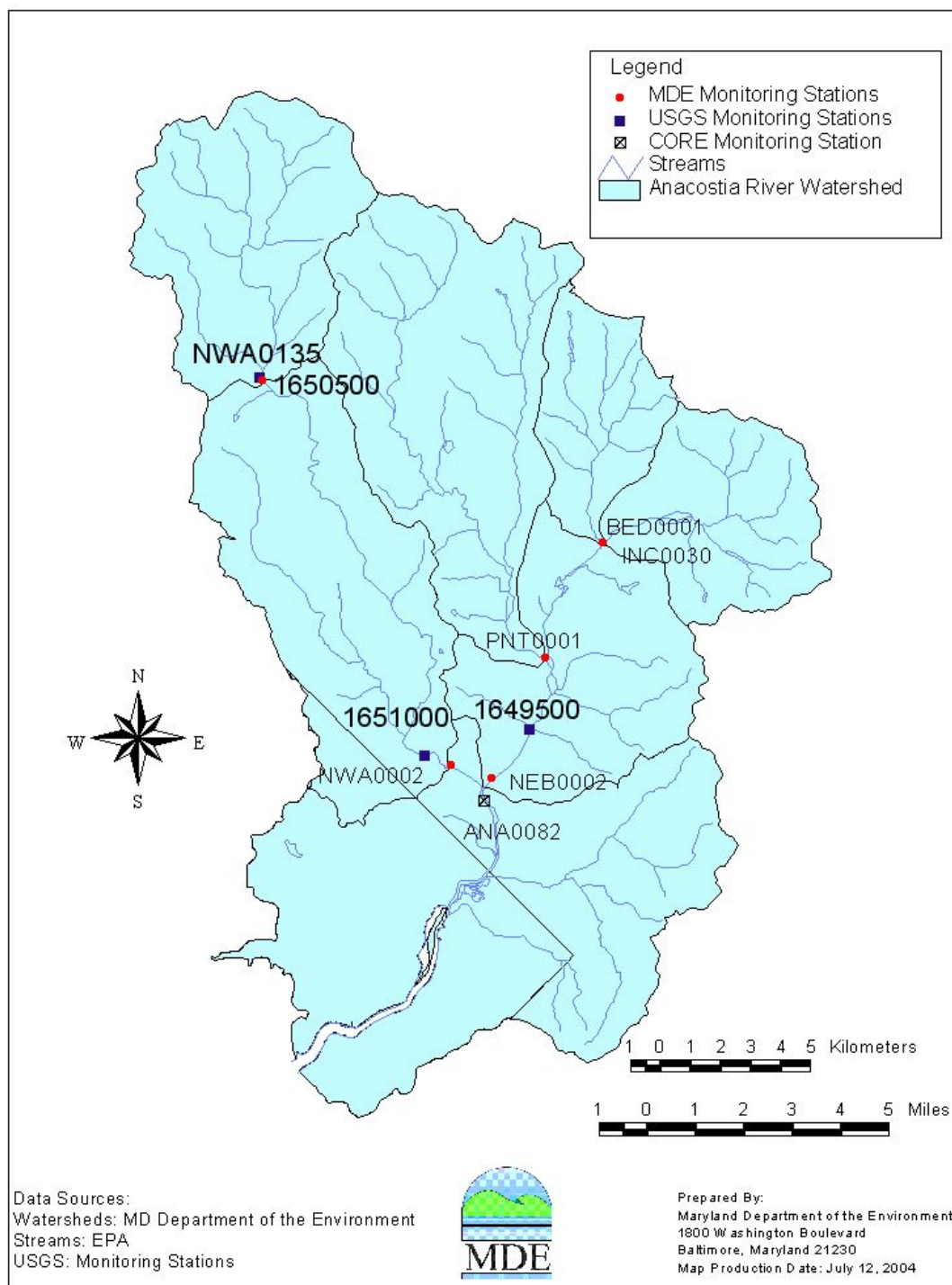
Figure 4.2.2.1: Monitoring Stations and Subwatersheds in the Anacostia River Basin

4.2.3 Critical Condition and Seasonality for the Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence

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 various hydrological conditions (high flow/low flow) including 30-day high flow and 30-day low flow conditions to be protective of designated uses for DC waters. DC's water quality standards are based on a 30-day geometric mean.

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 30% high flow and 70% low flow as defined in Appendix B. Using the definition of a high flow condition occurring when the daily flow duration interval is less than 30% and a low flow condition occurring when the daily flow duration interval is greater than 70%, critical hydrological conditions can be estimated by the percent of high or low flows during a specific period.

Maryland's proposed fecal bacteria TMDL for Anacostia River watershed located upstream of the confluence has been determined by assessing various hydrological conditions to account for critical conditions and seasonality. Furthermore, both Maryland and DC fecal bacteria water quality standards, independent of the bacterial densities or the indicator organism used in their corresponding analyses[2], are based on EPA's recommendations in "Quality Criteria for Water" of an accepted illness rate of 8 illnesses/1,000 swimmers. Therefore, Maryland's proposed TMDLs have been established to meet both Maryland and DC water quality standards, and will be protective of downstream designated uses under any hydrological condition.

Table 4.2.3.1 presents the seven hydrological conditions used to account for the critical condition and include the effects of seasonality.

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2 MD's criteria: 33 enterococci MPN/100ml with an acceptable swimming associated gastroenteritis rate of 8 illnesses per 1,000 swimmers. D. C.'s criteria: 200 fecal coliform MPN/100 ml with an acceptable swimming associated gastroenteritis rate of 8 illnesses per 1,000 swimmers.

Table 4.2.3.1: Hydrological Conditions Used to Account for Critical Condition and Seasonality in the Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence

Hydrological Condition		Averaging Period	Water Quality Data Used	Subwatershed	Fraction High Flow	Fraction Low Flow	Period
Annual	Average Condition	365 days	All	All	0.30	0.70	Long Term Average
	High Flow	365 days	All	BED0001; INC0030; NWA0135	0.55	0.45	April 8th, 1996 – March 23rd, 1997
				PNT0001; NEB0002; NEB0002sub	0.53	0.47	Nov 1st, 2002 – Oct 31st, 2003
				NWA0002; NWA0002sub	0.55	0.45	Jan 8th, 1996 – Jan 7th, 1997
	Low Flow	365 days	All	BED0001; INC0030; NWA0135	0.07	0.93	October 1st, 2002 – Sept 30th, 2003
				PNT0001; NEB0002; NEB0002sub	0.08	0.92	Sept 28th, 2002 – Sept 27th, 2003
				NWA0002; NWA0002sub	0.09	0.91	Sept 28th, 2002 – Sept 27th, 2003
Season	High Flow	May 1st – Sept 30th	May 1st – Sept 30th	BED0001; INC0030; NWA0135	0.52	0.48	May 1st - Sept 30th, 2003
				PNT0001; NEB0002; NEB0002sub	0.50	0.50	May 1st - Sept 30th, 2003
				NWA0002; NWA0002sub	0.52	0.48	May 1st - Sept 30th, 2003
	Low Flow	May 1st – Sept 30th	May 1st – Sept 30th	BED0001; INC0030; NWA0135	0.11	0.89	May 1st – Sept 30th, 2002
				PNT0001; NEB0002; NEB0002sub	0.12	0.88	May 1st – Sept 30th, 1991
				NWA0002; NWA0002sub	0.13	0.87	May 1st – Sept 30th, 1991
30-day	High Flow	30 days	All	BED0001; INC0030; NWA0135	1.00	0.00	Several occurrences during both Winter and Summer
				PNT0001; NEB0002; NEB0002sub	1.00	0.00	
				NWA0002; NWA0002sub	1.00	0.00	
	Low Flow	30 days	All	BED0001; INC0030; NWA0135	0.00	1.00	Several occurrences during both Winter and Summer
				PNT0001; NEB0002; NEB0002sub	1.00	0.00	
				NWA0002; NWA0002sub	1.00	0.00	

The critical condition is determined by the maximum reduction per source that satisfies all seven conditions, and 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 attributed to a bacteria source category will be constant through all conditions (*e.g.,* pets waste can be reduced to 75%).

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If the monitoring data cover a sufficient temporal span (at least one year), seasonality will also be included. The monitoring data used in this TMDL analysis cover a sufficient temporal span to estimate annual and critical conditions loads. The monitoring data for all stations located in the Anacostia River watershed have at least one year of data under varying hydrologic conditions.

Table 4.2.3.2 shows the reductions required in each non-tidal subwatershed of the Anacostia River located upstream of the confluence to meet water quality standards for both Maryland and DC's designated uses.

Table 4.2.3.2: Reductions Required to Meet Water Quality Standards in the Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence

Subwatershed	Hydrological Condition		Domestic Animals %	Human %	Livestock %	Wildlife %
BED0001	Annual	High Flow	98%	98%	98%	76%
		Low Flow	98%	98%	98%	75%
	Seasonal	High Flow	98%	97%	98%	69%
		Low Flow	98%	98%	98%	77%
	30-day	High Flow	98%	98%	98%	77%
		Low Flow	98%	98%	98%	75%
	Maximum Source Reduction		98%	98%	98%	77%
INC0030	Annual	High Flow	98%	98%	98%	45%
		Low Flow	98%	98%	97%	8%
	Seasonal	High Flow	98%	98%	98%	18%
		Low Flow	98%	98%	98%	24%
	30-day	High Flow	98%	96%	98%	66%
		Low Flow	98%	98%	97%	1%
	Maximum Source Reduction		98%	98%	98%	66%
PNT0001	Annual	High Flow	98%	98%	96%	15%
		Low Flow	0%	9%	41%	0%
	Seasonal	High Flow	66%	98%	72%	0%
		Low Flow	0%	62%	59%	0%
	30-day	High Flow	98%	98%	98%	72%
		Low Flow	0%	0%	0%	0%
	Maximum Source Reduction		98%	98%	98%	72%
NEB0002sub	Annual	High Flow	98%	95%	92%	0%
		Low Flow	49%	93%	14%	0%
	Seasonal	High Flow	90%	94%	79%	0%
		Low Flow	43%	94%	52%	0%
	30-day	High Flow	98%	95%	98%	49%
		Low Flow	29%	93%	0%	0%
	Maximum Source Reduction		98%	98%	98%	49%

Subwatershed	Hydrological Condition		Domestic Animals %	Human %	Livestock %	Wildlife %
NWA0135	Annual	High Flow	98%	98%	3%	0%
		Low Flow	27%	98%	63%	0%
	Seasonal	High Flow	72%	98%	69%	0%
		Low Flow	42%	98%	0%	0%
	30-day	High Flow	98%	98%	98%	14%
		Low Flow	11%	98%	59%	0%
	Maximum Source Reduction		98%	98%	98%	14%
NWA0002sub	Annual	High Flow	98%	98%	97%	18%
		Low Flow	43%	96%	71%	0%
	Seasonal	High Flow	98%	98%	98%	53%
		Low Flow	98%	98%	94%	49%
	30-day	High Flow	98%	98%	98%	51%
		Low Flow	17%	96%	68%	0%
	Maximum Source Reduction		98%	98%	98%	53%

4.2.4   Margin of Safety in the Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence

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 the TMDL of Anacostia River Watershed area located upstream of the confluence, the second approach was used by estimating the loading capacity of the stream based on a more stringent water quality criterion concentration. The enterococci water quality criterion concentration was reduced by 5%, from 33 enterococci MPN/100ml to 31.35 enterococci MPN/100ml.

4.2.5 TMDL Loading Caps for the Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence

The TMDL loading cap is an estimate of the assimilative capacity of the monitored watershed and is provided in MPN/day. The loading cap presented in this section is for the watershed located upstream of monitoring stations NWA0002 and NEB0002, on Northwest Branch and Northeast Branch, respectively.

The TMDL for this area is based on a long-term geometric mean of bacteria levels, and therefore the loads are not literal daily limits. Estimation of the TMDL 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 loading cap is estimated by first determining the baseline or current condition load and the associated geometric mean from the available monitoring data. The baseline load is estimated using the geometric mean concentration and average daily flow for each flow stratum. The loads from these two strata are then weighted (same as the estimated concentration, see Table 4.2.2.1), 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 accounting for the critical conditions. It is assumed that a reduction in concentration is proportional to a reduction in load and thus the TMDL is equal to the current baseline load multiplied by one minus the required reduction.

$$TMDL = L_b * (1 - R) \tag{14}$$
where

L_b = Current or baseline load estimated from monitoring data
R = Reduction required from baseline to meet water quality criterion

FINAL

The bacteria TMDL for the watershed upstream of monitoring stations NEB0002 and NWA0002 is:

Table 4.2.5.1: TMDL Summary for the Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence

Station	Baseline Load enterococci (Billion MPN/day)	TMDL Load enterococci (Billion MPN/day)	% Target Reduction
BED0001	473	42	91%
INC0030	163	20	88%
PNT0001	545	68	87%
NEB0002sub	259	53	79%
NWA0135	478	57	88%
NWA0002sub	318	70	78%
Total	2,236	310	

4.2.6 TMDL Scenarios Descriptions for the Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence

Source Distribution for the Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence

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 stratum (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).

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

$$MS_{i,k} = \frac{\sum_{j=1}^{n_i} \log_{10}(C_{i,j}) * S_{i,j,k}}{\sum_{j=1}^{n_i} \log_{10}(C_{i,j})} \tag{13}$$

where

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

$$MS_k = \sum_{i=1}^{2} M_{i,k} * W_i \tag{14}$$

MS = weighted mean proportion of isolates of source k
W_i = Proportion covered by stratum i

The complete distributions of the annual and seasonal periods source loads are listed in Tables 4.2.6.1 and 4.2.6.2. Details of the BST data and tables with the BST analysis results can be found in Appendix C.

Table 4.2.6.1: Distribution of Fecal Bacteria Source Loads in the Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence for the Annual Period

STATION	Flow Stratum	% Domestic Animals	% Human	% Livestock	% Wildlife	% Unknown
BED0001	High Flow	35.6	3.0	4.2	24.3	32.9
BED0001	Low Flow	24.3	11.7	6.2	17.7	40.2
BED0001	Weighted	27.7	9.1	5.6	19.7	38.0
INC0030	High Flow	26.3	2.9	15.1	29.0	26.7
INC0030	Low Flow	20.9	23.5	7.6	22.5	25.5
INC0030	Weighted	22.5	17.3	9.9	24.4	25.9
NEB0002	High Flow	35.6	3.6	8.0	33.7	19.1
NEB0002	Low Flow	9.4	7.9	25.2	23.7	33.7
NEB0002	Weighted	17.3	6.6	20.1	26.7	29.3
NWA0002	High Flow	21.6	9.0	9.4	23.6	36.4
NWA0002	Low Flow	18.5	11.0	2.8	28.9	38.8
NWA0002	Weighted	19.4	10.4	4.8	27.3	38.1
NWA0135	High Flow	28.4	26.0	3.9	7.0	34.6
NWA0135	Low Flow	14.6	40.9	3.7	8.0	32.8
NWA0135	Weighted	18.8	36.4	3.7	7.7	33.3
PNT0001	High Flow	12.5	12.4	8.4	34.5	32.2
PNT0001	Low Flow	23.8	18.0	4.0	26.7	27.6
PNT0001	Weighted	20.4	16.3	5.3	29.0	29.0

Table 4.2.6.2: Distribution of Fecal Bacteria Source Loads in the Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence for the Seasonal Period (May 1st – September 30th)

STATION	Flow Stratum	% Domestic Animals	% Human	% Livestock	% Wildlife	% Unknown
BED0001	High Flow	26.7%	1.1%	6.4%	26.6%	39.2%
	Low Flow	29.3%	6.6%	6.2%	31.3%	26.5%
	Weighted	28.5%	5.0%	6.3%	29.9%	30.3%
INC0030	High Flow	26.9%	16.1%	4.3%	10.5%	42.2%
	Low Flow	14.0%	30.1%	11.6%	14.0%	30.3%
	Weighted	17.9%	25.9%	9.4%	13.0%	33.8%
NEB0002	High Flow	24.7%	3.4%	7.9%	44.4%	19.7%
	Low Flow	18.7%	4.9%	15.6%	9.8%	50.9%
	Weighted	20.5%	4.5%	13.3%	20.2%	41.5%
NWA0002	High Flow	32.8%	5.7%	10.1%	24.1%	27.2%
	Low Flow	23.4%	17.5%	2.2%	36.4%	20.5%
	Weighted	26.2%	14.0%	4.6%	32.7%	22.5%
NWA0135	High Flow	32.7%	38.8%	1.1%	5.5%	22.0%
	Low Flow	9.7%	71.0%	0.0%	0.0%	19.3%
	Weighted	16.5%	61.3%	0.34%	1.6%	20.1%
PNT0001	High Flow	13.5%	15.9%	8.1%	21.1%	41.4%
	Low Flow	12.6%	30.2%	1.8%	23.5%	31.9%
	Weighted	12.8%	25.9%	3.7%	22.8%	34.7%

The final source distribution is derived from the source proportions listed in the above tables. 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 final source distribution for the annual period is presented in Table 4.2.6.3. As stated in Section 4.2.2, the source distribution for stations NEB0002sub and NWA0002sub, was based on the sources identified at stations NEW0002 and NWA0002, respectively.

Table 4.2.6.3: Annual Period Source Distributions Used in the TMDL Analysis for the Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence

Station	% Domestic Animals	% Human	% Livestock	% Wildlife	% Total
BED0001	45%	15%	9%	32%	100%
INC0030	30%	23%	13%	33%	100%
PNT0001	29%	23%	7%	41%	100%
NEB0002sub	24%	9%	28%	38%	100%
NWA0135	28%	55%	6%	12%	100%
NWA0002sub	31%	17%	8%	44%	100%

Practicable Reduction Targets

The MPR for each of the four source categories is listed in Table 4.2.6.4. These values are based on review of the available literature and best professional judgment. It is assumed that human sources would potentially have the highest risk of causing gastrointestinal illness and therefore should have the highest reduction. If a domestic WWTP is located in the upstream watershed, this is considered 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 4.2.6.4: Maximum Practicable Reduction Targets

Max Practicable Reduction per Source	Human	Domestic Animals	Livestock	Wildlife
	95%	75%	75%	0%
Rationale	(1) Direct source inputs. (2) Human pathogens more prevalent in humans than animals. (3) 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 presents a public health risk that is orders of magnitude less than that associated with human waste.[4]

1. EPA. 1984. Health Effects Criteria for Fresh Recreational Waters. EPA-600/1-84-004. U.S. Environmental Protection Agency, Washington, DC.
2. EPA. 1999. Preliminary Data Summary of Urban Storm Water Best Management Practices. EPA-821-R-99-012. U.S. Environmental Protection Agency, Washington, DC.
3. EPA. 2004. Agricultural BMP Descriptions as Defined for The Chesapeake Bay Program Watershed Model. Nutrient Subcommittee Agricultural Nutrient Reduction Workshop.
4. Environmental 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 MPR 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 assigned 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 4.2.6.4). The model was defined as follows:

$$\text{Min} \sum_{i=1}^{7} (P_h*5 + P_d*3 + P_l*3 + P_w*1) \qquad i = \text{hydrological condition}$$

Subject to

$C = C_{cr}$
$0 <= R_h <= 95\%$
$0 <= R_l <= 75\%$
$0 <= R_d <= 75\%$
$R_w = 0$
$P_h >= 1\%$, $P_h >= 3\%$ for BED0001,
$P_h, P_l, P_d, P_w >= 1\%$

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

The last two constraints do not allow the point source reduction to go beyond the permit limits. In all watersheds upstream of the confluence, the constraints of this scenario could not be satisfied, indicating there was not a practicable solution. A summary of the analysis is presented in the following Table 4.2.6.5.

Table 4.2.6.5: Practicable Reduction Results for the Subwatersheds Located Upstream of the Northwest Branch and Northeast Branch Confluence

Station	Applied Reductions				Achievable With MPRs?
	Domestic Animals %	Human %	Livestock %	Wildlife %	
BED0001	75%	95%	75%	0%	No
INC0030	75%	95%	75%	0%	No
PNT0001	75%	95%	75%	0%	No
NEB0002sub	75%	95%	75%	0%	No
NWA0135	75%	95%	75%	0%	No
NWA0002sub	75%	95%	75%	0%	No

The TMDL must specify load allocations that will meet the water quality standards. In the practicable reduction targets scenario none of the watersheds located upstream of the NWB and NEB could meet water quality standards based on MPRs. To further develop the TMDL, the constraints on the MPRs were relaxed in all subwatersheds where the water quality attainment was not achievable with the MPRs. In all 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 maximum practicable reduction constraints. The model was defined as follows:

$$\text{Min} \sum_{i=1}^{7} (P_h*5 + P_d*3 + P_l*3 + P_w*1) \qquad i = \text{hydrological condition}$$

Subject to

$C = C_{cr}$
$0 <= R_h <= 98\%$
$0 <= R_l <= 98\%$
$0 <= R_d <= 98\%$
$0 <= R_w <= 98\%$
$P_h >= 1\%$, $P_h >= 3\%$ for BED0001,
$P_h, P_l, P_d, P_w >= 1\%$

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
R_d = Reduction applied to domestic animal sources

C_{cr} = Water quality criterion
R_h = Reduction applied to human sources
R_l = Reduction applied to livestock sources
C = In-stream concentration

The summary of the analysis for the Anacostia River watershed upstream of the confluence is presented in the Table 4.2.6.6.

Table 4.2.6.6: TMDL Reduction Results for the Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence: Optimization Model Allowing Up to 98% Reduction

Station	% Domestic Animals	% Human	% Livestock	% Wildlife	% Target Reduction
BED0001	98%	98%	98%	81%	91%
INC0030	98%	98%	98%	66%	88%
PNT0001	98%	98%	98%	72%	87%
NEB0002sub	98%	95%	98%	49%	79%
NWA0135	98%	98%	98%	14%	88%
NWA0002sub	98%	98%	98%	53%	78%

4.2.7 TMDL Allocation for the Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence

The TMDL allocation includes waste load allocations (WLA) for point sources and stormwater (where MS4 permits are required), and the load allocation (LA) for nonpoint sources. The margin of safety is explicit and has been incorporated in the analysis by estimating the loading capacity of the stream based on a more stringent water quality endpoint concentration. The enterococci water quality criterion concentration was reduced by 5%, from 33 MPN/100ml to 31.35 MPN/100ml. The MOS is not specific as a separate term. TMDL allocations in the Anacostia River watershed located upstream of the confluence are based on critical conditions and meet both Maryland and DC bacteria water quality criteria, by taking into account a 30-day hydrological condition as specified in DC's water quality standards. The final loads are based on average hydrological conditions. The load reduction scenario results in a load allocation that will achieve water quality standards in Maryland and DC. The State reserves the right to revise these allocations provided such allocations 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 4.2.7.1. This table identifies how the TMDL will be allocated among WWTPs, MS4 permits and the LA.

Table 4.2.7.1: Potential Source Contributions for TMDL Allocations

Allocation Category	Human	Domestic Animals	Livestock	Wildlife
WWTP	X		X[1]	
MS-4		X		X
LA	X		X	X

1. Special condition for USDA treatment plant

For the human sources, the nonpoint source contribution (LA) is estimated by subtracting the WWTP load from the final human load. Where the entire watershed is covered by a MS4 permit(s), the domestic pet allocation is assigned to the MS4 WLA. Livestock is not covered by MS4 permits and will therefore be part of the LA when it is not included as part of a CAFO. Under special permit conditions, a WWTP may receive livestock sewage. This is the case for the Northeast Branch of the Anacostia River and therefore the approximate percentage of human vs. livestock is estimated from the WWTP and used only to get the final livestock LA. Based on personal communication with the USDA WWTPs (MDE, 2005) it is assumed that 30% of the total inflow to the plant is from livestock. Wildlife is split between MS4 and LA. This wildlife ratio is estimated based on the amount of urban pervious land (*e.g.*, residential) compared to other pervious land (*e.g.*, pasture, forest). Note that only the final LA or WLA is reported in this TMDL.

Municipal Separate Storm Sewer System (MS4) Allocations

Both individual and general NPDES MS4 Phase I and Phase II permits are point sources subject to WLA assignment in the TMDL. Quantification of rainfall-driven nonpoint source loads is uncertain. EPA recognized this in its guidance document entitled "Establishing Total Maximum Daily Load (TMDL) Wasteload Allocations (WLAs) for Storm Water Sources and NPDES Permit Requirements Based on Those WLAs" (November 2002), which states that available data and information usually are not detailed enough to determine WLAs for NPDES-regulated stormwater discharges on an outfall-specific basis. Therefore, in watersheds with an existing MS4 permit, domestic animal bacteria loads will be lumped into a single WLA-MS4 load. In watersheds with no existing individual MS4 permits, these loads will be included in the LA.

The jurisdictions within the Anacostia watershed, Montgomery and Prince George's Counties, are covered by individual Phase I MS4 program regulations. Based on EPA's guidance, the MS4 WLA is presented as one combined load for the entire land area of each county. In the future, when more detailed data and information become available, it is anticipated that MDE will revise the WLA into appropriate WLAs and LAs, and may also revise the LAs accordingly. Note that the overall reductions in the TMDL will not change.

Table 4.2.7.2 presents the MS4 loads by jurisdictions within the Anacostia River watershed.

Table 4.2.7.2: MS4 Stormwater Allocations for the Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence

Station	MS4 - Loads			
	Montgomery County (MD0068349)	Prince Georges County (MD0068284)	D.C.*	Total
	(Billion MPN/day)			
BED0001		9		9
INC0030		9		9
PNT0001	26	15		41
NEB0002sub		34		34
NWA0135	32			32
NWA0002sub*	32	17	5*	54
Total	90	84	5*	179

*Subwatershed NWA002sub has 9% of its area in D.C.

Municipal WWTP

There are two point source facilities with permits regulating the discharge of bacteria directly into the Anacostia River watershed. See Table 4.2.7.3. The flow used in the TMDL allocation is based on the flow specified in the NPDES permit. Since Maryland has now adopted new indicator bacteria organisms, it is expected that the revised permit will now specify geometric mean concentrations for enterococci instead of fecal coliform.

Table 4.2.7.3: Municipal Waste Water Treatment Plants

Permittee	NPDES Permit No.	County	Permit Flow (MGD)	Permit Enterococci Concentration (MPN/100ml)	Permit Load (Billion MPN/day)	% of TMDL
BARC East Side	MD0020842	Prince George's	0.62	33	0.77	1.89%
Beltsville USDA West	MD0020851	Prince George's	0.20	33	0.25	0.36%

4.2.8 Anacostia River Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence – TMDL Summary

The TMDL for the Anacostia River watershed located upstream of the NWB and NEB confluence is presented below.

Table 4.2.8.1: TMDL for the Anacostia River Watershed Located Upstream of the Northwest Branch and Northeast Branch Confluence

Station	TMDL Load Enterococci (Billion MPN/day)	LA Load Enterococci (Billion MPN/day)	WLA-PS Load Enterococci (Billion MPN/day)	WLA-MS4 Load Enterococci (Billion MPN/day)
BED0001	42	32	0.8	9
INC0030	20	11	0	9
PNT0001	68	27	0.2	41
NEB0002sub	55	21	0	34
NWA0135	57	25	0	32
NWA0002sub	68	14	0	54
Total	310	130	1	179

In all six non-tidal subwatersheds located upstream of the NWB and NEB confluence, based on the maximum practicable reduction rates specified, water quality standards cannot be achieved. This can occur in watersheds with wildlife as a significant component or 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.

TMDL Analysis for the Anacostia River Watershed Located Between the Northwest Branch (NWB) and Northeast Branch (NEB) Confluence and Maryland/DC Line

This section presents the TMDL analysis for the Anacostia River watershed area located between the NWB and NEB confluence and the Maryland/DC line.   This region begins where the NWB and NEB meet to form the Anacostia River, and includes the tidal portion of the subwatershed. The area is approximately 13,726 acres and is highly urbanized.

4.3.1 Analysis Framework for the Watershed Located Between the Northwest Branch and Northeast Branch Confluence and Maryland/DC Line

Background

The TMDL for the Anacostia River watershed area located between the NWB and NEB confluence and the Maryland/DC line was developed on the basis of the allocation to MD specified in DC's fecal bacteria TMDL. In October 2003, EPA approved DC's "TMDLs for fecal coliform bacteria in the tidal Anacostia River and its tributaries". DC's TMDL includes an allocation to Maryland and this allocation is based on meeting water quality standards in DC waters.

A summary of DC's approved TMDLs for fecal coliform bacteria including the allocation to Maryland is presented in Table 4.3.1.1.

Table 4.3.1.1: DC's Fecal Coliform TMDL Summary

Segment	TMDL	WLA	LA	Upstream (Allocation to MD)	MOS
	MPN/year				
Upper Anacostia	1.99×10^{15}	1.63×10^{15}	1.11×10^{13}	0.348×10^{15}	Implicit
Lower Anacostia	0.827×10^{15}	0.821×10^{15}	0.598×10^{13}		Implicit
Total	2.83×10^{15}	2.46×10^{15}	1.71×10^{13}	0.348×10^{15}	

4.3.2 TMDL Analysis for the Watershed Located Between the Northwest Branch and Northeast Branch Confluence and MD/DC Line

Maryland and DC use different pathogen indicator organisms in their bacteriological water quality standards. To estimate the TMDL for this area based on DC's allocation to Maryland, first it is necessary to convert DC's fecal coliform TMDL to an enterococci-based TMDL. The pathogen indicator organism used in DC's TMDL analysis was fecal coliform, whereas Maryland, which recently adopted EPA recommended bacteria indicator organisms (*E. coli* and enterococci), used enterococci for its bacteria TMDL analysis (See Section 2.2). Although using different indicator organisms, both Maryland and DC fecal bacteria water quality standards, independent of the bacterial densities and indicator organism used in their corresponding analyses, are based on EPA's recommendations in "Quality Criteria for Water" of an accepted illness rate of 8 illnesses/1,000 swimmers.

A correlation analysis between *E. coli* and enterococci was performed using data collected from designated Use I sites in Maryland during late May-September, 1999 and late May-September,

2000. These data were originally collected to compare fecal coliform and enterococci in Maryland's waters, primarily beaches. These data were incorporated in MDE's study entitled "A Quantitative Evaluation of the Impact of Adopting the EPA's Recommended Recreational Water Quality Criteria for Enterococci and *E. coli* in Maryland" (MDE, 2001), still in draft form. A total of 173 samples were tested for both organisms and paired fecal coliform and enterococci results were obtained. Thirty-day running geometric means were calculated for each site and for each indicator organism. The resulting 173 paired thirty-day running geometric means were based on 5 or more samples. With the paired geometric means, ratios of enterococci to *E. coli* were then calculated. Statistics were run for these ratios and the results are as follows:

Table 4.3.2.1: Statistical Parameters for Enterococci/Fecal Coliform Ratios from Correlation analysis

	Ratio Enterococci/Fecal Coliform
Median	0.30
Mean	0.25
Geomean	**0.34**
Minimum	12.53
Maximum	0.03
St Dev	1.19
Count	173

The results in Table 4.3.2.1 show that correlation between enterococci and fecal coliform in a waterbody varies significantly. Ratios of enterococci to fecal coliform range from 0.03 to 12.53 with median, mean and geometric means of 0.30, 0.25 and 0.34, respectively. The geometric mean of 0.34 was the most appropriate to use in the conversion because it more accurately represents the bacteria loading rates in the three areas of concern of this analysis: 1) the Anacostia River watershed area within DC boundaries, 2) the Anacostia River watershed area upstream of the confluence, and 3) the Anacostia River watershed area downstream of the confluence. Using a different ratio (*i.e.*, the median of 0.30 or the mean of 0.25) would result in different loading rates for the three subwatersheds mentioned.

The loading rates analysis results and the TMDL for the area are shown below in Table 4.3.2.2.

Table 4.3.2.2: Enterococci/Fecal Coliform Correlation and Loading Rates Analysis Results

	TMDL Load	÷	Ratio Enterococci/FC	=	TMDL Load (Billion Fecal Coliform/year)	Area Covered by TMDL (acres)	TMDL Loading Rate (billion fecal coliform MPN/Ac/yr)
DC TMDL Allocation to MD	348,000 Billion Fecal Coliform/year	÷	**N/A**	=	348,000 Billion Fecal Coliform/year	94,387	**3.7**
MD TMDL for Area Upstream of Confluence of Northwest Branch and Northeast Branch	99,687 Billion Enterococci/year	÷	0.34	=	296,688 Billion Fecal Coliform/year	80,661	**3.7**

The TMDL for the Anacostia River watershed located downstream of the confluence is calculated by subtracting Maryland's TMDL estimate from DC's allocation to Maryland:

MD TMDL (Between NWB and NEB confluence and MD/DC line)	=	**DC TMDL** Allocation to MD	-	**MD TMDL** (Upstream of Confluence of Northwest Branch and Northeast Branch)
51,312 Billion fecal coliform MPN/year	=	348,000 Billion fecal coliform MPN/year	-	296,688 Billion fecal coliform MPN/year

Finally, the TMDL for the area is converted to the enterococci based TMDL as follows:

Fecal Coliform MD TMDL (Between NWB and NEB confluence and MD/DC line)				X	**Ratio Ecocc/FC**	=	**Enterococci MD TMDL** (Between NWB and NEB confluence and MD/DC line)
51,312 Billion fecal coliform MPN/year	÷	365 days	=	141 Billion fecal coliform MPN/day	X	0.34	=	47.2 Billion enterococci MPN/day

4.3.3 TMDL Allocation

This section details how the TMDL for the Anacostia watershed area downstream of the confluence is allocated between waste load allocations (WLA) for point sources and stormwater (where MS4 permits are required), and load allocation (LA) for nonpoint sources. Critical conditions and seasonality are implicitly accounted for in this area TMDL as it was derived from DC's TMDL. The same critical conditions and seasonality used in DC and Maryland TMDLs apply to this area's TMDL. The margin of safety is implicit for the same reasons and is not specified as a separate term.

The bacteria source distribution for this area is derived from the source proportions from DC's BST study. As explained in Section 2.4, the fecal bacteria sources were obtained at a monitoring station, located at the Maryland/DC line, used in DC's bacteria source tracking study. DC's BST methodology analysis includes ARA coupled with Pulsed Field Gel Electrophoresis (PFGE). The average bacteria source proportions in this area are as follows:

Table 4.3.3.1: DC Average Bacteria Source Distribution for Anacostia Watershed Area Downstream of the NWB and NEB Confluence and Upstream of the Maryland/DC Line

Source Category	Birds	Human	Livestock	Pets	Wildlife	Total
%	27.5	22.1	0.3	21.1	29.0	100.0

For the TMDL analysis and allocations, and to be consistent with Maryland's source distribution, the percentage of sources identified as "birds" were added to the wildlife. The average annual final source distribution is presented in Table 4.3.3.2.

Table 4.3.3.2: DC Final Average Bacteria Source Distribution for Anacostia Watershed Downstream of the NWB and NEB Confluence and Upstream of the Maryland/DC Line

Source Category	Domestic Animals	Human	Livestock	Wildlife	Total
%	21.1%	22.2%	0.3%	56.5%	100.0

The TMDL for this area will be allocated among WWTPs, MS4 permits and the LA in the same manner as the TMDL for the area located upstream of the confluence. (See Section 4.2.3 and Table 4.2.3.1). There are no WWTPs located in this area of the Anacostia River watershed.

4.3.4   Anacostia River Watershed Downstream of the NWB and NEB Confluence and Upstream of the Maryland/DC Line - TMDL Summary

The TMDL for the Anacostia River watershed located <u>downstream of the NWB and NEB confluence</u> and upstream of the Maryland/DC Line is presented below.

Table 4.3.4.1: TMDL for the Anacostia River Watershed Downstream of the NWB and NEB Confluence and Upstream of the Maryland/DC Line

Subwatershed	TMDL Load Enterococci (Billion MPN/day)	LA Load Enterococci (Billion MPN/day)	WLA-PS Load Enterococci (Billion MPN/day)	WLA-MS-4 Load Enterococci (Billion MPN/day)
Area downstream of the NWB and NEB Confluence and upstream of the MD/DC line	47	16	0	31

4.4   Anacostia River Watershed TMDL Summary

The fecal bacteria TMDL for the entire Anacostia River watershed located within Maryland boundaries is 357 billion MPN enterococci/day. The TMDLs are distributed between load allocation (LA) for non-point sources and waste load allocations (WLA) for point sources, including National Pollutant Discharge Elimination System (NPDES) wastewater treatment plants (WWTPs) and NPDES municipal separate storm sewer systems (MS4). The total LA is 146 billion MPN/day.   The WWTPs' WLA is 1.0 billion MPN/day and the MS4 WLA is 210 billion MPN/day. The TMDL has been allocated among the non-tidal watershed located upstream of the confluence of NWB and NEB and the watershed located downstream of that same confluence and upstream of the Maryland/DC line, and is presented in Table 4.4.1.

Table 4.4.1: Anacostia River Fecal Bacteria TMDL Allocations

Subwatershed	TMDL	LA	WLA-MS4	WLA-WWTP
	Billion MPN Enterococci/day			
Upstream of Confluence of Northwest Branch and Northeast Branch	310	130	179	1
Downstream of Confluence of Northwest Branch and Northeast Branch and Upstream of MD/DC line	47	16	31	0
TOTAL (Derived from DC's TMDL)	357	146	210	1

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 Anacostia River watershed, the TMDL analysis indicates that reduction of fecal bacteria loads from all sources including wildlife are beyond the maximum practicable reduction (MPR) targets. The extent of the fecal bacteria load reductions required to meet water quality criteria in the six subwatersheds of the non-tidal Anacostia River and in downstream waters are not feasible by effluent limitations (there are no point sources in the tidal watershed), nor by implementing cost-effective and reasonable best management practices to nonpoint sources. Therefore, MDE proposes a staged approach of implementation beginning with the MPR scenario, with regularly scheduled follow-up monitoring to assess the effectiveness of the implementation plan.

For all subwatersheds, the final scenario is based on reductions that are beyond the MPR targets. These MPR targets were defined based on a literature review of BMP effectiveness and assuming a zero reduction for wildlife sources. The uncertainty of BMP effectiveness for bacteria, reported within the literature, is quite large. As an example, pet waste education programs have varying results based on stakeholder involvement. Additionally, the extent of wildlife reduction associated with various BMP methods (*e.g.*, structural, non-structural, *etc.*) is uncertain. Therefore, MDE intends for the required reductions to be implemented in a staged 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.

In 1983, the EPA Nationwide Urban Runoff Program found that stormwater runoff from urban areas contains the same general types of pollutants found in wastewater, and that 30% of identified cases of water quality impairment were attributable to stormwater discharges. In November 1990, EPA required jurisdictions with a population greater than 100,000 to apply for NPDES Permits for stormwater discharges. The two Maryland jurisdictions where the Anacostia River watershed is located, Montgomery and Prince George's Counties, are required to participate in the stormwater NPDES program, and have to comply with the NPDES Permit regulations for stormwater discharges. The permit-required management programs are being implemented in both counties to meet locally established watershed protection and restoration goals and to control stormwater discharges to the maximum extent. Potential funding available for local governments includes the State Water Quality Revolving Loan Fund and the Stormwater Pollution Cost Share Program. Details of these programs and additional funding sources can be found at http://www.dnr.state.md.us/bay/services/summaries.html

Additional potential funding sources for implementation include 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 result in 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 implementing 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 20 WRAS projects in which local people identify local watershed priorities for restoration, protection and implementation. The WRAS project area in Prince George's County, Maryland totals about 86 square miles including portions of municipalities that are in the watershed. For this part of the watershed, Prince George's County is working on a WRAS project to be completed in 2005. In the WRAS, the County will identify and prioritize local restoration and protection needs associated with water quality and habitat (DNR - WRAS Program, 2005).

The WRAS also includes a stream corridor assessment where the locations of exposed pipes in the Anacostia River were identified. This information provides guidance for locating potential input from infrastructure. However, additional information is required to determine if there is a bacteria input from an exposed or failing pipe. A stream corridor assessment was done for Sligo Creek, in the Northwest portion of the Anacostia River (Figure 5.1).

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, 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 taking appropriate steps to address the cause(s) of the overflows. See COMAR 26.08.10.03.

> 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. Under Section 106 of the Clean

Water Act, EPA is authorized to issue grants to states for the purpose of assisting in establishing and carrying out pollution control programs.

In 2004, the United States and the State of Maryland brought suit against WSSC in the U.S. District Court for the District of Maryland to remedy recurrent SSOs from the WSSC system, *United States et al. v. Washington Suburban Sanitary Commission,* C.A. No. PJM 04-3679 (Greenbelt Division). A consent decree was negotiated among the United States, Maryland, several intervenor citizen groups and WSSC, and lodged on July 26, 2005. It is now before the court for approval. WSSC already reports overflows to MDE as required by Environment Article, Section 9-331.1, Annotated Code of Maryland, and COMAR 26.08.10.
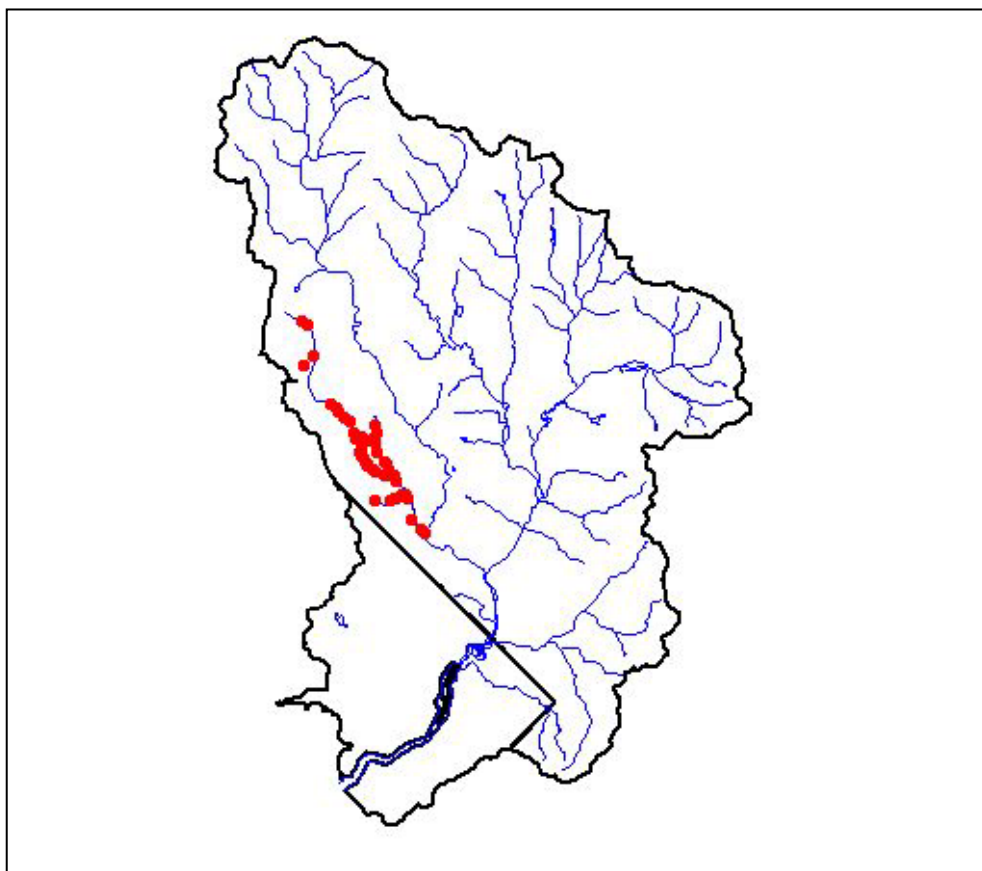
Figure 5.1: Exposed Pipes Found in Sligo Creek in the Anacostia River Watershed

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. However, while neither Maryland nor EPA is proposing the elimination of wildlife to allow for the attainment of water quality standards, managing the overpopulation of wildlife remains an option for state and local stakeholders.

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

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Appendix A – Bacteria Concentration Raw Data per Sampling Date with Corresponding Daily Flow Frequency and Water Quality Data Figures

Table A-1: Enterococci Concentrations per Water Quality Stations with Corresponding Daily Flow Frequency.

SAMPLING STATION IDENTIFIER	Date	Daily Flow Frequency	Enterococci MPN/100ml
BED0001	10/07/2002	97.8	1860
BED0001	10/21/2002	87.2	840
BED0001	11/06/2002	8.0	8660
BED0001	11/18/2002	6.1	4110
BED0001	12/02/2002	70.0	210
BED0001	12/16/2002	28.6	750
BED0001	01/06/2003	20.8	1400
BED0001	01/21/2003	50.6	660
BED0001	02/03/2003	47.1	780
BED0001	03/03/2003	5.2	520
BED0001	03/17/2003	24.2	20
BED0001	04/21/2003	26.9	110
BED0001	05/05/2003	32.9	190
BED0001	05/19/2003	17.9	200
BED0001	06/02/2003	25.5	50
BED0001	06/16/2003	25.5	370
BED0001	06/24/2003	24.2	100
BED0001	07/07/2003	11.2	290
BED0001	07/21/2003	50.6	100
BED0001	08/04/2003	39.7	470
BED0001	08/18/2003	43.8	270
BED0001	08/25/2003	70.2	470
BED0001	09/08/2003	66.1	460
BED0001	09/22/2003	22.7	100
BED0001	10/06/2003	55.2	70
BED0001	10/20/2003	51.9	230
INC0030	10/07/2002	97.8	570

SAMPLING STATION IDENTIFIER	Date	Daily Flow Frequency	Enterococci MPN/100ml
INC0030	10/21/2002	87.2	130
INC0030	11/06/2002	8.0	7270
INC0030	11/18/2002	6.1	990
INC0030	12/16/2002	28.6	190
INC0030	01/06/2003	20.8	100
INC0030	01/21/2003	50.6	10
INC0030	02/03/2003	47.1	10
INC0030	03/03/2003	5.2	50
INC0030	03/17/2003	24.2	30
INC0030	04/21/2003	26.9	140
INC0030	05/05/2003	32.9	70
INC0030	05/19/2003	17.9	7700
INC0030	06/02/2003	25.5	110
INC0030	06/16/2003	25.5	130
INC0030	06/24/2003	24.2	60
INC0030	07/07/2003	11.2	110
INC0030	07/21/2003	50.6	280
INC0030	08/04/2003	39.7	300
INC0030	08/18/2003	43.8	310
INC0030	08/25/2003	70.2	160
INC0030	09/08/2003	66.1	60
INC0030	09/22/2003	22.7	100
INC0030	10/06/2003	55.2	50
INC0030	10/20/2003	51.9	150
NEB0002	10/07/2002	98.7	20
NEB0002	10/21/2002	89.3	60
NEB0002	11/06/2002	6.3	7700
NEB0002	11/18/2002	6.4	1240
NEB0002	12/02/2002	78.5	60
NEB0002	12/16/2002	24.0	190
NEB0002	01/06/2003	16.0	230

SAMPLING STATION IDENTIFIER	Date	Daily Flow Frequency	Enterococci MPN/100ml
NEB0002	01/21/2003	58.6	10
NEB0002	02/03/2003	53.3	390
NEB0002	03/03/2003	10.1	200
NEB0002	03/17/2003	29.1	60
NEB0002	04/21/2003	39.6	50
NEB0002	05/05/2003	47.6	30
NEB0002	05/19/2003	16.5	500
NEB0002	06/02/2003	23.2	170
NEB0002	06/16/2003	9.0	160
NEB0002	06/23/2003	16.5	310
NEB0002	07/07/2003	9.9	70
NEB0002	07/21/2003	33.0	10
NEB0002	08/04/2003	35.3	260
NEB0002	08/18/2003	40.6	360
NEB0002	08/25/2003	66.8	10
NEB0002	09/08/2003	62.7	50
NEB0002	09/22/2003	27.7	300
NEB0002	10/06/2003	61.1	120
NEB0002	10/20/2003	61.1	100
NWA0002	10/07/2002	99.1	30
NWA0002	10/21/2002	88.6	170
NWA0002	11/06/2002	6.4	4110
NWA0002	11/18/2002	6.7	2600
NWA0002	12/02/2002	68.6	140
NWA0002	12/16/2002	29.9	420
NWA0002	01/06/2003	16.9	290
NWA0002	01/21/2003	50.6	110
NWA0002	02/03/2003	63.2	70
NWA0002	03/03/2003	5.8	400
NWA0002	03/17/2003	29.9	20
NWA0002	04/21/2003	40.3	40

SAMPLING STATION IDENTIFIER	Date	Daily Flow Frequency	Enterococci MPN/100ml
NWA0002	05/05/2003	38.1	20
NWA0002	05/19/2003	17.3	380
NWA0002	06/02/2003	25.6	90
NWA0002	06/16/2003	21.2	160
NWA0002	06/23/2003	17.3	120
NWA0002	07/07/2003	10.6	4110
NWA0002	07/21/2003	53.7	30
NWA0002	08/04/2003	36.9	1580
NWA0002	08/18/2003	42.9	310
NWA0002	08/25/2003	66.7	30
NWA0002	09/08/2003	63.2	40
NWA0002	09/22/2003	39.1	1500
NWA0002	10/06/2003	53.7	10
NWA0002	10/20/2003	50.6	70
NWA0135	10/07/2002	97.8	390
NWA0135	10/21/2002	87.2	110
NWA0135	11/06/2002	8.0	10460
NWA0135	11/18/2002	6.1	2720
NWA0135	12/02/2002	70.0	100
NWA0135	12/16/2002	28.6	170
NWA0135	01/06/2003	20.8	100
NWA0135	01/21/2003	50.6	10
NWA0135	02/03/2003	47.1	30
NWA0135	03/03/2003	5.2	430
NWA0135	03/17/2003	24.2	20
NWA0135	04/21/2003	26.9	10
NWA0135	05/05/2003	32.9	10
NWA0135	05/19/2003	17.9	630
NWA0135	06/02/2003	25.5	100
NWA0135	06/16/2003	25.5	100
NWA0135	06/23/2003	18.7	90

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SAMPLING STATION IDENTIFIER	Date	Daily Flow Frequency	Enterococci MPN/100ml
NWA0135	07/07/2003	11.2	19860
NWA0135	07/21/2003	50.6	160
NWA0135	08/04/2003	39.7	640
NWA0135	08/18/2003	43.8	1510
NWA0135	08/25/2003	70.2	190
NWA0135	09/08/2003	66.1	60
NWA0135	09/22/2003	22.7	170
NWA0135	10/06/2003	55.2	40
NWA0135	10/20/2003	51.9	30
PNT0001	10/07/2002	98.7	40
PNT0001	10/21/2002	89.3	70
PNT0001	11/06/2002	6.3	4350
PNT0001	11/18/2002	6.4	1180
PNT0001	12/02/2002	78.5	20
PNT0001	12/16/2002	24.0	60
PNT0001	01/06/2003	16.0	170
PNT0001	01/21/2003	58.6	10
PNT0001	02/03/2003	53.3	10
PNT0001	03/17/2003	29.1	10
PNT0001	04/21/2003	39.6	10
PNT0001	05/05/2003	47.6	10
PNT0001	05/19/2003	16.5	560
PNT0001	06/02/2003	23.2	70
PNT0001	06/16/2003	9.0	120
PNT0001	06/23/2003	16.5	170
PNT0001	07/07/2003	9.9	170
PNT0001	07/21/2003	33.0	10
PNT0001	08/04/2003	35.3	120
PNT0001	08/18/2003	40.6	500
PNT0001	08/25/2003	66.8	20
PNT0001	09/08/2003	62.7	20

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SAMPLING STATION IDENTIFIER	Date	Daily Flow Frequency	Enterococci MPN/100ml
PNT0001	09/22/2003	27.7	400
PNT0001	10/06/2003	61.1	30
PNT0001	10/20/2003	61.1	50
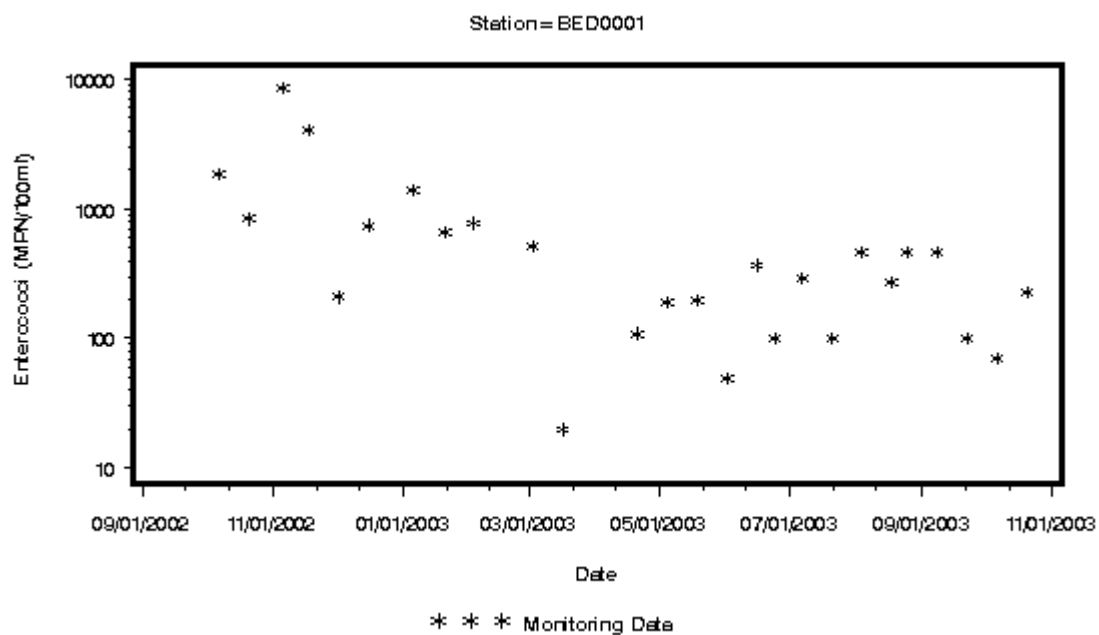
Figure A-1: Enterococci Concentration vs. Time for Anacostia River Monitoring Station BED0001

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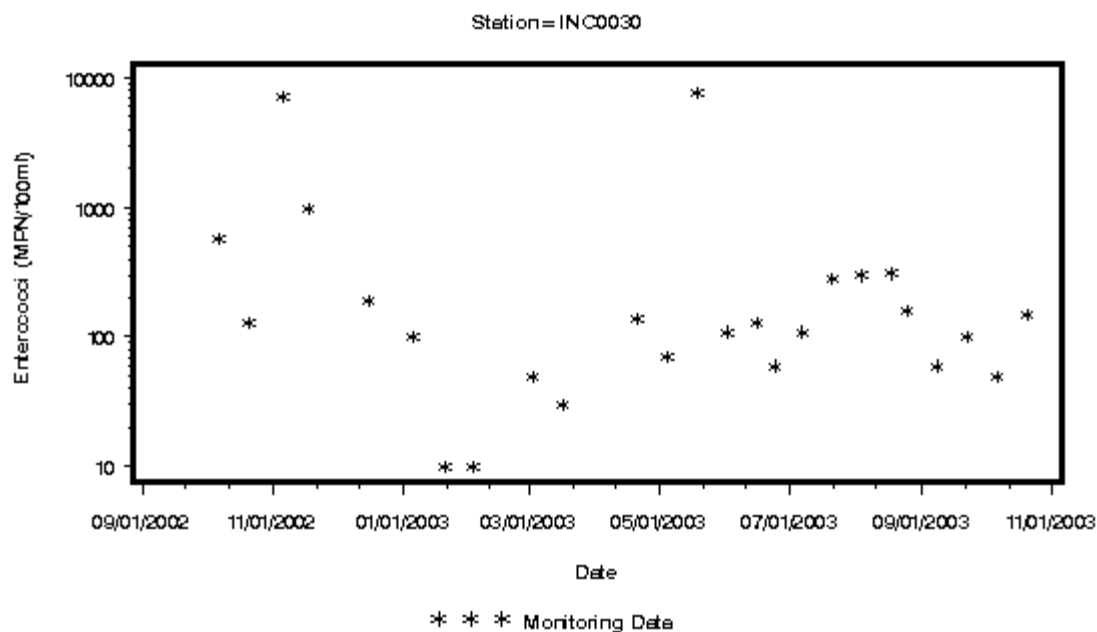
Figure A-2: Enterococci Concentration vs. Time for Anacostia River Monitoring Station INC0030
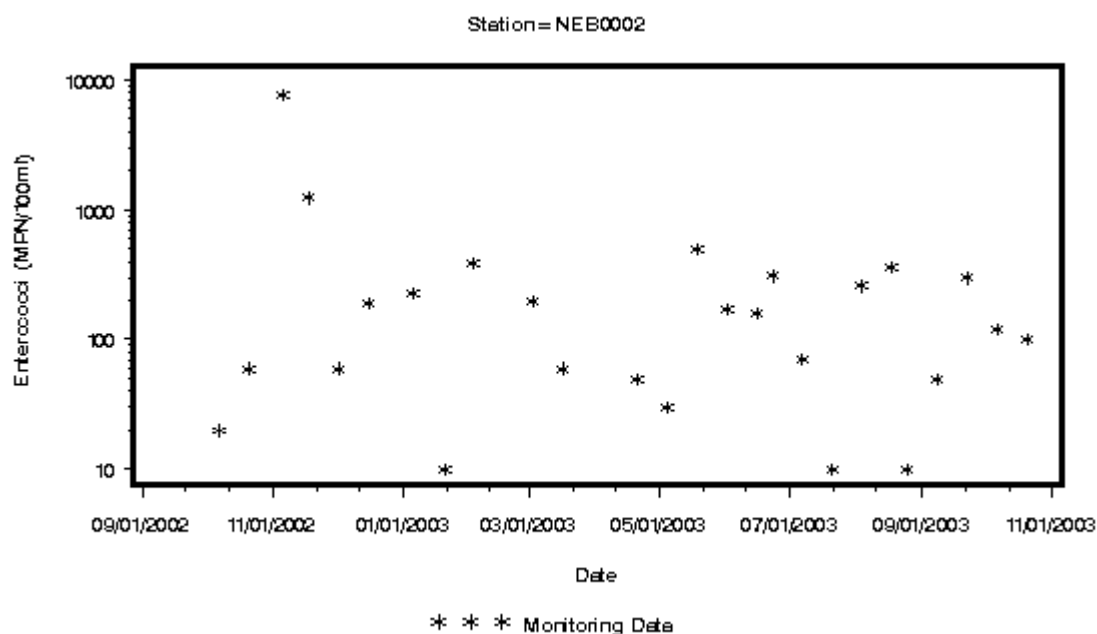
Figure A-3: Enterococci Concentration vs. Time for Anacostia River Monitoring Station NEB0002
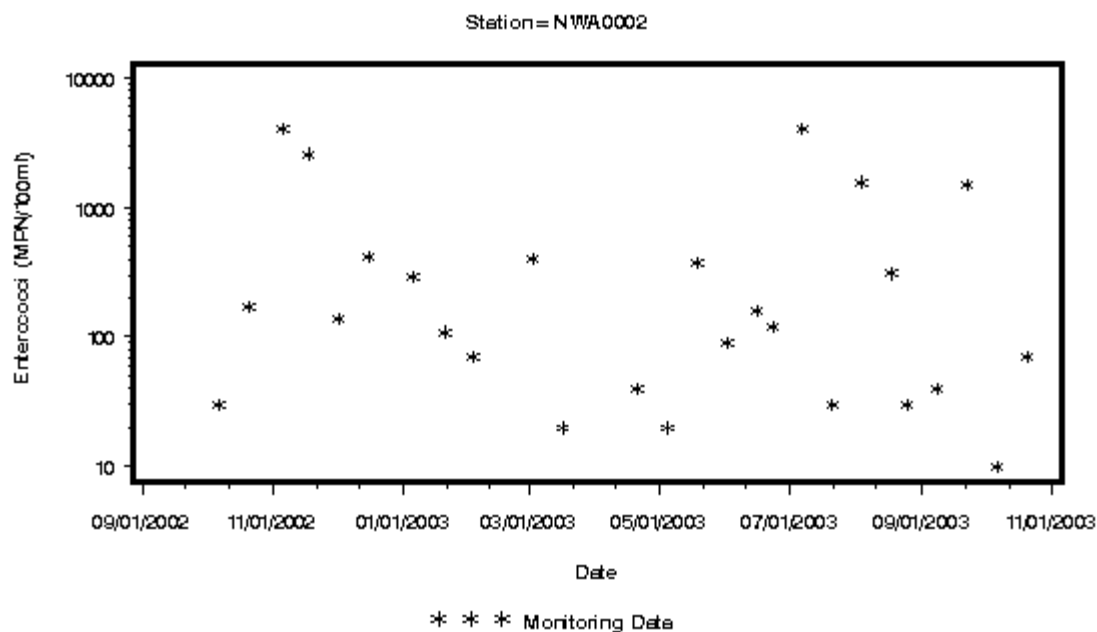
Station = NWA0002

Figure A-4: Enterococci Concentration vs. Time for Anacostia River Monitoring Station NWA0002

Station = NWA0135

Figure A-5: Enterococci Concentration vs. Time for Anacostia River Monitoring Station NWA0135

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Station= PNT0001

Figure A-6: Enterococci Concentration vs. Time for Anacostia River Monitoring Station PNT0001

FINAL

Appendix B - Flow Duration Curve Analysis to Define Strata

The Anacostia River watersheds were assessed to determine hydrologically significant strata. The purpose of these strata are 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 Anacostia River Watershed has three active USGS flow gauges. The gauges and dates of information used are as follows:

Table B-1: USGS Gauges in the Anacostia River Watershed

USGS Gage #	Dates used	Description
01649500	Oct 1, 1988 to Sep 30, 2003	
01651000	Oct 1, 1988 to Sep 30, 2003	
01650500	Nov 27, 1997 to Sep 30, 2003	
01650500 (estimate)	Oct 1, 1988 to Sep 30, 2003	Estimated flow based on USGS Gage 0165100 using MOVE.1 (Hirsch, 1982)

Flow duration curves for these three gauges are presented in Figure B-1.

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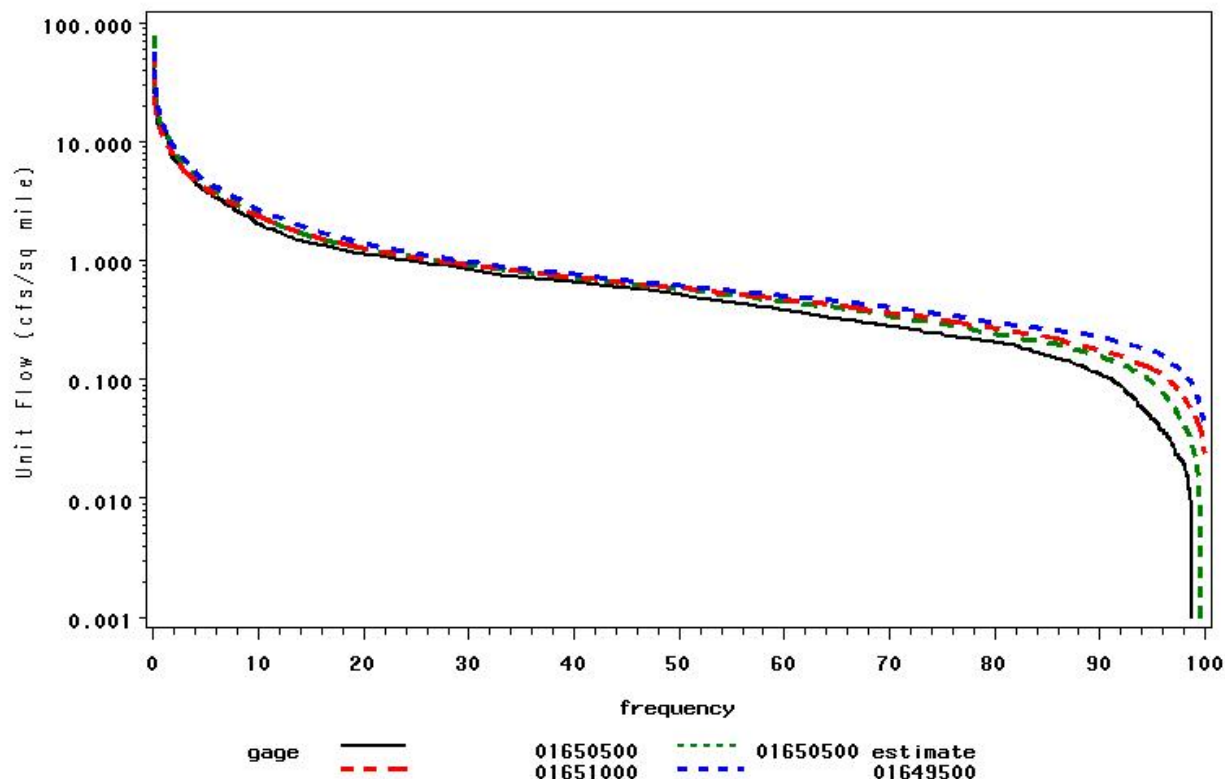
Figure B-1: Anacostia River Flow Duration Curves

The separation of high flow and low flow was based on the analysis of flow data for three USGS gauges located in the Anacostia River watershed. The hydrograph separation technique is equivalent to the sliding interval technique used in the USGS HYSEP program (USGS, 1996) and the interval is based on the duration of surface runoff estimated from Linsley *et. al.* (1982) and Pettyjohn and Henning (1979). Following hydrograph separation, the percent of surface runoff vs. the daily flow duration interval is plotted and a non-parametric smoothing method (LOESS) was used to identify general patterns.
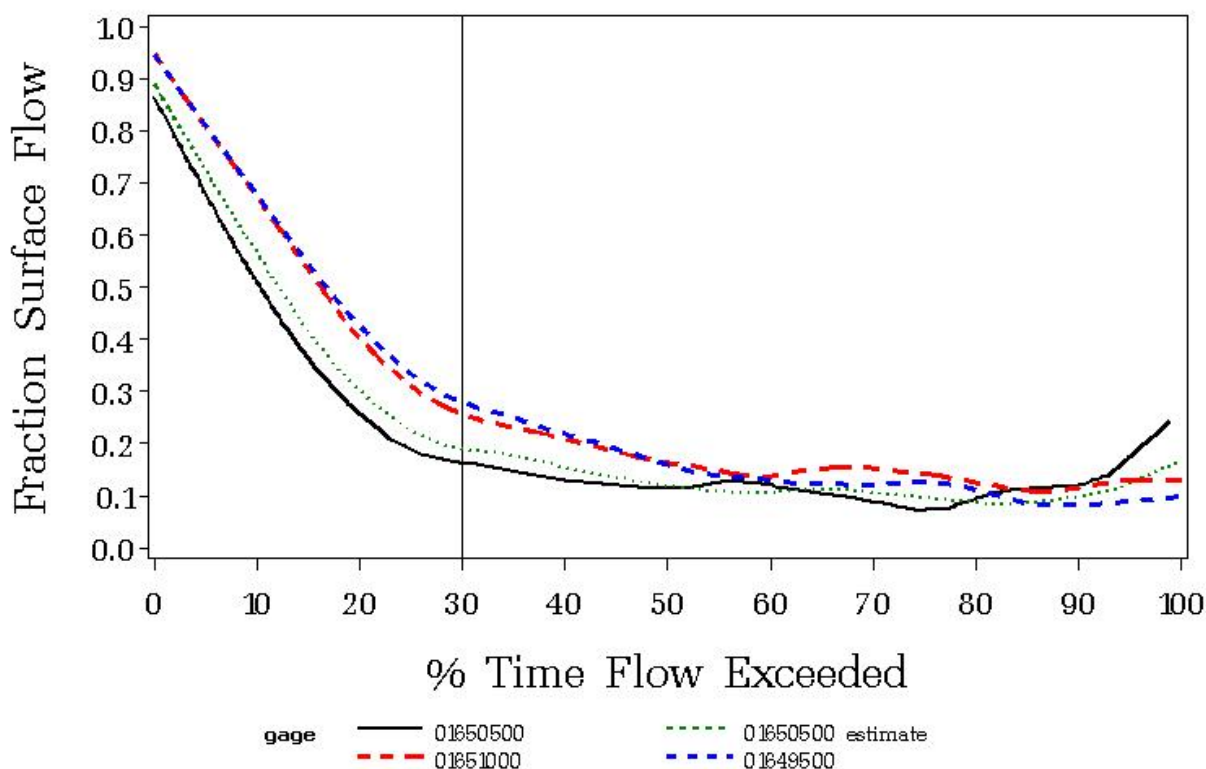
Figure B-2: Anacostia River: LOESS Smoothing of Hydrograph Separation

These patterns are illustrated in Figure B-2. From this figure it can be seen that a significant change in slope occurs at approximately the 30 percent daily flow interval for the two gauges located near the confluence of the Northeast (01649500) and Northwest Branches (01651000) of the Anacostia River. The predominant inflection point for the station located on the upstream section of the Northwest Branch occurs near the 25th percentile. For consistency among the stations, the inflection point was based on the stations with the most monitoring data, station 01651000 and station 01649500.

It was observed that no significant change in slope occurs in the Anacostia River *below* the 30th percentile daily flow interval and that this area is representative of a region of significant and increasing surface flow contribution to the stream. Above the 30th percentile, a small change of slope was observed between the 50th – 60th percentiles, however, given the similarity in the mean fraction of surface flow for the 30th – 100th percentile stratum, an additional stratum was not defined. Therefore, the 30th percentile threshold was used to define the limits between high flow and mid-range flows and low flows as appropriate.   Using these thresholds, definitions of high and low range flows are presented in Table B-2.

Table B-2: Definition of Flow Strata

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 (enteroccocci or *E. coli*) monitoring data are "placed" within the regions (stratum) based on the daily flow duration percentile of the date of sampling. Figures B-3 to B-8 show the Anacostia River enterococci monitoring data with corresponding flow frequency.

Maryland's water quality standards for bacteria state that 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 fewer than five representative sampling events for an area being assessed are available, data from the previous two years will be evaluated. In the Anacostia River, there are sufficient samples in both the high and low flow strata to estimate the geometric means.

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) Annual average 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
  (6) 30-day High Flow Condition
  (7) 30-day Low Flow Condition

Weighted geometric means are plotted with the monitoring data on Figures B-3 to B-8.

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

| Hydrological Condition		Subwatershed	Weighting Factor High Flow	Weighting Factor Low Flow
Annual	Average Condition	All Subwatersheds	0.30	0.70
	High Flow	BED0001; INC0030; NWA0135	0.55	0.45
		PNT0001; NEB0002; NEB0002sub	0.53	0.47
		NWA0002; NWA0002sub	0.55	0.45
	Low Flow	BED0001; INC0030; NWA0135	0.07	0.93
		PNT0001; NEB0002; NEB0002sub	0.08	0.92
		NWA0002; NWA0002sub	0.09	0.91
Season	High Flow	BED0001; INC0030; NWA0135	0.52	0.48
		PNT0001; NEB0002; NEB0002sub	0.50	0.50
		NWA0002; NWA0002sub	0.52	0.48
	High Flow	BED0001; INC0030; NWA0135	0.11	0.89
		PNT0001; NEB0002; NEB0002sub	0.12	0.88
		NWA0002; NWA0002sub	0.13	0.87
30-day	High Flow	BED0001; INC0030; NWA0135	1.00	0.00
		PNT0001; NEB0002; NEB0002sub	1.00	0.00
		NWA0002; NWA0002sub	1.00	0.00
	High Flow	BED0001; INC0030; NWA0135	0.00	1.00
		PNT0001; NEB0002; NEB0002sub	1.00	0.00
		NWA0002; NWA0002sub	1.00	0.00
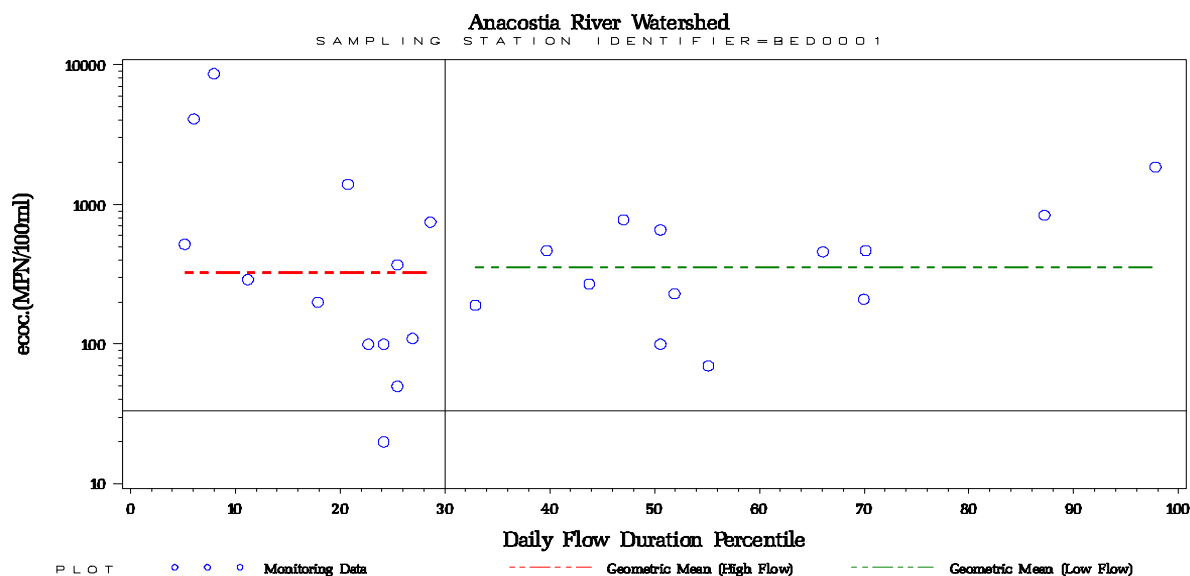
Figure B-3: Enterococci Concentration vs. Flow Duration for Anacostia River Monitoring Station BED0001
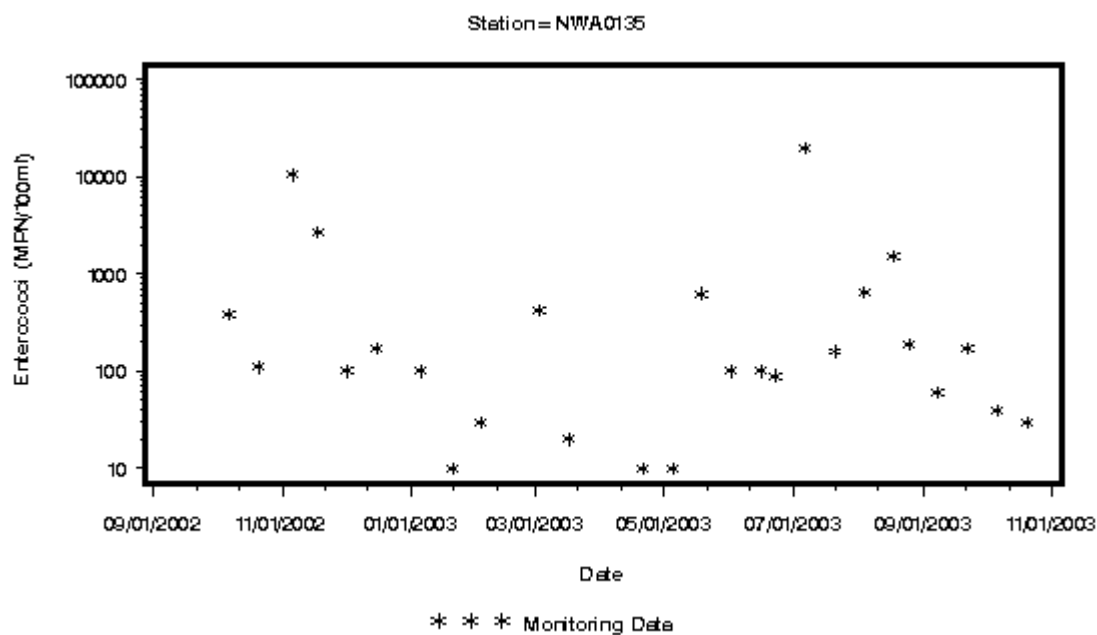

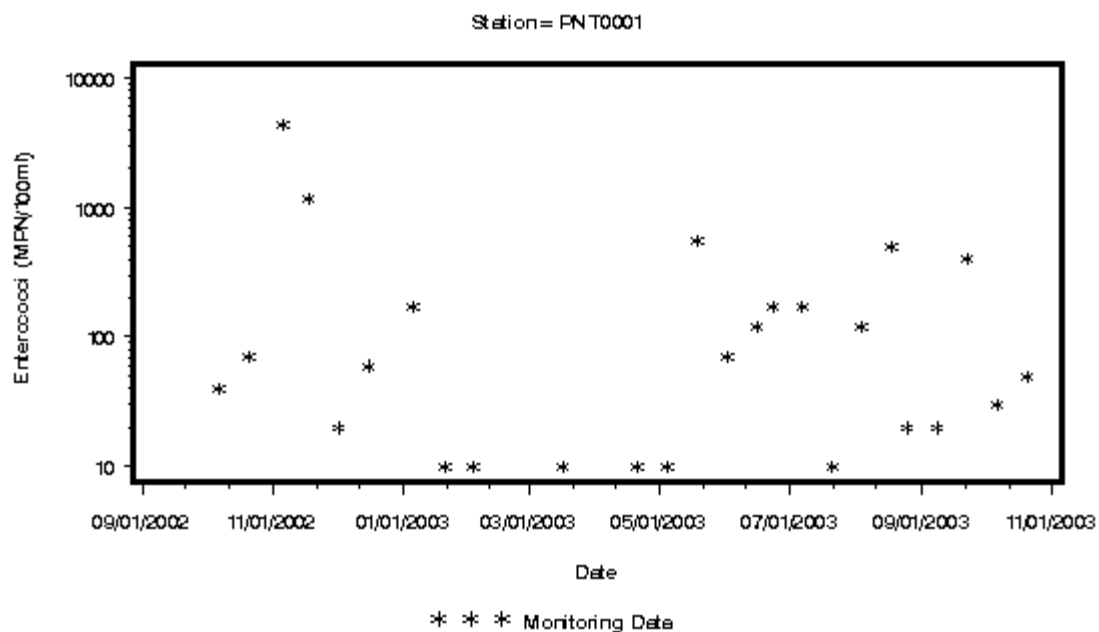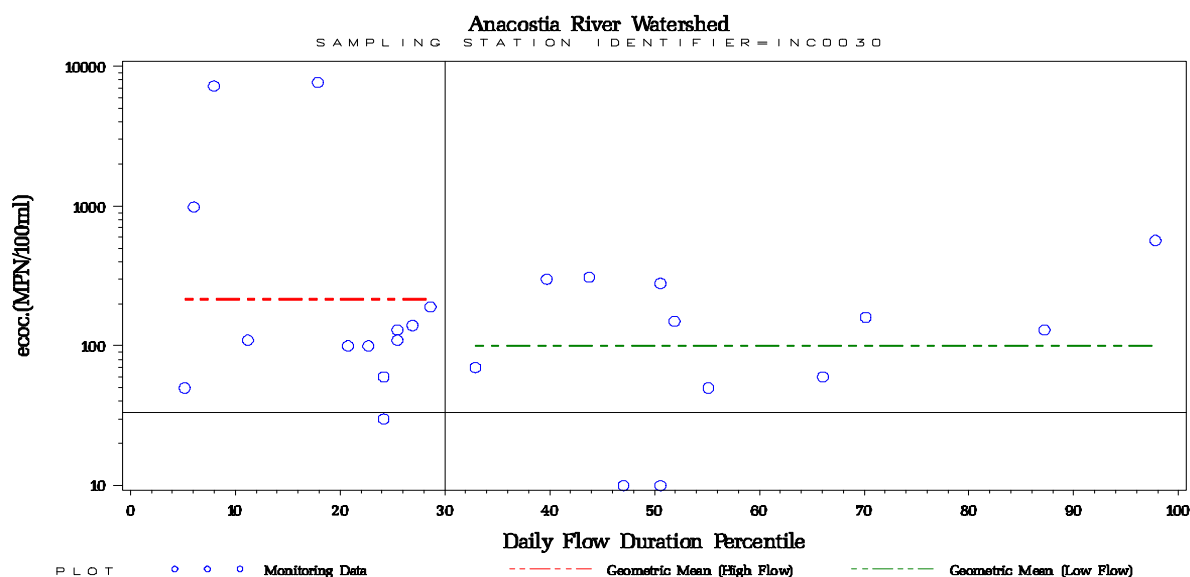**Figure B-4: Enterococci Concentration vs. Flow Duration for Anacostia River Monitoring Station INC0030**
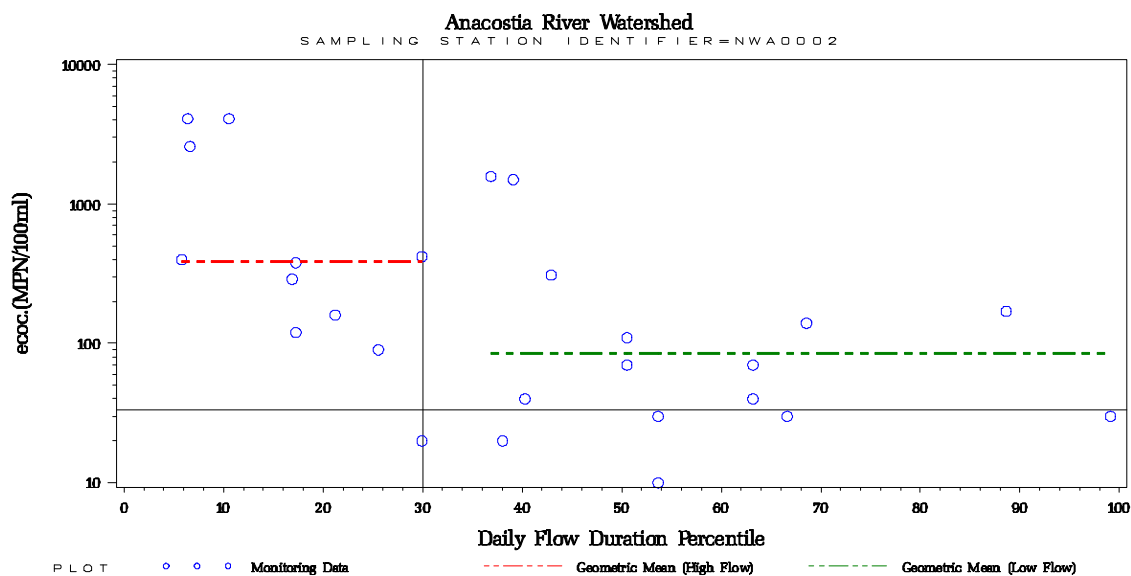
Figure B-5: Enterococci Concentration vs. Flow Duration for Anacostia River Monitoring Station NWA0002
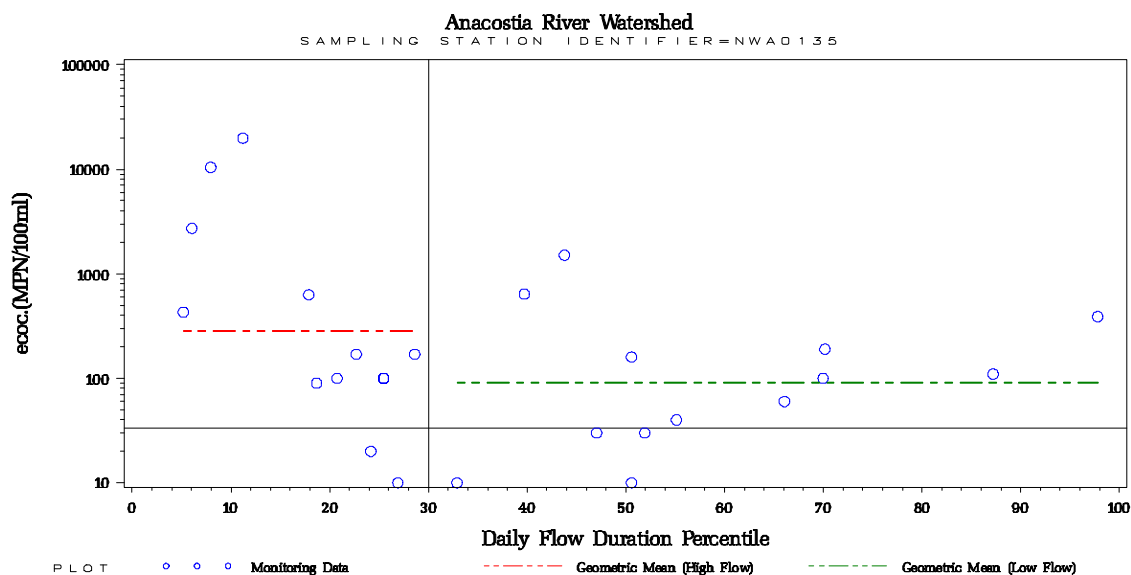
Figure B-6: Enterococci Concentration vs. Flow Duration for Anacostia River Monitoring Station NWA0135
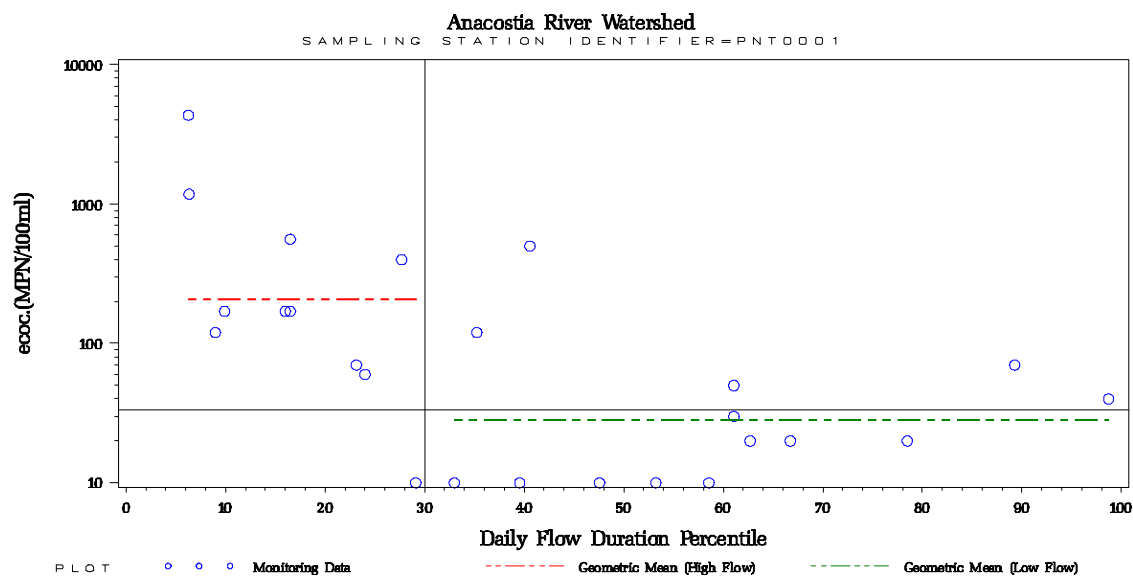
Figure B-7: Enterococci Concentration vs. Flow Duration for Anacostia River Monitoring Station PNT0001
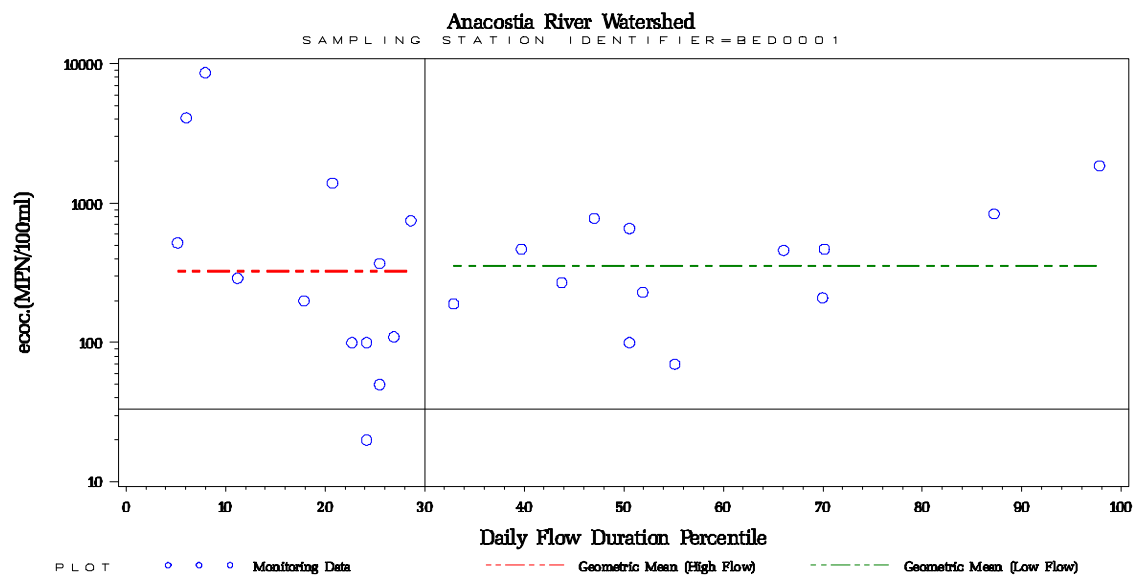
Figure B-8: Enterococci Concentration vs. Flow Duration for Anacostia River Monitoring Station BED0001

Appendix C – Anacostia River Bacterial Source Tracking

Probable Sources of Enterococci Contamination

November 2002 – October 2003

Bacterial Source Tracking Report:

**Identifying Sources of Fecal Pollution in the
Anacostia River Watershed, Maryland**

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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 study of the Anacostia River Watershed, we used the ARA method 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).

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

specific antibiotics used in the analysis, isolates from humans, livestock and pets could be differentiated from each other.

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

LABORATORY METHODS

Isolation of Enterococci 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 Enterococci 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 enterococci isolates were collected from each water sample and all isolates were then shipped to the SU BST lab.

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

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

The following table includes the antibiotics and concentrations used for isolates in the Anacostia River watershed analysis.

Table C-1: Antibiotics and concentrations used for ARA

Antibiotic	Concentration (µg/ml)
Amoxicillin	0.625
Cephalothin	10, 15, 30, 50
Chloramphenicol	1, 2.5, 5, 10
Chlortetracycline	60, 80, 100
Erythromycin	50
Gentamycin	5, 10, 15, 20
Neomycin	40, 60, 80
Oxytetracycline	20, 40, 60, 80, 100
Salinomycin	1, 2.5, 5, 10
Streptomycin	40, 60, 80, 100
Tetracycline	15, 30, 50, 100
Vancomycin	2.5

KNOWN-SOURCE LIBRARY

Construction and Use. Fecal samples (scat) from known sources in the watershed were collected during the study period by MDE personnel and delivered to the BST Laboratory at SU. Enterococci isolates were obtained from known sources, which included human, dog, cow, goat, horse, pig, sheep, chicken, deer, rabbit, fox, and goose.   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).   Enterococci isolate response patterns were also obtained from bacteria in water samples collected at the six (6) monitoring stations in the basin. Using statistical techniques, these patterns were then compared to those in the library to identify the probable source of each water isolate.

STATISTICAL ANALYSIS

We applied a tree classification method, [1]CART®, to build a model that classifies isolates into source categories based on ARA data. CART® builds a classification tree by recursively splitting the library of isolates into two nodes. Each split is determined by the antibiotic variables (antibiotic resistance measured for a collection of antibiotics at varying concentrations).

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

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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. For the Anacostia River tree-classification model, the *acceptable source identification probability* was set at 0.70 (70%).

RESULTS

Known-Source Library. The 1,155 known-source isolates in the library were grouped into four categories: domestic (pets, specifically dogs), human, livestock (horse, pig, goat, sheep, chicken, cow), and wildlife (goose, deer, rabbit, fox) (Table C-2). The library was analyzed for its ability to take a subset of the library isolates and correctly predict the identity of their host sources when they were treated as unknowns. Average rates of correct classification (ARCC) for the library were found by repeating this analysis using several probability cutoff points, as described above. The number-not-classified for each probability was determined. From these results, the percent unknown and percent correct classification (ARCC) was calculated (Table C-3).

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

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

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Table C-2: Category, total number, and number of unique patterns in the known-source library

Category	Potential Source	Total Isolates	Unique Patterns
Domestic	Dogs	236	152
Human	Humans	399	206
Livestock	Horses, pigs, goats, Sheep, chicken, cow	245	172
Wildlife	Goose, deer, Rabbit, fox	194	191
Total		1074	611

Table C-3: Number of isolates not classified, percent unknown, and percent correct for six (6) cutoff probabilities

Cutoff Probability	Number Not Classified	Percent Unknown	Percent Correct
0.25	0	0.0%	81.8%
0.375	7	0.7%	82.2%
0.50	86	8.0%	85.0%
0.60	204	19.0%	88.7%
0.70	324	30.2%	92.7%
0.80	355	33.1%	93.3%

A cutoff probability of 0.70 (70%) was shown to yield a high ARCC of 93%. An increase to a 0.80 (80%) cutoff did not increase the rate of correct classification as much as it increased the percent unknown (Figure C-1). Therefore, using a cutoff probability of 0.70 (70%), the 324 isolates that were not useful in the prediction of probable sources were removed, leaving 1074 isolates remaining in the library. This library was then used in the statistical prediction of probable sources of bacteria in water samples collected from the Anacostia River. The rates of correction classification for the four categories of sources in the library are shown in Table C-4 below.
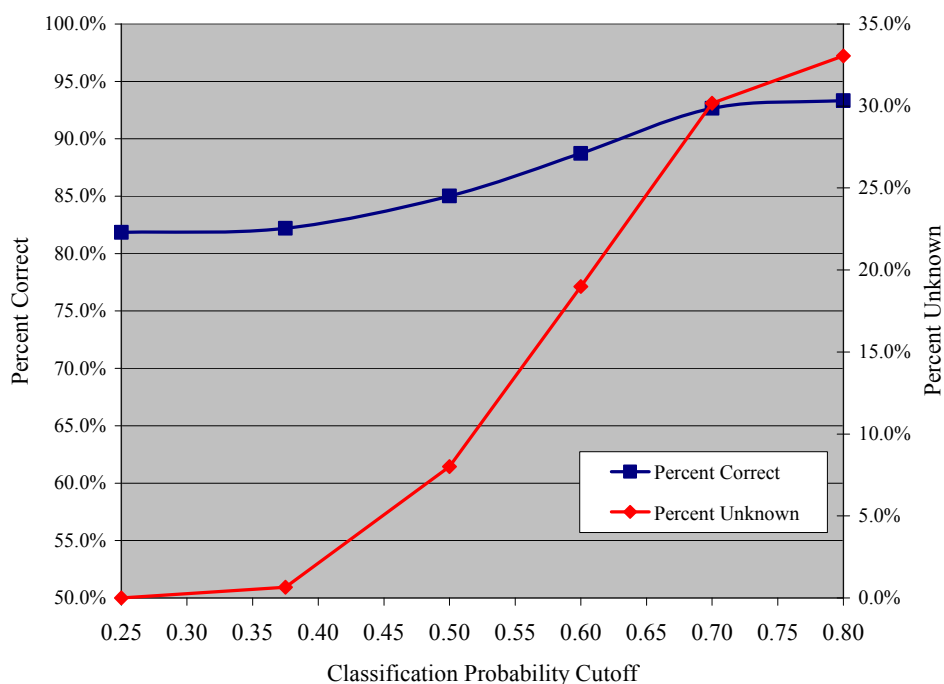
Figure C-1: Classification Model: Percent Correct versus Percent Unknown

Table C-4: Actual species categories versus predicted categories, with rates of correct classification (RCC) for each category.

	Predicted →					
Actual ↓	DOMESTIC	HUMAN	LIVESTOCK	WILDLIFE	TOTAL	RCC[1]
DOMESTIC	**163**	6	2	4	175	93%
HUMAN	4	**335**	7	0	346	97%
LIFESTOCK	5	5	**146**	1	157	93%
WILDLIFE	7	13	1	**51**	72	71%
Total	179	359	156	56	750	

[1]RCC = 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%.

Anacostia River Water Samples.   Monthly monitoring from six (6) stations on the Anacostia River was the source of water samples. The maximum number of enterococci isolates per water sample was 24, although the number of isolates that actually grew was sometimes fewer than 24. A total of 1565 enterococci isolates were analyzed by statistical analysis. The BST results by species category, shown below in Table C-5, indicate that there is little difference in the cutoff probabilities of 0.70 (70%) and 0.80 (80%).

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Table C-5: Potential host sources of water isolates by species category, number of isolates, percent isolates classified at cutoff probabilities of 70% and 80%.

Category	No.	% Isolates Classified 70% Prob.	No.	% Isolates Classified 80% Prob.
DOMESTIC	363	23.2%	357	22.8%
HUMAN	231	14.8%	214	13.7%
LIVESTOCK	133	8.5%	130	8.3%
WILDLIFE	350	22.4%	350	22.4%
UNKNOWN	488	31.2%	514	32.8%
Missing Data	0		0	
Total w/ Complete Data	1565		1565	
Total	1565		1565	
% Classified	**68.8%**		**67.5%**	

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

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

Station	Fall	Winter	Spring	Summer	Total
NEB0002	45	69	71	64	249
NWA0002	54	72	70	59	255
PNT0001	59	44	72	85	260
INC0030	59	68	48	69	244
BED0001	54	72	95	70	291
NWA0135	62	69	70	65	266
Total	333	394	426	412	1565

Tables C-7 through C-11 on the following pages show the results of BST analysis from the estimation of number of isolates per station per date to the final estimation of the overall percentage of bacteria sources by subwatershed.

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

Station	Date	% domestic animals	% human	% livestock	% wildlife	% unknown
BED0001	11/18/2002	2	0	0	4	1
BED0001	12/02/2002	2	7	0	4	11
BED0001	01/06/2003	13	3	0	1	7
BED0001	02/03/2003	9	3	0	0	12
BED0001	03/03/2003	9	0	0	1	14
BED0001	04/21/2003	6	0	4	1	13
BED0001	05/05/2003	4	0	1	3	15
BED0001	06/02/2003	20	2	3	11	12
BED0001	07/07/2003	6	0	2	10	6
BED0001	08/04/2003	1	2	0	11	8
BED0001	09/08/2003	13	1	3	3	4
BED0001	10/06/2003	4	4	6	3	6
INC0030	11/18/2002	5	0	3	14	0
INC0030	12/02/2002	0	3	0	17	0
INC0030	01/06/2003	5	0	5	9	5
INC0030	02/03/2003	3	1	2	9	5
INC0030	03/03/2003	6	2	4	2	10
INC0030	04/21/2003	11	1	5	2	5
INC0030	05/05/2003	9	7	1	3	4
INC0030	07/07/2003	4	1	1	2	15
INC0030	08/04/2003	5	3	4	5	7
INC0030	09/08/2003	1	12	1	1	7
INC0030	10/06/2003	4	2	0	7	4
NEB0002	11/18/2002	10	0	1	9	1
NEB0002	12/02/2002	3	4	2	9	6
NEB0002	01/06/2003	6	1	4	10	3
NEB0002	02/03/2003	0	1	14	0	9
NEB0002	03/03/2003	5	3	1	4	8

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Station	Date	% domestic animals	% human	% livestock	% wildlife	% unknown
NEB0002	04/21/2003	4	2	8	2	8
NEB0002	05/05/2003	2	1	5	2	14
NEB0002	06/02/2003	7	0	3	8	5
NEB0002	07/07/2003	12	0	0	6	6
NEB0002	08/04/2003	0	2	2	15	3
NEB0002	09/08/2003	5	1	2	2	8
NWA0002	11/18/2002	3	2	0	12	5
NWA0002	12/02/2002	4	0	0	12	8
NWA0002	01/06/2003	3	2	1	1	17
NWA0002	02/03/2003	3	1	2	4	14
NWA0002	03/03/2003	4	4	6	0	10
NWA0002	04/21/2003	3	0	1	1	18
NWA0002	05/05/2003	4	0	0	11	8
NWA0002	06/02/2003	7	2	5	3	7
NWA0002	07/07/2003	8	1	1	7	6
NWA0002	08/04/2003	5	8	0	7	4
NWA0002	09/08/2003	4	0	1	5	2
NWA0002	10/06/2003	1	2	0	0	5
NWA0135	11/18/2002	7	5	1	1	7
NWA0135	12/02/2002	9	6	1	3	5
NWA0135	01/06/2003	6	2	4	0	11
NWA0135	02/03/2003	0	0	5	0	19
NWA0135	03/03/2003	3	5	0	2	12
NWA0135	04/21/2003	1	0	0	7	14
NWA0135	05/05/2003	2	10	0	1	11
NWA0135	06/02/2003	19	1	1	0	3
NWA0135	07/07/2003	4	13	0	2	5
NWA0135	08/04/2003	2	18	0	0	4
NWA0135	09/08/2003	2	11	0	0	4
NWA0135	10/06/2003	3	2	0	6	6
PNT0001	11/18/2002	3	0	0	20	1

Station	Date	% domestic animals	% human	% livestock	% wildlife	% unknown
PNT0001	12/02/2002	8	1	0	11	3
PNT0001	01/06/2003	4	2	5	4	9
PNT0001	02/03/2003	6	3	3	1	7
PNT0001	04/21/2003	14	1	0	4	5
PNT0001	05/05/2003	5	3	1	13	2
PNT0001	06/02/2003	3	0	4	4	13
PNT0001	06/23/2003	0	10	1	2	8
PNT0001	07/07/2003	5	2	1	6	10
PNT0001	08/04/2003	5	1	2	8	8
PNT0001	09/08/2003	1	7	0	0	8
PNT0001	10/06/2003	1	5	0	3	3

Table C-8: Percentage of Sources per Station per Date

Station	Date	% domestic animals	% human	% livestock	% wildlife	% unknown
BED0001	11/18/2002	28.6	0.0	0.0	57.1	14.3
BED0001	12/02/2002	8.3	29.2	0.0	16.7	45.8
BED0001	01/06/2003	54.2	12.5	0.0	4.2	29.2
BED0001	02/03/2003	37.5	12.5	0.0	0.0	50.0
BED0001	03/03/2003	37.5	0.0	0.0	4.2	58.3
BED0001	04/21/2003	25.0	0.0	16.7	4.2	54.2
BED0001	05/05/2003	17.4	0.0	4.3	13.0	65.2
BED0001	06/02/2003	41.7	4.2	6.3	22.9	25.0
BED0001	07/07/2003	25.0	0.0	8.3	41.7	25.0
BED0001	08/04/2003	4.5	9.1	0.0	50.0	36.4
BED0001	09/08/2003	54.2	4.2	12.5	12.5	16.7
BED0001	10/06/2003	17.4	17.4	26.1	13.0	26.1
INC0030	11/18/2002	22.7	0.0	13.6	63.6	0.0

Station	Date	% domestic animals	% human	% livestock	% wildlife	% unknown
INC0030	12/02/2002	0.0	15.0	0.0	85.0	0.0
INC0030	01/06/2003	20.8	0.0	20.8	37.5	20.8
INC0030	02/03/2003	15.0	5.0	10.0	45.0	25.0
INC0030	03/03/2003	25.0	8.3	16.7	8.3	41.7
INC0030	04/21/2003	45.8	4.2	20.8	8.3	20.8
INC0030	05/05/2003	37.5	29.2	4.2	12.5	16.7
INC0030	07/07/2003	17.4	4.3	4.3	8.7	65.2
INC0030	08/04/2003	20.8	12.5	16.7	20.8	29.2
INC0030	09/08/2003	4.5	54.5	4.5	4.5	31.8
INC0030	10/06/2003	23.5	11.8	0.0	41.2	23.5
NEB0002	11/18/2002	47.6	0.0	4.8	42.9	4.8
NEB0002	12/02/2002	12.5	16.7	8.3	37.5	25.0
NEB0002	01/06/2003	25.0	4.2	16.7	41.7	12.5
NEB0002	02/03/2003	0.0	4.2	58.3	0.0	37.5
NEB0002	03/03/2003	23.8	14.3	4.8	19.0	38.1
NEB0002	04/21/2003	16.7	8.3	33.3	8.3	33.3
NEB0002	05/05/2003	8.3	4.2	20.8	8.3	58.3
NEB0002	06/02/2003	30.4	0.0	13.0	34.8	21.7
NEB0002	07/07/2003	50.0	0.0	0.0	25.0	25.0
NEB0002	08/04/2003	0.0	9.1	9.1	68.2	13.6
NEB0002	09/08/2003	27.8	5.6	11.1	11.1	44.4
NWA0002	11/18/2002	13.6	9.1	0.0	54.5	22.7
NWA0002	12/02/2002	16.7	0.0	0.0	50.0	33.3
NWA0002	01/06/2003	12.5	8.3	4.2	4.2	70.8
NWA0002	02/03/2003	12.5	4.2	8.3	16.7	58.3
NWA0002	03/03/2003	16.7	16.7	25.0	0.0	41.7
NWA0002	04/21/2003	13.0	0.0	4.3	4.3	78.3
NWA0002	05/05/2003	17.4	0.0	0.0	47.8	34.8
NWA0002	06/02/2003	29.2	8.3	20.8	12.5	29.2
NWA0002	07/07/2003	34.8	4.3	4.3	30.4	26.1
NWA0002	08/04/2003	20.8	33.3	0.0	29.2	16.7

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Station	Date	% domestic animals	% human	% livestock	% wildlife	% unknown
NWA0002	09/08/2003	33.3	0.0	8.3	41.7	16.7
NWA0002	10/06/2003	12.5	25.0	0.0	0.0	62.5
NWA0135	11/18/2002	33.3	23.8	4.8	4.8	33.3
NWA0135	12/02/2002	37.5	25.0	4.2	12.5	20.8
NWA0135	01/06/2003	26.1	8.7	17.4	0.0	47.8
NWA0135	02/03/2003	0.0	0.0	20.8	0.0	79.2
NWA0135	03/03/2003	13.6	22.7	0.0	9.1	54.5
NWA0135	04/21/2003	4.5	0.0	0.0	31.8	63.6
NWA0135	05/05/2003	8.3	41.7	0.0	4.2	45.8
NWA0135	06/02/2003	79.2	4.2	4.2	0.0	12.5
NWA0135	07/07/2003	16.7	54.2	0.0	8.3	20.8
NWA0135	08/04/2003	8.3	75.0	0.0	0.0	16.7
NWA0135	09/08/2003	11.8	64.7	0.0	0.0	23.5
NWA0135	10/06/2003	17.6	11.8	0.0	35.3	35.3
PNT0001	11/18/2002	12.5	0.0	0.0	83.3	4.2
PNT0001	12/02/2002	34.8	4.3	0.0	47.8	13.0
PNT0001	01/06/2003	16.7	8.3	20.8	16.7	37.5
PNT0001	02/03/2003	30.0	15.0	15.0	5.0	35.0
PNT0001	04/21/2003	58.3	4.2	0.0	16.7	20.8
PNT0001	05/05/2003	20.8	12.5	4.2	54.2	8.3
PNT0001	06/02/2003	12.5	0.0	16.7	16.7	54.2
PNT0001	06/23/2003	0.0	47.6	4.8	9.5	38.1
PNT0001	07/07/2003	20.8	8.3	4.2	25.0	41.7
PNT0001	08/04/2003	20.8	4.2	8.3	33.3	33.3
PNT0001	09/08/2003	6.3	43.8	0.0	0.0	50.0
PNT0001	10/06/2003	8.3	41.7	0.0	25.0	25.0

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Table C-9: Enterococci Concentration and Percentage of Sources by Stratum

STATION	DATE	Flow Stratum (1=high 2=low)	Ecocc Conc MPN/ 100ml	log mean conc	% domestic animals	% human	% livestock	% wildlife	% unknown
BED0001	10/07/2002	2	1860	3.26951	.	.	.	.	.
BED0001	10/21/2002	2	840	2.92428	.	.	.	.	.
BED0001	11/06/2002	1	8660	3.93752	.	.	.	.	.
BED0001	11/18/2002	1	4110	3.61384	28.5714	0.0000	0.0000	57.1429	14.2857
BED0001	12/02/2002	2	210	2.32222	8.3333	29.1667	0.0000	16.6667	45.8333
BED0001	12/16/2002	1	750	2.87506	.	.	.	.	.
BED0001	01/06/2003	1	1400	3.14613	54.1667	12.5000	0.0000	4.1667	29.1667
BED0001	01/21/2003	2	660	2.81954	.	.	.	.	.
BED0001	02/03/2003	2	780	2.89209	37.5000	12.5000	0.0000	0.0000	50.0000
BED0001	03/03/2003	1	520	2.71600	37.5000	0.0000	0.0000	4.1667	58.3333
BED0001	03/17/2003	1	20	1.30103	.	.	.	.	.
BED0001	04/21/2003	1	110	2.04139	25.0000	0.0000	16.6667	4.1667	54.1667
BED0001	05/05/2003	2	190	2.27875	17.3913	0.0000	4.3478	13.0435	65.2174
BED0001	05/19/2003	1	200	2.30103	.	.	.	.	.
BED0001	06/02/2003	1	50	1.69897	41.6667	4.1667	6.2500	22.9167	25.0000
BED0001	06/16/2003	1	370	2.56820	.	.	.	.	.
BED0001	06/24/2003	1	100	2.00000	.	.	.	.	.
BED0001	07/07/2003	1	290	2.46240	25.0000	0.0000	8.3333	41.6667	25.0000
BED0001	07/21/2003	2	100	2.00000	.	.	.	.	.
BED0001	08/04/2003	2	470	2.67210	4.5455	9.0909	0.0000	50.0000	36.3636
BED0001	08/18/2003	2	270	2.43136	.	.	.	.	.
BED0001	08/25/2003	2	470	2.67210	.	.	.	.	.
BED0001	09/08/2003	2	460	2.66276	54.1667	4.1667	12.5000	12.5000	16.6667
BED0001	09/22/2003	1	100	2.00000	.	.	.	.	.
BED0001	10/06/2003	2	70	1.84510	17.3913	17.3913	26.0870	13.0435	26.0870

STATION	DATE	Flow Stratum (1=high 2=low)	Ecocc Conc MPN/ 100ml	log mean conc	% domestic animals	% human	% livestock	% wildlife	% unknown
BED0001	10/20/2003	2	230	2.36173	.	.	.	.	.
INC0030	10/07/2002	2	570	2.75587	.	.	.	.	.
INC0030	10/21/2002	2	130	2.11394	.	.	.	.	.
INC0030	11/06/2002	1	7270	3.86153	.	.	.	.	.
INC0030	11/18/2002	1	990	2.99564	22.7273	0.0000	13.6364	63.6364	0.0000
INC0030	12/02/2002	.	.	.	0.0000	15.0000	0.0000	85.0000	0.0000
INC0030	12/16/2002	1	190	2.27875	.	.	.	.	.
INC0030	01/06/2003	1	100	2.00000	20.8333	0.0000	20.8333	37.5000	20.8333
INC0030	01/21/2003	2	10	1.00000	.	.	.	.	.
INC0030	02/03/2003	2	10	1.00000	15.0000	5.0000	10.0000	45.0000	25.0000
INC0030	03/03/2003	1	50	1.69897	25.0000	8.3333	16.6667	8.3333	41.6667
INC0030	03/17/2003	1	30	1.47712	.	.	.	.	.
INC0030	04/21/2003	1	140	2.14613	45.8333	4.1667	20.8333	8.3333	20.8333
INC0030	05/05/2003	2	70	1.84510	37.5000	29.1667	4.1667	12.5000	16.6667
INC0030	05/19/2003	1	7700	3.88649	.	.	.	.	.
INC0030	06/02/2003	1	110	2.04139	.	.	.	.	.
INC0030	06/16/2003	1	130	2.11394	.	.	.	.	.
INC0030	06/24/2003	1	60	1.77815	.	.	.	.	.
INC0030	07/07/2003	1	110	2.04139	17.3913	4.3478	4.3478	8.6957	65.2174
INC0030	07/21/2003	2	280	2.44716	.	.	.	.	.
INC0030	08/04/2003	2	300	2.47712	20.8333	12.5000	16.6667	20.8333	29.1667
INC0030	08/18/2003	2	310	2.49136	.	.	.	.	.
INC0030	08/25/2003	2	160	2.20412	.	.	.	.	.
INC0030	09/08/2003	2	60	1.77815	4.5455	54.5455	4.5455	4.5455	31.8182
INC0030	09/22/2003	1	100	2.00000	.	.	.	.	.
INC0030	10/06/2003	2	50	1.69897	23.5294	11.7647	0.0000	41.1765	23.5294
INC0030	10/20/2003	2	150	2.17609	.	.	.	.	.
NEB0002	10/07/2002	2	20	1.30103	.	.	.	.	.
NEB0002	10/21/2002	2	60	1.77815	.	.	.	.	.
NEB0002	11/06/2002	1	7700	3.88649	.	.	.	.	.

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STATION	DATE	Flow Stratum (1=high 2=low)	Ecocc Conc MPN/ 100ml	log mean conc	% domestic animals	% human	% livestock	% wildlife	% unknown
NEB0002	11/18/2002	1	1240	3.09342	47.6190	0.0000	4.7619	42.8571	4.7619
NEB0002	12/02/2002	2	60	1.77815	12.5000	16.6667	8.3333	37.5000	25.0000
NEB0002	12/16/2002	1	190	2.27875	.	.	.	.	.
NEB0002	01/06/2003	1	230	2.36173	25.0000	4.1667	16.6667	41.6667	12.5000
NEB0002	01/21/2003	2	10	1.00000	.	.	.	.	.
NEB0002	02/03/2003	2	390	2.59106	0.0000	4.1667	58.3333	0.0000	37.5000
NEB0002	03/03/2003	1	200	2.30103	23.8095	14.2857	4.7619	19.0476	38.0952
NEB0002	03/17/2003	1	60	1.77815	.	.	.	.	.
NEB0002	04/21/2003	2	50	1.69897	16.6667	8.3333	33.3333	8.3333	33.3333
NEB0002	05/05/2003	2	30	1.47712	8.3333	4.1667	20.8333	8.3333	58.3333
NEB0002	05/19/2003	1	500	2.69897	.	.	.	.	.
NEB0002	06/02/2003	1	170	2.23045	30.4348	0.0000	13.0435	34.7826	21.7391
NEB0002	06/16/2003	1	160	2.20412	.	.	.	.	.
NEB0002	06/23/2003	1	310	2.49136	.	.	.	.	.
NEB0002	07/07/2003	1	70	1.84510	50.0000	0.0000	0.0000	25.0000	25.0000
NEB0002	07/21/2003	2	10	1.00000	.	.	.	.	.
NEB0002	08/04/2003	2	260	2.41497	0.0000	9.0909	9.0909	68.1818	13.6364
NEB0002	08/18/2003	2	360	2.55630	.	.	.	.	.
NEB0002	08/25/2003	2	10	1.00000	.	.	.	.	.
NEB0002	09/08/2003	2	50	1.69897	27.7778	5.5556	11.1111	11.1111	44.4444
NEB0002	09/22/2003	1	300	2.47712	.	.	.	.	.
NEB0002	10/06/2003	2	120	2.07918	.	.	.	.	.
NEB0002	10/20/2003	2	100	2.00000	.	.	.	.	.
NWA0002	10/07/2002	2	30	1.47712	.	.	.	.	.
NWA0002	10/21/2002	2	170	2.23045	.	.	.	.	.
NWA0002	11/06/2002	1	4110	3.61384	.	.	.	.	.
NWA0002	11/18/2002	1	2600	3.41497	13.6364	9.0909	0.0000	54.5455	22.7273
NWA0002	12/02/2002	2	140	2.14613	16.6667	0.0000	0.0000	50.0000	33.3333
NWA0002	12/16/2002	1	420	2.62325	.	.	.	.	.
NWA0002	01/06/2003	1	290	2.46240	12.5000	8.3333	4.1667	4.1667	70.8333

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STATION	DATE	Flow Stratum (1=high 2=low)	Ecocc Conc MPN/ 100ml	log mean conc	% domestic animals	% human	% livestock	% wildlife	% unknown
NWA0002	01/21/2003	2	110	2.04139	.	.	.	.	.
NWA0002	02/03/2003	2	70	1.84510	12.5000	4.1667	8.3333	16.6667	58.3333
NWA0002	03/03/2003	1	400	2.60206	16.6667	16.6667	25.0000	0.0000	41.6667
NWA0002	03/17/2003	1	20	1.30103	.	.	.	.	.
NWA0002	04/21/2003	2	40	1.60206	13.0435	0.0000	4.3478	4.3478	78.2609
NWA0002	05/05/2003	2	20	1.30103	17.3913	0.0000	0.0000	47.8261	34.7826
NWA0002	05/19/2003	1	380	2.57978	.	.	.	.	.
NWA0002	06/02/2003	1	90	1.95424	29.1667	8.3333	20.8333	12.5000	29.1667
NWA0002	06/16/2003	1	160	2.20412	.	.	.	.	.
NWA0002	06/23/2003	1	120	2.07918	.	.	.	.	.
NWA0002	07/07/2003	1	4110	3.61384	34.7826	4.3478	4.3478	30.4348	26.0870
NWA0002	07/21/2003	2	30	1.47712	.	.	.	.	.
NWA0002	08/04/2003	2	1580	3.19866	20.8333	33.3333	0.0000	29.1667	16.6667
NWA0002	08/18/2003	2	310	2.49136	.	.	.	.	.
NWA0002	08/25/2003	2	30	1.47712	.	.	.	.	.
NWA0002	09/08/2003	2	40	1.60206	33.3333	0.0000	8.3333	41.6667	16.6667
NWA0002	09/22/2003	2	1500	3.17609	.	.	.	.	.
NWA0002	10/06/2003	2	10	1.00000	12.5000	25.0000	0.0000	0.0000	62.5000
NWA0002	10/20/2003	2	70	1.84510	.	.	.	.	.
NWA0135	10/07/2002	2	390	2.59106	.	.	.	.	.
NWA0135	10/21/2002	2	110	2.04139	.	.	.	.	.
NWA0135	11/06/2002	1	10460	4.01953	.	.	.	.	.
NWA0135	11/18/2002	1	2720	3.43457	33.3333	23.8095	4.7619	4.7619	33.3333
NWA0135	12/02/2002	2	100	2.00000	37.5000	25.0000	4.1667	12.5000	20.8333
NWA0135	12/16/2002	1	170	2.23045	.	.	.	.	.
NWA0135	01/06/2003	1	100	2.00000	26.0870	8.6957	17.3913	0.0000	47.8261
NWA0135	01/21/2003	2	10	1.00000	.	.	.	.	.
NWA0135	02/03/2003	2	30	1.47712	0.0000	0.0000	20.8333	0.0000	79.1667
NWA0135	03/03/2003	1	430	2.63347	13.6364	22.7273	0.0000	9.0909	54.5455
NWA0135	03/17/2003	1	20	1.30103	.	.	.	.	.

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STATION	DATE	Flow Stratum (1=high 2=low)	Ecocc Conc MPN/ 100ml	log mean conc	% domestic animals	% human	% livestock	% wildlife	% unknown
NWA0135	04/21/2003	1	10	1.00000	4.5455	0.0000	0.0000	31.8182	63.6364
NWA0135	05/05/2003	2	10	1.00000	8.3333	41.6667	0.0000	4.1667	45.8333
NWA0135	05/19/2003	1	630	2.79934	.	.	.	.	.
NWA0135	06/02/2003	1	100	2.00000	79.2	4.2	4.2	0.0	12.5
NWA0135	06/16/2003	1	100	2.00000	.	.	.	.	.
NWA0135	06/23/2003	1	90	1.95424	.	.	.	.	.
NWA0135	07/07/2003	1	19860	4.29798	16.7	54.2	0.0	8.3	20.8
NWA0135	07/21/2003	2	160	2.20412	.	.	.	.	.
NWA0135	08/04/2003	2	640	2.80618	8.3	75.0	0.0	0.0	16.7
NWA0135	08/18/2003	2	1510	3.17898	.	.	.	.	.
NWA0135	08/25/2003	2	190	2.27875	.	.	.	.	.
NWA0135	09/08/2003	2	60	1.77815	11.8	64.7	0.0	0.0	23.5
NWA0135	09/22/2003	1	170	2.23045	.	.	.	.	.
NWA0135	10/06/2003	2	40	1.60206	17.6	11.8	0.0	35.3	35.3
NWA0135	10/20/2003	2	30	1.47712	.	.	.	.	.
PNT0001	10/07/2002	2	40	1.60206	.	.	.	.	.
PNT0001	10/21/2002	2	70	1.84510	.	.	.	.	.
PNT0001	11/06/2002	1	4350	3.63849	.	.	.	.	.
PNT0001	11/18/2002	1	1180	3.07188	12.5	0.0	0.0	83.3	4.2
PNT0001	12/02/2002	2	20	1.30103	34.8	4.3	0.0	47.8	13.0
PNT0001	12/16/2002	1	60	1.77815	.	.	.	.	.
PNT0001	01/06/2003	1	170	2.23045	16.7	8.3	20.8	16.7	37.5
PNT0001	01/21/2003	2	10	1.00000	.	.	.	.	.
PNT0001	02/03/2003	2	10	1.00000	30.0	15.0	15.0	5.0	35.0
PNT0001	03/17/2003	1	10	1.00000	.	.	.	.	.
PNT0001	04/21/2003	2	10	1.00000	58.3	4.2	0.0	16.7	20.8
PNT0001	05/05/2003	2	10	1.00000	20.8	12.5	4.2	54.2	8.3
PNT0001	05/19/2003	1	560	2.74819	.	.	.	.	.
PNT0001	06/02/2003	1	70	1.84510	13	0	17	17	54
PNT0001	06/16/2003	1	120	2.07918	.	.	.	.	.

STATION	DATE	Flow Stratum (1=high 2=low)	Ecocc Conc MPN/ 100ml	log mean conc	% domestic animals	% human	% livestock	% wildlife	% unknown
PNT0001	06/23/2003	1	170	2.23045	0.0	47.6	4.8	9.5	38.1
PNT0001	07/07/2003	1	170	2.23045	20.8	8.3	4.2	25.0	41.7
PNT0001	07/21/2003	2	10	1.00000	.	.	.	.	.
PNT0001	08/04/2003	2	120	2.07918	20.8	4.2	8.3	33.3	33.3
PNT0001	08/18/2003	2	500	2.69897	.	.	.	.	.
PNT0001	08/25/2003	2	20	1.30103	.	.	.	.	.
PNT0001	09/08/2003	2	20	1.30103	6.3	43.8	0.0	0.0	50.0
PNT0001	09/22/2003	1	400	2.60206	.	.	.	.	.
PNT0001	10/06/2003	2	30	1.47712	8.3	41.7	0.0	25.0	25.0
PNT0001	10/20/2003	2	50	1.69897	.	.	.	.	.

Table C-10: Percentage of Sources per Station by Stratum

STATION	Flow Stratum (1=high/2=low)	% domestic animals	% human	% livestock	% wildlife	% unknown
BED0001	1	35.6	3.0	4.2	24.3	32.9
BED0001	2	24.3	11.7	6.2	17.7	40.2
INC0030	1	26.3	2.9	15.1	29.0	26.7
INC0030	2	20.9	23.5	7.6	22.5	25.5
NEB0002	1	35.6	3.6	8.0	33.7	19.1
NEB0002	2	9.4	7.9	25.2	23.7	33.7
NWA0002	1	21.6	9.0	9.4	23.6	36.4
NWA0002	2	18.5	11.0	2.8	28.9	38.8
NWA0135	1	28.4	26.0	3.9	7.0	34.6
NWA0135	2	14.6	40.9	3.7	8.0	32.8
PNT0001	1	12.5	12.4	8.4	34.5	32.2
PNT0001	2	23.8	18.0	4.0	26.7	27.6

Table C-11: Overall Percentage of Sources per Station

Subwatershed / Station	% domestic animals	% human	% livestock	% wildlife	% unknown	total
BED0001	27.7	9.1	5.6	19.7	38.0	100.0
INC0030	22.5	17.3	9.9	24.4	25.9	100.0
NEB0002	17.3	6.6	20.1	26.7	29.3	100.0
NWA0002	19.4	10.4	4.8	27.3	38.1	100.0
NWA0135	18.8	36.4	3.7	7.7	33.3	100.0
PNT0001	20.4	16.3	5.3	29.0	29.0	100.0

SUMMARY

The use of ARA was successful for identification of bacterial sources in the Anacostia River Watershed as evidenced by the high RCCs (> 93% except for wildlife) in the library. The lower RCC for wildlife of 71% is still acceptable. When water isolates were compared to the library and potential sources predicted, 69% of the isolates were classified by statistical analysis. The largest two categories of potential sources in the watershed as a whole were domestic animals and wildlife (23% and 22% of the classified isolates, respectively). Human potential sources were 15% of those classified, while livestock made up 9%. These results were consistent with field observations.

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Appendix D - Assigning Flow Frequency to Ungauged Watersheds

The Anacostia River Watershed has three USGS gauges within the watershed boundary and they are listed in Table D-1.

Table D-1: USGS Gauges in the Anacostia River Watershed

USGS Gage #	Dates used	Description
01649500	Oct 1, 1988 to Sep 30, 2003	
01651000	Oct 1, 1988 to Sep 30, 2003	
01650500	Nov 27, 1997 to Sep 30, 2003	
01650500 (estimate)	Oct 1, 1988 to Sep 30, 2003	Estimated flow based on USGS Gage 0165100 using MOVE.1 (Hirsch, 1982)

There are a total of six bacteria monitoring stations within the watershed and three of these stations (NEB0002, NWA0002 and NWA0135) have USGS flow data available. As noted in Table D-1, USGS station 01650500, located in the Northwest Branch of the Anacostia River near Colesville, only has a partial record with the time series beginning on November 27, 1997 and ending on September 30, 2003. Therefore, this record was extended using the station with the highest cross-correlation results, station 0165100 located downstream on Northwest Branch.

Three bacteria monitoring stations remain that do not have daily flow information. They are BED0001, INC0030 and PNT0001. To plot the bacteria monitoring data in a flow duration curve format, flow frequencies must be estimated for monitoring dates at these locations. Typical methods for estimating flows at ungauged locations include using regional regression equations or a drainage area ratio approach with a gauged basin.

Previous regression studies for predicting flows in Maryland are by Dillow (1995), Moglen et. al. (2002) and Versar (2004). All of these studies identify that the most statistically significant watershed characteristic for predicting flow is the watershed area. Soil and land use characteristics, when added to the equations, add some predictive power. Results from Versar (2004) indicated that the flow regression equations described more of the variability found in high flows than for low flows. Reiss *et. al.* (2000) provides a summary of recent literature and notes that when using the drainage area ratio approach, evidence suggests that the ratio of the ungauged basin to gauged basin should be between 0.33 and 3.0.

The cross-correlation of daily flow frequency and daily flow rate were analyzed using an n-lag model. The purpose of this was to determined wether two watersheds will have similar flows and frequencies for the same day. Results for three stations indicated the highest correlation occurred with the 0-lag model, suggesting that daily flows and frequencies are similar for the same days. Results for the zero lag correlations are as follows:

Table D-2: Cross-Correlation of Flow Frequency

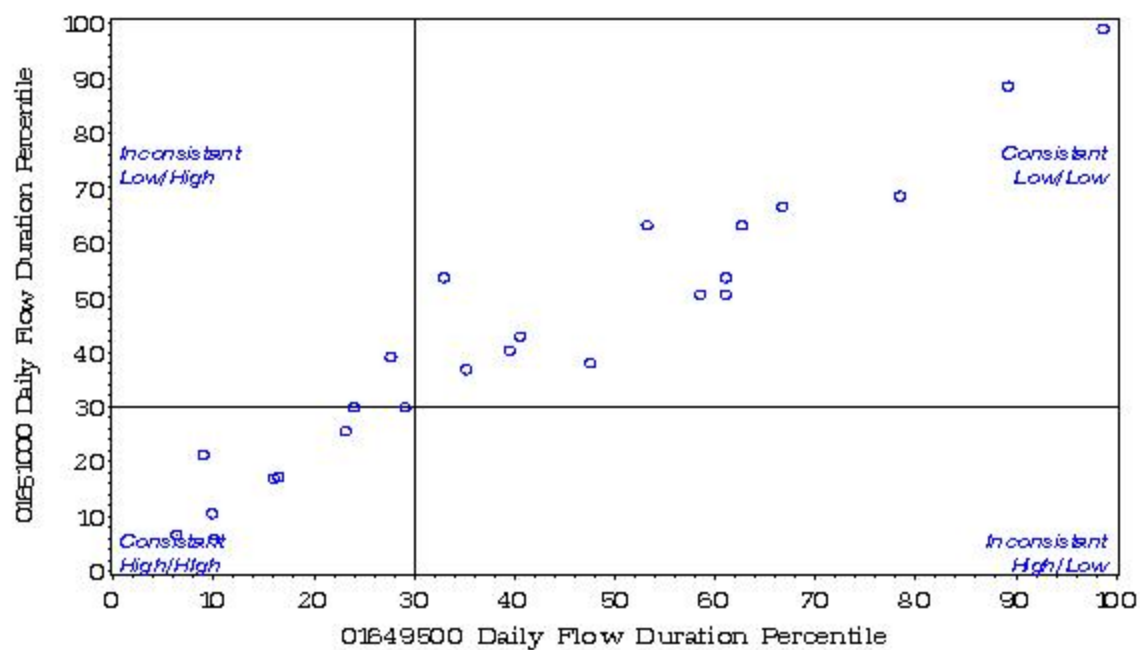|	01649500	01651000	01650500
01649500	1		
01651000	0.958	1	
01650500	0.945	0.954	1

Using primarily watershed area ratio as the criterion, gages were assigned as follows:

**Table D-3: Bacteria Monitoring Stations and Reference Flow Gages**

Station	Watershed Area (sq. miles)	USGS Reference Gauge	USGS Gauge Area (sq. miles)	Area Ratio
BED0001	14.5	1650500	21.1	0.69
INC0030	9.8	1650500	21.1	0.47
PNT0001	32.2	1649500	72.8	0.44
NEB0002	76.8	1649500	72.8	1.06
NWA0135	21.8	1650500	21.1	1.03
NWA0002	52.2	1651000	49.9	1.05

A visual comparison among the USGS flow gauges is presented in Figures D-1 through D-3. Note that the separation of the flow strata is added to identify potential misclassification of a sample. The four quadrants of the graphs are defined and labels identify zones based on consistent and inconsistent placement of stations in flow strata between gauges. These figures support the conclusion that the flow frequency between the gauges, especially for bacteria sample dates, is very similar.
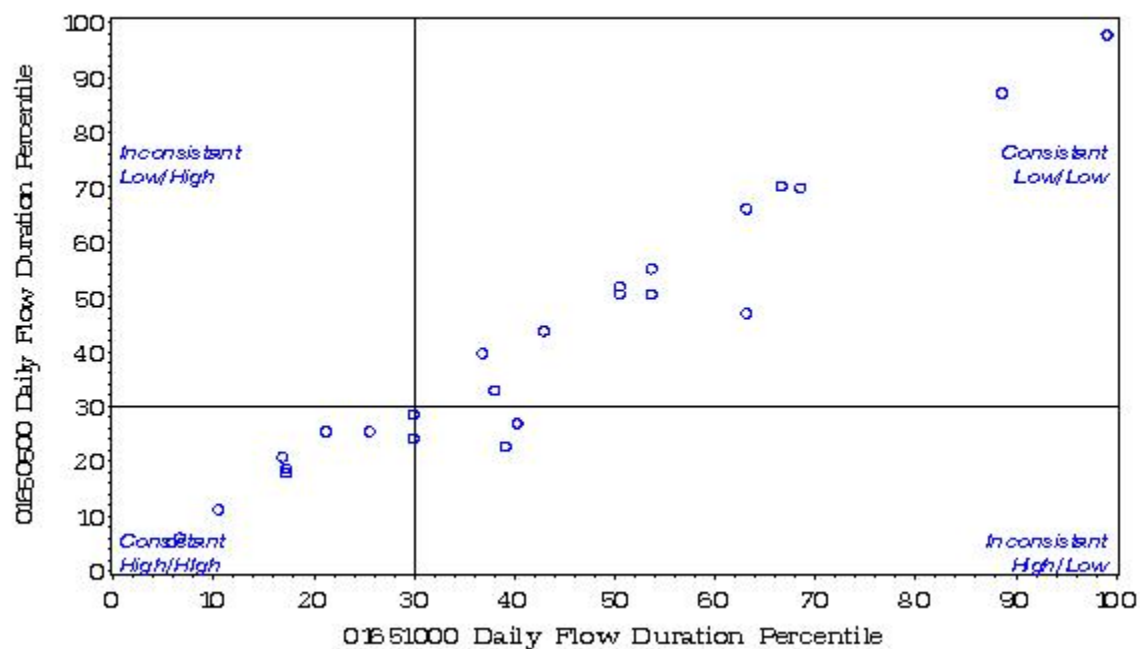
Figure D-1: Comparison of Flow Frequency Between 01651000 and 01649500 for Anacostia River Bacteria Monitoring Dates
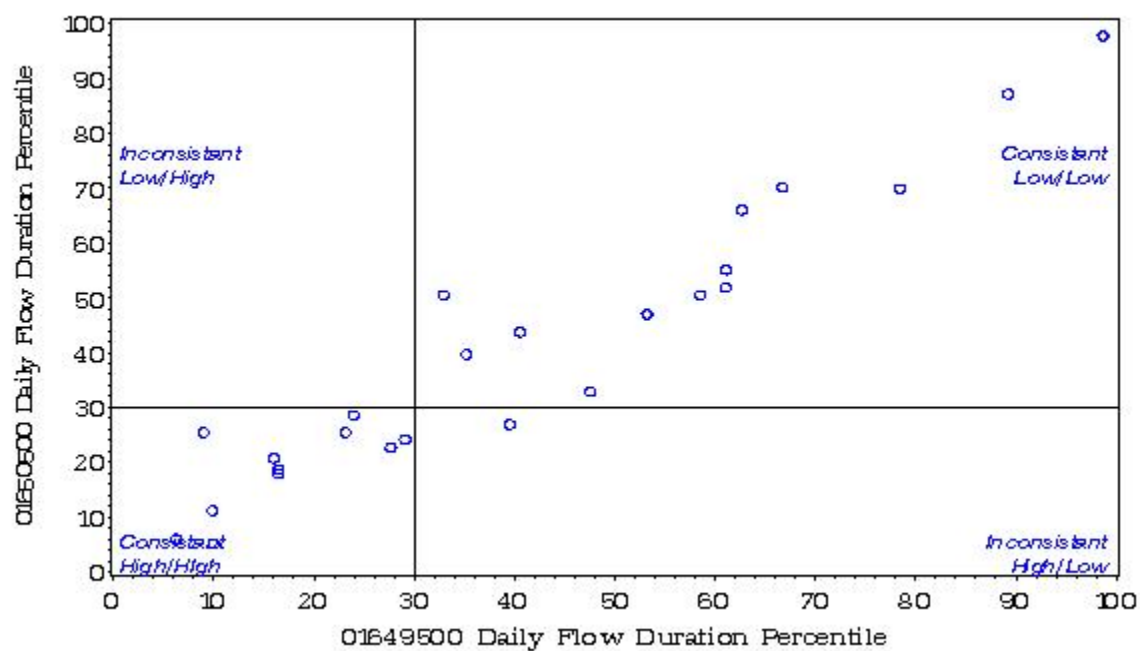
Figure D-2: Comparison of Flow Frequency Between 01650500 and 01649500 for Anacostia River Bacteria Monitoring Dates

Figure D-3: Comparison of Flow Frequency Between 0160500 and 01651000 for Anacostia River Bacteria Monitoring Dates

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