Total Maximum Daily Loads of Fecal Bacteria for the Non-Tidal Piscataway Creek Basin in Prince George's County, Maryland

REVISED FINAL

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Submitted to:

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List of Abbreviations

ARCC	Average rates of correct classification
ARA	Antibiotic Resistance Analysis
AFB	Air Force Base
BMP	Best Management Practice
BPA	Blue Plains Advanced WWTP
BST	Bacteria Source Tracking
CAFO	Confined Animal Feeding Operations
cfs	Cubic Feet per Second
CFR	Code of Federal Regulations
CFU	Colony Forming Units
COMAR	Code of Maryland Regulations
CSO	Combined Sewer Overflow
CSU	Carbon Source Utilization
CWA	Clean Water Act
CWP	Center for Watershed Protection
DNR	Department of Natural Resources
EPA	Environmental Protection Agency
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
MRLC	Multi-Resolution Land Cover
MPR	Maximum Practicable Reduction
MS4	Municipal Separate Storm Sewer System
MST	Microbial Source Tracking
NPDES	National Pollutant Discharge Elimination System
PFGE	Pulsed-Field Gel Electrophoresis
RAPD	Randomly-Amplified Polymorphic DNA
RCC	Rates of Correct Classification
SSO	Sanitary Sewer Overflows
SU	Salisbury University
TARSA	Technical and Regulatory Services Administration
TMDL	Total Maximum Daily Load
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

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 non-tidal portion of Piscataway Creek (basin number 02-14-02-03). Section 303(d) of the federal Clean Water Act (CWA) and the EPA 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 non-tidal portion of Piscataway Creek, Use IP – Water Contact Recreation, and Protection of Aquatic Life and Public Water Supply [Code of Maryland Regulations (COMAR) 26.08.02.08O(1)] in the State's 303(d) as impaired by nutrients (1996), sediments (1996), bacteria (fecal coliform) (2002), and impacts to biological communities (2004). The listings for nutrients and sediments are in the tidal portion of Piscataway Creek. This document proposes to establish a TMDL of fecal bacteria in the non-tidal portions of Piscataway Creek that will allow for the attainment of the designated primary contact recreation. The listings for nutrients, sediments, and impacts to biological communities 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 was considered.

To establish baseline and allowable pollutant loads for this TMDL, a flow duration curve approach, using flow strata estimated from United States Geological Survey (USGS) daily flow monitoring data and bacteria monitoring data, was used. The pollutant loads set forth in this document are for the non tidal area of the Piscataway Creek watershed. The sources of fecal bacteria are estimated at two representative stations in the Piscataway Creek watershed where samples were collected for one year. Multiple antibiotic resistance analysis (ARA) source tracking was used to determine the relative proportion of domestic (pets and human associated animals), human (human waste), livestock (agricultural related animals), and wildlife (mammals and waterfowl) source categories.

The allowable load is determined by estimating a baseline load from current monitoring data. The baseline load is estimated using a long-term geometric mean and weighting factors from the flow duration curve. The TMDL load for fecal bacteria entering the Piscataway Creek is established after considering four different hydrological conditions: wet and dry annual conditions; and wet and dry seasonal conditions (the period between May 1st and September 30th where water contact recreation is more prevalent). This allowable load is reported in the 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.

Two scenarios were developed; the first assessing whether 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 the two subwatersheds, it was estimated that water quality standards could not be attained with the MPRs. Thus, for these subwatersheds, a second scenario with greater reductions, which may not be feasible, was applied.

The fecal bacteria TMDL developed for the Piscataway Creek non-tidal watershed is 201 billion MPN *E. coli*/day. The TMDL is distributed between load allocation (LA) for non-point sources and waste load allocations (WLA) for point sources, including National Pollutant Elimination System (NPDES) wastewater treatment plants (WWTPs) and NPDES municipal separate storm sewer systems (MS4). The LA is 118 billion MPN/day. The MS4 WLA is 83 billion MPN/day. The WWTP WLA is 0.1 billion MPN/day. The margin of safety (MOS) is implicit in this 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. MDE intends for the required reduction to be implemented in an iterative process that first addresses those sources with the largest impacts to 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 implementation efforts. As previously stated, water quality standards cannot be attained in the Piscataway Creek 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. In these cases, it is expected that the first stage of TMDL implementation will be to implement the MPR scenario. MDE cannot provide EPA reasonable assurance at this time that the TMDL allocations can be met given the magnitude of the MS4 allocation and known efficiencies for relevant urban Best Management Practices. However, progress will be made through the iterative implementation process described above and the situation will be reevaluated in the future.

1.0 INTRODUCTION

This document, upon approval by the U.S. Environmental Protection Agency (EPA), establishes a Total Maximum Daily Load (TMDL) for fecal bacteria in the Piscataway Creek (basin number 02-14-02-03). Section 303(d)(1)(C) of the federal Clean Water Act (CWA) and the 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 Maryland Department of the Environment (MDE) has identified Piscataway Creek in the State’s 303(d) list as impaired by nutrients (1996), sediments (1996), fecal bacteria (2002), and impacts to biological communities (2004). The listings for nutrients and sediments are in the tidal portion of the Piscataway Creek. This document proposes to establish fecal bacteria TMDL in the non-tidal portions of the Piscataway Creek that will allow for attainment of its designated uses. All other impairments in the tidal and non-tidal portions of Piscataway Creek will be addressed at a future date. A data solicitation for fecal bacteria was conducted by MDE in 2003, and all readily available data from the past five years were considered in the TMDL analysis.

Fecal bacteria are microscopic single-celled organisms (primarily fecal 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 are a subgroup of total coliform bacteria and *E. coli* are a subgroup of fecal coliform. 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 Piscataway Creek watershed was listed on the Maryland 303(d) list using fecal coliform as the indicator organism. Based on EPA's guidance (EPA, 1986), adopted by Maryland in 2004, the State has revised the bacteria water quality criteria and it is now based on water column limits for either *E. coli* or enterococci. Because multiple monitoring datasets are available within this watershed for various pathogen indicators, the general term fecal bacteria will be used to refer to the impairing substance throughout this document. The TMDL will be based on the pathogen indicator organisms specified in Maryland's current bacteria water quality criteria, either *E. coli* or enterococci. The indicator organism used in the Piscataway Creek TMDL analysis was *E. coli*.

2.0 SETTING AND WATER QUALITY DESCRIPTION

2.1 General Setting

Location

The Piscataway Creek watershed encompasses 69 square miles in Prince George's County (See Figure 2.1.1). Headwaters originate to the west and east of Andrews Air Force Base (AFB) (in the vicinity of Camp Springs, Clinton and Woodyard). On the southwest side of Andrews AFB two branches join to form Tinkers Creek, the major tributary to Piscataway Creek. Surface water runoff flows into Tinkers Creek, to Piscataway Creek, and eventually into the Potomac River. From the southeast of Andrews AFB, the mainstem receives drainage from nearly 1,500 acres of the base and is partially redirected to a man-made lake (Base Lake) on base. The Piscataway Creek mainstem has two named tributaries: Dower House Branch to the northeast and Butler Branch to the southwest. There are several small unnamed tributaries supplying input to Piscataway Creek.

Northern Region

This area is the more developed portion of the region. It is the northern region of the Piscataway Creek between Andrews AFB and Louise F. Cosca Regional Park. The major land use in this region is Andrews AFB. The base sits atop a north-south drainage divide, in the vicinity of the runways, that separates the Potomac River Basin to the west and the Patuxent River Basin to the east. The area surrounding Andrews AFB to the east is residential, commercial, light and heavy industrial, agricultural and some open land. The land use to the west is residential, commercial, and industrial. The area to the north is commercial and light industrial. The population density here is high. A source of potential microbial loading is from failing septic systems in older homes and facilities.

Southern Region

This region comprises the area between Louise F. Cosca Regional Park to Piscataway Creek drainage. The land use to the south is mostly forested, some open and row-crop agricultural land, residential, commercial, and light industrial. Butler Branch (tributary to Piscataway Creek) flows through Louise F. Cosca Regional Park and it forms a lake within the park. This park has extensive facilities: shelters, grills, restrooms, athletic fields, tennis courts and nature trails. To the south the land is more forested and agricultural with the encroachment of rural development; many new home estates. Along Accokeek Road (Route 373) between Dyson Road and Bealle Road there are older homes with septic systems. To the south along Indian Head Highway (Route 210) there is extensive urban development and homes with septic systems. There are farms in this region (row-crop and horse) but neither are sources of significant microbial loading. Potential sources of microbial loading are failing septic systems and wildlife.

Geology/Soils

The Piscataway Creek watershed is in the Coastal Plain Province, draining to the Potomac River. A wedge of unconsolidated sediments including gravel, sand, silt and clay underlies this physiographic province. The topography varies from level to hilly in the watershed, with slopes ranging from sea level to 200 feet. The creek and its tributaries follow a dendritic pattern; a branching tree-like effect. The main source of water in the Coastal Plain is groundwater. Because unconsolidated sediments underlie the region, precipitation usually sinks in easily.

The mainstem of the non tidal Piscataway Creek and its tributaries lie predominantly in the Beltsville, Bibb soil series. A small portion of the watershed at the headwaters of the Creek lies in the Westphalia soil series. Beltsville soils are moderately deep, well drained to poorly drained, dominantly gently sloping soils that have a compact subsoil or substratum. The Bibb marsh association series consists of poorly drained soils of the flood plains and soils in marshes that are subject to tidal flooding. The Westphalia soil series are deep, well drained to excessively drained soils of uplands that are mostly moderately sloping to steep. The tidal part of the creek lies mostly in the Othello soil series. The Othello series are deep, poorly drained soils that have gray, highly silty subsoils through which water moves slowly. The Othello soils are on nearly level to gently sloping uplands of the Coastal Plain (Soil Conservation Service, 1967). The spatial distributions for each soil series are shown in Figure 2.1.2.

Land Use

The non-tidal Piscataway Creek basin has an area of approximately 36,000 acres. The land use in the watershed is diverse. The 2002 Maryland Department of Planning (MDP) land use/land cover data shows that the Piscataway Creek watershed can be characterized as residential and forested. There are 15,590 acres (24.4 square miles) of park and forest lands evenly dispersed throughout the watershed, such as the Fort Washington Forest, Piscataway Creek Park, Tinkers Creek Park and L. F. Cosca Regional Park. The watershed contains 10,728 acres (16.8 square miles) of residential land use that represents 30% of the total area. The commercial land use is largely confined to the northeast region of the basin south of Andrews AFB. This region contains approximately 5,014 acres (7.8 square miles) of commercial land (including the base).

Crops and Pasture lands are dispersed through the watershed with higher concentration of croplands towards the southwest region of the watershed. Total crops and pasture areas constitute 3,230 (5.0 square miles) and 1,367 acres (2.1 square miles) respectively. The land use percentage distribution for Piscataway Creek Basin is shown in Table 2.1.1, and spatial distributions for each land use are shown in Figure 2.1.3.

Table 2.1.1: Land Use Percentage Distribution for Piscataway Creek Basin

Land Type	Acreage	Percentage
Forest	15,590	43%
Residential	10,728	30%
Commercial	5,014	14%
Crops	3,230	9%
Pasture	1,367	4%
Water	77	0.2%
Totals	**36,006**	**100%**

Population

The total population in the Piscataway Creek watershed is estimated to be 58,991 people. Figure 2.1.4 describes the population density in the watershed. The human population and the number of households were estimated based on a weighted average from the GIS 2000 Census Block and the Maryland Department of Planning (MDP) Land Use 2002 Cover that includes the Piscataway Creek watershed. Since the Piscataway Creek watershed is a sub-area of the Census Block, the Geographic Information System (GIS) tool was used to extract the areas from the 2000 Census Block within the watershed. Based on the Land Use for Residential Density (low, medium, high) from the MDP Land Use Cover, the number of dwellings per acre was calculated using Table 2.1.2 in the Piscataway Creek watershed.

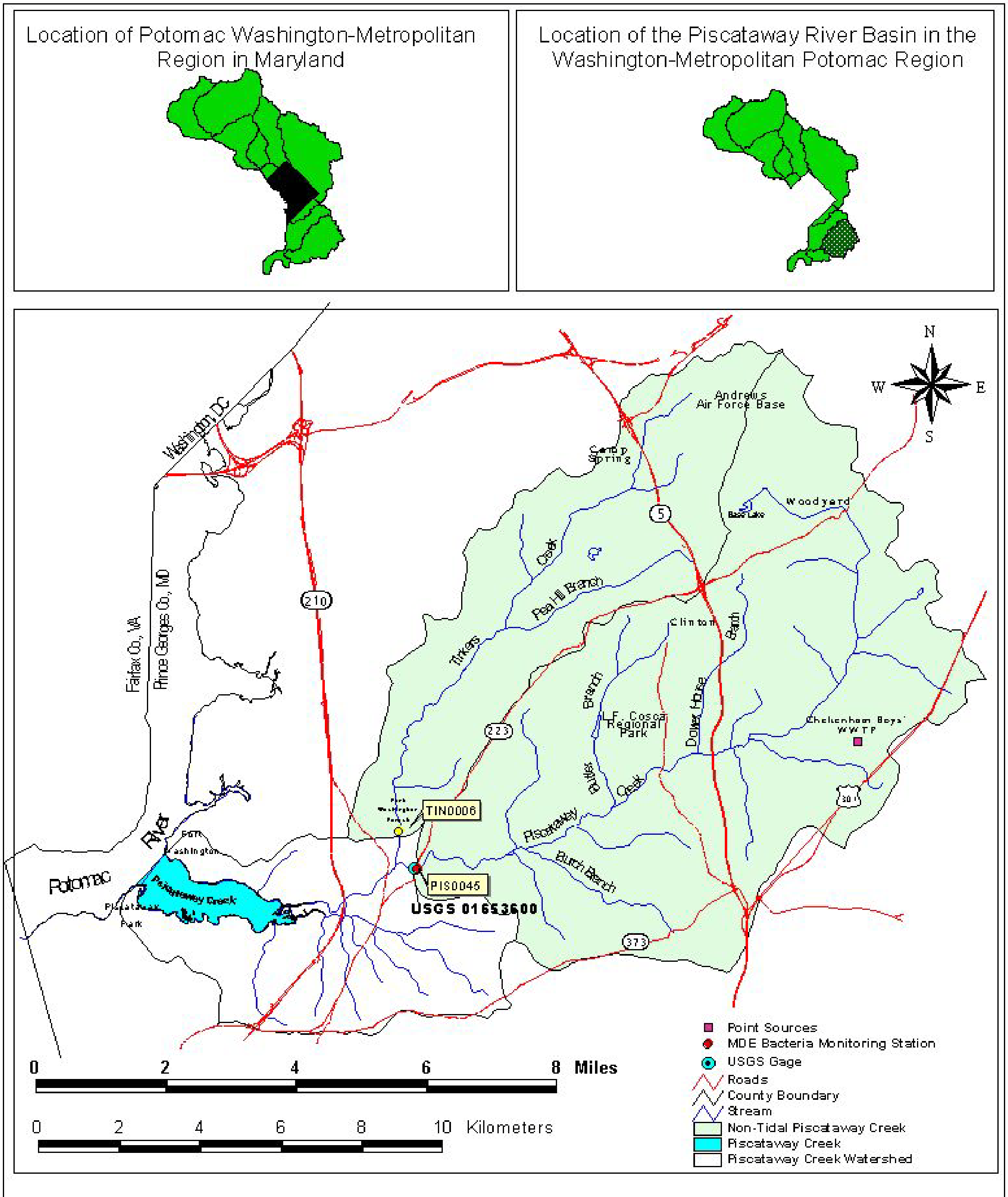
Table 2.1.2: Number of Dwellings Per Acre

Land use Code	Dwellings Per Acre
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 subwatershed was calculated (Table 2.1.3.)

Table 2.1.3: Total Population Per Subwatershed in Piscataway Creek Watershed

Tributary	Station	Population
Piscataway Creek	PIS0045	33,745
Tinkers Creek	TIN0006	25,246



Data Sources:
Watershed: MD Department of Natural Resources
Monitoring Stations: MD Department of the Environment
Streams: EPA
Roads: MD State Highway Administration

MDE

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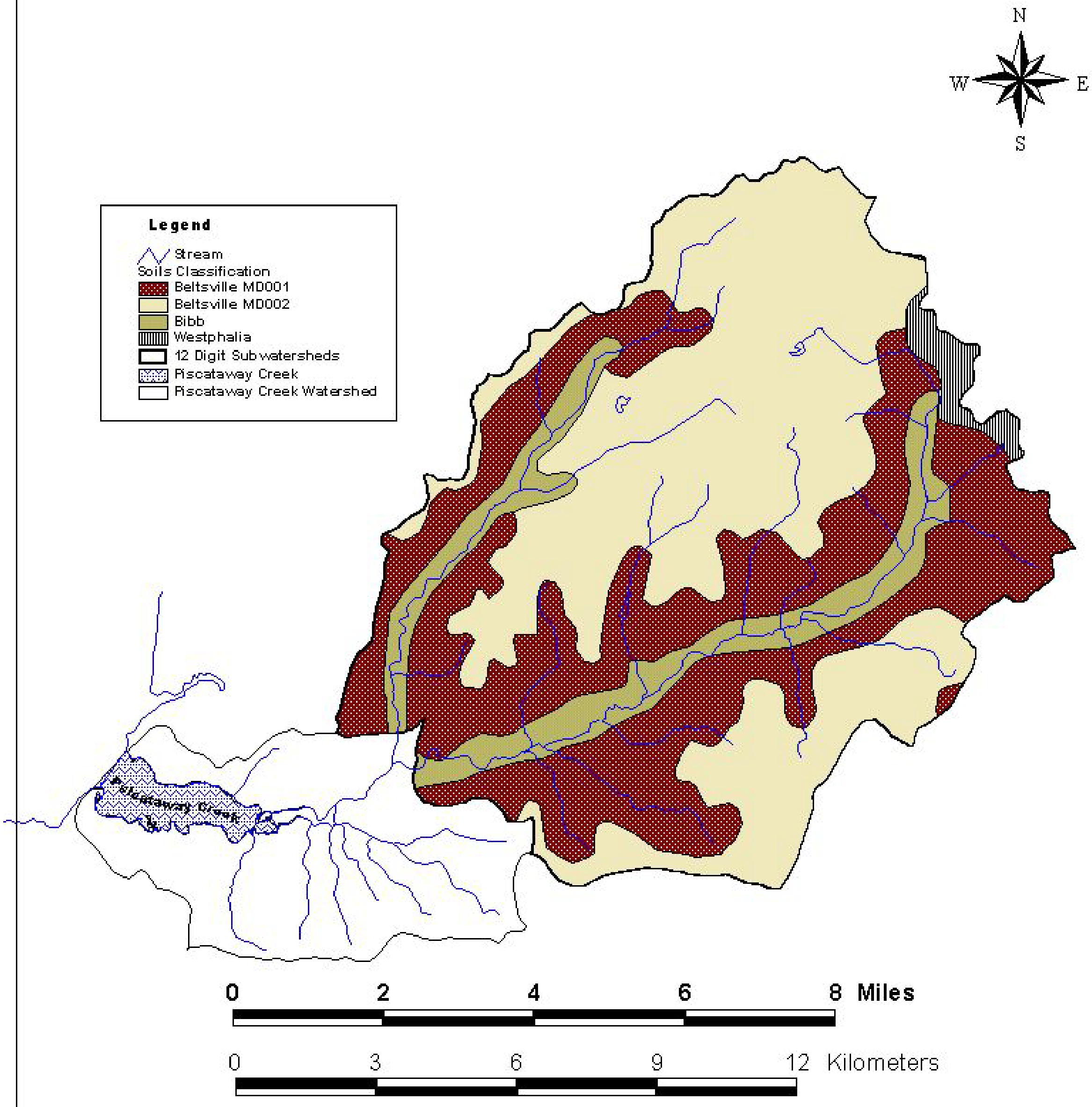
Map Production Date: February 7, 2005

Figure 2.1.1: Location Map of the Piscataway Creek Basin



Data Sources:
Watersheds: MD Dept of Natural Resources
Streams: EPA
Soils: Statsgo

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Figure 2.1.2: General Soil Series in the Piscataway Creek Basin

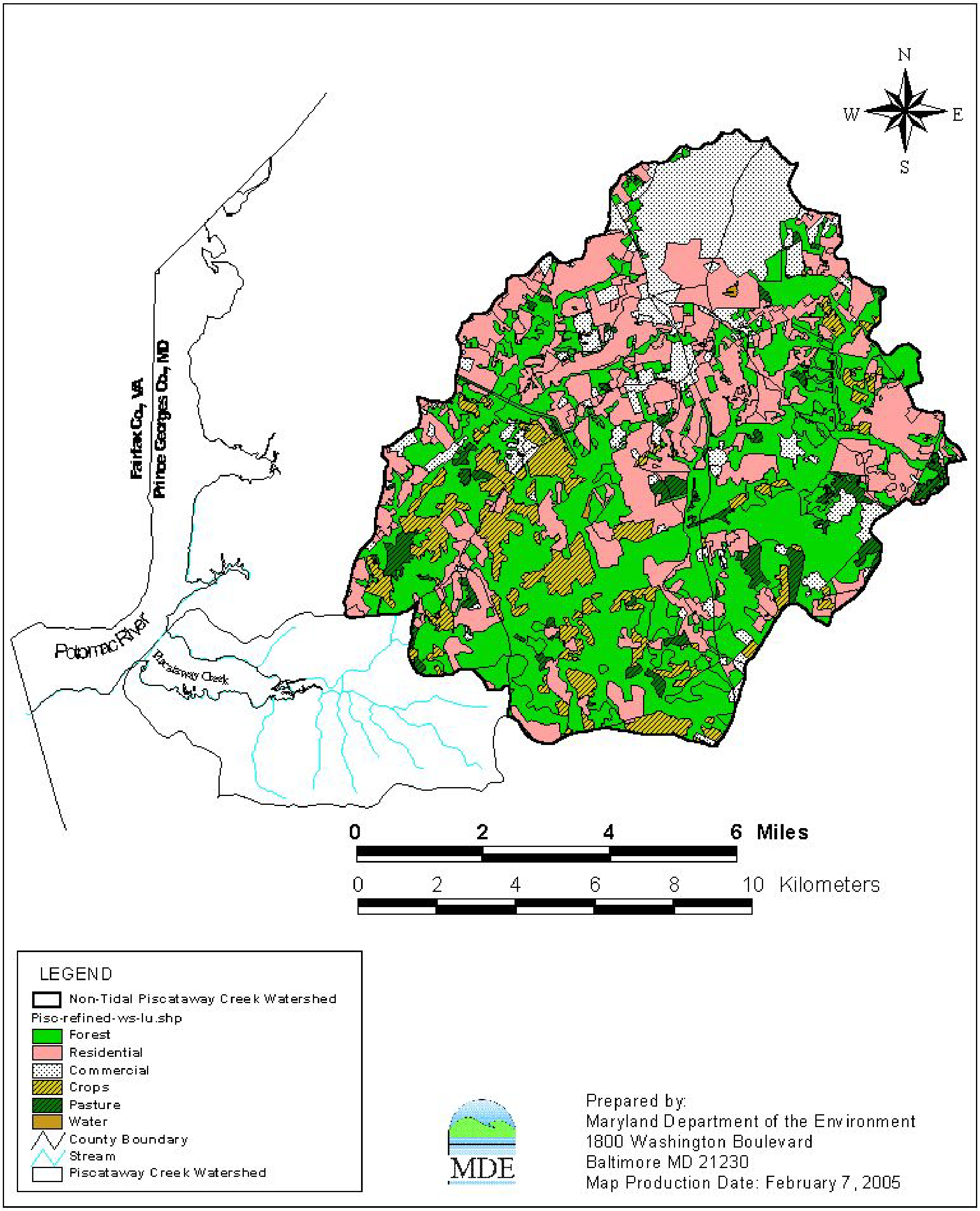

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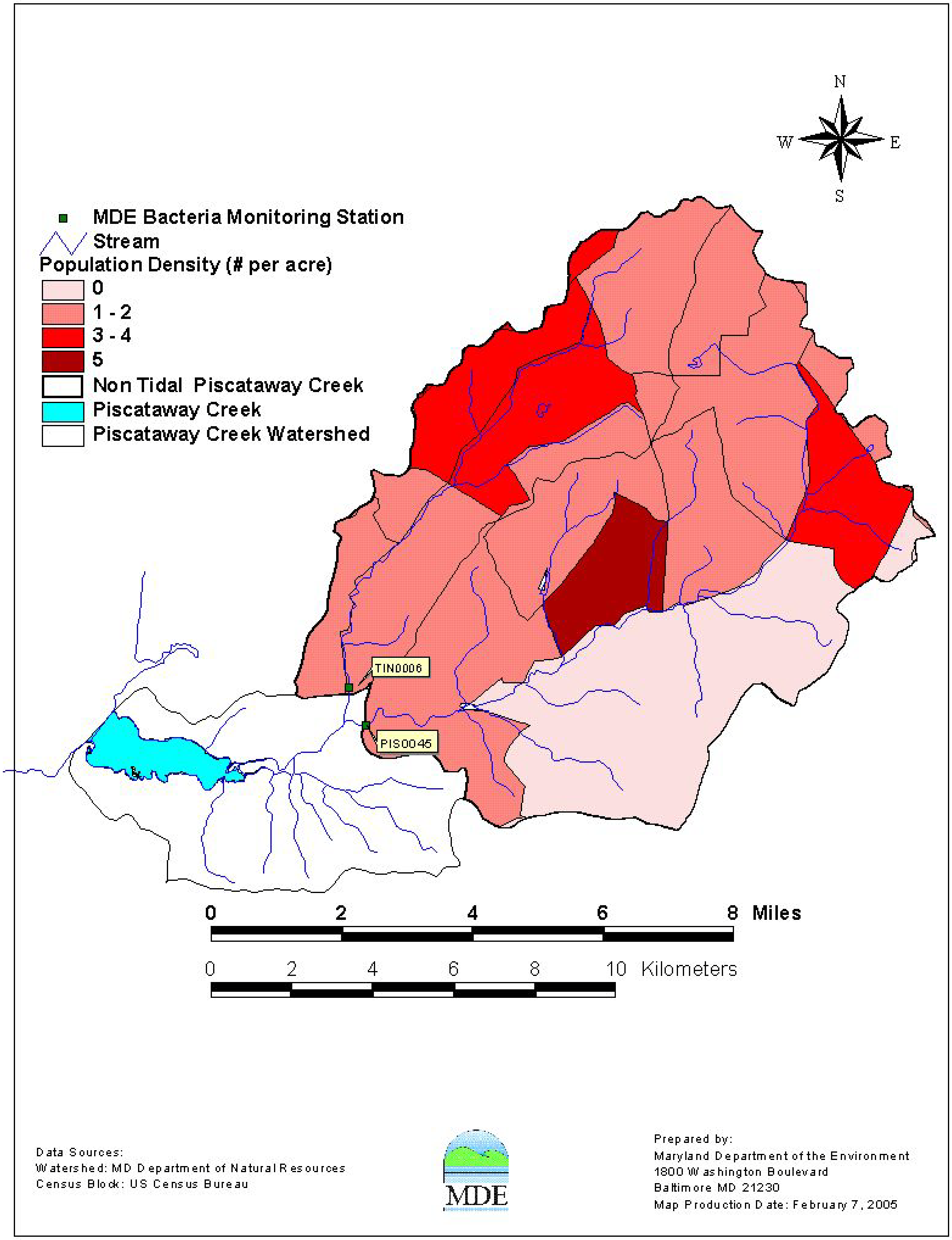
Figure 2.1.3: Land Use of the Piscataway Creek Watershed



Data Sources:
Watershed: MD Department of Natural Resources
Census Block: US Census Bureau

Prepared by:
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Figure 2.1.4: Population Density in Piscataway Creek Basin

2.2 Water Quality Characterization

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

As per EPA's guidance, Maryland has adopted the new indicator organisms, *E. coli* and enterococci, for the protection of public health in Use I, II, and IV waters. These bacteria listings were originally assessed using fecal coliform bacteria. The 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 (USEPA, 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 Piscataway Creek watershed. Monitoring Station PIS0033 (CORE) was used by the Maryland Department of Natural Resources (DNR) to identify the bacterial impairment. MDE conducted bacteria monitoring at two stations from October 2002 through October 2003. In addition to the bacteria monitoring stations, there is one United States Geological Survey (USGS) gauging station located in the Piscataway Creek watershed that was used in deriving the surface flow in Piscataway Creek. The locations of these stations are shown in Table 2.2.2 – Table 2.2.4 and Figure 2.2.1. Observations recorded during 2002-2003 from the two 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 impairment decreases with watershed size. Appendix A has a table that lists the monitoring results from the Piscataway Creek watershed.

Bacteria counts are highly variable in Piscataway Creek. This is typical for all streams due to the nature of bacteria and its relationship to flow. Results of bacteria counts for the two monitoring stations are shown in Appendix A. Data were collected from September 2002 through November 2003. Ranges were typically between 10 and 2,010 MPN/100 ml.

Table 2.2.1: Historical Monitoring Data in the Piscataway Creek Watershed

Sponsor	Location	Date	Design	Summary
Maryland Department of Natural Resources (DNR) Core Monitoring	MD	1/8/97 – 4/1/98	Fecal Coliform	PIS0033 at Indian Head Highway
MDE	MD	11/02 to 10/03	*E. coli*	2 station 2x per month
MDE	MD	11/02 to 10/03	BST (*E. coli*)	2 station ARA Bacterial Source Tracking (BST) 1x per month

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

Monitoring Station	Obs. Period	Total Obs.	LATITUDE Decimal Degrees	LONGITUDE Decimal Degrees
PIS0033	3/17/97 – 4/6/98	13	38.6985	-76.9866

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

Monitoring Station	Obs. Period	Total Observations	LATITUDE Decimal Degrees	LONGITUDE Decimal Degrees
PIS0045	2002-2003	26	38.7056	-76.9656
TIN0006	2002-2003	26	38.7141	-76.9706

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

Monitoring Station	Obs. Period	Total Observations	LATITUDE Decimal Degrees	LONGITUDE Decimal Degrees
1653600	2002 - 2003	5967	38.7058	-76.9662

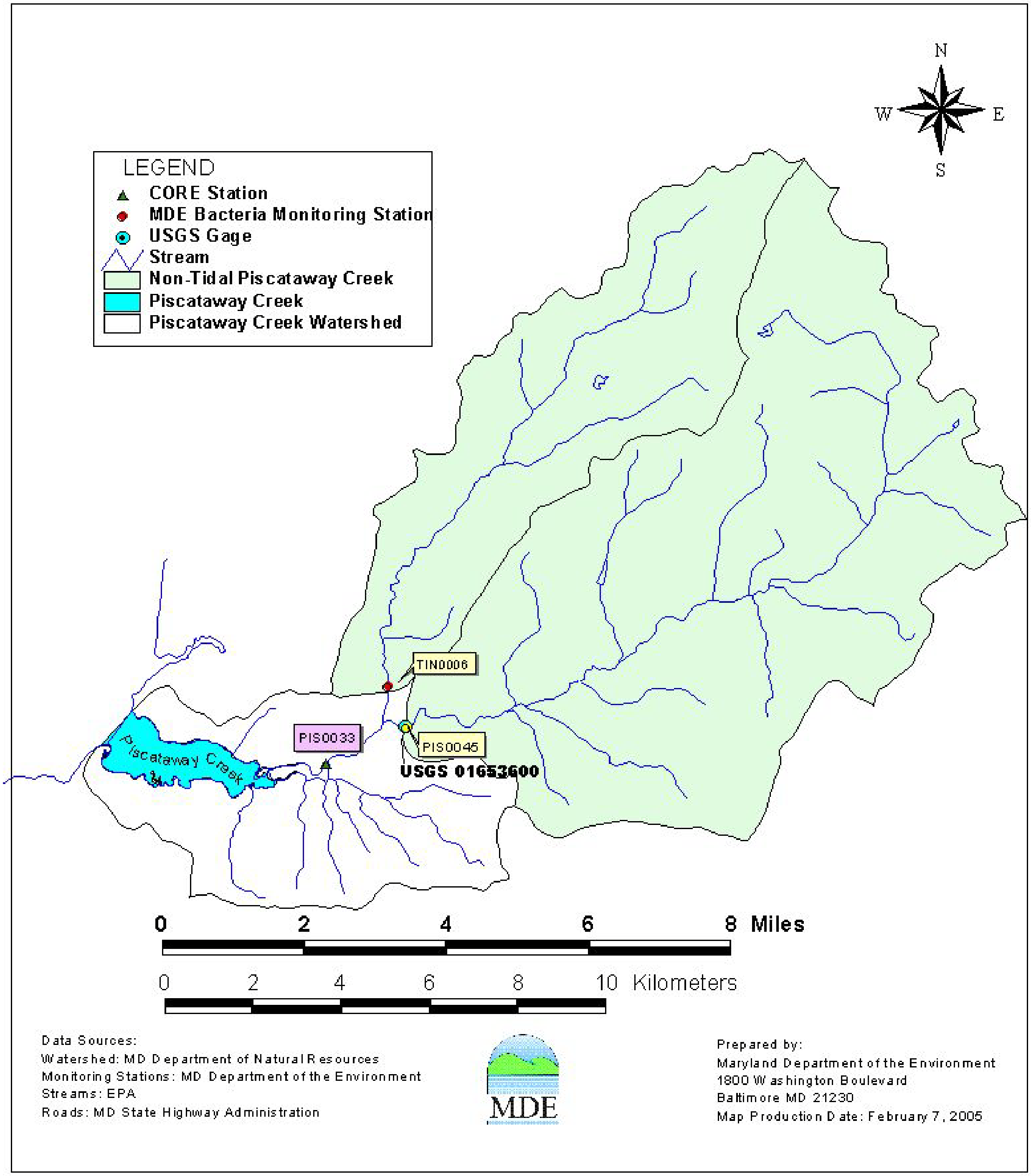


Figure 2.2.1: Monitoring Stations in the Piscataway Creek Basin

2.3 Water Quality Impairment

Designated Uses and Water Quality Standard

The Maryland water quality standards Surface Water Use Designations for the non-tidal portion of this watershed area is Use I-P – Water Contact Recreation, and Protection of Aquatic Life and Public Water Supply (COMAR 26.08.02.08O(1)). The Piscataway Creek has been included on the final 2004 Integrated 303(d) List as impaired by fecal coliform bacteria.

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 Piscataway Creek 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.

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 *E. coli* concentrations with the water quality criterion. 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 on an annual and seasonal basis.

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 Piscataway Creek watershed. To estimate the steady state geometric means, the monitoring data were first reviewed by plotting the sample results versus their corresponding daily flow duration percentile. Graphs illustrating these results can be found in Appendix B.

To calculate the steady state geometric means 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.

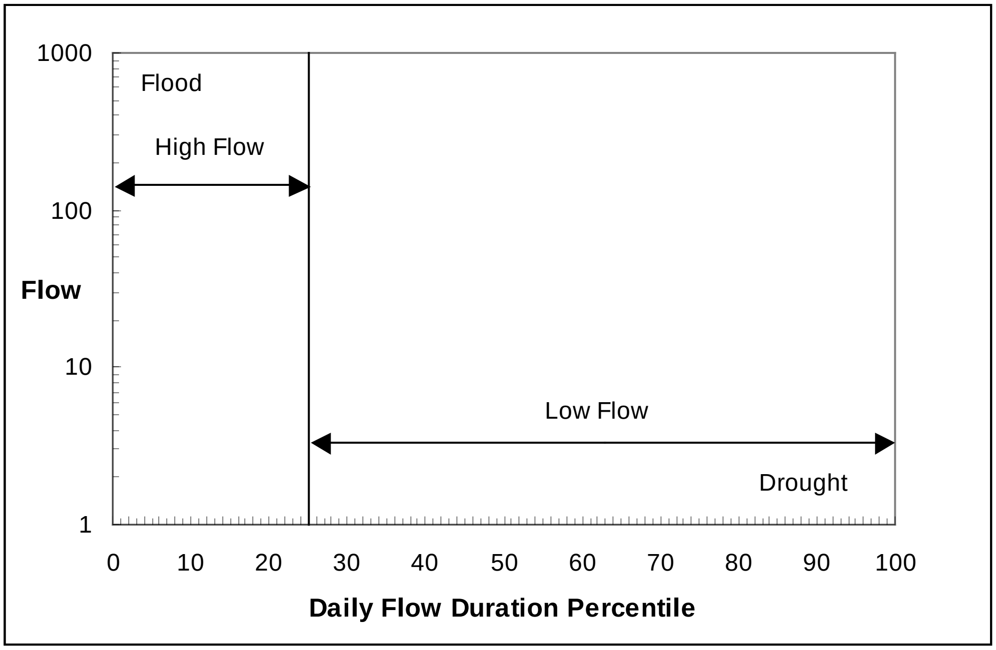


Figure 2.3.1: Conceptual Diagram of Flow Duration Zones

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 Piscataway Creek TMDL analysis are presented in the following table (Table 2.3.2).

Table 2.3.2: Weighting factors for Average Hydrology Year Used for Estimation of Geometric Means in the Piscataway Creek Watershed (Average Hydrology Year)

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

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

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

where

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

Mi = log mean concentration for stratum i
Ci,j = Concentration for sample j in stratum i
ni = number of samples in stratum I
M = weighted mean
Wi= Proportion of stratum i

Finally the weighted log mean is back transformed from log space using the following equation.

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

Cgm = Steady state geometric mean concentration

Tables 2.3.3 and 2.3.4 present the geometric means by stratum and the weighted steady state geometric mean for the Piscataway Creek subwatersheds for the annual and the seasonal (May 1st –September 30th) periods.

Table 2.3.3: Piscataway Creek Annual Steady State Geometric Mean by Stratum per Subwatersheds

Tributary	Station	Flow Stratum	Annual Steady State Geometric Mean (MPN/100ml)	Annual Weighted Geometric Mean (MPN/100ml)
Piscataway Creek	PIS0045	High	180	123
		Low	109	
Tinkers Creek	TIN0006	High	203	108
		Low	87	

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

Tributary	Station	Flow Stratum	Seasonal Steady State Geometric Mean (MPN/100ml)	Seasonal Weighted Geometric Mean (MPN/100ml)
Piscataway Creek	PIS0045	High	358	232
		Low	200	
Tinkers Creek	TIN0006	High	395	183
		Low	141	

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: Piscataway Creek Monitoring Data and Steady State Geometric Mean per Subwatershed for Annual Period

Tributary	Station	# Samples	*E. coli* Minimum (MPN/100ml)	*E. coli* Maximum (MPN/100ml)	*E. coli* Geometric Mean (MPN/100ml)	*E. coli* Criterion (MPN/100ml)
Piscataway Creek	PIS0045	25	10	1350	123	126
Tinkers Creek	TIN0006	25	10	2010	108	126

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

Tributary	Station	# Samples	*E. coli* Minimum (MPN/100ml)	*E. coli* Maximum (MPN/100ml)	*E. coli* Geometric Mean (MPN/100ml)	*E. coli* Criterion (MPN/100ml)
Piscataway Creek	PIS0045	12	110	1350	232	126
Tinkers Creek	TIN0006	12	10	2010	183	126

2.4 Source Assessment

Nonpoint Source Assessment

Nonpoint sources of fecal bacteria do not have one discharge point but occur over the entire length of a stream or waterbody. Many types of nonpoint sources introduce fecal bacteria to the land surface including the manure spreading process, direct deposition from livestock during the grazing season, and excretions from pets and wildlife. As the runoff occurs during rain events, surface runoff transports water and fecal bacteria over the land surface and discharges to the stream system. The deposition of non-human fecal bacteria directly to the stream occurs when livestock or wildlife have direct access to the waterbody. Nonpoint source contributions from human activities generally arise from failing septic systems and their associated drain fields or leaking infrastructure (*i.e.*, sewer systems). In summary, the transport of fecal bacteria from the land surface to the stream system is dictated by the rainfall, soil type, land use, and topography of the watershed.

Sewer 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.

There are two municipal treatment plants in the Piscataway Creek watershed. The Piscataway wastewater treatment plant (WWTP) and the Cheltenham Boys' WWTP are municipal point sources located in the Piscataway Creek watershed. The Piscataway Creek WWTP receives wastewater from the entire sewer collection system within Piscataway Creek. The Piscataway WWTP has not discharged into Piscataway Creek for over 20 years. There is a pipeline that transports its effluent to the middle of the Potomac River (basin number 02140201). Only Cheltenham Boys' WWTP, which serves a juvenile delinquent school facility, currently discharges into one of the tributaries of the Piscataway Creek. The plant is located in the northeastern part of the Piscataway Creek near the town of Cheltenham.

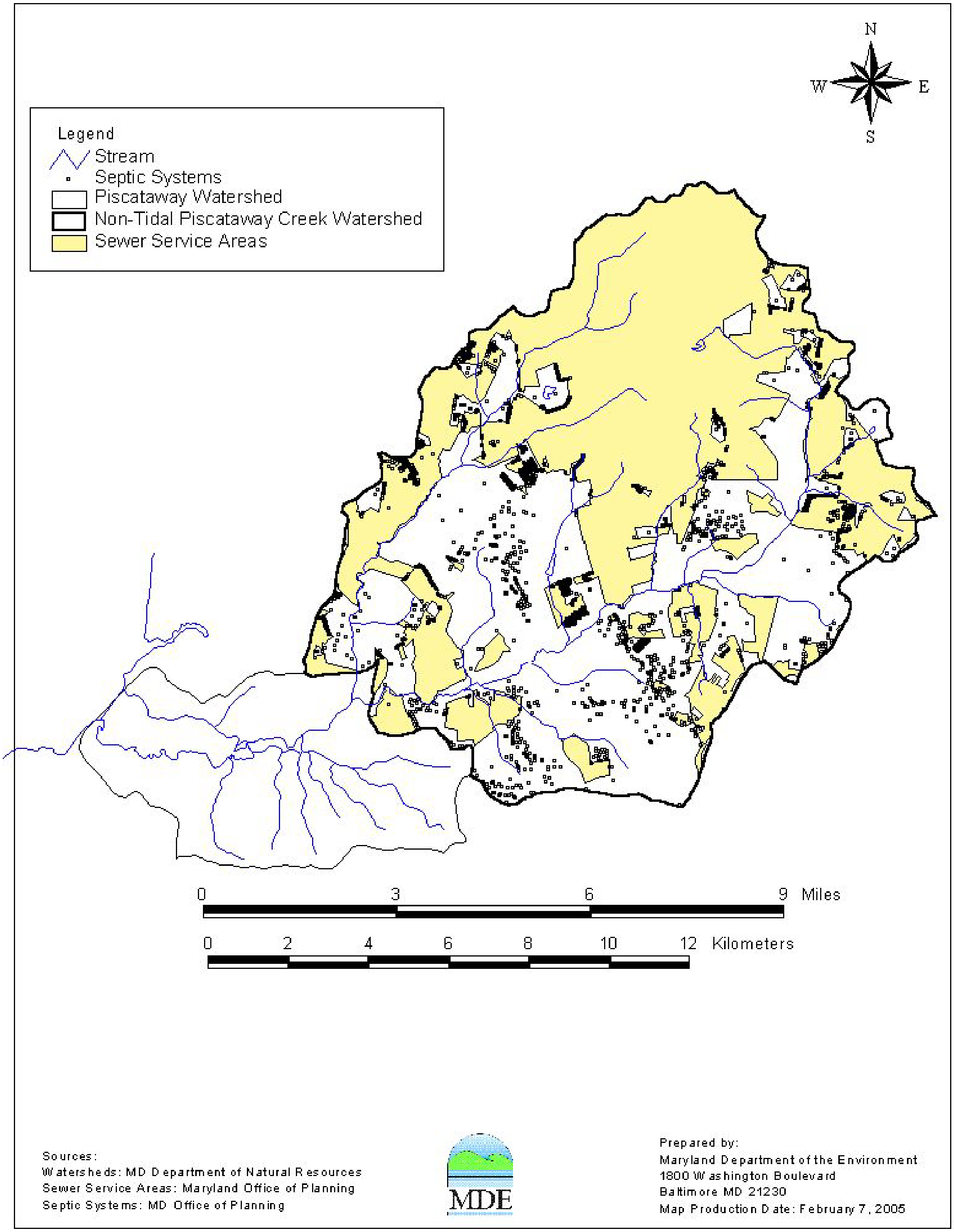
There are also septic systems found in the east and in the southern half of the Piscataway Creek watershed. Andrews AFB and the region near the base are mostly residential and serviced by sewer systems. Figure 2.4.1 depicts the areas that are serviced by sewers and septic systems. Table 2.4.1 displays the number of septic systems and households per sub watershed.

Sanitary Sewer Overflows (SSOs) occur when the capacity of a separate sanitary sewer is exceeded. There are several factors that may contribute to SSOs from a sewerage system, including pipe capacity, operations and maintenance effectiveness, sewer design, age of system, pipe materials, geology and building codes. SSOs are prohibited by the facilities' permit and therefore 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 total of 25 sanitary sewers overflow reported between July 27, 2001 and September 14, 2004, in the Prince George's County portion of Piscataway Creek watershed. Approximately 3,196,000 gallons of SSO discharge was released through various waterways (surface water, groundwater, sanitary sewers, etc.) in the Piscataway Creek watershed (MDE, Water Management Administration). Figure 2.4.2 depicts the location of sanitary sewer overflows, from 2001 to 2004 in the Piscataway Creek watershed.

Table 2.4.1: Septic Systems and Households Per Sub-Watershed in Piscataway Creek Watershed

Tributary	**Station**	**Septic Systems (units)**	**Households per Subwatershed**
Piscataway Creek	PIS0045	1,810	15,398
Tinkers Creek	TIN0006	661	23,730
	TOTAL	**2,471**	**39,128**



Sources:
Watersheds: MD Department of Natural Resources
Sewer Service Areas: Maryland Office of Planning
Septic Systems: MD Office of Planning

Prepared by:
Maryland Department of the Environment
1800 Washington Boulevard
Baltimore MD 21230
Map Production Date: February 7, 2005

Figure 2.4.1: Sanitary Sewer Service and Septic Systems in the Piscataway Creek Watershed

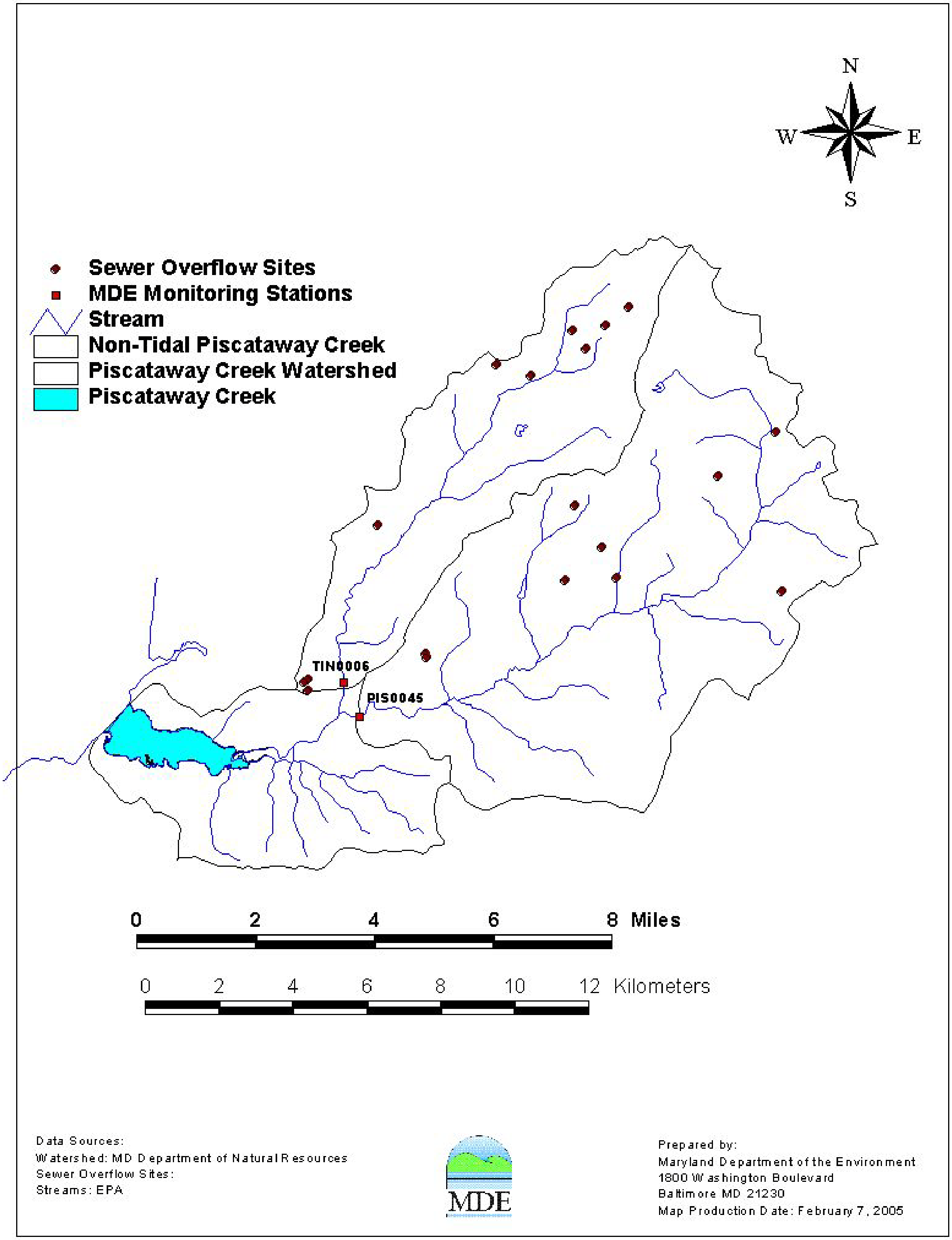


Figure 2.4.2: Sanitary Sewer Overflows in the Piscataway Creek Watershed

Point Source Assessment

Stormwater

The Piscataway Creek watershed is located in Prince George's County, a Phase I National Pollutant Discharge Elimination System (NPDES) Municipal Separate Storm Sewer System (MS4) permit jurisdiction. The MS4 permit covers stormwater discharges from the municipal separate stormwater sewer system in the County.

Municipal and Industrial WWTPs

Based on the point source permitting information, there are two WWTPs located in the watershed but only one facility has a permit regulating discharge of fecal bacteria directly into the Piscataway Creek watershed (Table 2.4.2 and Figure 2.4.1). Piscataway WWTP (permit MD0021539) is located within the Piscataway Creek watershed but its permit regulates the discharge of fecal bacteria into the tidal portion of the Potomac River. Cheltenham Boy's Village WWTP (permit MD0023931) discharges into a free-flowing tributary of Piscataway Creek. Human source can be obtained at the latter location.

Table 2.4.2: NPDES Permit Holders discharging directly in the Piscataway Creek Watershed (02-14-02-03)

Permittee	NPDES Permit No.	County	Average Annual Flow (MGD)	Average Annual Fecal Coliform Concentrations (MPN/100ml)	Load Per Day (MPN/100ml)
Cheltenham Boy's Village	MD0023931	Prince George's	0.036	13.63	1.87E+07

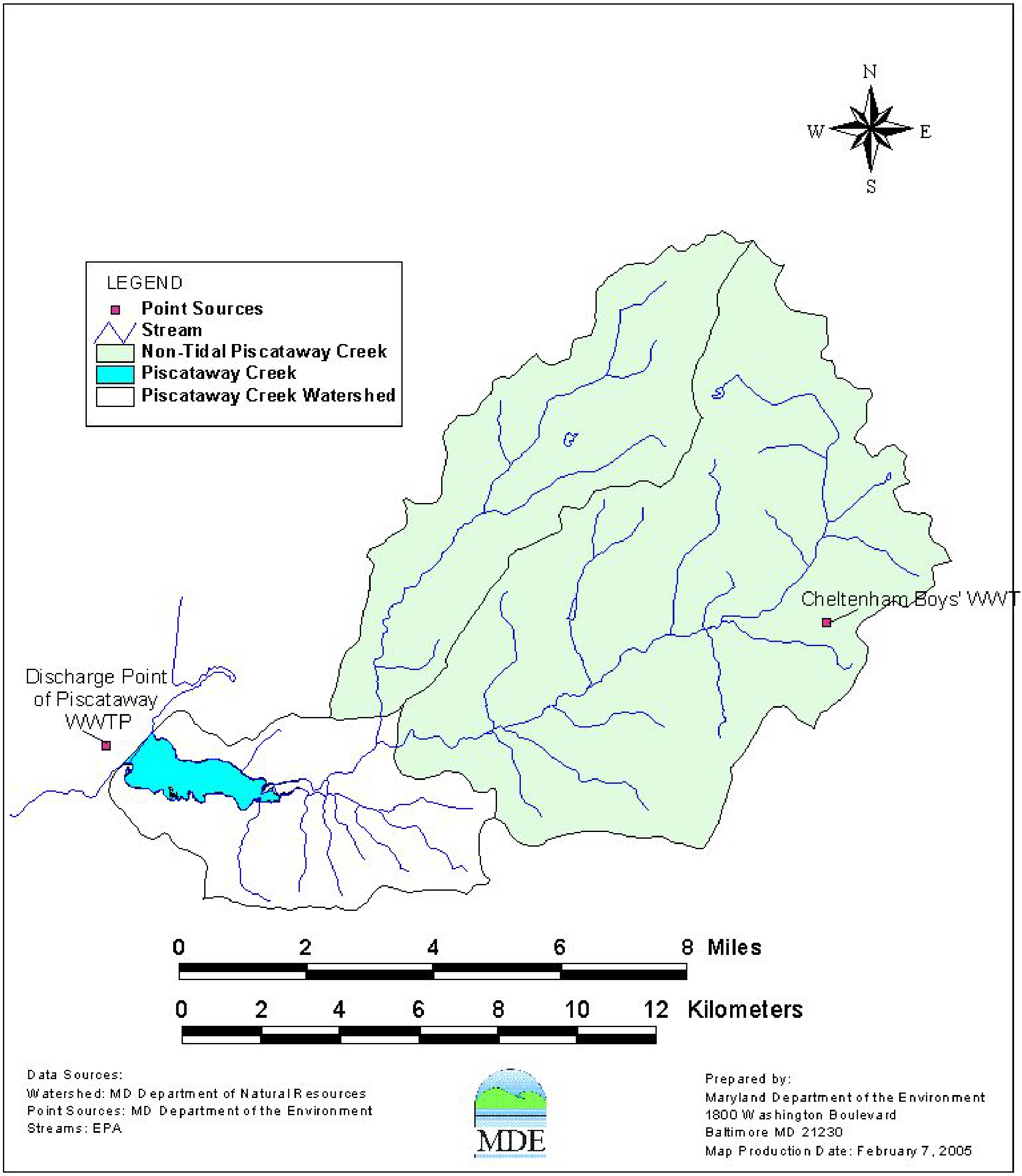


Figure 2.4.1: Permitted Point Sources in the Piscataway Creek Watershed

Bacteria Source Tracking

Bacteria source tracking (BST) was used to identify the relative contribution of bacteria from different sources in in-stream water samples. BST Monitoring was conducted at two stations throughout the Piscataway Creek watershed with 12 samples (one per month) collected for a one-year duration. Sources are defined as domestic (pets and human associated animals), human (human waste), livestock (agricultural animals), and wildlife (mammals and waterfowl). To identify sources, samples are collected within the watershed from known fecal sources and the patterns of antibiotic resistance of these known sources are compared to isolates of unknown bacteria from ambient samples. Details of the BST methodology and data can be found in Appendix C.

An accurate representation of the expected average source at each station is estimated by using a stratified weighted mean of the identified sample results over the specified period. The weighting factors are based on the log10 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 as follows:

1. Calculate the percentage of isolates per source per each sample date (S).
2. Calculate the weighted percentage (MS) of each source per flow strata (high/low) (see Section 4). The weighting is based on the log10 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 (see Appendix C).

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

$$MS_{i,k} = \frac{\sum_{j=1}^{n_i} \log_{10}(C_{i,j}) * S_{i,j,k}}{n_i} \qquad (4)$$

where

MSi,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)
Ci,j = Concentration for sample j in stratum i
Si,j,k = Proportion of isolates for sample j, of source k in stratum i
ni = number of samples in stratum I

$$M_k = \sum_{i=1}^{2} MS_{i,k} * W_i \qquad (5)$$

M = weighted mean proportion of isolates of source k
Wi= Proportion covered by stratum i

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

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

STATION	Flow Stratum	% Domestic Animals	% Human	% Livestock	% Wildlife	% Unknown
PIS0045	**High Flow**	23	37	8	27	5
	Low Flow	5	29	20	42	5
	Weighted	9	31	17	38	5
TIN0006	**High Flow**	38	23	2	29	7
	Low Flow	5	29	11	45	10
	Weighted	14	28	9	41	9

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

STATION	Flow Stratum	% Domestic Animals	% Human	% Livestock	% Wildlife	% Unknown
PIS0045	**High Flow**	26	25	7	40	3
	Low Flow	2	31	12	51	3
	Weighted	8	29	11	48	3
TIN0006	**High Flow**	38	21	2	37	2
	Low Flow	5	31	7	55	2
	Weighted	13	29	6	51	2

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 “ensure” attainment of water quality standards in the Piscataway Creek watershed. These standards are described fully in Section 2.3, “Water Quality Impairment”.

4.0 TOTAL MAXIMUM DAILY LOADS AND SOURCE ALLOCATION

4.1 Overview

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

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

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

Bacteria concentrations, determined through laboratory analysis of in-stream water samples for bacteria indicators (*e.g.*, *E. coli*), are expressed in either colony forming units (CFU) or most probable number (MPN) of colonies. The first method (Method 1600) is a direct estimate of the bacteria colonies (EPA, 1985), and the second (Method 9223B) is a statistical estimate of the number of colonies (APHA, 1998.) Enumeration results indicate the extreme variability in the total bacteria counts. The distribution of the enumeration results from water samples 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 stream (near-field 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 analysis, appears to provide reasonable results (Cleland, 2003). Using this approach, Maryland can address more impaired streams in the same time period than using the traditional water quality modeling methods.

4.2 Analysis Framework

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

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

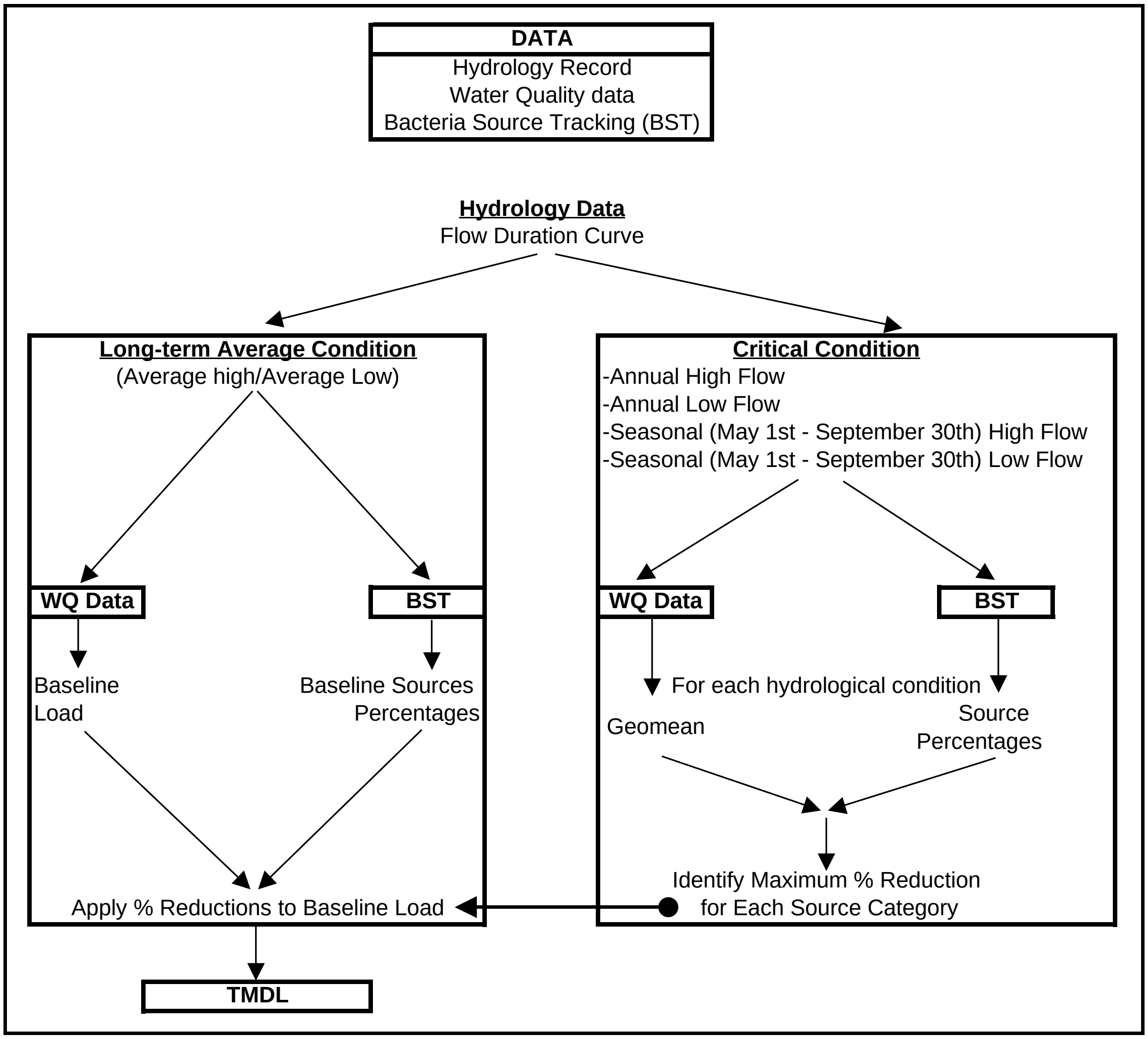


Figure 4.2.1: Diagram of Non-tidal Bacteria TMDL Analysis Framework

4.3 Estimating Baseline Loads

Baseline loads estimated in this TMDL analysis are reported as 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 flow monitoring data are available.

The loads for each stratum are estimated as follows:

$$L_i = Q_i * C_i * F_1 * F_2 \qquad (6)$$

where

Li = Daily average load (MPN/day) at each station for stratum i
Qi = Daily average flow (cfs) for stratum i
Ci = long term annual geometric mean for stratum i
F1= Unit conversion factor from cfs*MPN/100ml to MPN/day (2.4466x107)
F2= Bias correction factor

To total baseline load is estimated as follows:

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

Lt = Daily average load at station (MPN/day)
Wi= Proportion or weighting factor of stratum i

In the Piscataway Creek watershed, a weighting factor of 0.25 for high flow and 0.75 for low flow were used to estimate the annual baseline load expressed as billion MPN *E. coli*/day. Results are as follows:

Table 4.3.1: Baseline Load Calculations

Station	Area (sq. miles)	USGS Reference Gage	High Flow			Low Flow			Baseline Load (Billion MPN/day)
			Unit flow (cfs/sq, mile)	Q (cfs)	*E. coli* Concentration (MPN/100ml)	Unit flow (cfs/sq. mile)	Q (cfs)	*E. coli* Concentration (MPN/100ml)	
PIS0045	39.2	1653600	3.36	131.9	180	0.45	17.9	109	351.51
TIN0006	17.1	1653600	3.36	57.4	203	0.45	7.8	87	138.84

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. Piscataway Creek has two monitoring stations (Refer back to Figure 2.1.1).

4.4 Critical Condition and Seasonality

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

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

As stated above, Maryland's proposed fecal bacteria TMDL for Piscataway Creek has been determined by assessing various hydrological conditions to account for seasonal and annual averaging periods. The five conditions listed in Table 4.4.1were used to account for the critical condition.

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

Hydrological Condition		Averaging Period	Water Quality Data Used	Fraction High Flow	Fraction Low Flow	Condition Period
Annual	Average	365 days	All	0.25	0.75	Long Term Average
	Wet	365 days	All	0.56	0.44	May 1996 – May 1997
	Dry	365 days	All	0.03	0.97	Nov 2001 - Nov 2002
Seasonal	Wet	May 1st – Sept 30th	May 1st – Sept 30th	0.58	0.42	May 2003 - Sep 2003
	Dry	May 1st – Sept 30th	May 1st – Sept 30th	0.01	0.99	May 2002 - Sep 2002

The critical condition is determined by the maximum reduction per source that satisfy all five 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 implemented to a bacteria source category will be constant through all conditions (*e.g.*, pet waste can be reduced by 75%).

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

Table 4.4.2: Required Reductions to Meet Water Quality Standards

Station	Hydrological Condition		Domestic %	Human %	Livestock %	Wildlife %
PIS0045	Annual	Average	30.9%	0.0%	0.0%	0.0%
		Wet	39.0%	14.0%	43.5%	0.0%
		Dry	0.0%	0.0%	0.0%	0.0%
	Seasonal	Wet	82.3%	95.0%	79.3%	20.7%
		Dry	74.7%	91.4%	73.6%	0.0%
	Maximum Source Reduction		**82.3%**	**95.0%**	**79.3%**	**20.7%**
TIN0006	Annual	Average	0.0%	0.0%	0.0%	0.0%
		Wet	12.4%	27.6%	59.4%	0.0%
		Dry	0.0%	0.0%	0.0%	0.0%
	Seasonal	Wet	81.6%	95.0%	76.2%	12.4%
		Dry	64.1%	27.7%	59.1%	0.0%
	Maximum Source Reduction		**81.6%**	**95.0%**	**76.2%**	**12.4%**

4.5 Margin of Safety

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

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

4.6 TMDL Loading Caps

The TMDL loading 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 PIS0045 and TIN0006.

The TMDL is based on a long-term average hydrological condition, 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 the 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 to represent average conditions (see Table 4.3.1), based on the proportion of each stratum, to estimate the total long-term loading rate.

Next, the percent reduction (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.

$$TMDL = L_b * (1 - R) \tag{12}$$

where

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

The bacteria TMDL for the subwatersheds are:

Table 4.6.1: Piscataway Creek Watershed TMDL Summary

Station	Baseline Load *E. coli* (Billion MPN/day)	TMDL Load *E. coli* (Billion MPN/day)	% Target Reduction
PIS0045	352	136	61.2%
TIN0006	139	64	53.8%
Total	490	201	

4.7 Scenario Descriptions

Source Distribution

The final source distribution is derived from the source proportions listed in Table 2.4.3. For the purposes of the TMDL analysis and allocations, the percentage of sources identified as “unknown” were removed and the known sources were then scaled up proportionally so that they totaled 100%. The source distribution used in this scenario is presented in Table 4.7.1. .

Table 4.7.1: Baseline Source Distributions

STATION	% Domestic Animals	% Human	% Livestock	% Wildlife	% Total
PIS0045	9.7%	32.5%	17.7%	40.1%	100.0%
TIN0006	15.0%	30.3%	9.4%	45.3%	100.0%

Practicable Reduction Targets

The maximum practicable reduction (MPR) for each of the four source categories is listed in Table 4.7.2. These values are based on best professional judgment and a review of the available literature. It is assumed that human sources would potentially confer the highest risk of 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 4.7.2: Maximum Practicable Reduction Targets

	Human	**Domestic**	**Livestock**	**Wildlife**
Max Practical Reduction per Source	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	Target goal reflects uncertainty in effectiveness of urban BMPs[1] and is also based on best professional judgment	Target goal based on sediment reductions from BMPs[2] and best professional judgment	No programmatic approaches for wildlife reduction to meet water quality standards Waters contaminated by wild animal waste offer a public health risk that is orders of magnitude less than that associated with human waste.[4]

1. USEPA. 1984. Health Effects Criteria for Fresh Recreational Waters. EPA-600/1-84-004. U.S. Environmental Protection Agency, Washington, DC.
2. USEPA. 1999. Preliminary Data Summary of Urban Storm Water Best Management Practices. EPA-821-R-99-012. U.S. Environmental Protection Agency, Washington, DC.
3. USEPA. 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 (BMPs). The BMP efficiency for bacteria reduction ranged from –6% to +99% based on a total of 10 observations (USEPA, 1999). The MPR to agricultural lands was based on sediment reductions identified by the EPA (EPA, 2004).

The practicable reduction scenario was developed based on an optimization analysis whereby a subjective estimate of risk was minimized, and constraints were set on maximum reduction and allowable background conditions. Risk was defined on a scale of one to five, where it was assumed that human sources had the highest risk (5), domestic animal and livestock next (3) and wildlife the lowest (1) (see Table 4.7.2). 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:

$$\text{Min} \sum_{i=1}^{7} (Ph*5 + Pd*3 + Pl*3 + Pw*1) \qquad i = \text{hydrological condition}$$

Subject to

$C = Ccr$
$0 <= Rh <= 95\%$
$0 <= Rl <= 75\%$
$0 <= Rd <= 75\%$
$Rw = 0$
$Ph ,Pl, Pd, Pw >= 1\%$

Where

Ph = % human source in final allocation
Pd = % domestic animal source in final allocation
Pl = % livestock source in final allocation
Pw = % wildlife source in final allocation
C = In-stream concentration
Ccr = Water quality criterion
Rh = Reduction applied to human sources
Rl = Reduction applied to livestock sources
Rd = Reduction applied to domestic animal sources

In the two subwatersheds, the constraints of this scenario could not be satisfied indicating there was not a practicable solution. A summary of the analysis is presented in Table 4.7.3.

Table 4.7.3: Practicable Reduction Results

Station	Applied Reductions				Achievable
	Domestic %	Human %	Livestock %	Wildlife %	
PIS0045	75.0%	95.0%	75.0%	0.0%	No
TIN0006	75.0%	95.0%	75.0%	0.0%	No

The TMDL must specify load allocations that will meet the water quality standards. In the practicable reduction targets scenario the two subwatersheds could not meet water quality standards based on MPRs.

To further develop the TMDL, the constraints on the MPRs were relaxed in the two subwatersheds where the water quality attainment was not achievable with the MPRs. The maximum allowable reduction was increased to 99% 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:

$$\text{Min} \sum_{i=1}^{7} (Ph*5 + Pd*3 + Pl*3 + Pw*1) \qquad i = \text{hydrological condition}$$

Subject to

$C = Ccr$
$0 <= Rh <= 99\%$
$0 <= Rl <= 99\%$
$0 <= Rd <= 99\%$
$0 <= Rw <= 99\%$
$Ph , Pl, Pd, Pw >= 1\%$

Where

Ph = % human source in final allocation
Pd = % domestic animal source in final allocation
Pl = % livestock source in final allocation
Pw = % wildlife source in final allocation
C = In-stream concentration
Ccr = Water quality criterion
Rh = Reduction applied to human sources
Rl = Reduction applied to livestock sources

Rd = Reduction applied to domestic animal sources

The summary of the analysis is presented in Table 4.7.4.

Table 4.7.4: TMDL Reduction Results: Optimization Model Up to 99% Reduction

Station	Domestic %	Human %	Livestock %	Wildlife %	Target Reduction
PIS0045	82.3%	95.0%	79.3%	20.7%	61.2%
TIN0006	81.6%	95.0%	76.2%	12.4%	53.8%

4.8 TMDL Allocation

The TMDL allocation includes waste load allocations (WLA) for point sources, for stormwater (where MS4 permits are required), and the load allocation (LA) for nonpoint sources. The margin of safety is implicit and not a separate term. TMDL allocations in the Piscataway Creek watershed are based on critical conditions. The final loads represent loads based on average hydrological conditions. The load reduction scenario results in a load allocation that will achieve water quality standards. The State reserves the right to revise these allocations provided such 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 4.8.1. This table identifies how the TMDL will be allocated among WWTPs, MS4 permits and the LA.

Table 4.8.1: Potential Source Contributions for Piscataway Creek TMDL Allocations

Allocation Category	Human	Domestic	Livestock	Wildlife
WWTP	X			
MS4		X		X
LA	X		X	X

For the human sources, the non-point source contribution is estimated by subtracting the WWTP load from the final human load. Where the entire watershed is covered by an 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 Confined Animal Feeding Operation (CAFO). 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.

Stormwater

In November 2002, EPA advised States that NPDES-regulated storm water discharges must be addressed by the WLA component of a TMDL (40 C.F.R. § 130.2(h)). NPDES-regulated storm water discharges may not be addressed by the LA component of a TMDL.

Current stormwater Phase I general permits and new stormwater Phase II general permits are point sources subject to WLA assignment in the TMDL. The stormwater WLA is expressed as a gross allotment, rather than individual allocations for separate pipes, ditches, construction sites, etc.

Waste load allocations from stormwater point source dischargers are based on the relative contribution of pollutant load to the waterbody. Estimating a load contribution to a particular waterbody from the stormwater Phase I and II sources is imprecise, given the variability in sources, runoff volumes, and pollutant loads over time. Therefore, any stormwater WLA portion of the TMDL is based on an estimate.

Table 4.8.2: MS4 Stormwater Allocations

Station	WLA – MS4 Load (Billion MPN/day)
PIS0045	46.0
TIN0006	36.8
Total	82.8

Municipal and Industrial WWTPs

There is one point source facility with a permit regulating the discharge of bacteria directly into the Piscataway Creek watershed. See Table 4.8.3. 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 *E. coli* instead of fecal coliform.

Table 4.8.3: Municipal Waste Water Treatment Plants

Permittee	**NPDES Permit No.**	**County**	**Permit Flow (MGD)**	**Permit *E. coli* Concentration (MPN/100ml)**	**Permit Load (Billion MPN/day)**	**% of TMDL**
Cheltenham Boy's Village	MD0023931	Prince George's	0.07	126	0.334	0.24%

4.9 Summary

The TMDL for the Piscataway Creek watershed is presented below.

Table 4.9.1: Piscataway Creek Watershed TMDL

Station	**TMDL Load (Billion MPN/day)**	**LA Load (Billion MPN/day)**	**WLA-PS Load (Billion MPN/day)**	**WLA – MS-4 Load (Billion MPN/day)**
PIS0045	136.5	90.2	0.334	46.0
TIN0006	64.1	27.3	0.0	36.8
Total	200.6	117.7	0.1	82.8

In the two subwatersheds, based on the practicable reduction rates specified, water quality standards cannot be achieved. This may occur in watersheds where wildlife is 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 this case, it is expected that the first stage of implementation will be to implement the MPR scenario.

5.0 ASSURANCE OF IMPLEMENTATION

Section 303(d) of the Clean Water Act and current EPA regulations require reasonable assurance that the TMDL load and wasteload allocations can and will be implemented. In the Piscataway Creek watershed, the TMDL analysis indicates that reduction of fecal bacteria loads from all sources including wildlife are beyond the MPR targets. The Piscataway Creek and its tributary North Branch may not be able to attain water quality standards. The extent of the fecal bacteria load reductions required to meet water quality criteria in the two subwatersheds of the non-tidal Piscataway Creek and in downstream waters are not feasible by effluent limitations (there are no point sources) and also by implementing cost-effective and reasonable best management practices to nonpoint sources. Therefore, MDE cannot assure that the TMDL load and wasteload allocations can be implemented.

Based on the above, the final scenario, for each subwatershed, is based on reductions that are beyond the MPR targets. These MPR targets were defined based on a literature review of BMPs effectiveness and assuming a zero reduction for wildlife sources. The uncertainty of BMPs 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 BMPs methods (*e.g.*, structural, non-structural, etc.) is uncertain. Therefore, MDE intends for the required reductions to be implemented in an iterative process that first addresses those sources with the greatest 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 jurisdiction where the Piscataway Creek watershed is located, Prince George’s County, is required to participate in the stormwater NPDES program, and has to comply with the NPDES Permit regulations for stormwater discharges. The permit-required management programs are being implemented in the County to meet locally established watershed protection and restoration goals and to control stormwater discharges to the maximum extent practicable. These jurisdiction-wide programs are designed to control stormwater discharges to the maximum extent practical. Funding sources for implementation include the State Water Quality Revolving Loan Fund and the Stormwater Pollution Cost Share Program. Details of this program 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 the Maryland’s Agricultural Cost Share Program (MACS) which provides grants to farmers to help protect natural resources and the Environmental Quality and Incentives Program which focuses on implementing conservation practices and BMPs on land involved with livestock and production. Though not directly linked, it is assumed that the nutrient management plans from the Water Quality Improvement Act of 1998 (WQIA) will have some reduction of bacteria from manure application practices.

Additionally, MDE's Managing for Results document states the following related to sewage overflows:

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

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

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

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

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.

Implementation and Wildlife Sources

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

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

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Appendix A – Table of Bacteria Concentration Raw Data per Sampling Date with Corresponding Daily Flow Frequency

Station	Date	Daily flow frequency	E. coli MPN/100ml
PIS0045	10/23/2002	90.8162	630
PIS0045	11/12/2002	17.5465	550
PIS0045	11/26/2002	65.0578	10
PIS0045	12/04/2002	74.0741	20
PIS0045	12/18/2002	41.8301	110
PIS0045	01/08/2003	20.4290	50
PIS0045	01/23/2003	52.4049	20
PIS0045	02/03/2003	51.0977	30
PIS0045	03/03/2003	1.3575	120
PIS0045	03/17/2003	20.8815	10
PIS0045	04/21/2003	26.0432	100
PIS0045	05/05/2003	48.5001	150
PIS0045	05/19/2003	9.8877	460
PIS0045	06/02/2003	17.9320	1350
PIS0045	06/16/2003	23.3786	180
PIS0045	06/23/2003	10.4910	330
PIS0045	07/07/2003	13.2060	160
PIS0045	07/21/2003	52.4049	130
PIS0045	08/04/2003	47.1426	530
PIS0045	08/18/2003	42.9194	590
PIS0045	08/25/2003	73.7892	120
PIS0045	09/08/2003	53.9132	160
PIS0045	09/22/2003	25.2891	110
PIS0045	10/06/2003	52.4049	260
PIS0045	10/20/2003	58.5554	140
TIN0006	10/23/2002	90.8162	200
TIN0006	11/12/2002	17.5465	410
TIN0006	11/26/2002	65.0578	100

Station	Date	Daily flow frequency	E. coli MPN/100ml
TIN0006	12/04/2002	74.0741	70
TIN0006	12/18/2002	41.8301	70
TIN0006	01/08/2003	20.4290	50
TIN0006	01/23/2003	52.4049	10
TIN0006	02/03/2003	51.0977	60
TIN0006	03/03/2003	1.3575	50
TIN0006	03/17/2003	20.8815	60
TIN0006	04/21/2003	26.0432	60
TIN0006	05/05/2003	48.5001	10
TIN0006	05/19/2003	9.8877	490
TIN0006	06/02/2003	17.9320	776
TIN0006	06/16/2003	23.3786	220
TIN0006	06/23/2003	10.4910	330
TIN0006	07/07/2003	13.2060	350
TIN0006	07/21/2003	52.4049	160
TIN0006	08/04/2003	47.1426	2010
TIN0006	08/18/2003	42.9194	410
TIN0006	08/25/2003	73.7892	50
TIN0006	09/08/2003	53.9132	90
TIN0006	09/22/2003	25.2891	190
TIN0006	10/06/2003	52.4049	40
TIN0006	10/20/2003	58.5554	70

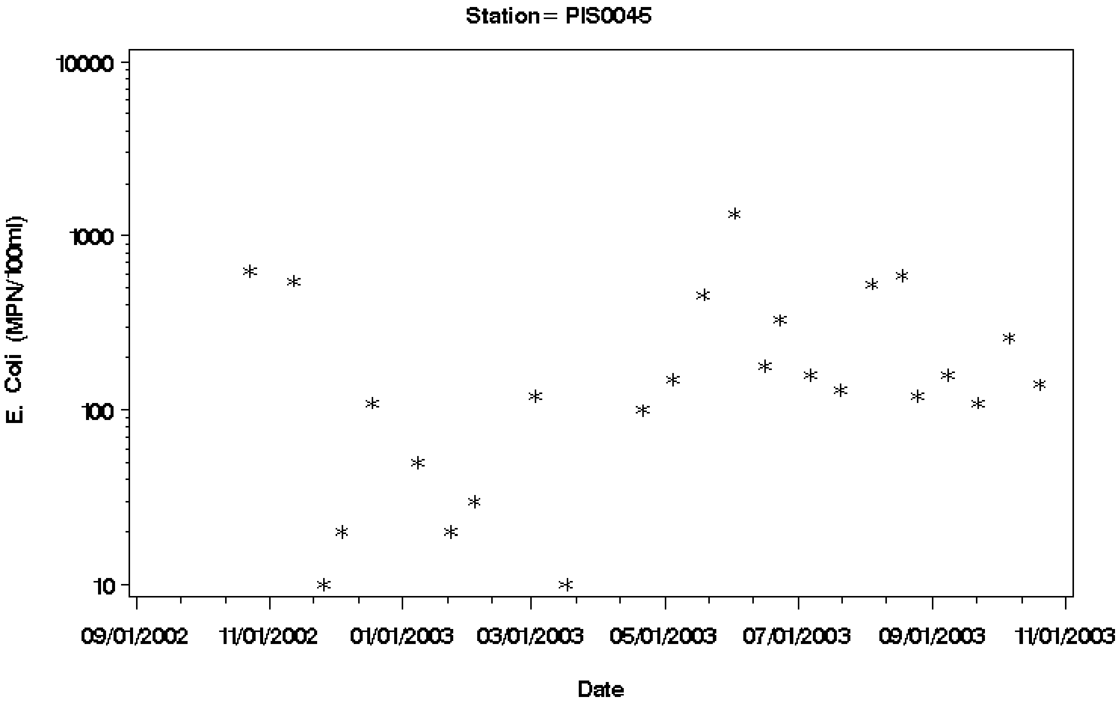

Figure A-1: *E. coli* Concentration vs. Time for Piscataway Creek Monitoring Station PIS0045

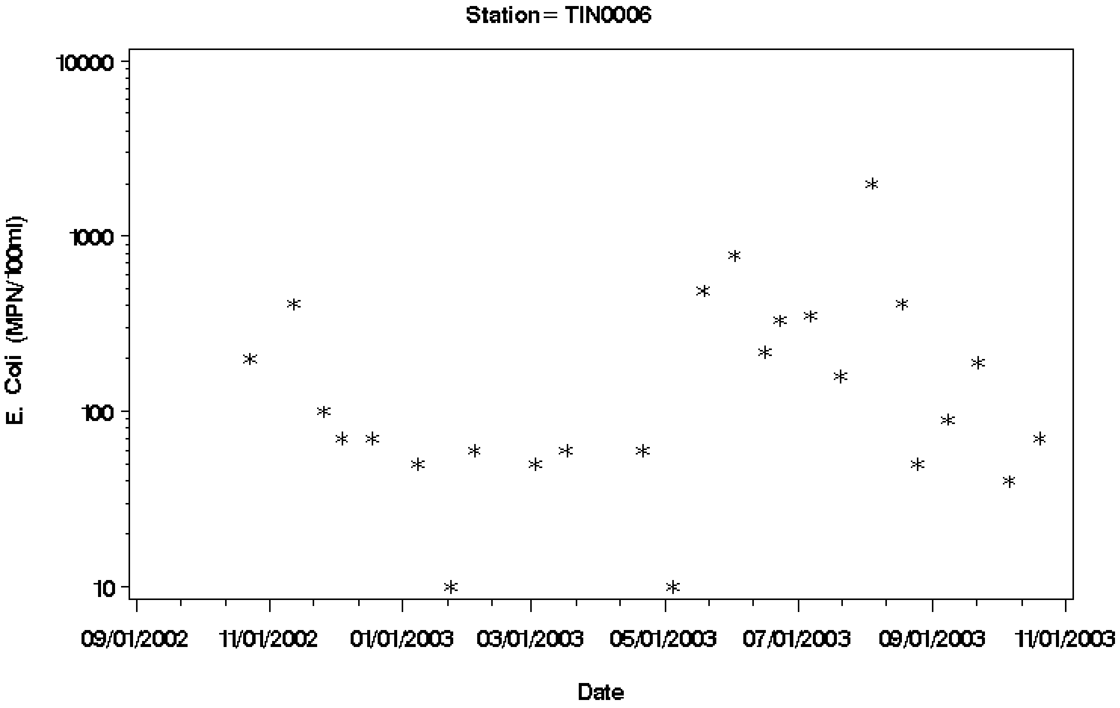

Figure A-2: *E. coli* Concentration vs. Time for Piscataway Creek Monitoring Station TIN0006

Appendix B - Flow Duration Curve Analysis to Define Strata

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

The flow duration curve for a watershed is a plot of all possible daily flows, ranked from highest to lowest, versus their probability of exceedence. 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 Piscataway Creek Watershed has one active (01653600) USGS flow gauge. The gauge and dates of information used are as follows:

Table B-1: USGS Gauges in the Piscataway Creek Watershed

USGS Gauge #	Dates used
01653600	Oct 1, 1988 to Sep 30, 2003

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

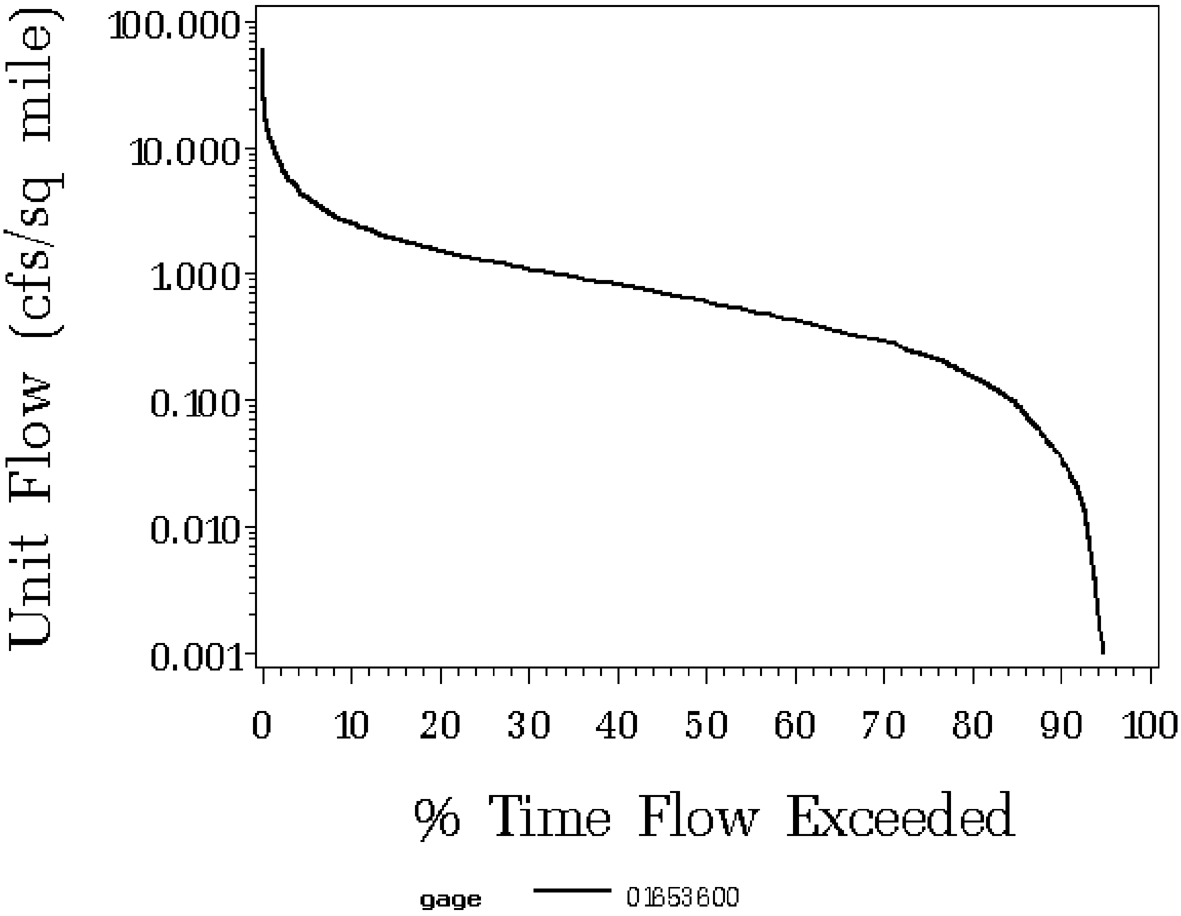

Figure B-1: Piscataway Creek Flow Duration Curves

The separation of high flow and low flow was based on the analysis of flow data for the referenced USGS gauges located in the Piscataway Creek watershed. The hydrograph separation technique is equivalent to the sliding interval technique use 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.

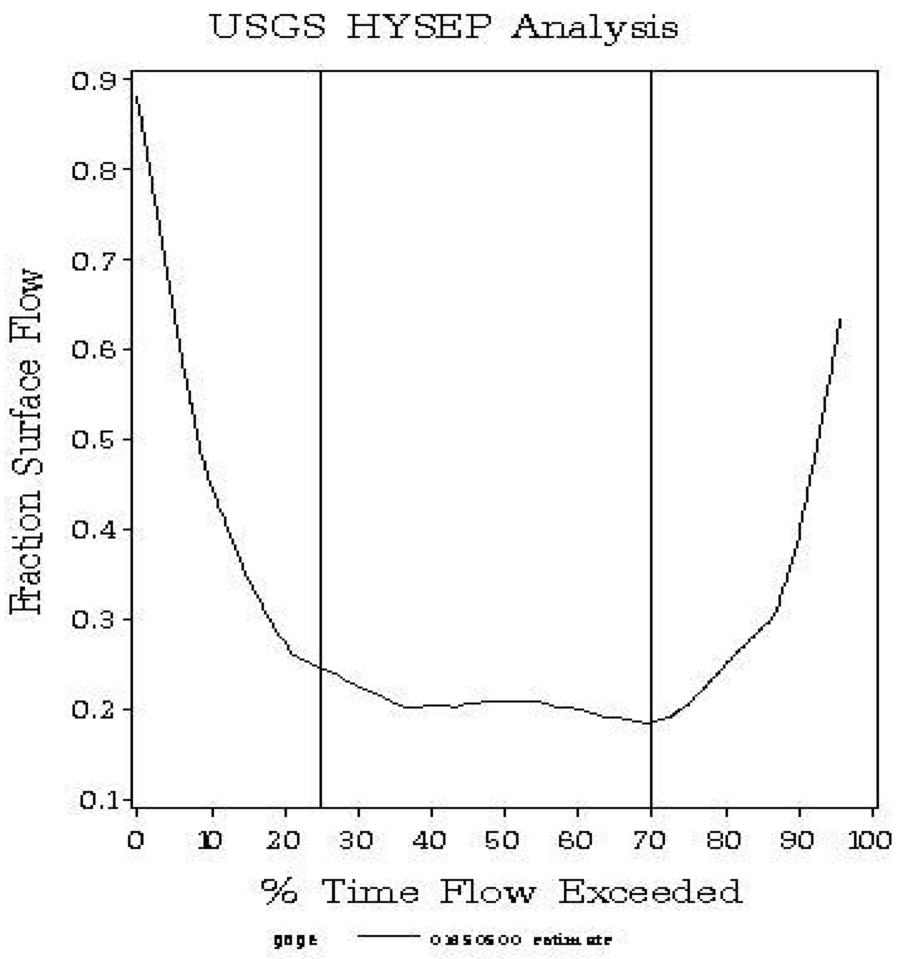

Figure B-2: Piscataway Creek: LOESS Smoothing of Hydrograph Separation

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 25 and 70 percent daily flow interval for the gauge located at Piscataway Creek (01653600) of the Piscataway Creek watershed. The area below the 25th percentile is representative of a region where surface runoff controls stream flow. The area between the 25th and 70th percentile is representative of a region where groundwater controls stream flow. The area above the 70th percentile is representative of drought conditions. These three thresholds were used to define the limits between high, mid and low range flows and are presented in Table B-2.

Table B-2: Definition of Flow Regimes

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

Flow-Data Analysis

The final analysis to define the daily flow duration intervals (flow regions, strata) includes the bacteria monitoring data. Bacteria (enterococci 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-4 show the Piscataway Creek *E. coli* monitoring data with corresponding flow frequency for the annual average and the seasonal conditions.

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 Piscataway Creek, there are sufficient samples in the high flow strata to estimate the geometric mean. For the low flow strata less than five samples exist; therefore, the mid and low flow strata will be combined to calculate the geometric mean.

Weighting factors for estimating a weighted geometric mean are based on the frequency of each flow stratum during the averaging period. The weighting factors for the averaging periods and hydrological conditions are presented in Table B-3. Averaging periods are defined in this report as:

(1) 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

Weighted geometric means for the average annual and the seasonal conditions are plotted with the monitoring data on Figures B-3 to B-4.

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

Hydrological Condition		Averaging Period	Water Quality Data Used	Fraction High Flow	Fraction Low Flow
Annual	Average	365 days	All	0.25	0.75
	Wet	365 days	All	0.56	0.44
	Dry	365 days	All	0.03	0.97
Seasonal	Wet	May 1st – Sept 30th	May 1st – Sept 30th	0.58	0.42
	Dry	May 1st – Sept 30th	May 1st – Sept 30th	0.01	0.99

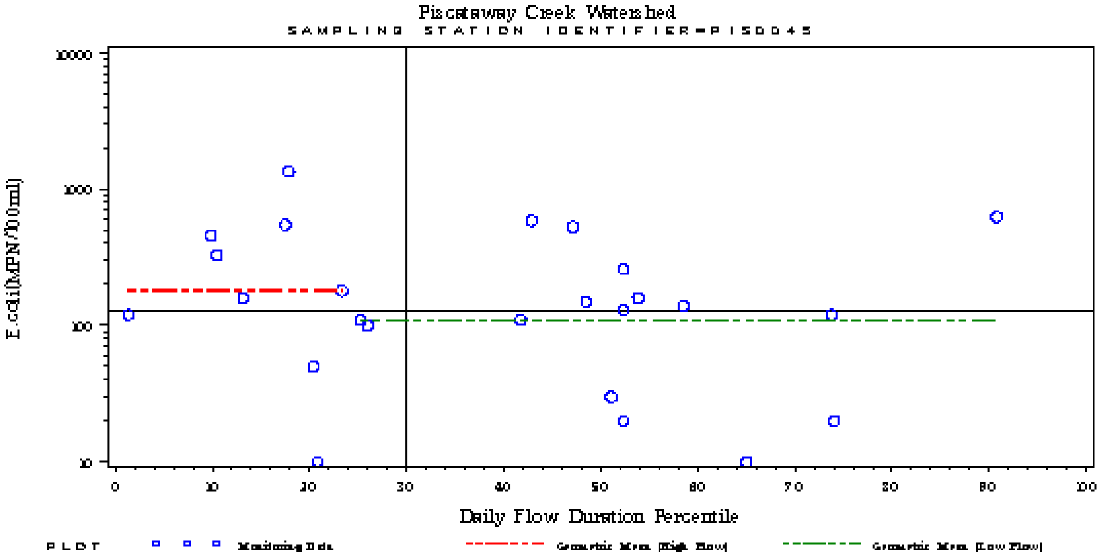

Figure B-3: ***E. coli*** **Concentration vs. Flow Duration for Piscataway Creek Monitoring Station PIS0045 (Average Annual Condition)**

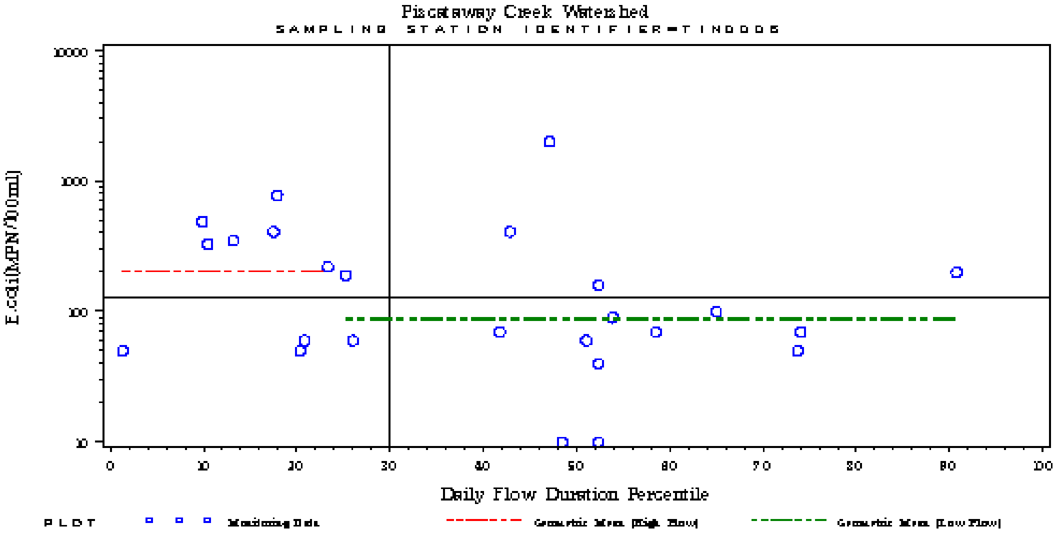

Figure B-4: ***E. coli*** **Concentration vs. Flow Duration for Piscataway Creek Monitoring Station TIN0006 (Average Annual Condition)**

Appendix C – Piscataway Creek Bacterial Source Tracking

Probable Source of Enterococci Contamination

November 2002 – October 2003

Bacterial Source Tracking Report:

Identifying Sources of Fecal Pollution in the Piscataway River Watershed, Maryland

to

Maryland Department of the Environment

from

Mark F. Frana, Ph.D. and Elichia A. Venso, Ph.D.
Department of Biological Sciences and Environmental Health Science
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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-source 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 Piscataway 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^{o} C, up to 10 enterococci 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 prepared for further analysis.

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^{o} C and isolates then scored for growth (resistance) or no growth (sensitivity). Data consisting of a “1” for resistance or “0” for sensitivity for each isolate at each concentration of each antibiotic was then entered into a spread-sheet for statistical analysis.

The following includes the antibiotics and concentrations used for isolates in the Piscataway River Watershed analysis.

Table C-1: Antibiotics and concentrations used for ARA

Antibiotic	Concentration (ug ml^{-1})
Amoxicillin	0.625
Cephalothin	10, 15, 30, 50
Chloramphenicol	1, 2.5, 5, 10
Chlortetracycline	60, 80, 100
Erythromycin	10, 15, 30, 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	10, 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, cat, dog, horse, beaver, deer, rabbit, raccoon, skunk, 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). The library consisted of response patterns of 774 enterococcus isolates from the Piscataway River Watershed. The Piscataway Creek watershed isolate library was not paired with another watershed after examination of possible library combinations (Figure C-1). The classification models in Figure C-1 show the percent correct classification of isolates for various combinations of libraries versus the percent unknown (unclassified) isolates for those combinations. The watersheds in those models were Rock Creek (RC), Anacostia (Ana), Cabin John (CJ), and Piscataway (Pis). “All Inland” was the combination of all four, RC, Ana, CJ, and Pis.

Enterococci isolate response patterns were also obtained from bacteria in water samples collected at the two (2) monitoring stations in the Piscataway Creek Watershed. Using statistical techniques, these patterns were then compared to those in the combined library to identify the probable source of each water isolate.

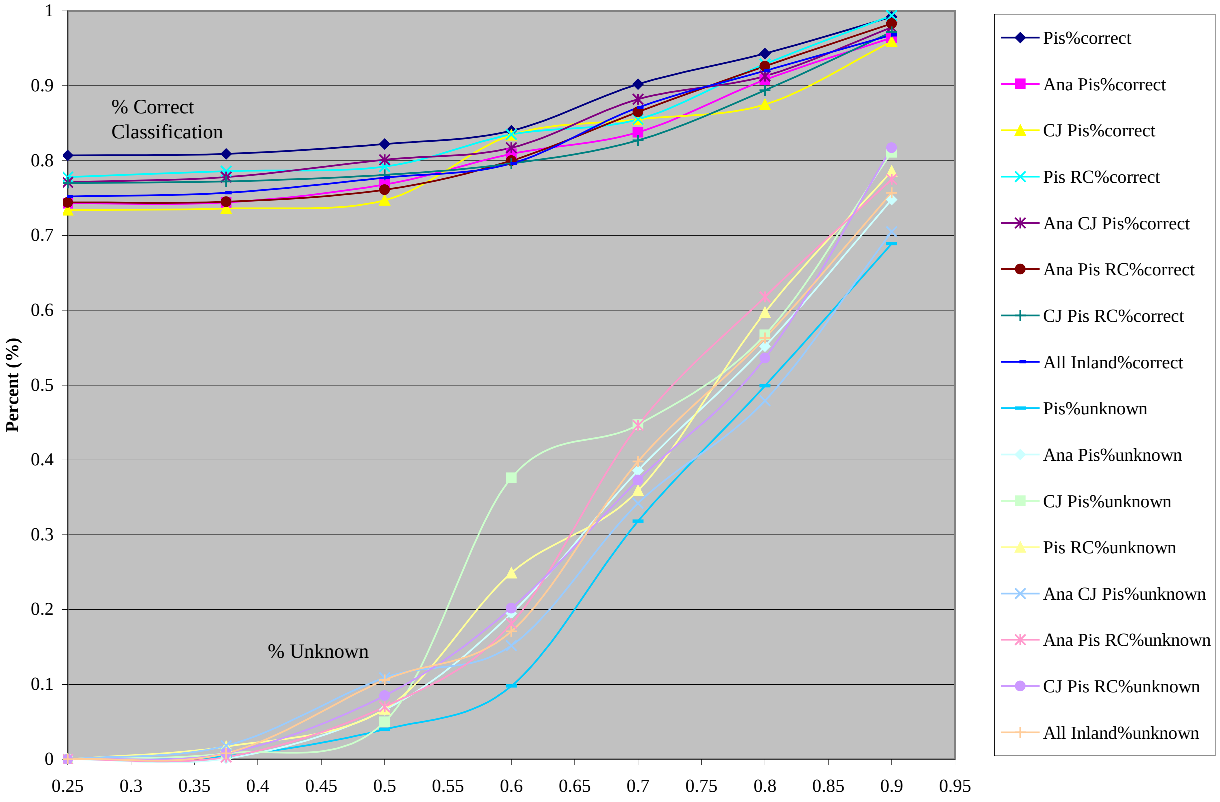

Figure C-1: Classification models for determination of composition of known-source library for identification of Piscataway Creek Watershed isolates

STATISTICAL ANALYSIS

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

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

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

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 Piscataway Creek Watershed tree-classification model, the *acceptable source identification probability* was set at 0.50 (50%).

RESULTS: LIBRARY

Known-Source Library. The known-source isolates in the Piscataway Creek Watershed known-source library were grouped into four categories: pet, human, livestock, and wildlife (Table C-2).

Table C-2: Category, total number of isolates and of unique isolate patterns in the Piscataway Creek Watershed known-source library

Category	Total Isolates	Unique Patterns
Pet	173	84
Human	154	124
Livestock	180	82
Wildlife	267	101
Total	774	391

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 combined library were found by repeating this analysis

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[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.

using several probability cutoff points, as described above. From these results, the percent unknown and percent correct classification (ARCC) was calculated (Table C-3).

Table C-3: Percent unknown and percent correct for seven (7) cutoff probabilities used to identify probable sources of Piscataway River Watershed water isolates

Cutoff Probability	Percent Unknown	(ARCC) Percent Correct
0.25	0.0%	80.7%
0.375	0.4%	80.9%
0.50	4.0%	82.2%
0.60	9.8%	84.0%
0.70	31.8%	90.2%
0.80	49.9%	94.3%
0.90	68.9%	99.2%

A cutoff probability of 0.50 (50%) was shown to yield an acceptable ARCC of 82%. The percent correct using no cutoff was 81%. Using a cutoff probability of 0.50 (50%), the library isolates that were not classified and thus unknown were removed. The library containing the remaining isolates was then used to test the ability of the library to correctly predict the known-source isolates obtained from the Piscataway Creek Watershed. The rates of correct classification for the four categories of sources in Piscataway Creek Watershed known-source isolate library are shown in Table C-4 below. The library was then used in the statistical prediction of probable sources of bacteria in water samples collected from the Piscataway Creek Watershed.

Table C-4: Actual source categories versus predicted categories of Piscataway Creek known-source isolate library, with total number of unknown isolates, total isolates, total classified, and rates of correct classification (RCC) for each category

	Predicted →							
Actual ↓	Pet	Human	Livestock	Wildlife	Unknown	Total	Total Classified	RCC[1]
Pet	**162**	3	0	5	3	173	170	95%
Human	13	**113**	11	6	11	154	143	79%
Livestock	8	9	**128**	26	9	180	171	75%
Wildlife	7	6	38	**208**	8	267	259	80%
Sum	190	131	177	245	31	774	743	

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[1] RCC = Number of correctly predicted species category / Total number classified (predicted). Example: One hundred sixty-two (162) Pet correctly predicted / 170 total number classified for Pet = 162/170 = 95% RCC.

RESULTS: WATER

Piscataway Creek Watershed Water Samples. Monthly monitoring from the Piscataway Creek monitoring stations was the source of water samples. If weather conditions prevented sampling at a station, a second collection(s) in a later month was performed. 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 529 enterococci isolates were analyzed by statistical analysis. The BST results by category, Table C-5 below shows the number of isolates and percent isolates classified at the 0.50 (50%) cutoff probability, as well as the percent classified overall.

TableC-5: Probable host sources of Piscataway Creek water isolates by category, number of isolates, percent isolates classified at cutoff probabilities of 50%

Category	No.	% Isolates Classified 50% Prob.
Pet	84	15.9%
Human	242	18.3%
Livestock	89	16.8%
Wildlife	97	45.7%
Unknown	17	3.2%
Missing Data	0	
Total w/ Complete Data	529	
Total	529	
% Classified		96.8%

The relative contributions of probable sources of Enterococci contamination in the watershed is shown below in Figure C-2.

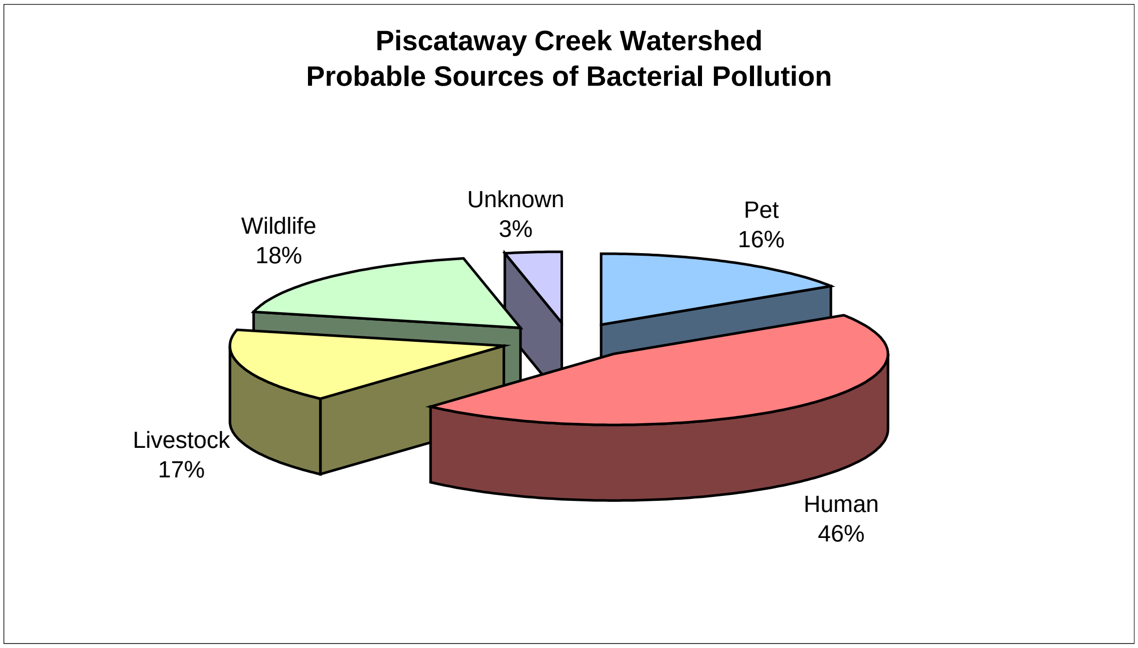

Figure C-2: Piscataway Creek Watershed contributions by probable sources of enterococci contamination

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

Table C-6: Enterococci isolates from water collected and analyzed during the fall, winter, spring, and summer seasons for Piscataway Creek monitoring stations

Station	Fall	Winter	Spring	Summer	Total
PIS0045	83	61	62	63	269
TIN0006	75	50	67	68	260
Total	158	111	129	131	529

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.

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

Station	Date	% domestic animals	% human	% livestock	% wildlife	% unknown
PIS0045	**12/04/2002**	7	8	4	3	1
PIS0045	**01/08/2003**	5	8	5	3	3
PIS0045	**02/03/2003**	0	7	0	5	3
PIS0045	**03/03/2003**	4	16	0	1	1
PIS0045	**04/21/2003**	1	4	0	10	0
PIS0045	**05/05/2003**	0	9	0	14	1
PIS0045	**06/02/2003**	10	7	2	3	1
PIS0045	**07/07/2003**	0	4	1	18	0
PIS0045	**08/04/2003**	2	7	0	14	1
PIS0045	**09/08/2003**	0	3	7	6	0
PIS0045	**09/22/2003**	0	9	2	11	1
PIS0045	**10/06/2003**	0	1	10	1	1
PIS0045	**11/12/2003**	2	11	3	4	4
TIN0006	**12/04/2002**	3	3	3	5	7
TIN0006	**01/08/2003**	4	3	0	4	5
TIN0006	**02/03/2003**	0	5	1	4	0
TIN0006	**03/03/2003**	13	8	1	2	0
TIN0006	**04/21/2003**	2	5	5	4	3
TIN0006	**05/05/2003**	6	5	4	8	1
TIN0006	**06/02/2003**	17	5	1	0	1
TIN0006	**07/07/2003**	0	5	0	19	0
TIN0006	**08/04/2003**	1	2	0	20	0
TIN0006	**09/08/2003**	0	1	5	15	0
TIN0006	**09/22/2003**	0	21	0	1	1
TIN0006	**10/06/2003**	0	2	1	7	4
TIN0006	**11/12/2003**	0	2	1	11	3

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

Station	Date	% domestic animals	% human	% livestock	% wildlife	% unknown
PIS0045	**12/04/2002**	30.4348	34.7826	17.3913	13.0435	4.3478
PIS0045	**01/08/2003**	20.8333	33.3333	20.8333	12.5000	12.5000
PIS0045	**02/03/2003**	0.0000	46.6667	0.0000	33.3333	20.0000
PIS0045	**03/03/2003**	18.1818	72.7273	0.0000	4.5455	4.5455
PIS0045	**04/21/2003**	6.6667	26.6667	0.0000	66.6667	0.0000
PIS0045	**05/05/2003**	0.0000	37.5000	0.0000	58.3333	4.1667
PIS0045	**06/02/2003**	43.4783	30.4348	8.6957	13.0435	4.3478
PIS0045	**07/07/2003**	0.0000	17.3913	4.3478	78.2609	0.0000
PIS0045	**08/04/2003**	8.3333	29.1667	0.0000	58.3333	4.1667
PIS0045	**09/08/2003**	0.0000	18.7500	43.7500	37.5000	0.0000
PIS0045	**09/22/2003**	0.0000	39.1304	8.6957	47.8261	4.3478
PIS0045	**10/06/2003**	0.0000	7.6923	76.9231	7.6923	7.6923
PIS0045	**11/12/2003**	8.3333	45.8333	12.5000	16.6667	16.6667
TIN0006	**12/04/2002**	14.2857	14.2857	14.2857	23.8095	33.3333
TIN0006	**01/08/2003**	25.0000	18.7500	0.0000	25.0000	31.2500
TIN0006	**02/03/2003**	0.0000	50.0000	10.0000	40.0000	0.0000
TIN0006	**03/03/2003**	54.1667	33.3333	4.1667	8.3333	0.0000
TIN0006	**04/21/2003**	10.5263	26.3158	26.3158	21.0526	15.7895
TIN0006	**05/05/2003**	25.0000	20.8333	16.6667	33.3333	4.1667
TIN0006	**06/02/2003**	70.8333	20.8333	4.1667	0.0000	4.1667
TIN0006	**07/07/2003**	0.0000	20.8333	0.0000	79.1667	0.0000
TIN0006	**08/04/2003**	4.3478	8.6957	0.0000	86.9565	0.0000
TIN0006	**09/08/2003**	0.0000	4.7619	23.8095	71.4286	0.0000
TIN0006	**09/22/2003**	0.0000	91.3043	0.0000	4.3478	4.3478
TIN0006	**10/06/2003**	0.0000	14.2857	7.1429	50.0000	28.5714
TIN0006	**11/12/2003**	0.0000	11.7647	5.8824	64.7059	17.6471

Table C-9: *E. coli* Concentration and Percentage of Sources by Stratum (Annual Period)

Station	Date	flow regime (1=high/2=low)	*E. coli* conc MPN/100ml	log mean conc	% domestic animals	% human	% livestock	% wildlife	% unknown
PIS0045	**10/23/2002**	2	630	2.79934	.	.	.	.	.
PIS0045	**11/12/2002**	1	550	2.74036	.	.	.	.	.
PIS0045	**11/26/2002**	2	10	1.00000	.	.	.	.	.
PIS0045	**12/04/2002**	2	20	1.30103	30.4348	34.7826	17.3913	13.0435	4.3478
PIS0045	**12/18/2002**	2	110	2.04139	.	.	.	.	.
PIS0045	**01/08/2003**	1	50	1.69897	20.8333	33.3333	20.8333	12.5000	12.5000
PIS0045	**01/23/2003**	2	20	1.30103	.	.	.	.	.
PIS0045	**02/03/2003**	2	30	1.47712	0.0000	46.6667	0.0000	33.3333	20.0000
PIS0045	**03/03/2003**	1	120	2.07918	18.1818	72.7273	0.0000	4.5455	4.5455
PIS0045	**03/17/2003**	1	10	1.00000	.	.	.	.	.
PIS0045	**04/21/2003**	2	100	2.00000	6.6667	26.6667	0.0000	66.6667	0.0000
PIS0045	**05/05/2003**	2	150	2.17609	0.0000	37.5000	0.0000	58.3333	4.1667
PIS0045	**05/19/2003**	1	460	2.66276	.	.	.	.	.
PIS0045	**06/02/2003**	1	1350	3.13033	43.4783	30.4348	8.6957	13.0435	4.3478
PIS0045	**06/16/2003**	1	180	2.25527	.	.	.	.	.
PIS0045	**06/23/2003**	1	330	2.51851	.	.	.	.	.
PIS0045	**07/07/2003**	1	160	2.20412	0.0000	17.3913	4.3478	78.2609	0.0000
PIS0045	**07/21/2003**	2	130	2.11394	.	.	.	.	.
PIS0045	**08/04/2003**	2	530	2.72428	8.3333	29.1667	0.0000	58.3333	4.1667
PIS0045	**08/18/2003**	2	590	2.77085	.	.	.	.	.
PIS0045	**08/25/2003**	2	120	2.07918	.	.	.	.	.
PIS0045	**09/08/2003**	2	160	2.20412	0.0000	18.7500	43.7500	37.5000	0.0000
PIS0045	**09/22/2003**	2	110	2.04139	0.0000	39.1304	8.6957	47.8261	4.3478
PIS0045	**10/06/2003**	2	260	2.41497	0.0000	7.6923	76.9231	7.6923	7.6923
PIS0045	**10/20/2003**	2	140	2.14613	.	.	.	.	.
PIS0045	**11/12/2003**	.	.	.	8.3333	45.8333	12.5000	16.6667	16.6667
TIN0006	**10/23/2002**	2	200	2.30103	.	.	.	.	.

Station	Date	flow regime (1=high/2=low)	*E. coli* conc MPN/100ml	log mean conc	% domestic animals	% human	% livestock	% wildlife	% unknown
TIN0006	**11/12/2002**	1	410	2.61278	.	.	.	.	.
TIN0006	**11/26/2002**	2	100	2.00000	.	.	.	.	.
TIN0006	**12/04/2002**	2	70	1.84510	14.2857	14.2857	14.2857	23.8095	33.3333
TIN0006	**12/18/2002**	2	70	1.84510	.	.	.	.	.
TIN0006	**01/08/2003**	1	50	1.69897	25.0000	18.7500	0.0000	25.0000	31.2500
TIN0006	**01/23/2003**	2	10	1.00000	.	.	.	.	.
TIN0006	**02/03/2003**	2	60	1.77815	0.0000	50.0000	10.0000	40.0000	0.0000
TIN0006	**03/03/2003**	1	50	1.69897	54.1667	33.3333	4.1667	8.3333	0.0000
TIN0006	**03/17/2003**	1	60	1.77815	.	.	.	.	.
TIN0006	**04/21/2003**	2	60	1.77815	10.5263	26.3158	26.3158	21.0526	15.7895
TIN0006	**05/05/2003**	2	10	1.00000	25.0000	20.8333	16.6667	33.3333	4.1667
TIN0006	**05/19/2003**	1	490	2.69020	.	.	.	.	.
TIN0006	**06/02/2003**	1	776	2.88986	70.8333	20.8333	4.1667	0.0000	4.1667
TIN0006	**06/16/2003**	1	220	2.34242	.	.	.	.	.
TIN0006	**06/23/2003**	1	330	2.51851	.	.	.	.	.
TIN0006	**07/07/2003**	1	350	2.54407	0.0000	20.8333	0.0000	79.1667	0.0000
TIN0006	**07/21/2003**	2	160	2.20412	.	.	.	.	.
TIN0006	**08/04/2003**	2	2010	3.30320	4.3478	8.6957	0.0000	86.9565	0.0000
TIN0006	**08/18/2003**	2	410	2.61278	.	.	.	.	.
TIN0006	**08/25/2003**	2	50	1.69897	.	.	.	.	.
TIN0006	**09/08/2003**	2	90	1.95424	0.0000	4.7619	23.8095	71.4286	0.0000
TIN0006	**09/22/2003**	2	190	2.27875	0.0000	91.3043	0.0000	4.3478	4.3478
TIN0006	**10/06/2003**	2	40	1.60206	0.0000	14.2857	7.1429	50.0000	28.5714
TIN0006	**10/20/2003**	2	70	1.84510	.	.	.	.	.
TIN0006	**11/12/2003**	.	.	.	0.0000	11.7647	5.8824	64.7059	17.6471

Table C-10: Percentage of Sources per Station by Stratum (Annual Period)

Station	flow regime (1=high/2=low)	% domestic animals	% human	% livestock	% wildlife	% unknown
PIS0045	**1**	22.9682	37.4700	7.9229	26.7777	4.86119
PIS0045	**2**	4.6289	28.6654	19.7427	41.8788	5.08413
TIN0006	**1**	38.4064	22.8372	2.1649	29.2167	7.37487
TIN0006	**2**	5.4337	29.0784	10.6549	45.2172	9.61584

Table C-11: Overall Percentage of Sources per Station (Annual Period)

Station	% domestic animals	% human	% livestock	% wildlife	% unknown	total
PIS0045	9.2137	30.8666	16.7877	38.1036	5.02839	100
TIN0006	13.6769	27.5181	8.5324	41.2170	9.05560	100

SUMMARY

The use of ARA was successful for identification of probable bacterial sources in the Piscataway Creek Watershed as evidenced by the RCCs in the library (a range of from a usable 75% for livestock to highs of 95% for pet, 80% for wildlife, and 79% for human). When water isolates were compared to the library and probable sources predicted, 97% the water isolates were classified by statistical analysis. The largest category of probable sources in the watershed was wildlife (46%). Seventeen percent (17%) of the water isolates were from livestock, pet (16%), and human (18%). Only three percent (3%) of the water isolates were unable to be classified and were thus unknown.

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