Total Maximum Daily Loads of Fecal Bacteria for the Non-Tidal Wicomico River Headwaters Basin in Wicomico County, Maryland

FINAL

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

Submitted to:

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

January 2006

EPA Submittal Date: Jan. 31, 2006
EPA Approval Date: Sept. 20, 2007

Table of Contents

List of Figures

List of Tables

List of Abbreviations

ARA	Antibiotic Resistance Analysis
ARCC	Average rates of correct classification
BMP	Best Management Practice
BST	Bacteria Source Tracking
CAFO	Confined Animal Feeding Operations
cfs	Cubic Feet per Second
CFR	Code of Federal Regulations
CFU	Colony Forming Units
COMAR	Code of Maryland Regulations
CSO	Combined Sewer Overflow
CWA	Clean Water Act
DNR	Department of Natural Resources
EPA	Environmental Protection Agency
FA	Future Allocation
FDC	Flow Duration Curve
GIS	Geographic Information System
LA	Load Allocation
MACS	Maryland Agricultural Cost Share Program
MDE	Maryland Department of the Environment
MDP	Maryland Department of Planning
ml	Milliliter(s)
MGD	Million Gallons per Day
MOS	Margin of Safety
MPN	Most Probable Number
MPR	Maximum Practicable Reduction
MRLC	Multi-Resolution Land Cover
MS4	Municipal Separate Storm Sewer System
NPDES	National Pollutant Discharge Elimination System
RCC	Rates of Correct Classification
SSO	Sanitary Sewer Overflows
TARSA	Technical and Regulatory Services Administration
TMDL	Total Maximum Daily Load
USGS	United States Geological Survey
WQIA	Water Quality Improvement Act
WLA	Waste Load Allocation
WQLS	Water Quality Limited Segment
WWTP	Wastewater Treatment Plant

EXECUTIVE SUMMARY

This document, upon approval by the U.S. Environmental Protection Agency (EPA), establishes a Total Maximum Daily Load (TMDL) for bacteria in Wicomico River Headwaters (basin number 02-13-03-04). Section 303(d) of the Federal Clean Water Act (CWA) and the EPA's implementing regulations direct each State to identify and list waters, known as water quality limited segments (WQLSs), in which current required controls of a specified substance are inadequate to achieve water quality standards. For each WQLS, the State is to either establish a TMDL for 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 Wicomico River Headwaters, a Use I waterbody (Code of Maryland Regulations (COMAR) 26.08.02.08D), in the State's 1996 303(d) List as impaired by fecal bacteria, and in the 2004 303(d) as impaired by impacts to biological communities. Johnson Pond, an impoundment within the Wicomico River Headwaters was identified in the State's 1996 303(d) List for nutrients and sediments. This document proposes to establish a TMDL for fecal bacteria in the non-tidal portions of the Wicomico River Headwaters to allow for the attainment of the beneficial use designation, primary contact recreation. A nutrient and sediment TMDL for Johnson Pond in the Wicomico River Headwaters basin was approved by EPA in 2001. The listing for impacts to non-tidal 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 load duration curve approach, using flow estimated from regional flow regression equations developed by Versar Inc. (2004) and bacteria monitoring data were used to establish baseline and allowable loads. The pollutant loads set forth in this document are for the watershed located upstream of Johnson Pond in the Wicomico River Headwaters. The sources of fecal bacteria are estimated at five stations throughout the Wicomico River Headwaters watershed where samples were collected for one year. Multiple antibiotic resistance analysis (ARA) was used to determine the relative proportion of the following source categories: domestic (pets and human associated animals), human (human waste), livestock (agricultural related animals), and wildlife (mammals and waterfowl).

The allowable load is determined by first estimating a baseline load from current monitoring data. The baseline load is estimated using a long-term geometric mean and average flows. 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 with the required reduction applied. The TMDL load for fecal bacteria entering the Wicomico River Headwaters is established after considering two different loading conditions, the annual condition period and the period between May 1 and September 30th, where water contact recreation is more prevalent. This allowable load is reported in the units of MPN/day and represents a long-term load estimated over average flow conditions and not a literal daily limit.

Two scenarios were developed; the first assessing if attainment of current water quality standards could be achieved with the maximum practicable reductions (MPRs) applied and the second with the maximum practicable reduction constraints relaxed. 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 three of the five subwatersheds, in order to meet water quality standards during any condition, it was estimated that water quality standards could not be attained with the maximum practicable reductions. Thus, for these subwatersheds, the second scenario, with relaxed constraints, was applied.

The fecal bacteria TMDL developed for the Wicomico River Headwaters non-tidal watershed is 101.3 billion Most Probable Number (MPN) *E. coli*/day. The TMDL is distributed between load allocation (LA) for nonpoint sources and waste load allocations (WLA) for point sources, including National Pollutant Elimination System (NPDES) wastewater treatment plants (WWTPs). The LA is 97.4 billion MPN/day. The WLA is 3.9 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 impact to water quality and risk to human health, with consideration given to ease and cost of implementation. As previously stated, when applying practical reduction rates the water quality standards cannot be attained in three of the five subwatersheds. This may occur in subwatersheds where wildlife is a significant component, or from subwatersheds that require very high reductions 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.

1.0 INTRODUCTION

Section 303(d)(1)(C) of the Federal Clean Water Act (CWA) and the U.S. Environmental Protection Agency (EPA) implementing regulations direct each State to develop a Total Maximum Daily Load (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 Wicomico River Headwaters (basin number 02-13-03-04), a Use I waterbody [Code of Maryland Regulations (COMAR) 26.08.02.08D], was first identified in the State's 1996 303(d) List by Maryland Department of the Environment (MDE) as impaired by bacteria (fecal coliform), and in the 2004 303(d) as impaired by impacts to biological communities. Johnson Pond, an impoundment within the Wicomico River Headwaters was identified in the State's 1996 303(d) List for nutrients and sediments. This document, upon approval by the EPA , establishes a TMDL of fecal bacteria in the non-tidal portions of the Wicomico River Headwaters to allow for the attainment of beneficial use designation, primary contact recreation. A nutrient and sediment TMDL for Johnson Pond in the Wicomico River Headwaters basin was approved by EPA in 2001. The listing for impacts to non-tidal 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.

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 used for body-contact recreation, molluscan bivalve (shellfish) consumption and 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 Wicomico River Headwaters was listed on the Maryland 303(d) list using fecal coliform as the indicator organism. The State of Maryland used the 1986 EPA guidance as the basis of a 2004 water quality standards change from an indicator organism of fecal coliform to *E.coli*/*Enterococci* to fulfill requirements of the Beaches Act of 2000. 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 Wicomico River Headwaters TMDL analysis was *E. coli.*

2.0 GENERAL SETTING AND WATER QUALITY DESCRIPTION

2.1 General Setting

Location

The Wicomico River Headwaters is a subwatershed of the Wicomico River located on Maryland's lower Delmarva Peninsula (see Figure 2.1.1). While the vast majority of the Wicomico River’s drainage is tidal, it does contain five distinct regions with freshwater drainage, all collecting into different impoundments, and ultimately discharging to various heads of tide. The analysis presented in this report is limited to consideration of two subsegments of the river's headwaters zones. These two subsegments are described below.

The region known as Wicomico River Headwaters is the largest of these freshwater drainage areas. This headwaters region begins just upstream of State Route 50, at the spillage of Johnson Pond, which is the largest of the five impoundments collecting freshwater flow from the Wicomico River Headwaters. It continues north for approximately 8 miles, on average six miles from east to west, for an approximate total drainage area of 24,540 acres (38.3 square miles). Johnson Pond receives the spillage of Leonard Mill Pond, another impoundment collecting Wicomico River Headwaters drainage. The Leonard Mill Pond drainage area reaches as far as the Delaware state line, just north of the jointly held community of Delmar.

Area Upstream of Leonard Mill Pond:

The extreme northern reach of this drainage area extends into Delaware, crossing the Maryland state line approximately two miles north of Leonard Mill Pond and to the East of the incorporated town of Delmar. Topography is flat and slopes downwards from the north, above the state line, toward Leonard Mill Pond. Two unnamed tributaries drain into Andrews Branch which feeds Leonard Mill Pond. Flow through these channels has never been observed. Repeated visits to multiple road crossing of streambeds in this area has never resulted in observable flow, either during 2002, or during an earlier water quality study conducted in 1998. Only surface runoff immediately after a storm event routes through these streams (MDE Field Office Operations, 2003).

Area between Leonard Mill Pond & Johnson Pond:

Leonard Mill Pond is a recreational pond of approximately 30 acres. The discharge of Leonard Mill Pond is controlled, with a mandatory minimum release necessary to sustain aquatic life resulting in a perennial but variable release from the pond. This flows eventually to the head of Johnson Pond. Johnson Pond is a fairly large impoundment located at the outlet of the Upper Wicomico River. The dam at Johnson Pond is the designated dividing line between tidal and non-tidal waters in the Wicomico River. Little Burnt Branch, Connelly Mill Branch, and Leonard's Pond Run merge to form the northernmost tributary of Johnson Pond, while Middle Neck Branch and Peggy Branch merge to form the easternmost tributary. Brewington Branch enters the northeast arm of the pond between the other two main tributaries. Under base flow conditions the tributaries are generally shallow (1-3 feet) at their point of discharge to the pond. Discharge from the pond is to the Wicomico River, which flows southwesterly to the Chesapeake Bay.

Geology/Soils

The Wicomico River Headwaters basin lies in the Coastal Plain physiographic province. The soils immediately surrounding Johnson Pond are the Evesboro-Klej association (Soil Conservation Service, 1970) and are easily erodible. These soils generally range from level to steep, excessively drained to somewhat poorly drained sands, and are characterized by loamy sands in upland areas.

The outer watershed area is comprised of soils of the Matawan-Norfolk association. These soils are typically level to gently sloping, moderately well-drained and well-drained uplands soils that have a subsoil of friable or firm sandy clay loam.

A portion of the extreme eastern section of the watershed contain soils of the Elkton-Matawan-Bayboro association. These are level to gently sloping, very poorly drained to moderately well-drained upland soils that have a subsoil of plastic silty clay, sandy clay loam, or sandy clay.

Land Use

The 2002 Maryland Department of Planning (MDP) land use/land cover data shows that the watershed is evenly distributed between developed, agricultural and forested land uses. Park and forestlands comprise 36% of the watershed and are more concentrated on the easternmost part of the watershed. Agricultural areas cover 31% of the watershed and are dispersed throughout the watershed but mostly confined to the northwestern side of the basin. Developed areas makeup approximately 28% of the total watershed area. Pasture, wetlands and water cover the remaining 5% of the watershed area. The land use percentage distribution for the Wicomico River Headwaters 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 Wicomico River Headwaters Basin

Land Type	Acreage	Percentage
Residential	4,530	18%
Commercial	2,340	10%
Cropland	7,600	31%
Pasture	840	3%
Forest	8,730	36%
Water	500	2%
Total	24,540	100%

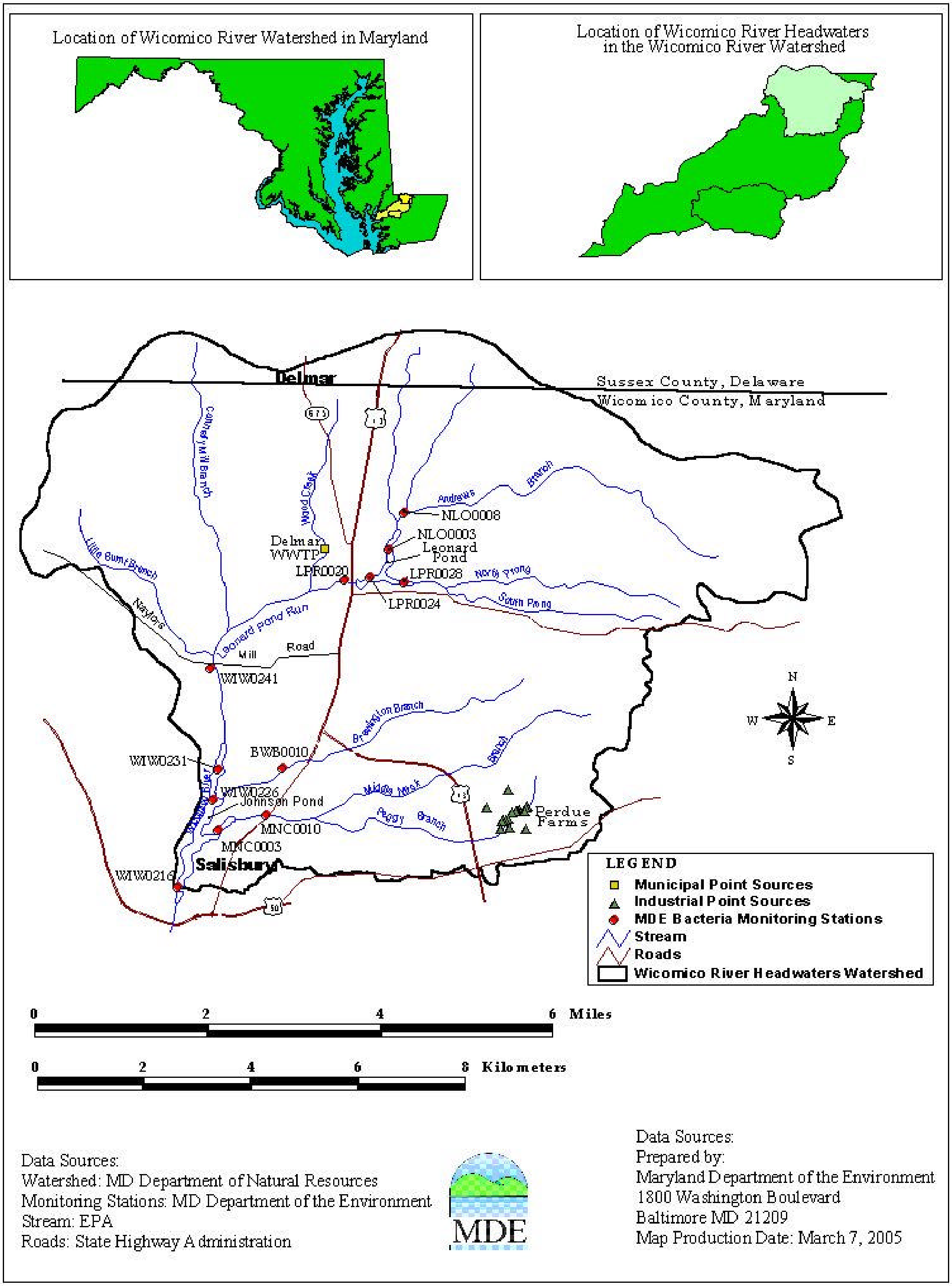


Figure 2.1.1: Location Map of the Wicomico River Headwaters Basin

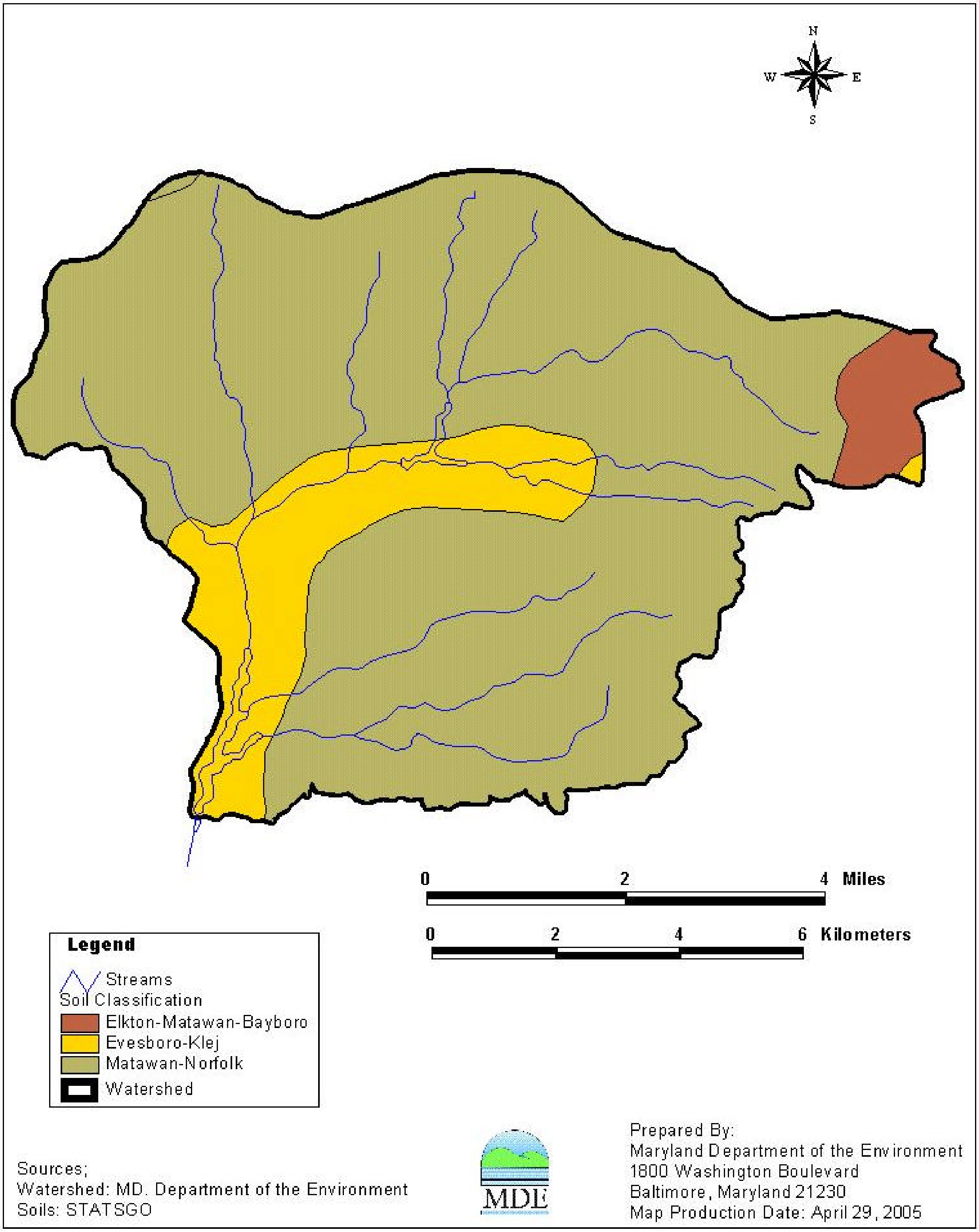
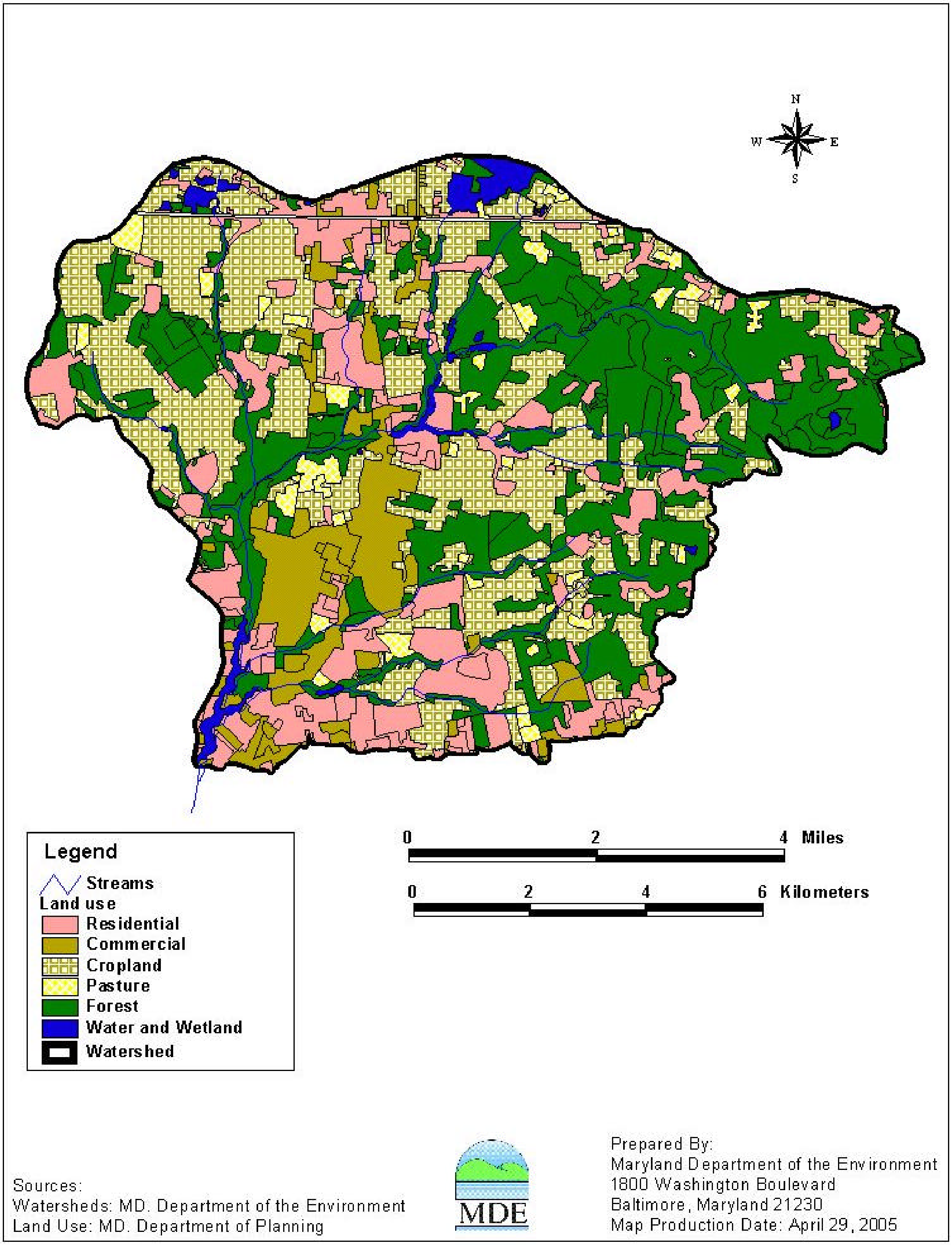


Figure 2.1.2: General Soil Series in the Wicomico River Headwaters Watershed



Sources:
Watersheds: MD. Department of the Environment
Land Use: MD. Department of Planning

Prepared By:
Maryland Department of the Environment
1800 Washington Boulevard
Baltimore, Maryland 21230
Map Production Date: April 29, 2005

Figure 2.1.3: Land Use of the Wicomico River Headwaters Watershed

Population

The total population in the Wicomico River Headwaters watershed is estimated to be 9,460. 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 Geographic Information System (GIS) 2000 Census Block and the MDP Land Use 2002 Cover that includes the Wicomico River watershed. Since the Wicomico River Headwaters watershed is a sub-area of the Census Block, percentages of each land use within the watershed were used to extract the areas from the 2000 Census Block within the watershed. Table 2.1.2 shows the number of dwellings per acre in the Wicomico River Headwaters watershed. The number of dwellings per acre was derived from information for residential density (low, medium, high) from the MDP land use cover.

Table 2.1.2: Number of Dwellings Per Acre

Landuse 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 sub-watershed was estimated (see Table 2.1.3).

Table 2.1.3: Total Population Per Subwatershed in Wicomico River Headwaters Watershed

Tributary	Station	Population
Wicomico River/ Leonard Pond Run	WIW0241	2,950
Andrews Branch	NLO0003	1,030
Middle Neck Branch	MNC0010	3,720
North/South Prong	LPR0028	600
Brewington Branch	BWB0010	1,160
	TOTAL	**9,460**

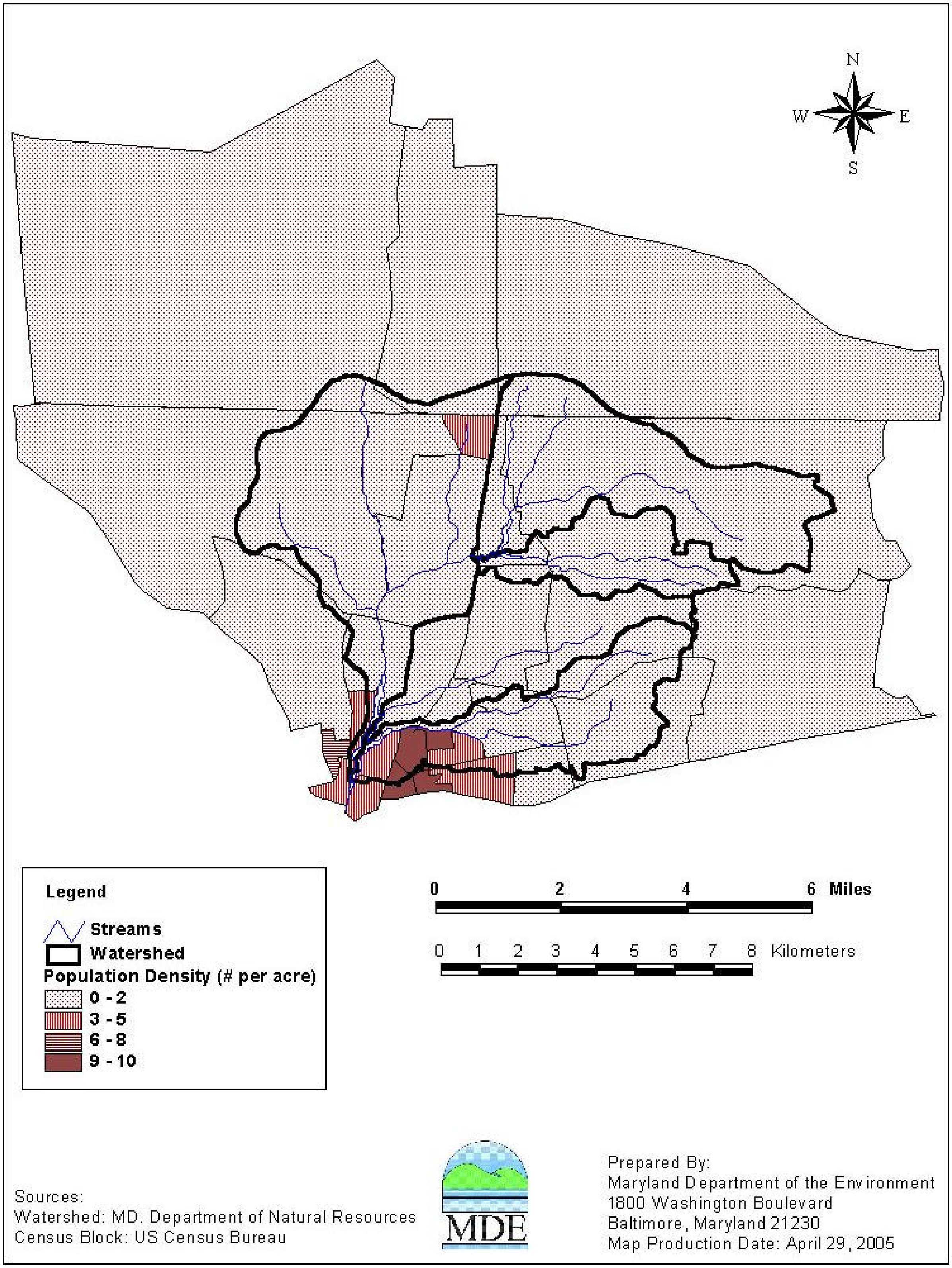


Figure 2.1.4: Population Density in the Wicomico River Headwaters Watershed

2.2 Water Quality Characterization

From EPA's guidance document (Ambient Water Quality Criteria for Bacteria, 1986), fecal bacteria, *E. coli* and *Enterococci* were assessed as indicator organisms for predicting human health impacts. A statistical analysis found that the highest correlation to gastrointestinal illness was linked to elevated levels of *E. coli* and *Enterococci* in fresh water (*Enterococci* in salt water), leading EPA to propose that States use *E. coli* or *Enterococci* as pathogen indicators. Maryland has adopted the EPA recommended bacterial indicators, *E. coli* and *Enterococcus*. Although the criteria numbers are different, the risk to the recreational bathers at the criteria levels are the same, thus the new indicators can better address this impairment although the impairment was identified using fecal coliform.

Bacteria Monitoring

Table 2.2.1 lists the monitoring data sources for the Wicomico River Headwaters watershed. The 305(b) report served as the basis used to identify the bacteria impairment in the Wicomico River Headwaters watershed. MDE conducted monitoring from October 2002 through October 2003. There are twelve MDE monitoring stations in the Wicomico River Headwaters basin.

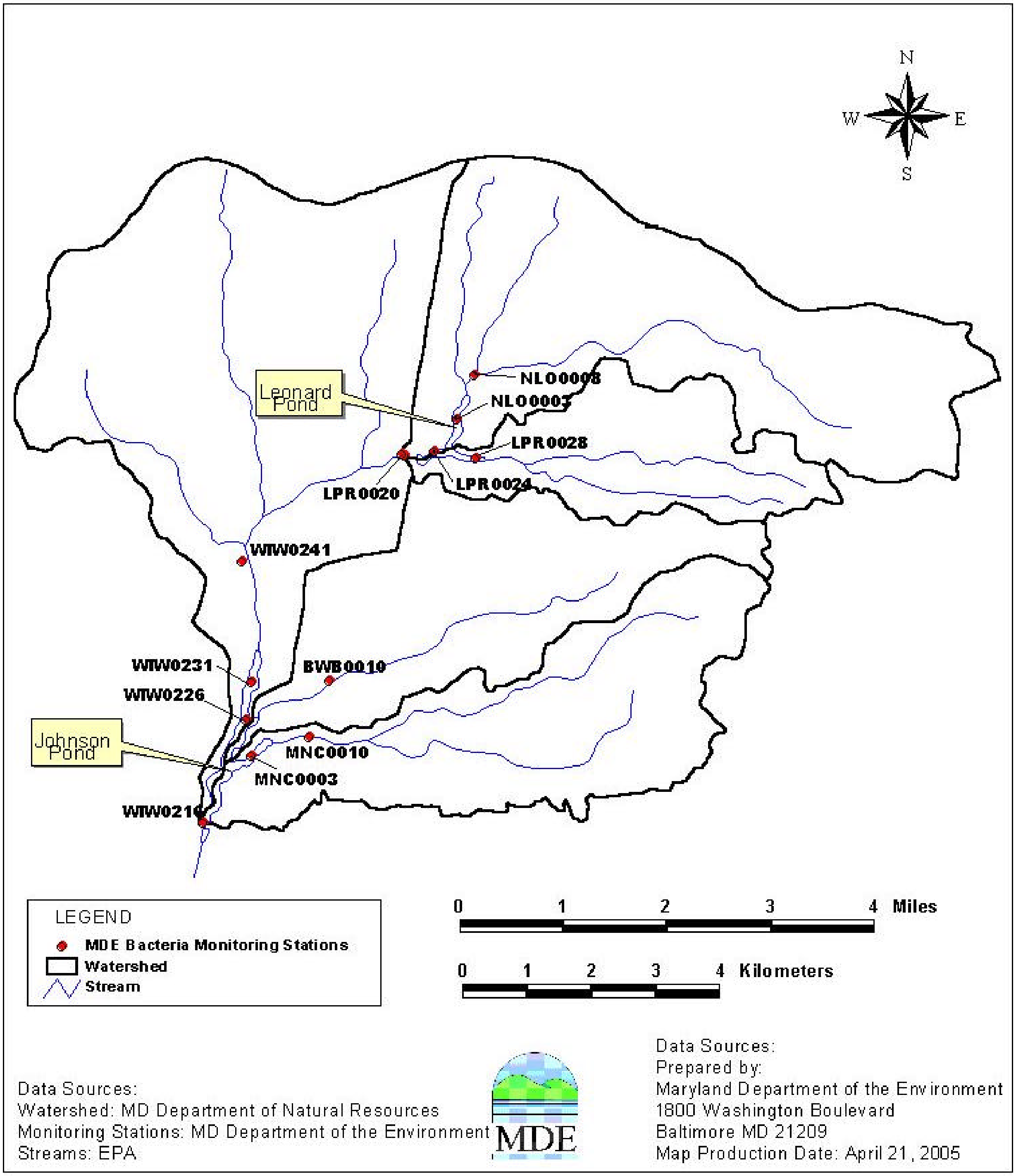
The locations of these stations are shown in Table 2.2.2 and Table 2.2.3 and illustrated in Figure 2.2.1. Observations recorded during the period 2002-2003 from MDE's monitoring station are displayed in Table A-1 and illustrated in Figure A-1 to Figure A-12 in Appendix A.

Table 2.2.1: Monitoring Data in the Wicomico River Headwaters Watershed

Sponsor	Location	Date	Design	Summary
MDE	MD	11/02 to 10/03	*E. coli*	12 stations 2 per month
MDE	MD	11/02 to 10/03	Bacteria Source Tracking (BST). Antibiotic Resistance Analysis (ARA).	9 stations ARA 1 per month

Table 2.2.2: Locations of MDE Monitoring Stations in the Wicomico River Headwaters Watershed

Tributary	Monitoring Station	Observation Period	Total Obs.	LATITUDE Dec-Deg	LONGITUDE Dec-Deg
Wicomico River	WIW0216	2002 - 2003	26	38^o 22.338’	75^o 36.161’
Wicomico River	WIW0226	2002 - 2003	24	38^o 23.207’	75^o 35.683’
Wicomico River	WIW0231	2002 - 2003	24	38^o 23.513’	75^o 35.619’
Wicomico River	WIW0241	2002 - 2003	22	38^o 24.528’	75^o 35.695’
Leonard Pond Run	LPR0020	2002 - 2003	26	38^o 25.397’	75^o 33.957’
Leonard Pond Run	LPR0024	2002 - 2003	23	38^o 25.426’	75^o 33.635’
Leonard Pond Run	LPR0028	2002 - 2003	23	38^o 25.368’	75^o 33.198’
North Prong	NLO0003	2002 - 2003	22	38^o 25.698’	75^o 33.385’
North Prong	NLO0008	2002 - 2003	21	38^o 26.070’	75^o 33.190’
Brewington Branch	BWB0010	2002 - 2003	21	38^o 23.517’	75^o 34.795’
Middle Neck Branch	MNC0003	2002 - 2003	24	38^o 22.902’	75^o 35.631’
Middle Neck branch	MNC0010	2002 - 2003	21	38^o 23.047’	75^o 35.015’



Data Sources:
Watershed: MD Department of Natural Resources
Monitoring Stations: MD Department of the Environment
Streams: EPA

Data Sources:
Prepared by:
Maryland Department of the Environment
1800 Washington Boulevard
Baltimore MD 21209
Map Production Date: April 21, 2005

Figure 2.2.1: Monitoring Stations in the Wicomico River Headwaters Watershed

2.3 Water Quality Impairment

Designated Uses and Water Quality Standard

The Maryland water quality standards Surface Water Use Designation for this watershed area is Use I – Water Contact Recreation, and Protection of Aquatic Life (COMAR 26.08.02.08D). The Wicomico River Headwaters have been included on the final 2004 Integrated 303(d) List as impaired by 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 Wicomico River analysis

Interpretation of Bacteria Data for General Recreational Use

The listing methodology as per 2006 integrated 303(d) list for all Use Waters - Water Contact Recreation and Protection of Aquatic Life is as follows:

Recreational Waters

A steady state geometric mean will be calculated with available data where there are at least 5 representative sampling events. The data shall be from samples collected during steady state conditions and during the beach season (Memorial Day through Labor Day) to be representative of the critical condition. If the resulting steady state geometric mean is greater than 35 coliform units (cfu)/100 ml enterococci in marine/estuarine waters, 33 cfu/100 ml enterococci in freshwater or 126 cfu/100 ml *E. coli* in freshwater, the water body will be listed as impaired. If fewer than 5 representative sampling events for an area being assessed are available, data from the previous two years will be evaluated. If the resulting steady state geometric mean of the available data for each year is greater than 35 cfu/100 ml enterococci in marine/estuarine waters, 33 cfu/100 ml enterococci in freshwater or 126 cfu/100 ml *E. coli* in freshwater, the water body or beach will be listed as impaired.

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

Water Quality Assessment

A water quality impairment was assessed by comparing the steady state geometric mean of *E. coli* concentrations for the annual and the May 1st – September 30th periods with the water quality criterion. May 1st – September 30th is the time period where water contact recreation is prevalent (May 1st through September 30th). 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 within the specified period. 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 for bacteria (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 mean can be reduced by weighting the samples results collected during high flow, mid flow and low flow regimes by the proportion of time each flow regime is expected to occur. This ensures that the high flow and low flow conditions are proportionally balanced.

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

A routine monitoring design was used to collect bacteria data in the Wicomico River Headwaters watershed.

In the Wicomico River Headwaters watershed, options 1 or 2 could not be used to calculate the steady state geometric means due to the absence of an appropriate United States Geological

Survey (USGS) gauging station in or nearby the watershed or any other reliable source of long term flow information.

The steady state geometric mean of the *E. coli* concentration for both annual and May 1st – September 30th periods at each monitoring station is calculated as follows:

$$M_k = \frac{\sum_{i=1}^{n} \log_{10}(C_{ki})}{n_k} \qquad (1)$$

where

M_k = log mean concentration at monitoring station k
C_{ki} = Concentration for sample i at station k
N_k = number of samples in station k

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

$$C_{gm} = 10^M \text{ = steady state geometric mean concentration} \qquad (2)$$

Summary of Water Quality Data

The water quality impairment was assessed by comparing the steady state geometric mean concentrations of *E. coli* with the water quality criterion. Graphs illustrating these results can be found in Appendix A. Steady State geometric means of the monitoring data for annual and critical conditions and the water quality criterion are shown in Tables 2.3.2 and 2.3.3.

Table 2.3.2: Wicomico River Headwaters Monitoring Data and Steady State Geometric Means for Annual Condition

Watershed	Tributary	Station	# Samples	Minimum *E. coli* Concentration MPN/100ml	Maximum *E. coli* Concentration MPN/100ml	Annual Condition Steady State Geometric Mean *E. coli* Concentration MPN/100ml	*E. coli* Criterion MPN/100 ml
02130304	Wicomico River	WIW0216	26	31	782	114	126
02130304	Wicomico River	WIW0226	24	10	2,005	94	126
02130304	Wicomico River	WIW0231	24	10	2,005	147	126
02130304	Wicomico River	WIW0241	22	64	1,445	184	126
02130304	Leonard Mill Pond	LPR0020	26	10	531	58	126
02130304	Leonard Pond Run	LPR0024	23	10	429	25	126
02130304	Leonard Pond Run	LPR0028	23	10	504	30	126
02130304	North Prong	NLO0003	22	10	478	32	126
02130304	North Prong	NLO0008	21	10	192	51	126
02130304	Brewington Branch	BWB0010	21	20	2,880	280	126
02130304	Middle Neck Branch	MNC0003	24	10	1,013	109	126
02130304	Middle Neck Branch	MNC0010	21	10	20,050	650	126

Table 2.3.3: Wicomico River Headwaters Monitoring Data and Steady State Geometric Means for May 1st – September 30th Period

Watershed	Tributary	Station	# Samples	Minimum *E. coli* Concentration MPN/100ml	Maximum *E. coli* Concentration MPN/100ml	May 1st – September 30th Steady State Geometric Mean *E. coli* Concentration MPN/100ml	*E. coli* Criterion MPN/100 ml
02130304	Wicomico River	WIW0216	14	53	271	124	126
02130304	Wicomico River	WIW0226	14	10	831	59	126
02130304	Wicomico River	WIW0231	14	10	2,005	107	126
02130304	Wicomico River	WIW0241	14	75	1,445	208	126
02130304	Leonard Mill Pond	LPR0020	14	10	531	84	126
02130304	Leonard Pond Run	LPR0024	14	10	99	24	126
02130304	Leonard Pond Run	LPR0028	14	10	344	31	126
02130304	North Prong	NLO0003	14	10	478	26	126
02130304	North Prong	NLO0008	13	10	192	65	126
02130304	Brewington Branch	BWB0010	14	42	2,880	495	126
02130304	Middle Neck Branch	MNC0003	14	10	384	97	126
02130304	Middle Neck Branch	MNC0010	14	10	20,050	1,086	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). 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.

Region Upstream of Leonard Mill Pond

Leonard Mill Pond and areas upstream of the pond have significantly large resident populations of Canadian geese. The number of geese multiplies many times over by the addition of migratory Canadian geese, which are present throughout late fall and early winter (Personal Communication with staff of Leonard Mill Pond visitor center, DNR, 2005). A minimal number of septic systems in the Leonard Mill Pond area are located at residences and farms in its headwaters. Properties located directly on the pond are serviced by the Delmar Wastewater Treatment Plant (WWTP).

The region upstream of Leonard Mill Pond is primarily forested, with some agricultural land use (predominately soybean). Communication with local farmers indicates fairly universal application of anhydrous ammonia for fertilizer purposes. Thus, poultry litter applications may not present a potential bacteria loading source. While a small number of poultry houses are found in the region, poultry litter is not applied locally, instead being marketed as resource to nearby row crop operations in Delaware (MDE Field Office, 2003).

Region Between Leonard Mill Pond & Johnson Pond

A potential loading source in the region between Leonard Mill and Johnson Ponds is the Leonard Mill Visitor Center, located just east of Route 13. This center receives extensive use by tourists and travelers, and contains a large pet exercise area located immediately on the banks of Leonard Mill Run. This pet exercise area is also a roosting zone for a large component of the resident goose population.

Potential sources in the area surrounding Johnson Pond are also present in a large migratory Canada geese population, which persists from the late fall to late winter. A much smaller resident population of these same birds is present year round. Communication with the City of Salisbury Board of Public Works indicates that the sewered areas surrounding Johnson Pond are in good condition.

Sewer and Septic Systems

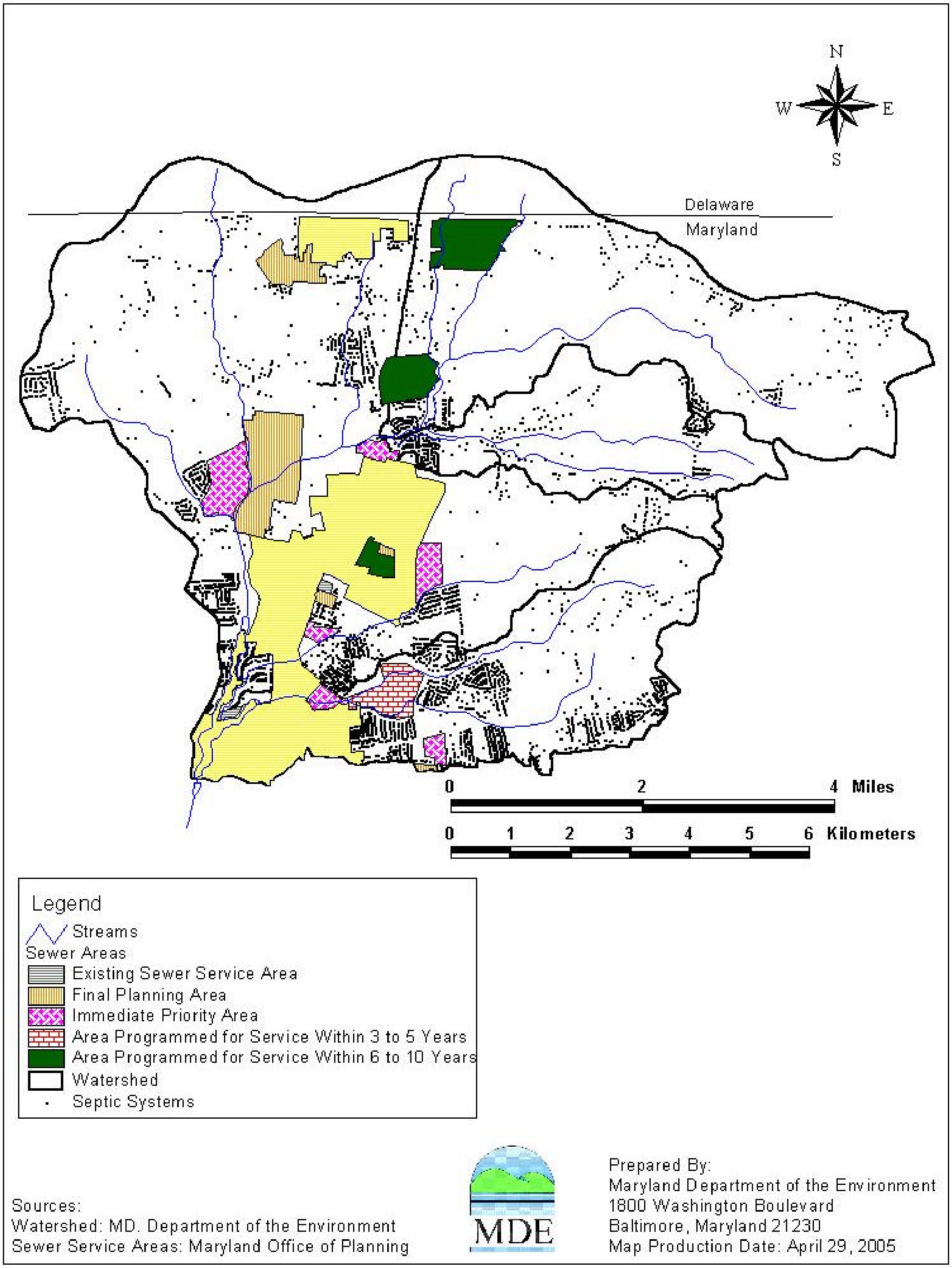
The Wicomico River Headwaters watershed is serviced by sewers primarily in the southwest area of the watershed covering approximately 10% (2,535 acres) of the land. On-site disposal (septic) systems are found throughout the entire watershed. Figure 2.4.1 depicts the areas that are serviced by sewers and septic systems. Table 2.4.1 presents the number of septic systems and total households per subwatershed.

Table 2.4.1: Septic Systems and Households per Subwatershed in the Wicomico River Headwaters Watershed

Tributary	Station	Septic Systems (units)	Households per Subwatershed
Wicomico River/ Leonard Pond Run	WIW0241	1,045	1,130
Andrews Branch	NLO0003	431	655
Middle Neck Branch	MNC0010	1,244	2,346
North/South Prong	LPR0028	309	601
Brewington Branch	BWB0010	749	792
	TOTAL	**3,778**	**5,524**

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.

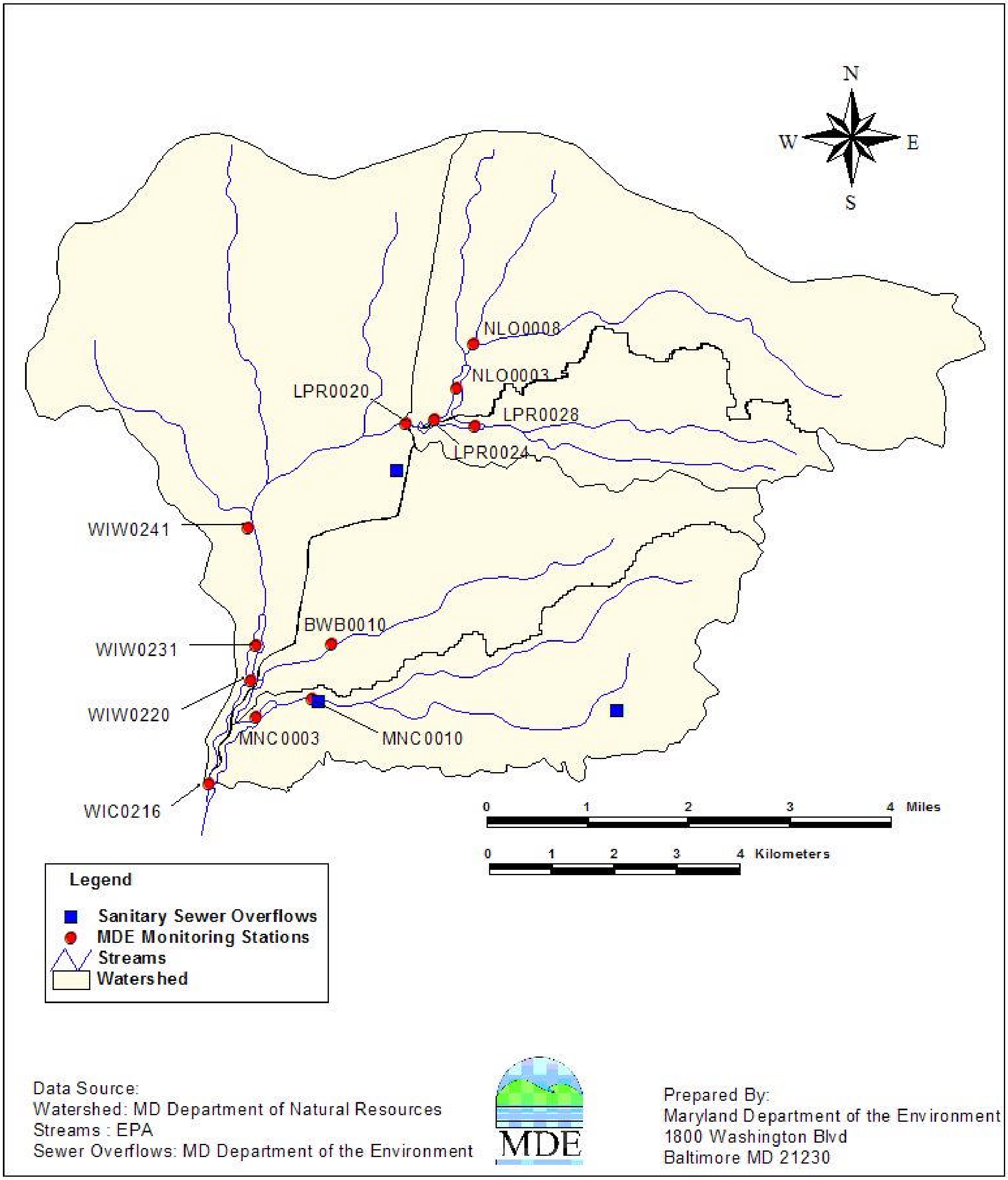
There were a total of 4 sanitary sewer overflows reported to MDE between March, 2001 and January, 2003. Approximately 60,200 gallons of sanitary sewer overflow discharge was released through various waterways in the Wicomico River Headwaters watershed (MDE, Water Management Administration). Two of those sewer overflows were due to equipment failure at the Perdue Farms treatment plant and the receiving waterbody was Peggy Branch, which discharges in Middle Neck Branch. The third and fourth overflows were located at Hammonds Street, close to monitoring station MNC0010. The overflows were caused by excessive rain and sewer blockage. The receiving waterbody in both cases was Middle Neck Branch. There was a fifth overflow reported during the same period, at a Wal-Mart pretreatment facility located in Salisbury Boulevard, but the sewage didn't reach any surface waters. Figure 2.4.2 shows the locations of sanitary sewer overflows in the Wicomico River Headwaters watershed reported in the last three years. The volume of untreated sewage to surface waters of the Wicomico River Headwaters is negligible relative to the existing volume of surface waters.



Sources:
Watershed: MD. Department of the Environment
Sewer Service Areas: Maryland Office of Planning

Prepared By:
Maryland Department of the Environment
1800 Washington Boulevard
Baltimore, Maryland 21230
Map Production Date: April 29, 2005

Figure 2.4.1: Sanitary Sewer Service and Septic Areas in the Wicomico River Headwaters Watershed



Data Source:
Watershed: MD Department of Natural Resources
Streams : EPA
Sewer Overflows: MD Department of the Environment

Prepared By:
Maryland Department of the Environment
1800 Washington Blvd
Baltimore MD 21230

Figure 2.4.2: Sanitary Sewer Overflows in the Wicomico River Headwaters Watershed

Point Source Assessment

Stormwater

In Wicomico County where the Wicomico River Headwaters watershed is located, there is no NPDES Municipal Separate Storm Sewer (MS4) permit to regulate stormwater discharges.

Municipal and Industrial WWTPs

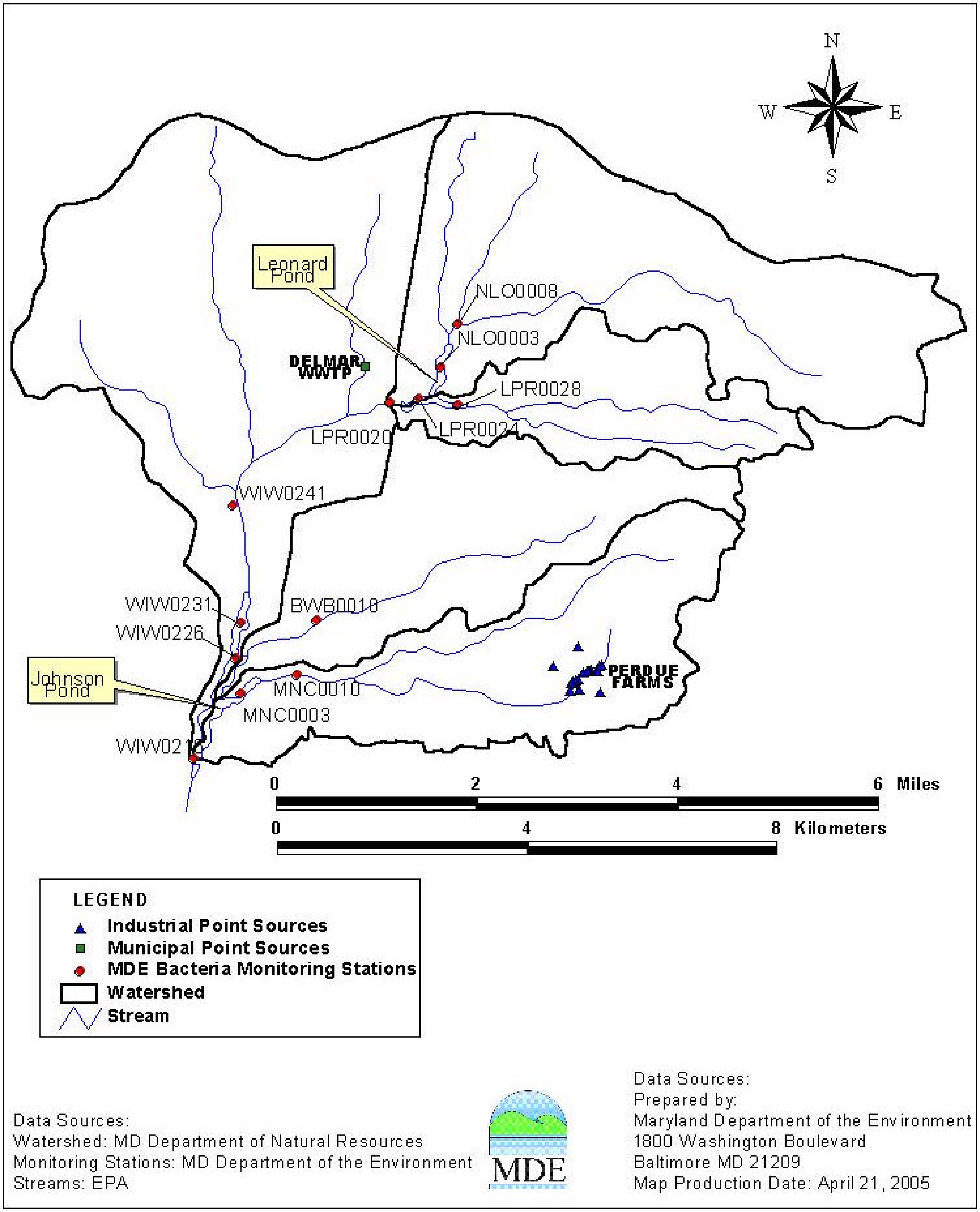
Based on the point source permitting information, there are two NPDES point source facilities with permits regulating the discharge of fecal bacteria directly into the Wicomico River Headwaters watershed. The Delmar Municipal WWTP is permitted to discharge 0.65 million gallons per day (mgd) of treated domestic wastewater into Wood Creek, which empties into Leonard Pond Run.

The Perdue Farms Industrial Wastewater Treatment Plant discharges an average of 0.17 mgd of treated wastewater into Peggy Branch, which empties into Middle Neck Branch. Perdue Farms is a relatively large hatchery operation (poultry) and it discharges treated floor drain wastewater to Peggy Branch, which in turn transports it to the east fork of Johnson Pond. This discharge is an NPDES industrial site. Self-monitoring reports indicate no permit limit violations. The overall operation is quite large, and is owned by a major regional company, Perdue Farms.

Table 2.4.2 and Figure 2.4.3 presents the point sources permitting information. Based on the Perdue Farms permit information, the facility treats domestic wastewater and industrial process wastewater and discharges into surface waters. Based on flow information from the plant, it is expected that there is approximately an 11% bacterial human contribution in the plant's effluent. The remaining 89% bacterial contribution is from processing water.

Table 2.4.2: NPDES Permit Holders in the Wicomico River Headwaters Watershed

Permittee	NPDES Permit No.	County	Average Annual Flow (MGD)	Fecal Coliform Concentrations Annual Avg. (MPN/100ml)	Fecal Coliform Load Per Day (billion MPN/day)
Delmar WWTP	MD0020532	Wicomico	0.47	2.43	4.3
Perdue Farms	MD0000060	Wicomico	0.11	30.65	12.8



Data Sources:
Watershed: MD Department of Natural Resources
Monitoring Stations: MD Department of the Environment
Streams: EPA

Data Sources:
Prepared by:
Maryland Department of the Environment
1800 Washington Boulevard
Baltimore MD 21209
Map Production Date: April 21, 2005

Figure 2.4.3: Point Sources with Permits Regulating the Discharge of Fecal Bacteria in the Wicomico River Headwaters Watershed

Bacteria Source Tracking

Bacteria source tracking (BST) was used to identify the relative contribution of bacteria in in-stream water samples. BST monitoring was conducted at nine stations in the Wicomico River Headwaters 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), wildlife (mammals and waterfowl) and unknown. 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.

An accurate representation of the expected average source observed at each monitoring station is estimated by using a weighted mean of the identified sample results over the specified averaging period. The procedure for calculating the weighted mean of the sources per monitoring station for the annual and the critical condition is as follow:

1. Calculate the percentage of isolates per source per each sample date (S).
2. Calculate the weighted percentage (MS) of each source. The weighting is based on the $\log_{10}$ bacteria concentration for the water sample.

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

$$MS_k = \frac{\sum_{j=1}^{n_i} \log_{10}(C_j) * S_{j,k}}{n} \qquad (4)$$

where

MS_k = Weighted mean proportion of isolates for source k
j = sample
k = Source category (1 = human, 2 = domestic, 3 = livestock, 4 = wildlife, 5 = unknown)
C_j = Concentration for sample j
$S_{j,k}$ = Proportion of isolates for sample j, of source k
n = number of samples

The complete distributions of average sources loads for the annual and critical conditions are also listed in Tables 2.4.3 and 2.4.4, respectively. Details of the BST data can be found in Appendix C.

Table 2.4.3: Distribution of Fecal Bacteria Source Loads in the Wicomico River Headwaters Basin for the Annual Condition

Tributary	Station	Domestic %	Human %	Livestock %	Wildlife %	Unknown %	Total %
Wicomico River	WIW0216	28.8%	22.9%	4.4%	32.9%	11.2%	100%
Wicomico River	WIW0226	20.5%	18.5%	16.3%	31.5%	13.4%	100%
Wicomico River	WIW0231	22.1%	25.9%	4.6%	29.9%	17.7%	100%
Wicomico River	WIW0241	20.4%	13.3%	5.1%	57.0%	4.3%	100%
Leonard Mill Pond	LPR0020	17.9%	14.5%	9.7%	48.9%	8.9%	100%
Leonard Pond Run	LPR0024	15.4%	19.1%	7.4%	45.2%	13.0%	100%
Leonard Pond Run	LPR0028	21.1%	8.8%	3.5%	53.5%	13.4%	100%
North Prong	NLO0003	15.1%	15.0%	6.6%	50.3%	13.1%	100%
North Prong	NLO0008	Use same BST % as NLO0003					
Brewington Branch	BWB0010	Use same BST % as WIW0231					
Middle Neck Branch	MNC0003	30.4%	21.6%	3.0%	34.0%	11.2%	100%
Middle Neck Branch	MNC0010	Use same BST % as MNC0003					

Table 2.4.4: Distribution of Fecal Bacteria Source Loads in the Wicomico River Headwaters Basin for the May 1st-September 30th Period

Tributary	Station	Domestic %	Human %	Livestock %	Wildlife %	Unknown %	Total %
Wicomico River	WIW0216	27.1%	24.1%	5.3%	39.0%	4.5%	100%
Wicomico River	WIW0226	19.1%	21.6%	12.0%	44.7%	2.6%	100%
Wicomico River	WIW0231	19.1%	21.6%	12.0%	44.4%	2.6%	100%
Wicomico River	WIW0241	21.5%	9.8%	1.3%	64.7%	2.7%	100%
Leonard Mill Pond	LPR0020	18.2%	11.6%	5.4%	57.5%	7.2%	100%
Leonard Pond Run	LPR0024	13.5%	6.3%	4.1%	60.3%	15.8%	100%
Leonard Pond Run	LPR0028	18.2%	26.8%	3.5%	48.2%	3.4%	100%
North Prong	NLO0003	17.4%	20.7%	10.3%	48.6%	3.1%	100%
North Prong	NLO0008	Use same BST % as NLO0003					
Brewington Branch	BWB0010	Use same BST % as WIW0231					
Middle Neck Branch	MNC0003	38.9%	28.2%	1.2%	31.8%	0.0%	100%
Middle Neck Branch	MNC0010	Use same BST % as MNC0003					

3.0 TARGETED WATER QUALITY GOAL

The overall objective of the fecal bacteria TMDL set forth in this document is to establish the loading caps needed to assure attainment of water quality standards in the Wicomico River Headwaters watershed area. These standards are described fully in Section 2.3, "Water Quality Impairment".

4.0 TOTAL MAXIMUM DAILY LOADS AND SOURCE ALLOCATION

4.1 Overview

This section provides an overview of the non-tidal fecal bacteria TMDL development, with a discussion 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 instream water samples for bacteria indicators (*e.g., Enterococci*), are expressed in either colony forming units (CFU) or most probable number (MPN) of colonies. The first method (EPA, 1985) is a direct estimate of the bacteria colonies (Method 1600), and the second is a statistical estimate of the number of colonies (ONPG MUG Standard Method 9223B, AOAC 991.15). Sample results indicate the extreme variability in the total bacteria counts (see Appendix A). The distribution of the sample results tends to be lognormal, with a strong positive skew of the data. Estimating loads of constituents that vary by orders of magnitude can introduce much uncertainty and result in large confidence intervals around the final results.

Estimating bacteria sources can be problematic due to the many assumptions required and the limited available data. For example, when considering septic systems, information is required on spatial location of failing septic systems, consideration of transport to instream 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 (for near-field 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 recognizes the inherent uncertainty in developing traditional water quality models for the calculation of bacteria TMDLs. In this TMDL, MDE applies an analytical method which, when combined with BST, provides reasonable results (Cleland, 2003); and allows impaired streams to be addressed expeditiously.

4.2 Analysis Framework

As explained previously, this analysis uses annual average flows and critical conditions to estimate the Wicomico River's non-tidal bacteria TMDL. The analytical method applied 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, water quality and BST data are linked together for the TMDL development.

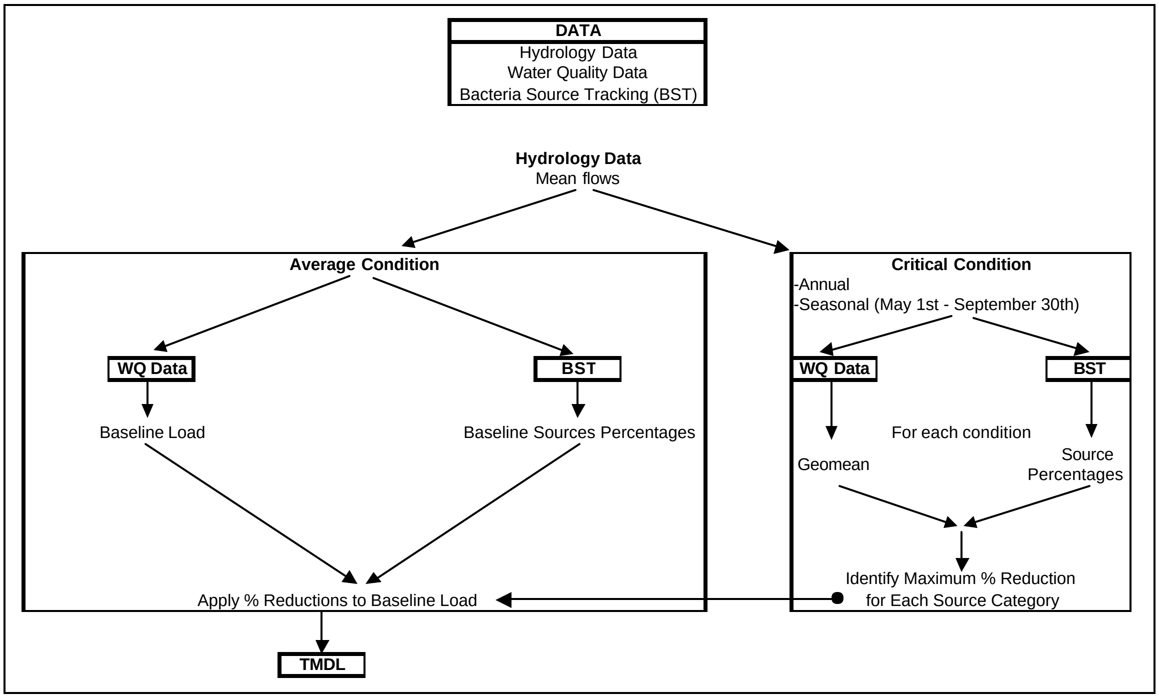


Figure 4.2.1: Diagram of the Wicomico River Headwaters Non-tidal Bacteria TMDL Analysis Framework

4.3 Estimating Baseline Loads

Due to the particular hydrological characteristics of the watershed (see Appendix B) and lack of available flow data, the daily average flows used in this analysis were from regression studies in Maryland (Versar, 2004). Flow regression equations specific to Maryland were developed by Versar using regression analysis in the manner used by Dillow (1998). Dillow developed a method to estimate peak flows for Maryland, grouped by three physiographic provinces: Blue Ridge and Piedmont regions (Piedmont Group); the Appalachian Plateau (Mountain Group); and the Western and Eastern Coastal Plain (Coastal Plain Group). Results for the Coastal Plain Group indicated that the flow regression equations described more of the variability found in high flows than the variability found for low flows. On average, the Coastal Plain Group model was able to predict average flows accurately with a mean flow R^2 value of 0.9794 and standard deviation of 0.0714. For details on how these flow regression equations were developed please refer to the document “Development of Regional Flow Duration Curves (FDC) in Maryland” (Versar, 2004).

The Wicomico River Headwaters watershed is located in the Coastal Plain physiographic province; thus, the FDC regression equation for coastal gauges was used to estimate the flows in the five subwatersheds of the Wicomico River Headwaters. A mean flow for each subwatershed was estimated using the following equation:

$$MeanFlow\ \ (cfs) = 10^{(-0.0194+1.0404\log_{10}(Area\ \ [Sq.Miles\,]))} \qquad (1)$$

Details the flow analysis, application of the regression equation and results of the flow estimation can be found in Appendix B.

With the mean flows estimated for each subwatershed, the bacteria baseline loads at each station are estimated as follows:

$$L_i = Q_i * C_i * F \qquad (5)$$

where

L_i = Average load at station i (MPN/day)
Q_i = Average flow at station i (cfs)
C_i = geometric mean at station i (MPN/100ml)
F = Unit conversion factor = $2.4466\text{x}10^9$ (MPN/day)/(cfs*MPN/100ml)

Results for the Wicomico River Headwaters are as follows:

Table 4.3.1: Baseline Load Calculations

Stations	**Area (miles^2)**	**Flow Source**	**Unit Flow (cfs/miles^2)**	**Q (cfs)**	**Annual Condition *E. coli* Geometric Mean Concentration MPN/100ml**	**Baseline Load in billions (or x10^9) MPN/day**
NLO0003	9.4	Versar	1.031	9.7	32	7.6
LPR0028	3.6	Versar	1.007	3.6	30	2.6
WIW0241sub	13.1	Versar	1.061	13.9	159	54.2
BWB0010	5.9	Versar	1.028	6.1	280	41.8
MNC0010	6.5	Versar	1.031	6.7	650	105.9

Watersheds with one or more subwatershed and a monitoring station were subdivided into unique watershed segments. This allowed for the treatment of each subwatershed as a separate entity with separate load calculations and reduction targets.

The subwatershed with an upstream monitoring station was defined with the extension sub to the station name (WIW0241sub, see Figure 4.3.1) and the load from this subwatershed was estimated using a steady state mass balance model with first order decay. The total baseline load from the upstream watershed, estimated from the monitoring data, was multiplied by a transport factor derived from first order decay. This transported load was then subtracted from the downstream cumulative load to estimate the adjacent subwatershed load. The general equation for the flow mass balance is:

$$\sum Q_{us} + Q_{sub} = Q_{ds} \tag{3}$$

where

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

and the general equations for bacteria loading mass balance:

$$\sum (e^{kt} Q_{us} C_{us}) + Q_{sub} C_{sub} = Q_{ds} C_{ds} \tag{4}$$

where

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

Source estimates from the bacteria source tracking analysis are completed for each station and are based on the contribution from the upstream watershed. Given the uncertainty in instream bacteria processes and the complexity involved in back-calculating an accurate source transport factor, the sources for station WIW0241 from the BST analysis were assigned to subwatershed station WIW0241sub.

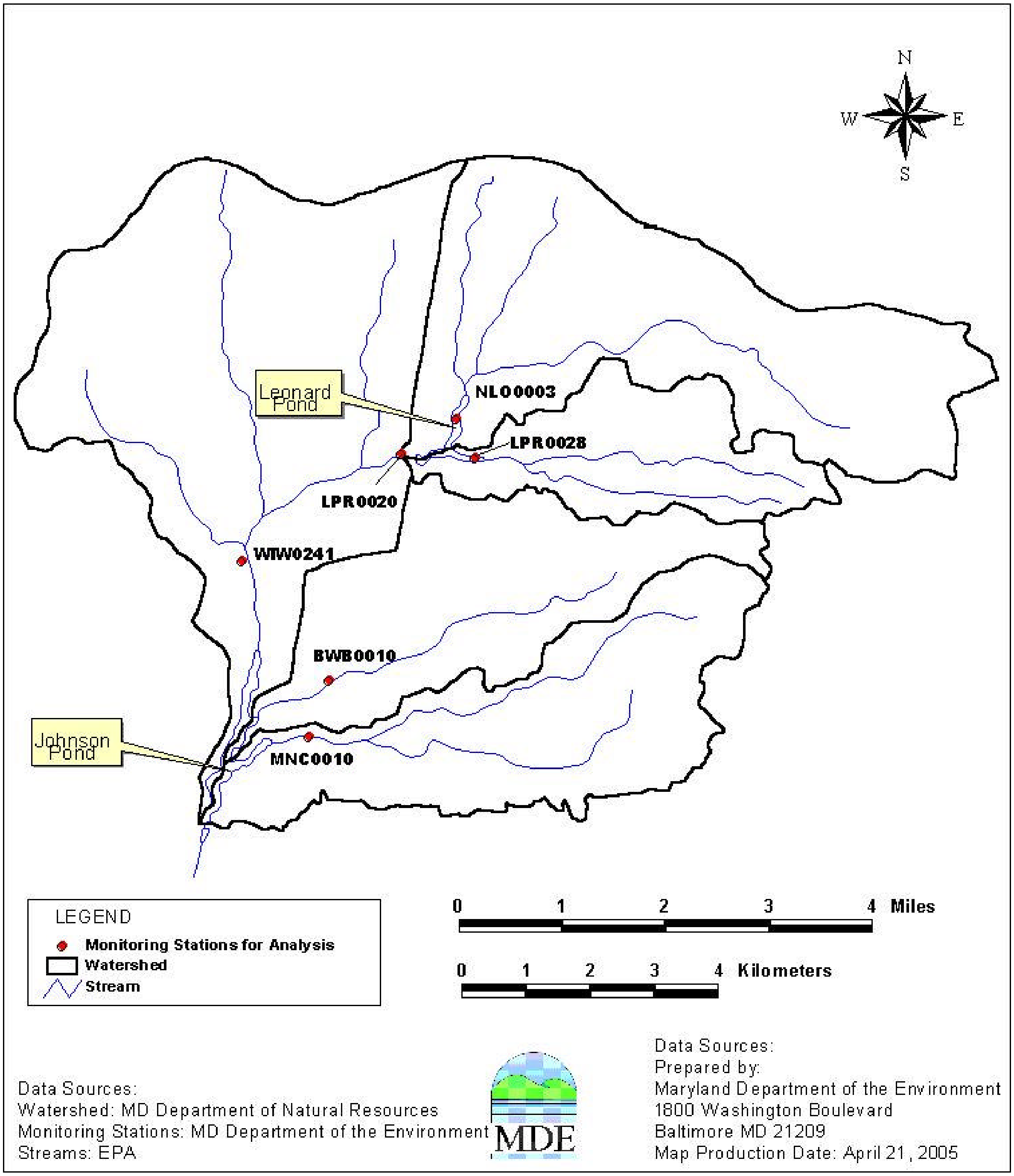


Figure 4.3.1: Monitoring Stations Used for TMDL Analysis and Subwatersheds in Wicomico River Headwaters Basin

4.4 Critical Condition and Seasonality

Federal regulations (40 CFR 130.7(c)(1)) require TMDLs to take into account critical conditions for stream flow, loading, and water quality parameters. The intent of this requirement is to ensure that the water quality of the waterbody is protected during times when it is most vulnerable. For this TMDL, the critical condition is determined by assessing both the annual and the seasonal (May 1st – September 30th) conditions. May 1st – September 30th is the time period when water contact recreation is expected. Using these two conditions, the critical condition is determined by the maximum reduction per source category that is required to meet the water quality standard while minimizing the risk to water contact recreation. It is assumed that the reduction that can be implemented to a bacteria source category will be constant through both conditions.

Critical conditions were also taken into account using Delmar's WWTP maximum design capacity flow of the plant and also assuming that maximum permit flows from Perdue Farms industrial and domestic plants discharge into the surface waters year round as opposed to spray irrigation during some periods of the year.

As explained above in Section 4.2, in the Wicomico River Headwaters Watershed, long term flow data was not available and thus average flows estimated from the regression equation was used. Seasonality in the Wicomico River Headwaters watershed was addressed with the monitoring data only. The monitoring data for all stations located in the Wicomico River Headwaters watershed cover a sufficient temporal span (at least one year), to estimate annual and seasonal conditions loads.

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 water quality indicators. As mentioned in Section 4.2, 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.

Loads estimated in this TMDL are based on the geometric mean concentration, which are calculated from the log transformation of the raw data and average daily flows. 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[a]; 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.

It was decided that the known low bias of the back transformed concentrations would be used as an implicit MOS when estimating the assimilative capacity of the stream systems. This bias will provide an environmentally conservative estimate of the load required to attain water quality standards.

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. This loading is for the watersheds upstream of monitoring stations WIW0241, BWB0010 and MNC0010, located on Wicomico River, Brewington Branch and Middle Neck Branch, respectively.

The TMDL is based on an average daily flow long term geometric mean bacteria concentration, and therefore the loads are not literal daily limits. 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 annual geometric mean concentration and the average daily flow as explained above, see Table 4.3.1.

Next the percent reduction is estimated from the observed bacteria concentrations accounting for the critical conditions. The percent reduction applied is the maximum reduction per source category that is required to meet the water quality standard (critical condition). 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.

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

where

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

The bacteria TMDL for the watersheds upstream of monitoring stations WIW0241, BWB0010 and MNC0010 is:

Table 4.6.1: Wicomico River Headwaters TMDL Summary

Station	**Baseline Load** *E. coli* **(billions MPN/day)**	**Annual Condition TMDL Load** *E. coli* **(billions MPN/day)**	**Annual Condition % Target Reduction**	**Critical Condition TMDL Load** *E. coli* **(billions MPN/day)**	**Critical Condition % Target Reduction**
NLO0003	7.6	29.9	0.0%	29.9	0.0%
LPR0028	2.6	11.0	0.0%	11.0	0.0%
WIW0241sub	54.2	43.0	20.6%	37.3	31.1%
BWB0010	41.8	18.8	55.0%	8.7	79.2%
MNC0010	105.9	20.6	80.6%	14.3	86.5%
Total	212.1	123.3		101.2	

4.7 Scenario Descriptions

Source Distribution

The final average 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" was removed and the known sources were then scaled up proportionally so that they totaled 100%. The source distribution used in this analysis is presented in Table 4.7.1. As stated in Section 4.3, the source distribution for stations WIW0241sub, BWB0010 and MNC0010, was based on the sources identified at stations WIW0241, WIW0226 and MNC0003, respectively.

Table 4.7.1: Baseline Average Source Distributions Used in the TMDL Analysis

Station	**% Domestic**	**% Human**	**% Livestock**	**% Wildlife**	**% Total**
NLO0003	17.4%	17.2%	7.6%	57.8%	100.0%
LPR0028	24.3%	10.2%	4.0%	61.5%	100.0%
WIW0241sub	21.3%	13.9%	5.3%	59.5%	100.0%
BWB0010	23.6%	21.4%	18.8%	36.3%	100.0%
MNC0010	34.2%	24.2%	3.3%	38.2%	100.0%

Practicable Reduction Targets

The maximum practicable reduction (MPR) per 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 have 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 Practical 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	(1) Target goal reflects uncertainty in effectiveness of urban BMP's[1] and is also based on best professional judgment	Target goal based on sediment reductions from BMP's[2] and best professional judgment	No programmatic approaches for wildlife reduction to meet water quality standards

1. USEPA. 1984. Health Effects Criteria for Fresh Recreational Waters. EPA-600/1-84-004. U.S. Environmental Protection Agency, Washington, D.C.
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., Mackney and Merle D. Pierson, Chapman & Hall.

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

For both annual and seasonal conditions, 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 risk for each condition while meeting the maximum practicable reduction constraints. The model was defined as follows:

Min (P_h*5 + P_d*3 + P_l*3 + P_w*1)

Subject to

$C = C_{cr}$
$0 <= R_h <= 95\%$
$0 <= R_l <= 75\%$
$0 <= R_d <= 75\%$
$R_w = 0$
P_h ,P_l, P_d, $P_w >= 1\%$
$P_h >= 7.5\%$ for WIW0241
$P_h >= 1.0\%$ for MNC0010
$P_l >= 3.5\%$ for MNC0010

Where

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

The last two constraints do not allow the point source reduction to go beyond the permit limits loads. In subwatersheds WIW0241sub, BWB0010 and MNC0010, the constraints of this scenario could not be satisfied indicating there was not a feasible solution. A summary of the analysis is presented in the following Table 4.7.3.

Table 4.7.3: Practicable Reduction Results

Station	Applied Reductions				Achievable during Average Annual Condition?	Achievable during Seasonal Condition?
	Domestic %	Human %	Livestock %	Wildlife %		
NLO0003	0.0%	0.0%	0.0%	0.0%	Yes	Yes
LPR0028	0.0%	0.0%	0.0%	0.0%	Yes	Yes
WIW0241sub	75.0%	95.0%	75.0%	0.0%	Yes	No
BWB0010	75.0%	95.0%	75.0%	0.0%	No	No
MNC0010	75.0%	95.0%	75.0%	0.0%	No	No

Final Reduction Targets

The TMDL must specify load allocations that will meet the water quality standards. In the practicable reduction targets scenarios, two subwatersheds (NLO0003 and LPR0028) met water quality standards. Subwatershed WIW0241sub met water quality standards based on MPRs during the annual condition but did not meet water quality statndards during the seasonal condition. Subwatersheds BWB0010 and MNC0010 (Brewington Branch and Middle Neck Branch) did not meet water quality standards based on MPRs during both annual and seasonal conditions.

To further develop the TMDL, in those subwatersheds not meeting criteria, the constraints on the MPRs were relaxed in those two subwatersheds where the water quality attainment was not achievable with the MPRs. In these three subwatersheds, the maximum allowable reduction was increased to 98% for all sources, including wildlife. A similar optimization procedure was used to minimize risk. The model was defined as follows:

Min $(P_h*5 + P_d*3 + P_l*3 + P_w*1)$

Subject to

$C = C_{cr}$
$0 <= R_h <= 98\%$
$0 <= R_l <= 98\%$
$0 <= R_d <= 98\%$
$0 <= R_w <= 98\%$
$P_h ,P_l, P_d, P_w >= 1\%$
$P_l >= 3.5\%$ for MNC0010
$P_H >= 1\%$ for MNC0010

Where

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

The final target reductions are determined by selecting the maximum reduction per source from both annual and seasonal conditions for each subwatershed.

The summary of the analysis is presented in Table 4.7.4:

Table 4.7.4: TMDL Reduction Results: Optimization Model Up to 98% Reduction

Station	Domestic %	Human %	Livestock %	Wildlife %	Target Reduction
NLO0003	0.0%	0.0%	0.0%	0.0%	0.0%
LPR0028	0.0%	0.0%	0.0%	0.0%	0.0%
WIW0241sub	95.1%	47.3%	69.9%	1.0%	31.11%
BWB0010	97.4%	97.7%	95.9%	47.7%	79.15%
MNC0010	98.0%	98.0%	79.7%	69.5%	86.50%

4.8 TMDL Allocation

The TMDL allocation includes waste load allocations (WLA) for point sources, for stormwater (where MS4 permits are required), and the LA for nonpoint sources. The margin of safety is implicit and not specific as a separate term. TMDL allocations are based on meeting Maryland's bacteria water quality criteria and represent loads based on average conditions. The load reduction scenario results in a load allocation by which the TMDL can be implemented to 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 municipal and industrial WWTPs and the LA.

Table 4.8.1: Potential Source Contributions for TMDL Allocations

Allocation Category	Human	Domestic	Livestock	Wildlife
Municipal WWTP	X			
Industrial WWTP	X		X[1]	
LA	X	X	X	X

1. Special condition for industrial treatment plant

For the human sources, the nonpoint source contribution is estimated by subtracting the WWTP load from the final human load. In the WIW0241sub, the Delmar municipal WWTP total contribution is allocated as human load. Wicomico County is not covered by a MS4 permit, therefore, the total domestic pet load is assigned to the LA. For the same reason, wildlife is also assigned to the LA. MS4 permits do not cover livestock and it will also be part of the LA when it is not designated as a CAFO.

Under special permit conditions, a WWTP may receive livestock sewage. Perdue Farms has both industrial and domestic treatment plants discharging waste into Peggy Branch, a tributary of the Wicomico River. The industrial discharge bacteria loading is from chicken hatchery waste and will be assigned in the WLA as livestock, while the domestic discharge will be assigned as human. The approximate percentage of human vs. livestock is estimated from the WWTPs flow data, and is used only to get the final livestock WLA. In addition to the dual source of bacteria, based on the permitted discharge, a percentage of the total effluent from the Perdue plants is irrigated to a nearby agricultural field. For this analysis, as a conservative assumption, the flow from the plants is discharged to the surface waters year round.

The headwaters zone of subwatersheds NLO0003 and WIW0241sub are located in the State of Delaware. For this reason the LA in those subwatersheds is distributed between the State of Maryland and the State of Delaware based on the percentage of the total area that each State occupies in each subwatershed. Subwatershed NLO0003 has 90% of its area in Maryland and 10% in Delaware, while subwatershed WIW0241sub has 91.1% of its area in Maryland and 8.9% in Delaware.

Stormwater

In November 2002, EPA advised States that NPDES regulated storm water discharges must be addressed by the WLA component of a TMDL. See 40 C.F.R. § 130.2(h). NPDES-regulated storm water discharges may not be addressed by the LA component of a TMDL. In Wicomico County, where the Wicomico River Headwaters watershed is located, there are no NPDES-regulated stormwater discharges. In this analysis, loads from urban areas (*i.e.*, domestic pets loads) are designated as LA.

Municipal and Industrial Waste Water Treatment Plant

There are two point source facilities with permits regulating the discharge of bacteria into the Wicomico River Headwaters watershed. Table 4.8.2 lists the permitting information. 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 4.8.2: Municipal and Industrial Waste Water Treatment Plants

Permittee	NPDES Permit No.	County	Permit Flow (MGD)		Permit *E. coli* (MPN/100ml)	Permit Load (billions MPN/day)		% of TMDL	
Municipal Delmar WWTP	MD0020532	Wicomico	0.65		126	3.1		8.3%	
Industrial Perdue Farms	MD0000060-001A	Wicomico	Total Flow 0.1668	Industrial Process WW 0.148	126	Total Load 0.80	0.71	5.5%	4.9%
				Domestic WW 0.0188	126		0.09		0.6%

4.9 Summary

The TMDL for the Wicomico River Headwaters watershed are presented below.

Table 4.9.1: Wicomico River Headwaters Watershed TMDL

Stations	TMDL	LA		WLA
	Load (billions MPN/day)	Load (billions MPN/day)		Load (billions MPN/day)
		Maryland	Delaware	
NLO0003	29.9	26.9	3.0	0.0
LPR0028	11.0	11.0	0.0	0.0
WIW0241sub	37.3	31.2	3.0	3.1
BWB0010	8.7	8.7	0.0	0.0
MNC0010	14.3	13.5	0.0	0.8
TOTAL	101.2	91.3	6.0	3.9

In three of the five subwatersheds, based on the practicable reduction rates specified, water quality standards could not be achieved. This has the potential to occur in watersheds where wildlife contributions are a significant component, or in watersheds that require very high reductions to meet water quality standards. However, if there is no practical TMDL scenario, then maximum practical reductions are increased to provide estimates of the reductions required to meet water quality standards. For these watersheds, it is noted that the reductions may be beyond practical limits. In these cases, it is expected that the first stage of implementation will be to implement the maximum practicable reduction 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 Wicomico River Headwaters watershed, the TMDL analysis indicates that in three of the five subwatersheds, reduction of fecal bacteria loads from all sources including wildlife are beyond the MPR targets. The Wicomico River Headwaters may not be able to attain water quality standards in the waterbody's segments downstream of Leonard Pond. The extent of the fecal bacteria load reductions required to meet water quality criteria in these three subwatersheds of the Wicomico River are not feasible by effluent limitations 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 three of the five subwatersheds 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 largest impact on water quality and human health risk, with consideration given to ease of implementation and cost. The iterative implementation of BMPs in the watershed has several benefits: tracking of water quality improvements following BMP implementation through follow-up stream monitoring; providing a mechanism for developing public support through periodic updates on BMP implementation; and helping to ensure that the most cost-effective practices are implemented first.

Potential funding sources for implementation include the Maryland's Agricultural Cost Share Program (MACS) which provides grants to farmers to help protect natural resources and the Environmental Quality and Incentives Program which focuses on implementing conservation practices and BMPs on land involved with livestock and production. Funding sources available for local governments include the State Water Quality Revolving Loan Fund and the Stormwater Pollution Cost Share Program. Details of these programs and additional funding sources can be found at http://www.dnr.state.md.us/bay/services/summaries.html.

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.

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 by the year 2010 through implementation of EPA's minimum control strategies, long term control plans (LTCP), and collection system improvements in capacity, inflow and infiltration reduction, operation and maintenance.

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

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

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

Implementation and Wildlife Sources

It is expected that in some waters for which TMDLs will be developed, the bacteria source analysis will indicate that after controls are in place for all anthropogenic sources, the waterbody does not meet water quality standards. However, while neither the State of 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 nonpoint controllable sources may also reduce some wildlife inputs to the waters.

REFERENCES

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

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

Code of Federal Regulations (CFR) 40 CFR 130.2(i). TMDL and individual water quality-based effluent limitations – Website http://www.access.gpo.gov/nara/cfr/waisidx_98/40cfr130_98.html

Code of Federal Regulations (CFR) 40 CFR 130.7(c)(1). Definitions - Website - http://www.access.gpo.gov/nara/cfr/waisidx_98/40cfr130_98.html 40 CFR 130.7(c)(1)

Code of Maryland Regulations, 26.08.02.03-3A(1). Bacteriological Criteria for Use I, III, IV-P Waters - Website http://www.dsd.state.md.us/comar/26/26.08.02.03-3.htm.

Code of Maryland Regulations (COMAR), 26.08.02.03-3A(1). Bacteriological Criteria for Use I, III, IV-P Waters - Website http://www.dsd.state.md.us/comar/26/26.08.02.03-3.htm.

Code of Maryland Regulations (COMAR), 26.08.02.08D. Stream Segment Designations – Website http://www.dsd.state.md.us/comar/26/26.08.02.08.htm

Code of Maryland Regulations, 26.08.10. Website http://www.dsd.state.md.us/comar/26/26.08.10.htm, last visited 07/29/05.

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

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

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

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

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

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

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

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

Rule, Timothy C. 1999 (Unpublished). Estimating Watershed Flow on the Delmarva Peninsula Using Linear Regression of Watershed Area Versus Known Flow.

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

Soil Conservation Service (SCS). *Soil Survey of WicomicoCounty, MD*, 1995.

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

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

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

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

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

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

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

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

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

Appendix A – Bacteria Monitoring Data

Table A-1: *E.coli* data for Wicomico Rivers Headwaters Monitoring Stations

Watershed	Date	Tributary	Station	E. Coli (MPN/100ml)
02130304	12/18/2002	Brewington Branch	BWB0010	53
02130304	01/08/2003	Brewington Branch	BWB0010	254
02130304	03/05/2003	Brewington Branch	BWB0010	238
02130304	03/19/2003	Brewington Branch	BWB0010	178
02130304	04/24/2003	Brewington Branch	BWB0010	75
02130304	05/07/2003	Brewington Branch	BWB0010	42
02130304	05/21/2003	Brewington Branch	BWB0010	164
02130304	06/04/2003	Brewington Branch	BWB0010	659
02130304	06/18/2003	Brewington Branch	BWB0010	738
02130304	06/25/2003	Brewington Branch	BWB0010	738
02130304	07/09/2003	Brewington Branch	BWB0010	1091
02130304	07/16/2003	Brewington Branch	BWB0010	1298
02130304	07/23/2003	Brewington Branch	BWB0010	2005
02130304	08/06/2003	Brewington Branch	BWB0010	2880
02130304	08/13/2003	Brewington Branch	BWB0010	697
02130304	08/20/2003	Brewington Branch	BWB0010	344
02130304	08/27/2003	Brewington Branch	BWB0010	504
02130304	09/10/2003	Brewington Branch	BWB0010	99
02130304	09/24/2003	Brewington Branch	BWB0010	222
02130304	10/08/2003	Brewington Branch	BWB0010	20
02130304	10/22/2003	Brewington Branch	BWB0010	53
02130304	10/02/2002	Leonard Mill Pond	LPR0020	31
02130304	10/23/2002	Leonard Mill Pond	LPR0020	10
02130304	11/06/2002	Leonard Mill Pond	LPR0020	124
02130304	11/18/2002	Leonard Mill Pond	LPR0020	478
02130304	12/04/2002	Leonard Mill Pond	LPR0020	10
02130304	12/18/2002	Leonard Mill Pond	LPR0020	20
02130304	01/08/2003	Leonard Mill Pond	LPR0020	64
02130304	03/05/2003	Leonard Mill Pond	LPR0020	42
02130304	03/19/2003	Leonard Mill Pond	LPR0020	31
02130304	04/24/2003	Leonard Mill Pond	LPR0020	64
02130304	05/07/2003	Leonard Mill Pond	LPR0020	207
02130304	05/21/2003	Leonard Mill Pond	LPR0020	111
02130304	06/04/2003	Leonard Mill Pond	LPR0020	111
02130304	06/18/2003	Leonard Mill Pond	LPR0020	192
02130304	06/25/2003	Leonard Mill Pond	LPR0020	99
02130304	07/09/2003	Leonard Mill Pond	LPR0020	150
02130304	07/16/2003	Leonard Mill Pond	LPR0020	164
02130304	07/23/2003	Leonard Mill Pond	LPR0020	20
02130304	08/06/2003	Leonard Mill Pond	LPR0020	531
02130304	08/13/2003	Leonard Mill Pond	LPR0020	10
02130304	08/20/2003	Leonard Mill Pond	LPR0020	53
02130304	08/27/2003	Leonard Mill Pond	LPR0020	20

Watershed	Date	Tributary	Station	E. Coli (MPN/100ml)
02130304	09/10/2003	Leonard Mill Pond	LPR0020	31
02130304	09/24/2003	Leonard Mill Pond	LPR0020	207
02130304	10/08/2003	Leonard Mill Pond	LPR0020	20
02130304	10/22/2003	Leonard Mill Pond	LPR0020	20
02130304	10/02/2002	Leonard Pond Run	LPR0024	20
02130304	10/02/2002	Leonard Pond Run	LPR0024	
02130304	10/02/2002	Leonard Pond Run	LPR0028	10
02130304	10/23/2002	Leonard Pond Run	LPR0024	10
02130304	10/23/2002	Leonard Pond Run	LPR0024	
02130304	10/23/2002	Leonard Pond Run	LPR0028	10
02130304	11/06/2002	Leonard Pond Run	LPR0024	64
02130304	11/06/2002	Leonard Pond Run	LPR0028	31
02130304	11/18/2002	Leonard Pond Run	LPR0024	429
02130304	11/18/2002	Leonard Pond Run	LPR0028	504
02130304	12/04/2002	Leonard Pond Run	LPR0024	
02130304	12/04/2002	Leonard Pond Run	LPR0028	
02130304	12/18/2002	Leonard Pond Run	LPR0024	20
02130304	12/18/2002	Leonard Pond Run	LPR0028	20
02130304	01/08/2003	Leonard Pond Run	LPR0024	10
02130304	01/08/2003	Leonard Pond Run	LPR0028	10
02130304	03/05/2003	Leonard Pond Run	LPR0024	10
02130304	03/05/2003	Leonard Pond Run	LPR0028	20
02130304	03/19/2003	Leonard Pond Run	LPR0024	60
02130304	03/19/2003	Leonard Pond Run	LPR0024	
02130304	03/19/2003	Leonard Pond Run	LPR0028	40
02130304	04/24/2003	Leonard Pond Run	LPR0024	10
02130304	04/24/2003	Leonard Pond Run	LPR0028	42
02130304	05/07/2003	Leonard Pond Run	LPR0024	20
02130304	05/07/2003	Leonard Pond Run	LPR0028	344
02130304	05/21/2003	Leonard Pond Run	LPR0024	99
02130304	05/21/2003	Leonard Pond Run	LPR0024	
02130304	05/21/2003	Leonard Pond Run	LPR0028	87
02130304	06/04/2003	Leonard Pond Run	LPR0024	10
02130304	06/04/2003	Leonard Pond Run	LPR0024	
02130304	06/04/2003	Leonard Pond Run	LPR0028	10
02130304	06/18/2003	Leonard Pond Run	LPR0024	53
02130304	06/18/2003	Leonard Pond Run	LPR0028	75
02130304	06/25/2003	Leonard Pond Run	LPR0024	20
02130304	06/25/2003	Leonard Pond Run	LPR0028	20
02130304	07/09/2003	Leonard Pond Run	LPR0024	64
02130304	07/09/2003	Leonard Pond Run	LPR0024	
02130304	07/09/2003	Leonard Pond Run	LPR0028	53
02130304	07/16/2003	Leonard Pond Run	LPR0024	31
02130304	07/16/2003	Leonard Pond Run	LPR0028	10
02130304	07/23/2003	Leonard Pond Run	LPR0024	24
02130304	07/23/2003	Leonard Pond Run	LPR0028	53
02130304	08/06/2003	Leonard Pond Run	LPR0024	42
02130304	08/06/2003	Leonard Pond Run	LPR0028	64

Watershed	Date	Tributary	Station	E. Coli (MPN/100ml)
02130304	08/13/2003	Leonard Pond Run	LPR0024	10
02130304	08/13/2003	Leonard Pond Run	LPR0024	
02130304	08/13/2003	Leonard Pond Run	LPR0028	10
02130304	08/20/2003	Leonard Pond Run	LPR0024	10
02130304	08/20/2003	Leonard Pond Run	LPR0028	10
02130304	08/27/2003	Leonard Pond Run	LPR0024	10
02130304	08/27/2003	Leonard Pond Run	LPR0028	10
02130304	09/10/2003	Leonard Pond Run	LPR0024	10
02130304	09/10/2003	Leonard Pond Run	LPR0024	
02130304	09/10/2003	Leonard Pond Run	LPR0028	20
02130304	09/24/2003	Leonard Pond Run	LPR0024	64
02130304	09/24/2003	Leonard Pond Run	LPR0028	53
02130304	10/08/2003	Leonard Pond Run	LPR0024	
02130304	10/08/2003	Leonard Pond Run	LPR0028	
02130304	10/22/2003	Leonard Pond Run	LPR0024	
02130304	10/22/2003	Leonard Pond Run	LPR0028	
02130304	10/02/2002	Middle Neck Branch	MNC0003	53
02130304	10/23/2002	Middle Neck Branch	MNC0003	75
02130304	11/06/2002	Middle Neck Branch	MNC0003	53
02130304	11/18/2002	Middle Neck Branch	MNC0003	1013
02130304	12/04/2002	Middle Neck Branch	MNC0003	192
02130304	12/18/2002	Middle Neck Branch	MNC0003	64
02130304	12/18/2002	Middle Neck Branch	MNC0010	99
02130304	01/08/2003	Middle Neck Branch	MNC0003	111
02130304	01/08/2003	Middle Neck Branch	MNC0010	192
02130304	03/05/2003	Middle Neck Branch	MNC0003	111
02130304	03/05/2003	Middle Neck Branch	MNC0010	222
02130304	03/19/2003	Middle Neck Branch	MNC0003	360
02130304	03/19/2003	Middle Neck Branch	MNC0010	222
02130304	04/24/2003	Middle Neck Branch	MNC0003	99
02130304	04/24/2003	Middle Neck Branch	MNC0010	453
02130304	05/07/2003	Middle Neck Branch	MNC0003	53
02130304	05/07/2003	Middle Neck Branch	MNC0010	10
02130304	05/21/2003	Middle Neck Branch	MNC0003	99
02130304	05/21/2003	Middle Neck Branch	MNC0010	324
02130304	06/04/2003	Middle Neck Branch	MNC0003	124
02130304	06/04/2003	Middle Neck Branch	MNC0010	1091
02130304	06/18/2003	Middle Neck Branch	MNC0003	164
02130304	06/18/2003	Middle Neck Branch	MNC0010	2005
02130304	06/25/2003	Middle Neck Branch	MNC0003	150
02130304	06/25/2003	Middle Neck Branch	MNC0010	738
02130304	07/09/2003	Middle Neck Branch	MNC0003	64
02130304	07/09/2003	Middle Neck Branch	MNC0010	831
02130304	07/16/2003	Middle Neck Branch	MNC0003	99
02130304	07/16/2003	Middle Neck Branch	MNC0010	1091
02130304	07/23/2003	Middle Neck Branch	MNC0003	64
02130304	07/23/2003	Middle Neck Branch	MNC0010	2005
02130304	08/06/2003	Middle Neck Branch	MNC0003	178

Watershed	Date	Tributary	Station	E. Coli (MPN/100ml)
02130304	08/06/2003	Middle Neck Branch	MNC0010	20050
02130304	08/13/2003	Middle Neck Branch	MNC0003	124
02130304	08/13/2003	Middle Neck Branch	MNC0010	1445
02130304	08/20/2003	Middle Neck Branch	MNC0003	10
02130304	08/20/2003	Middle Neck Branch	MNC0010	364
02130304	08/27/2003	Middle Neck Branch	MNC0003	238
02130304	08/27/2003	Middle Neck Branch	MNC0010	3840
02130304	09/10/2003	Middle Neck Branch	MNC0003	53
02130304	09/10/2003	Middle Neck Branch	MNC0010	697
02130304	09/24/2003	Middle Neck Branch	MNC0003	384
02130304	09/24/2003	Middle Neck Branch	MNC0010	11840
02130304	10/08/2003	Middle Neck Branch	MNC0003	
02130304	10/08/2003	Middle Neck Branch	MNC0010	271
02130304	10/22/2003	Middle Neck Branch	MNC0003	
02130304	10/22/2003	Middle Neck Branch	MNC0010	324
02130304	10/02/2002	North Prong	NLO0003	
02130304	10/23/2002	North Prong	NLO0003	10
02130304	10/23/2002	North Prong	NLO0003	
02130304	11/06/2002	North Prong	NLO0003	99
02130304	11/18/2002	North Prong	NLO0003	207
02130304	12/04/2002	North Prong	NLO0003	
02130304	12/18/2002	North Prong	NLO0008	10
02130304	12/18/2002	North Prong	NLO0003	64
02130304	01/08/2003	North Prong	NLO0003	42
02130304	01/08/2003	North Prong	NLO0008	31
02130304	03/05/2003	North Prong	NLO0003	31
02130304	03/05/2003	North Prong	NLO0008	42
02130304	03/19/2003	North Prong	NLO0003	100
02130304	03/19/2003	North Prong	NLO0008	137
02130304	04/24/2003	North Prong	NLO0003	10
02130304	04/24/2003	North Prong	NLO0008	20
02130304	05/07/2003	North Prong	NLO0003	478
02130304	05/07/2003	North Prong	NLO0008	10
02130304	05/21/2003	North Prong	NLO0003	10
02130304	05/21/2003	North Prong	NLO0008	42
02130304	06/04/2003	North Prong	NLO0003	10
02130304	06/04/2003	North Prong	NLO0008	20
02130304	06/18/2003	North Prong	NLO0003	20
02130304	06/18/2003	North Prong	NLO0008	164
02130304	06/25/2003	North Prong	NLO0003	10
02130304	06/25/2003	North Prong	NLO0008	150
02130304	07/09/2003	North Prong	NLO0003	42
02130304	07/09/2003	North Prong	NLO0008	137
02130304	07/16/2003	North Prong	NLO0003	20
02130304	07/16/2003	North Prong	NLO0008	137
02130304	07/23/2003	North Prong	NLO0003	150
02130304	07/23/2003	North Prong	NLO0008	192
02130304	08/06/2003	North Prong	NLO0003	20

Watershed	Date	Tributary	Station	E. Coli (MPN/100ml)
02130304	08/06/2003	North Prong	NLO0008	124
02130304	08/13/2003	North Prong	NLO0003	10
02130304	08/13/2003	North Prong	NLO0008	20
02130304	08/20/2003	North Prong	NLO0003	20
02130304	08/20/2003	North Prong	NLO0008	150
02130304	08/27/2003	North Prong	NLO0003	10
02130304	09/10/2003	North Prong	NLO0003	20
02130304	09/10/2003	North Prong	NLO0008	53
02130304	09/24/2003	North Prong	NLO0003	64
02130304	09/24/2003	North Prong	NLO0008	75
02130304	10/08/2003	North Prong	NLO0003	
02130304	10/08/2003	North Prong	NLO0008	53
02130304	10/22/2003	North Prong	NLO0003	
02130304	10/22/2003	North Prong	NLO0008	20
02130304	10/02/2002	Wicomico River	WIW0216	531
02130304	10/02/2002	Wicomico River	WIW0241	254
02130304	10/02/2002	Wicomico River	WIW0226	64
02130304	10/02/2002	Wicomico River	WIW0231	31
02130304	10/23/2002	Wicomico River	WIW0216	75
02130304	10/23/2002	Wicomico River	WIW0226	150
02130304	10/23/2002	Wicomico River	WIW0231	222
02130304	11/06/2002	Wicomico River	WIW0216	75
02130304	11/06/2002	Wicomico River	WIW0226	238
02130304	11/06/2002	Wicomico River	WIW0231	531
02130304	11/18/2002	Wicomico River	WIW0216	782
02130304	11/18/2002	Wicomico River	WIW0226	659
02130304	11/18/2002	Wicomico River	WIW0231	560
02130304	12/04/2002	Wicomico River	WIW0216	75
02130304	12/04/2002	Wicomico River	WIW0226	2005
02130304	12/04/2002	Wicomico River	WIW0231	591
02130304	12/18/2002	Wicomico River	WIW0216	64
02130304	12/18/2002	Wicomico River	WIW0241	64
02130304	12/18/2002	Wicomico River	WIW0226	111
02130304	12/18/2002	Wicomico River	WIW0231	150
02130304	01/08/2003	Wicomico River	WIW0216	111
02130304	01/08/2003	Wicomico River	WIW0241	192
02130304	01/08/2003	Wicomico River	WIW0226	75
02130304	01/08/2003	Wicomico River	WIW0231	254
02130304	03/05/2003	Wicomico River	WIW0216	137
02130304	03/05/2003	Wicomico River	WIW0241	124
02130304	03/05/2003	Wicomico River	WIW0226	75
02130304	03/05/2003	Wicomico River	WIW0231	137
02130304	03/19/2003	Wicomico River	WIW0216	124
02130304	03/19/2003	Wicomico River	WIW0241	164
02130304	03/19/2003	Wicomico River	WIW0226	170
02130304	03/19/2003	Wicomico River	WIW0231	190
02130304	04/24/2003	Wicomico River	WIW0216	53
02130304	04/24/2003	Wicomico River	WIW0241	271

Watershed	Date	Tributary	Station	E. Coli (MPN/100ml)
02130304	04/24/2003	Wicomico River	WIW0226	124
02130304	04/24/2003	Wicomico River	WIW0231	306
02130304	05/07/2003	Wicomico River	WIW0216	87
02130304	05/07/2003	Wicomico River	WIW0241	75
02130304	05/07/2003	Wicomico River	WIW0226	254
02130304	05/07/2003	Wicomico River	WIW0231	10
02130304	05/21/2003	Wicomico River	WIW0216	99
02130304	05/21/2003	Wicomico River	WIW0241	137
02130304	05/21/2003	Wicomico River	WIW0226	99
02130304	05/21/2003	Wicomico River	WIW0226	
02130304	05/21/2003	Wicomico River	WIW0231	150
02130304	05/21/2003	Wicomico River	WIW0231	
02130304	06/04/2003	Wicomico River	WIW0216	137
02130304	06/04/2003	Wicomico River	WIW0241	164
02130304	06/04/2003	Wicomico River	WIW0226	124
02130304	06/04/2003	Wicomico River	WIW0231	288
02130304	06/18/2003	Wicomico River	WIW0216	164
02130304	06/18/2003	Wicomico River	WIW0241	137
02130304	06/18/2003	Wicomico River	WIW0226	238
02130304	06/18/2003	Wicomico River	WIW0231	324
02130304	06/25/2003	Wicomico River	WIW0216	75
02130304	06/25/2003	Wicomico River	WIW0241	178
02130304	06/25/2003	Wicomico River	WIW0226	10
02130304	06/25/2003	Wicomico River	WIW0231	207
02130304	07/09/2003	Wicomico River	WIW0216	111
02130304	07/09/2003	Wicomico River	WIW0241	429
02130304	07/09/2003	Wicomico River	WIW0226	10
02130304	07/09/2003	Wicomico River	WIW0231	10
02130304	07/16/2003	Wicomico River	WIW0216	87
02130304	07/16/2003	Wicomico River	WIW0241	178
02130304	07/16/2003	Wicomico River	WIW0226	42
02130304	07/16/2003	Wicomico River	WIW0231	111
02130304	07/23/2003	Wicomico River	WIW0216	124
02130304	07/23/2003	Wicomico River	WIW0241	288
02130304	07/23/2003	Wicomico River	WIW0226	99
02130304	07/23/2003	Wicomico River	WIW0231	164
02130304	08/06/2003	Wicomico River	WIW0216	238
02130304	08/06/2003	Wicomico River	WIW0241	1445
02130304	08/06/2003	Wicomico River	WIW0226	75
02130304	08/06/2003	Wicomico River	WIW0231	2005
02130304	08/13/2003	Wicomico River	WIW0216	271
02130304	08/13/2003	Wicomico River	WIW0241	111
02130304	08/13/2003	Wicomico River	WIW0226	10
02130304	08/13/2003	Wicomico River	WIW0231	53
02130304	08/20/2003	Wicomico River	WIW0216	124
02130304	08/20/2003	Wicomico River	WIW0241	384
02130304	08/20/2003	Wicomico River	WIW0226	31
02130304	08/20/2003	Wicomico River	WIW0231	99

Watershed	Date	Tributary	Station	E. Coli (MPN/100ml)
02130304	08/27/2003	Wicomico River	WIW0216	53
02130304	08/27/2003	Wicomico River	WIW0241	150
02130304	08/27/2003	Wicomico River	WIW0226	10
02130304	08/27/2003	Wicomico River	WIW0231	10
02130304	09/10/2003	Wicomico River	WIW0216	207
02130304	09/10/2003	Wicomico River	WIW0241	207
02130304	09/10/2003	Wicomico River	WIW0226	99
02130304	09/10/2003	Wicomico River	WIW0226	
02130304	09/10/2003	Wicomico River	WIW0231	150
02130304	09/24/2003	Wicomico River	WIW0216	137
02130304	09/24/2003	Wicomico River	WIW0241	164
02130304	09/24/2003	Wicomico River	WIW0226	831
02130304	09/24/2003	Wicomico River	WIW0231	324
02130304	10/08/2003	Wicomico River	WIW0216	42
02130304	10/08/2003	Wicomico River	WIW0241	111
02130304	10/08/2003	Wicomico River	WIW0226	
02130304	10/08/2003	Wicomico River	WIW0231	
02130304	10/22/2003	Wicomico River	WIW0216	31
02130304	10/22/2003	Wicomico River	WIW0241	124
02130304	10/22/2003	Wicomico River	WIW0226	
02130304	10/22/2003	Wicomico River	WIW0226	
02130304	10/22/2003	Wicomico River	WIW0231	

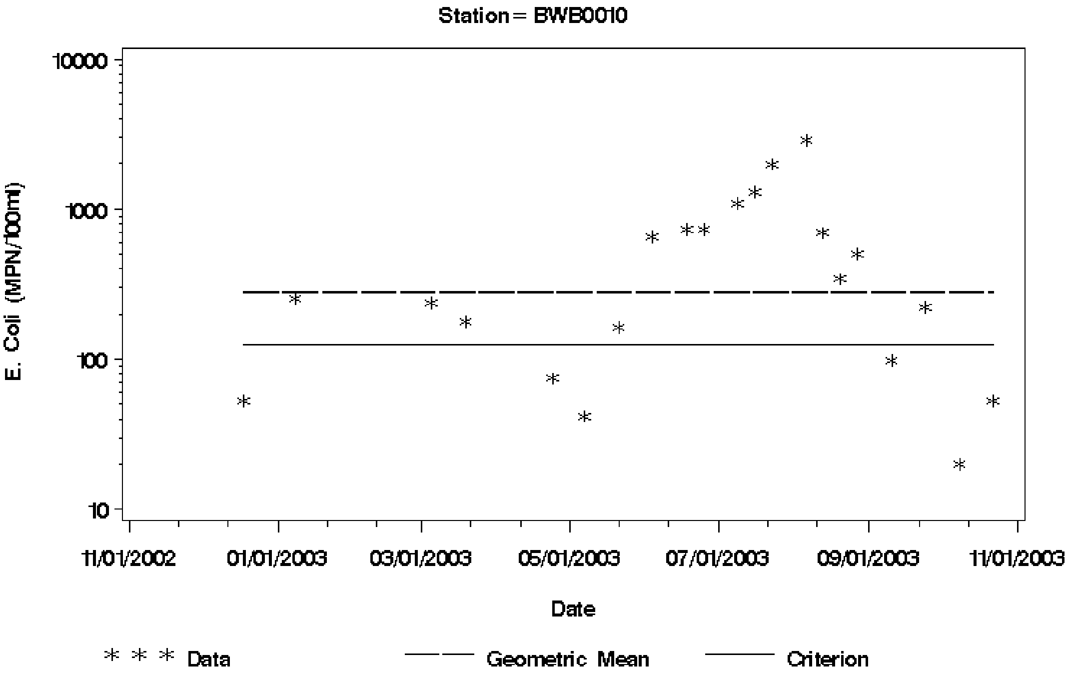


Figure A-1: *E. coli* **Concentration vs. Time for Wicomico River Headwaters Monitoring Station BWB0010 (Annual Condition)**

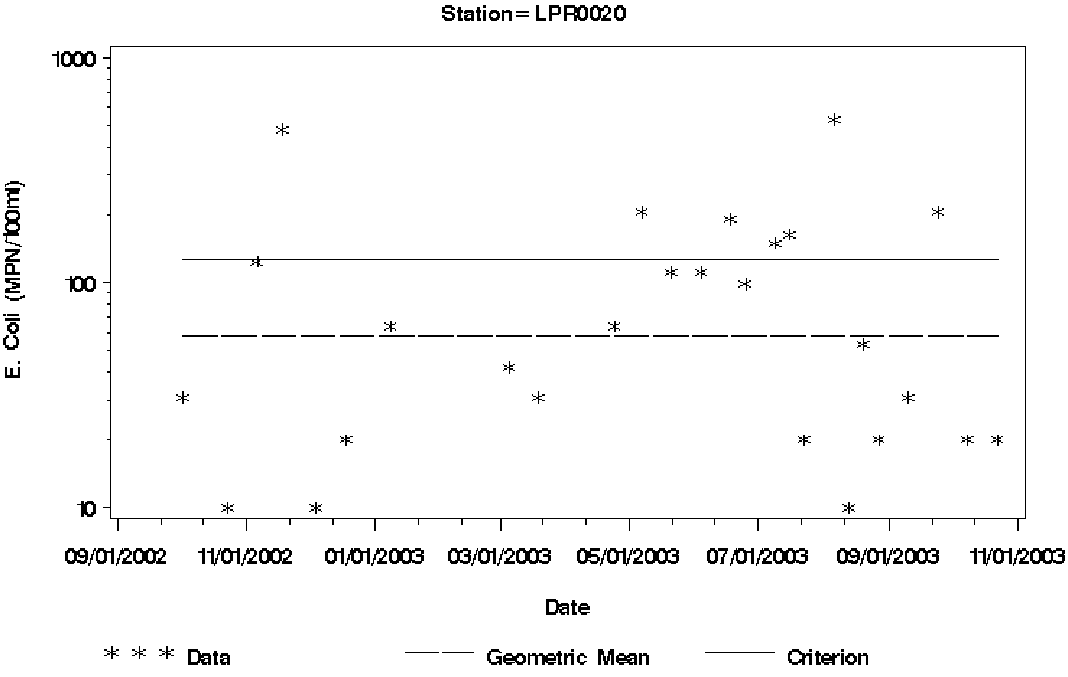


Figure A-2: *E. coli* **Concentration vs. Time for Wicomico River Headwaters Monitoring Station LPR0020 (Annual Condition)**

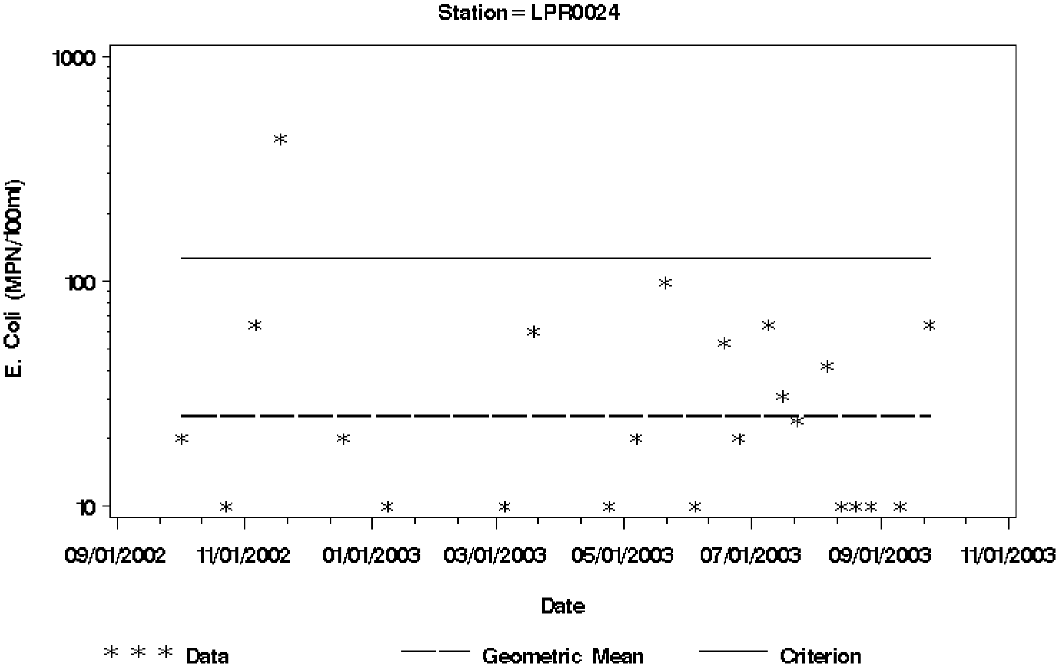


Figure A-3: ***E. coli*** **Concentration vs. Time for Wicomico River Headwaters Monitoring Station LPR0024 (Annual Condition)**

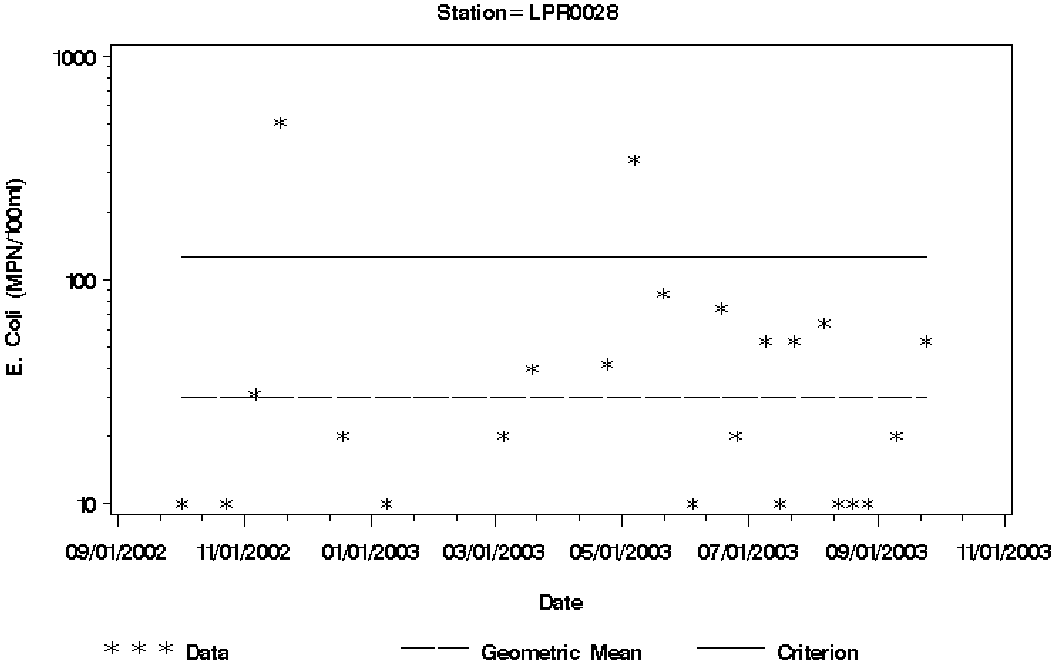


Figure A-4: ***E. coli*** **Concentration vs. Time for Wicomico River Headwaters Monitoring Station LPR0028 (Annual Condition)**

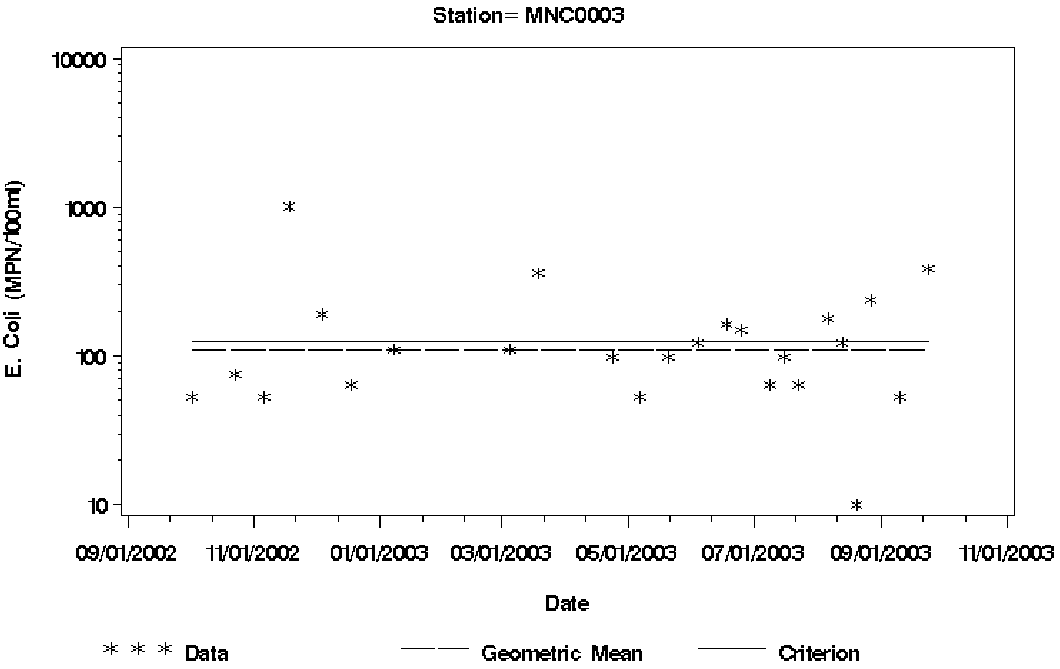


Figure A-5: ***E. coli*** **Concentration vs. Time for Wicomico River Headwaters Monitoring Station MNC0003 (Annual Condition)**

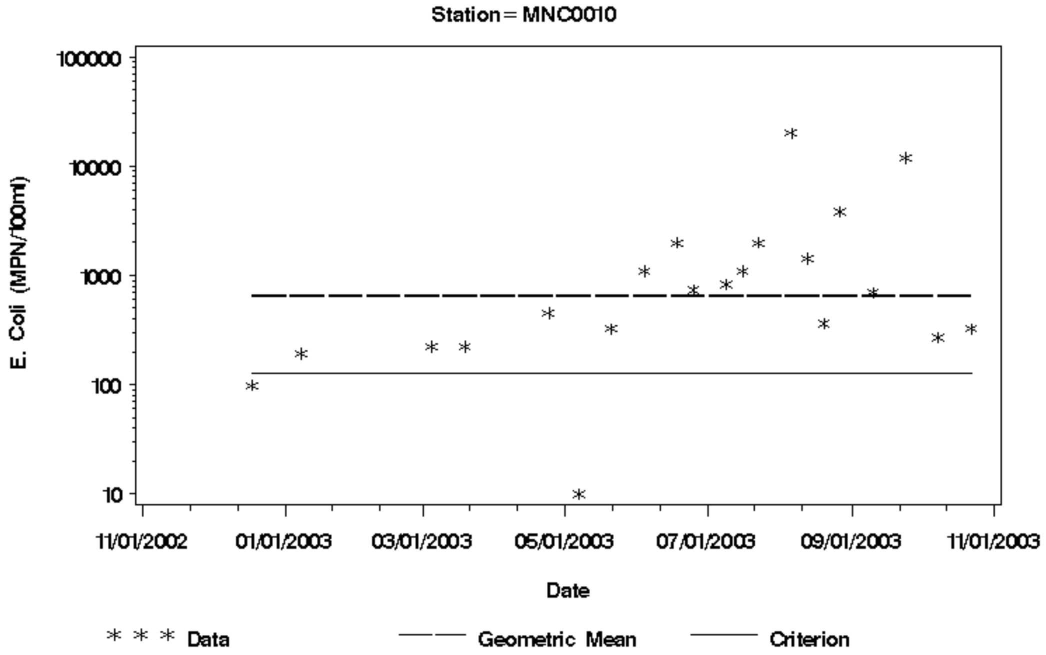


Figure A-6: ***E. coli*** **Concentration vs. Time for Wicomico River Headwaters Monitoring Station MNC0010 (Annual Condition)**

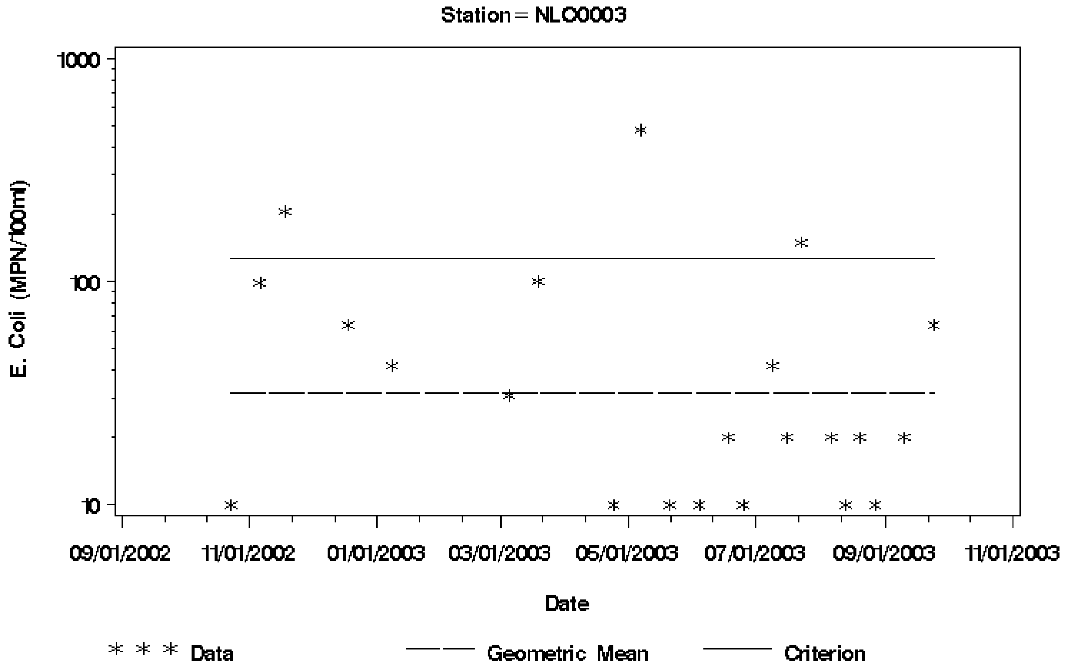


Figure A-7: ***E. coli*** **Concentration vs. Time for Wicomico River Headwaters Monitoring Station NLO0003 (Annual Condition)**

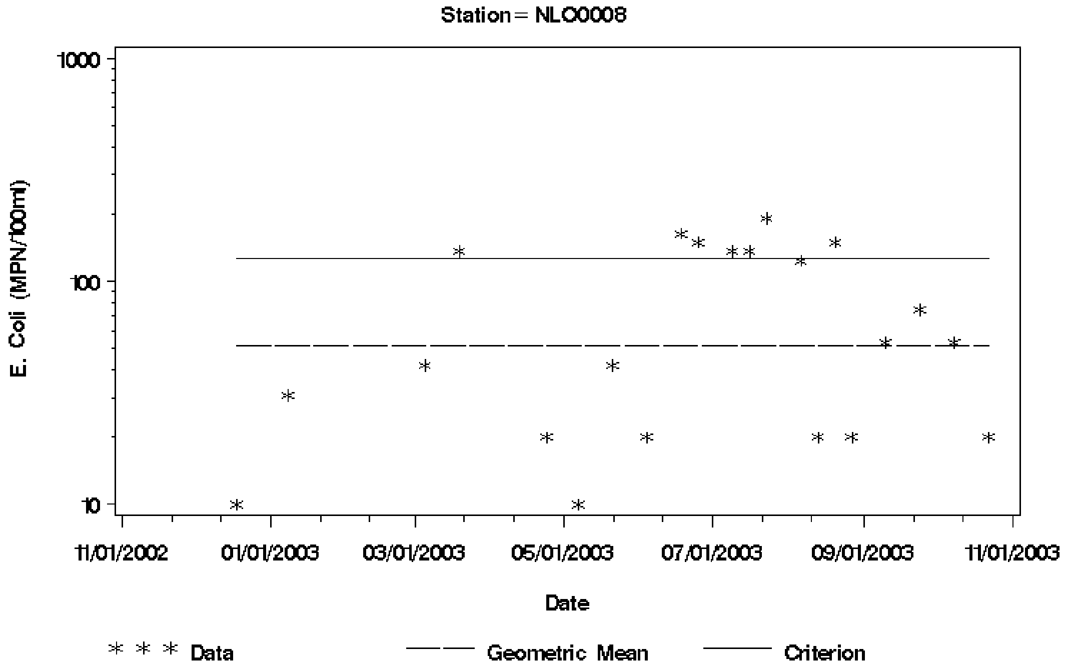


Figure A-8: ***E. coli*** **Concentration vs. Time for Wicomico River Headwaters Monitoring Station NLO0008 (Annual Condition)**

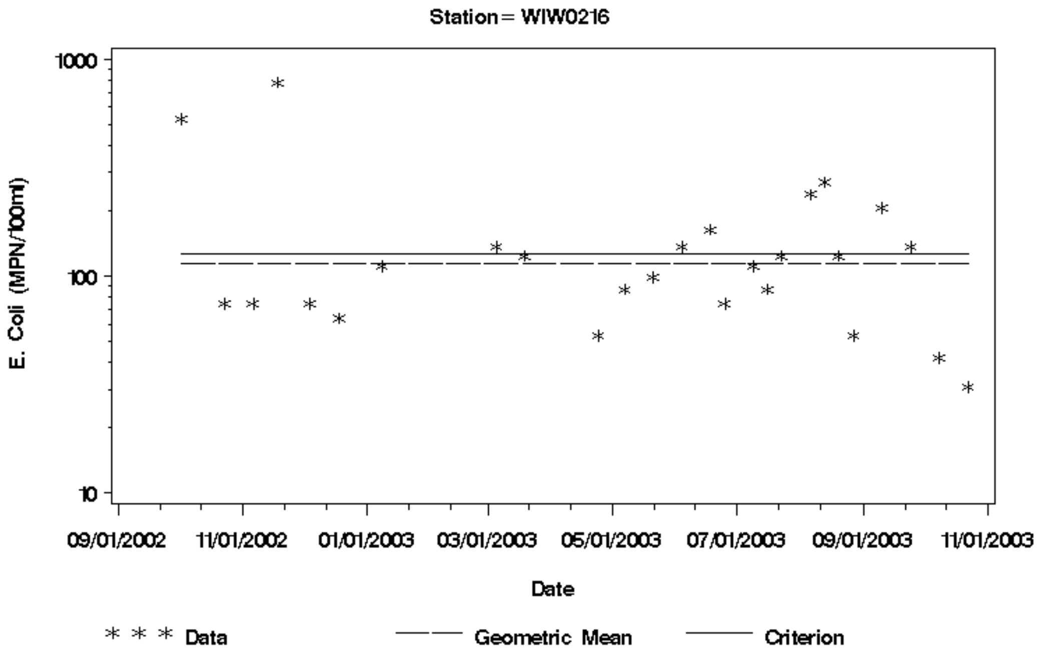


Figure A-9: *E. coli* Concentration vs. Time for Wicomico River Headwaters Monitoring Station WIW0216 (Annual Condition)

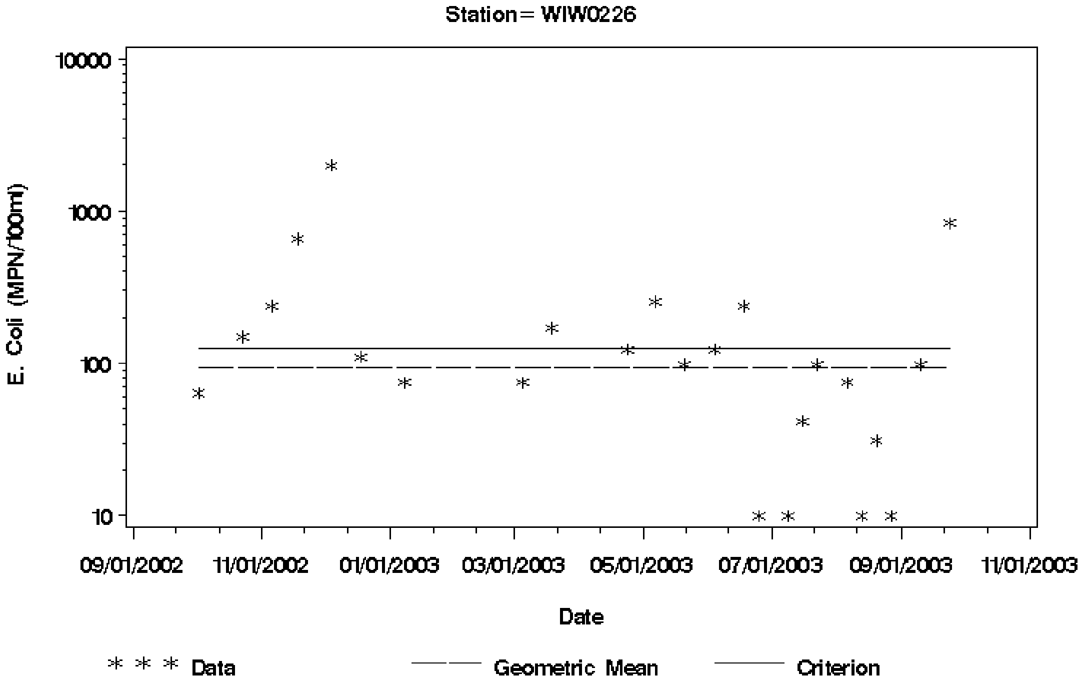


Figure A-10: *E. coli* Concentration vs. Time for Wicomico River Headwaters Monitoring Station WIW0226 (Annual Condition)

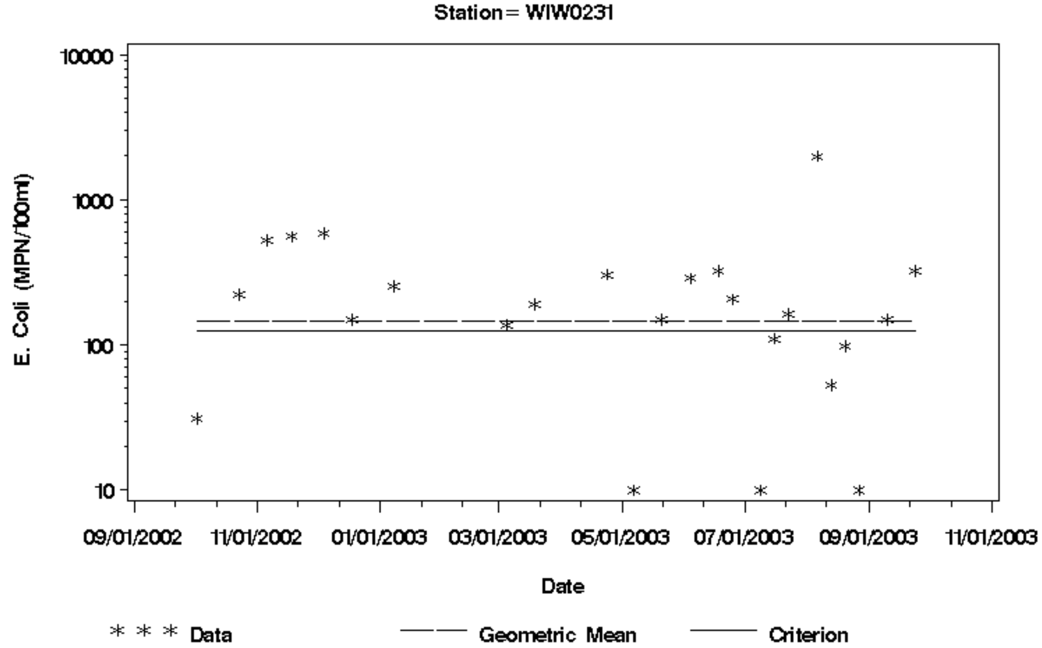


Figure A-11: ***E. coli*** **Concentration vs. Time for Wicomico River Headwaters Monitoring Station WIW0231 (Annual Condition)**

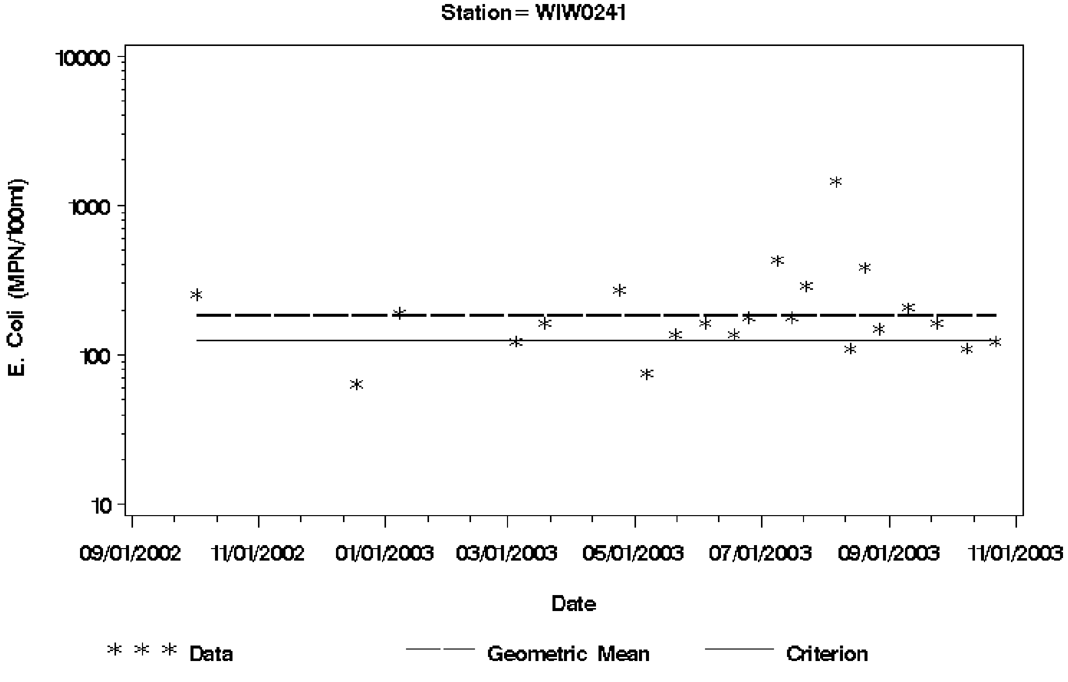


Figure A-12: ***E. coli*** **Concentration vs. Time for Wicomico River Headwaters Monitoring Station WIW0241 (Annual Condition)**

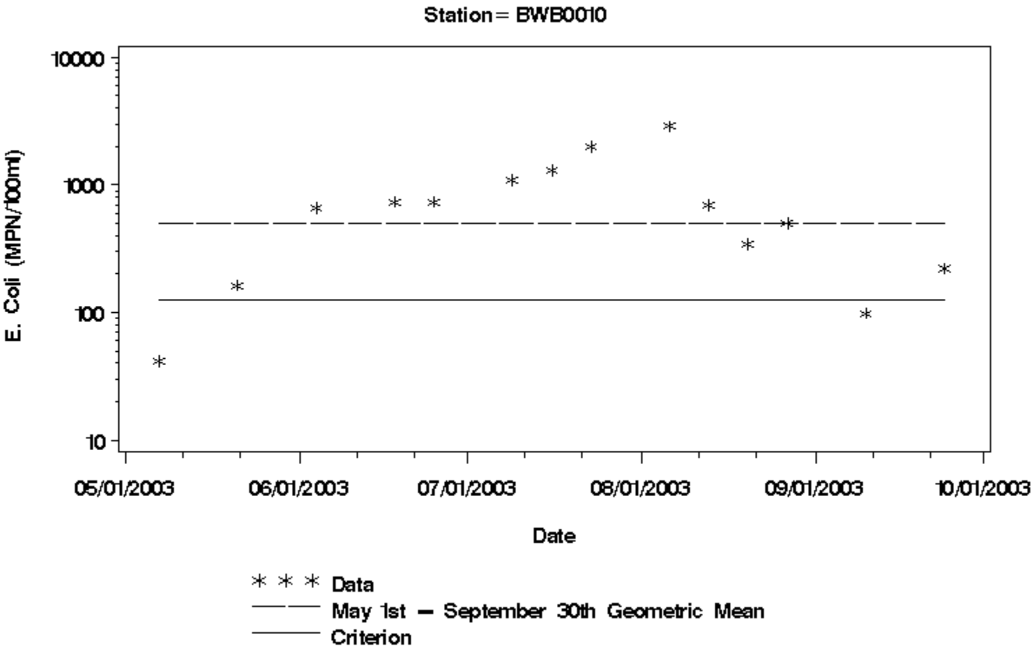


Figure A-13: *E. coli* Concentration vs. Time for Wicomico River Headwaters Monitoring Station BWB0010 (Seasonal Condition)

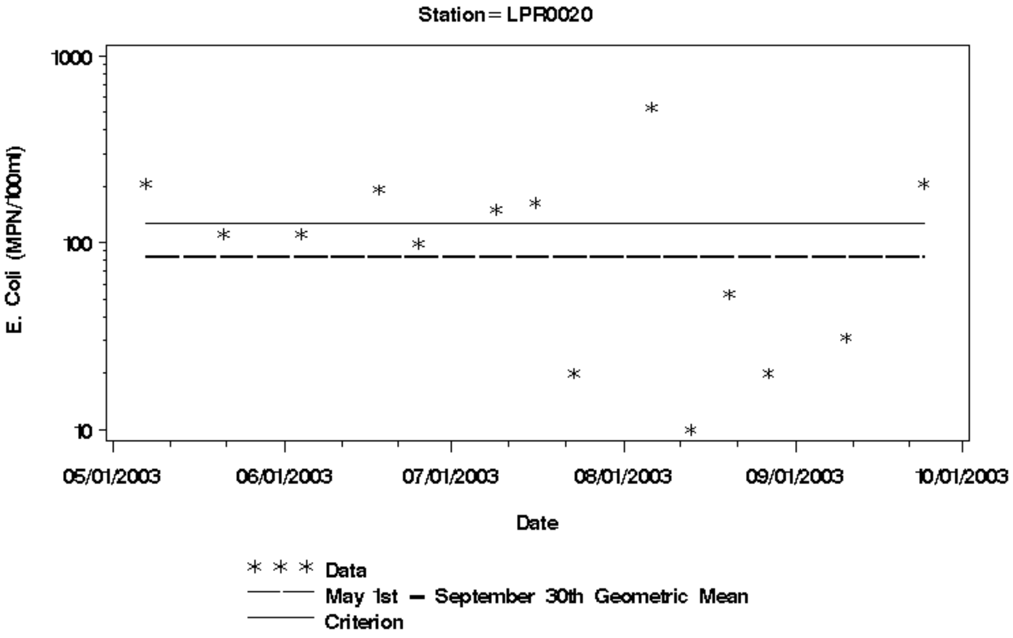


Figure A-14: *E. coli* Concentration vs. Time for Wicomico River Headwaters Monitoring Station LPR0020 (Seasonal Condition)

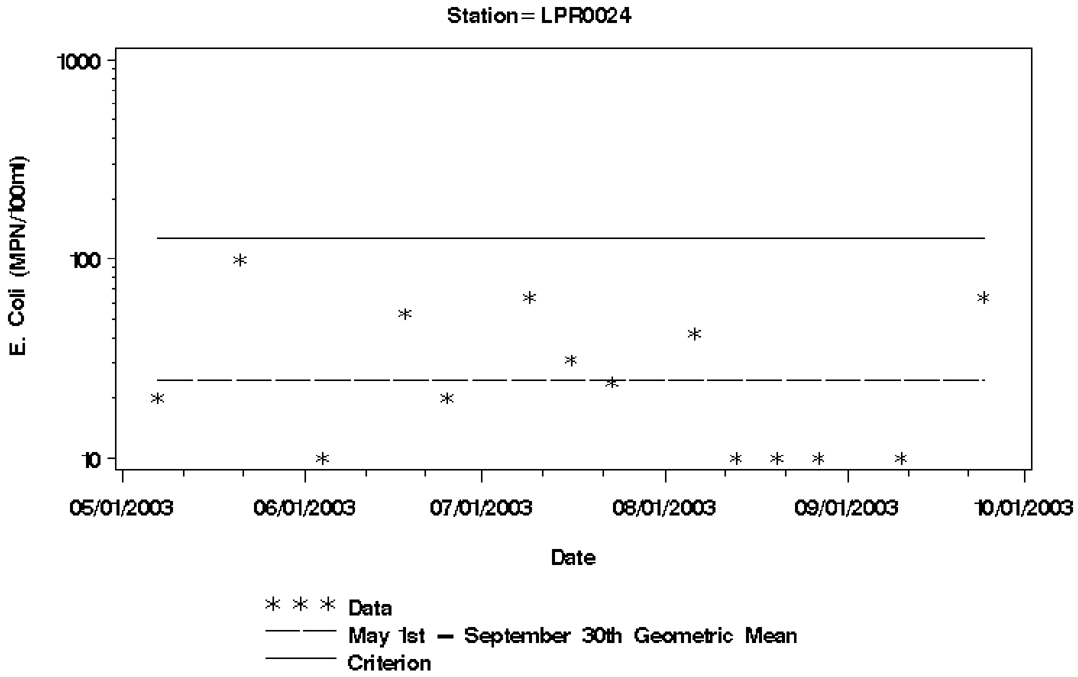


Figure A-15: *E. coli* Concentration vs. Time for Wicomico River Headwaters Monitoring Station LPR0024 (Seasonal Condition)

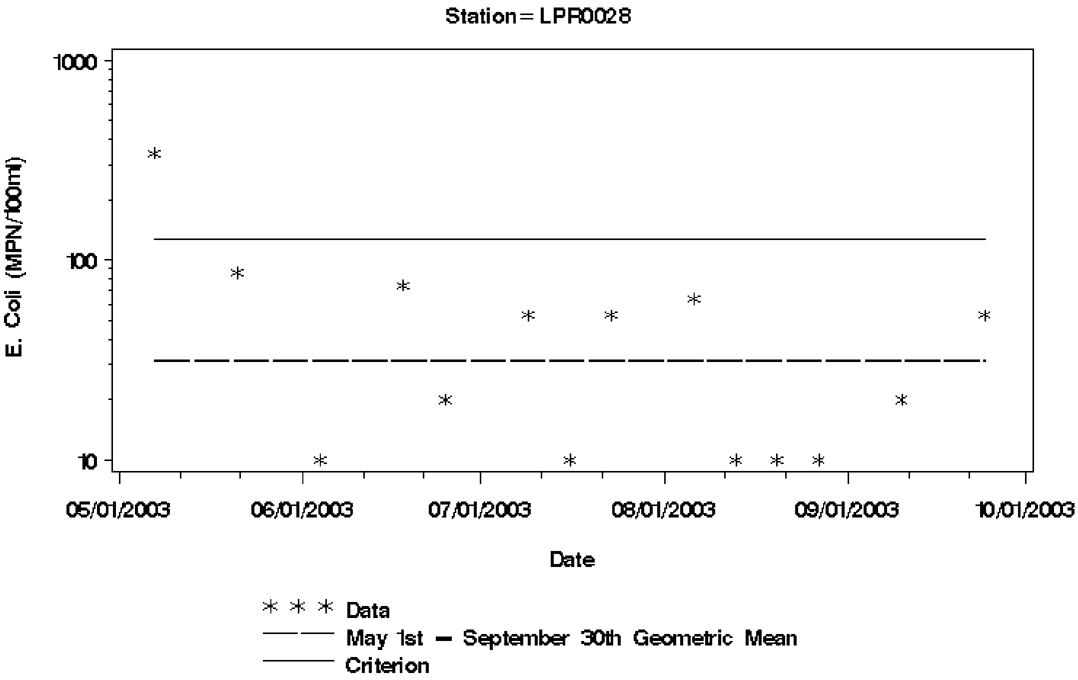


Figure A-16: *E. coli* Concentration vs. Time for Wicomico River Headwaters Monitoring Station LPR0028 (Seasonal Condition)

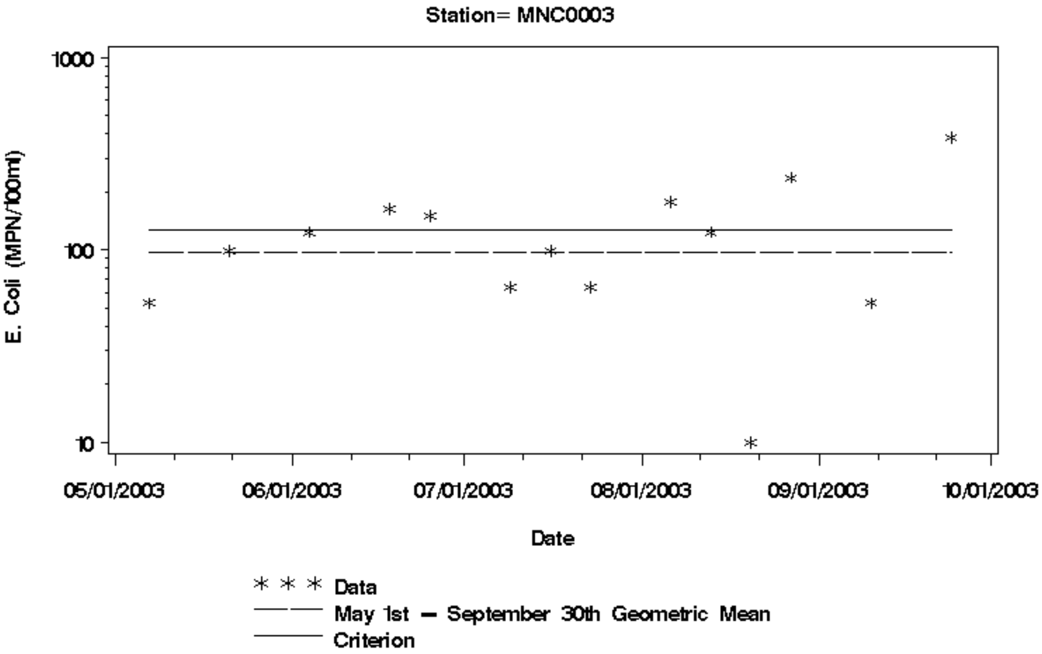


Figure A-17: ***E. coli*** **Concentration vs. Time for Wicomico River Headwaters Monitoring Station MNC0003 (Seasonal Condition)**

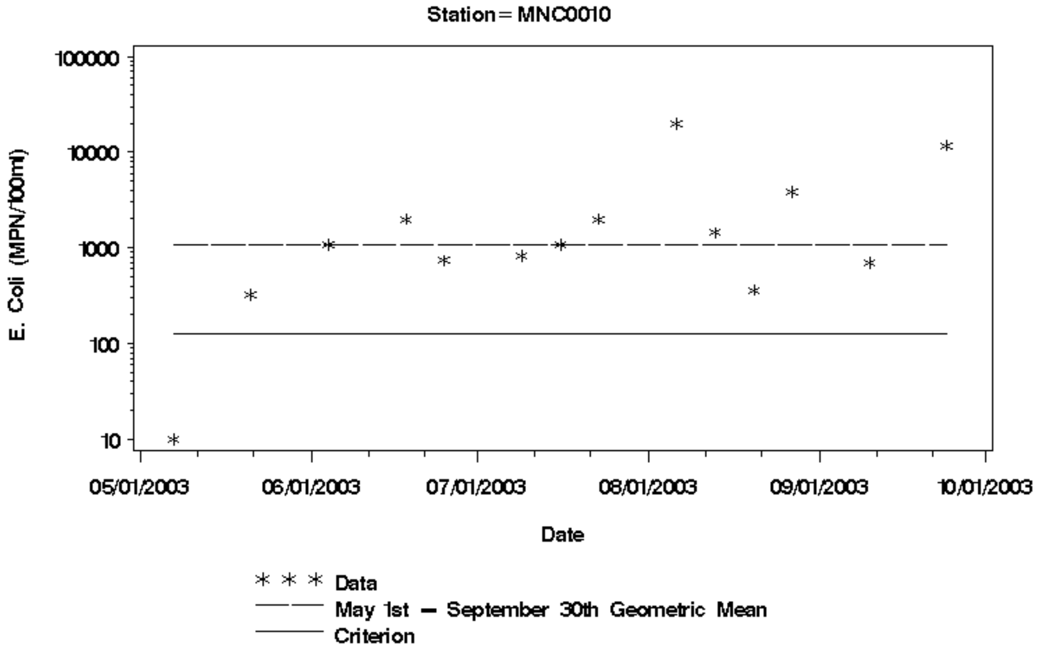


Figure A-18: ***E. coli*** **Concentration vs. Time for Wicomico River Headwaters Monitoring Station MNC0010 (Seasonal Condition)**

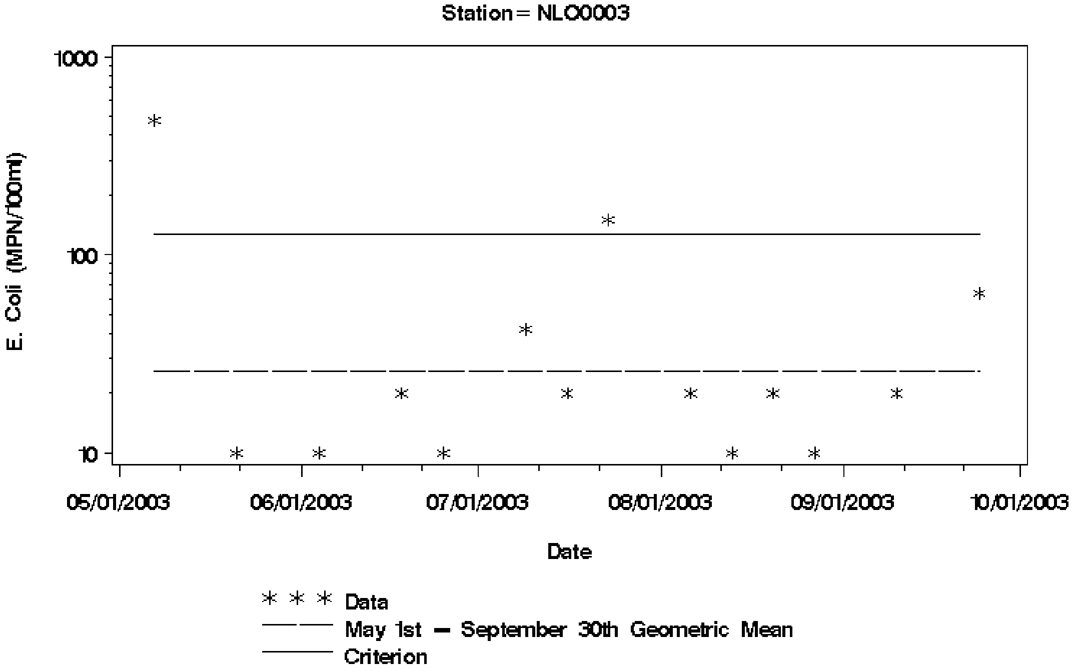


Figure A-19: ***E. coli*** **Concentration vs. Time for Wicomico River Headwaters Monitoring Station NLO0003 (Seasonal Condition)**

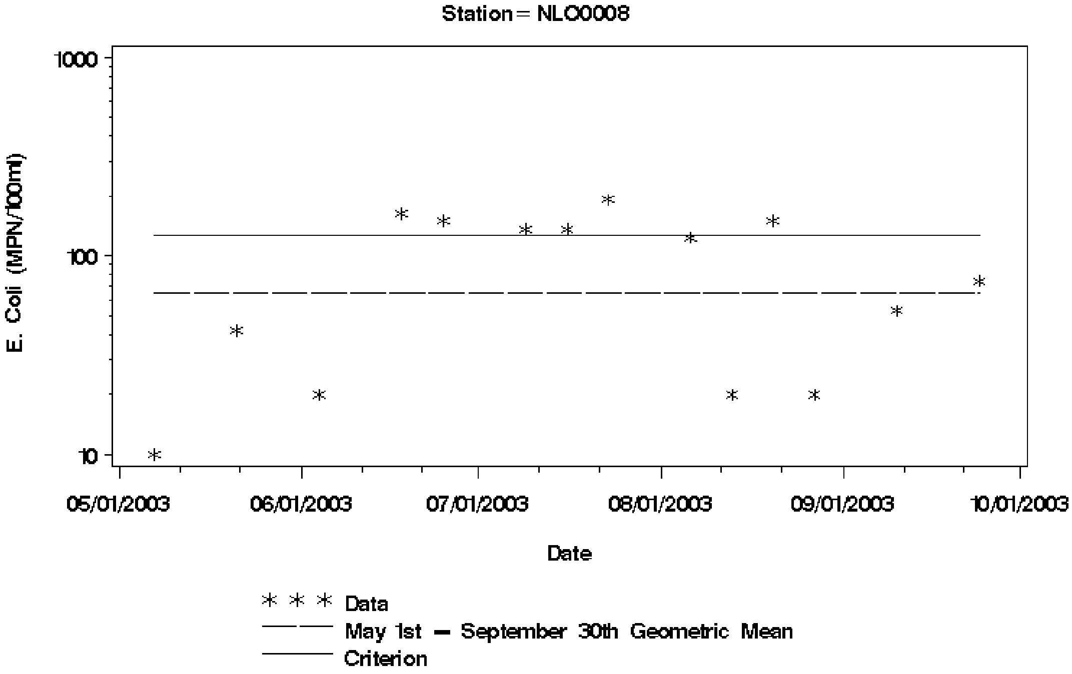


Figure A-20: ***E. coli*** **Concentration vs. Time for Wicomico River Headwaters Monitoring Station NLO0008 (Seasonal Condition)**

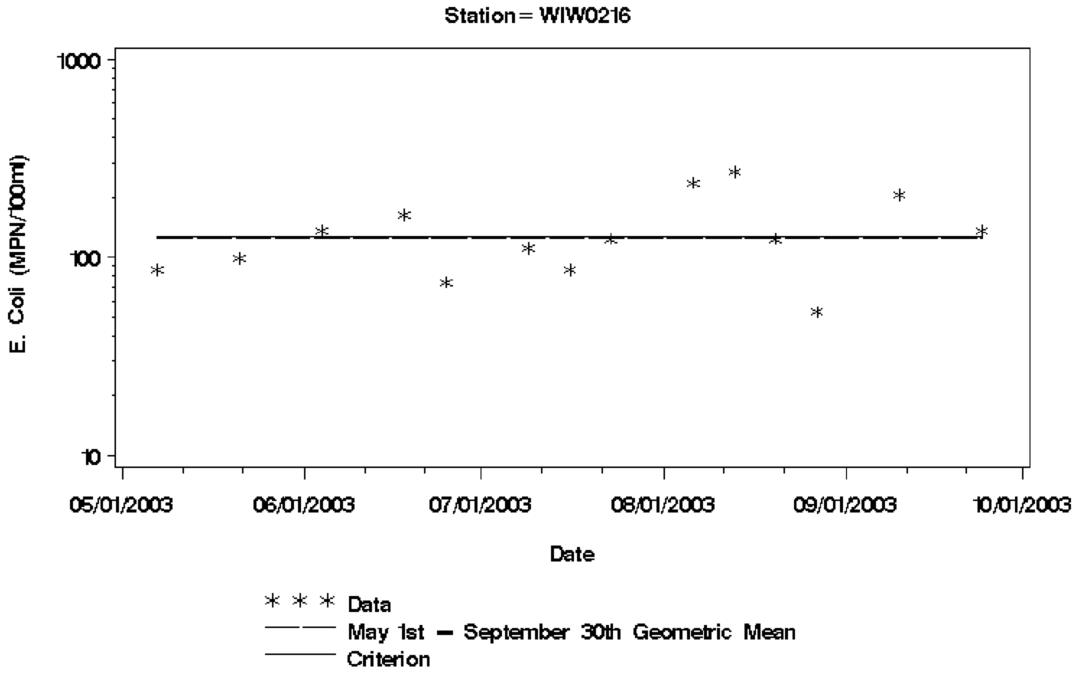


Figure A-21: ***E. coli*** **Concentration vs. Time for Wicomico River Headwaters Monitoring Station WIW0216 (Seasonal Condition)**

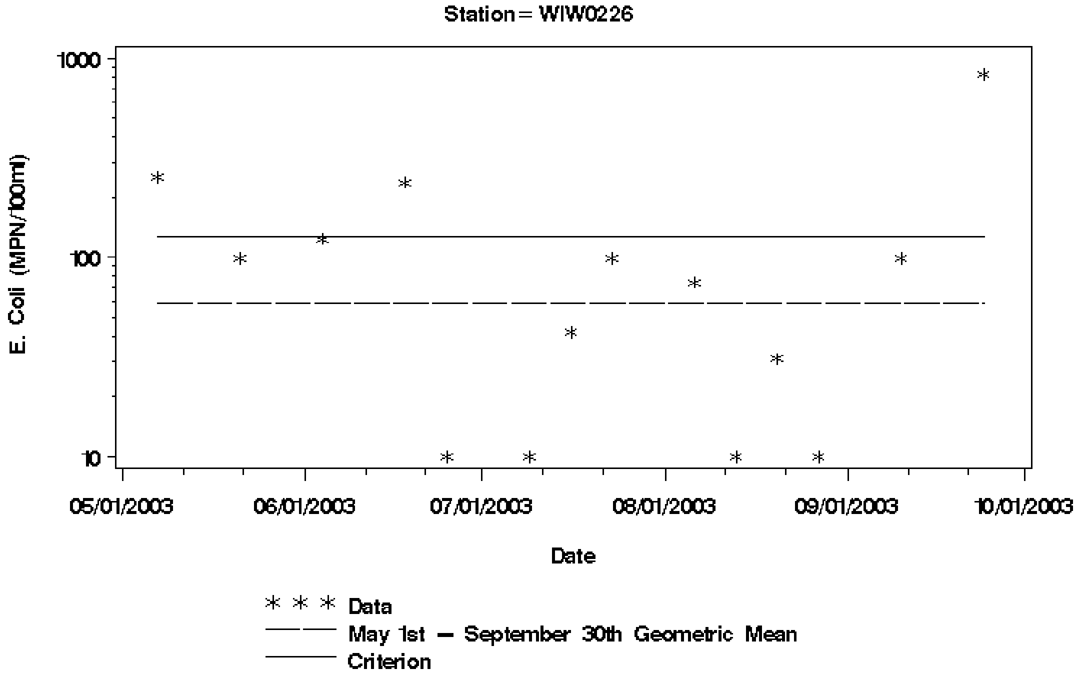


Figure A-22: ***E. coli*** **Concentration vs. Time for Wicomico River Headwaters Monitoring Station WIW0226 (Seasonal Condition)**

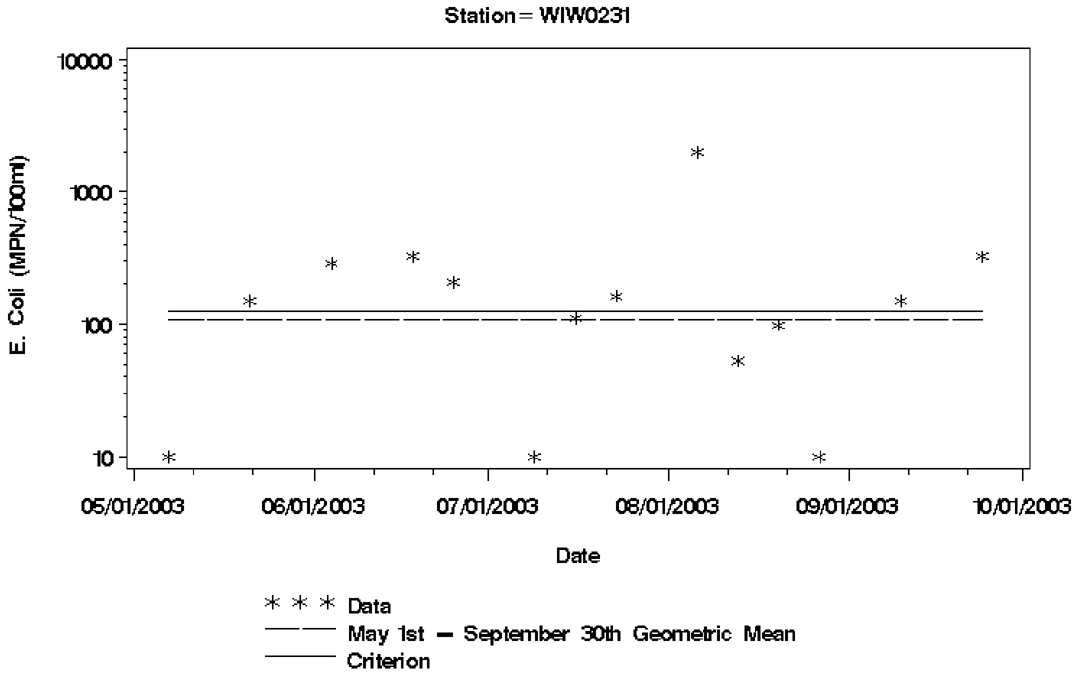


Figure A-23: *E. coli* Concentration vs. Time for Wicomico River Headwaters Monitoring Station WIW0231 (Seasonal Condition)

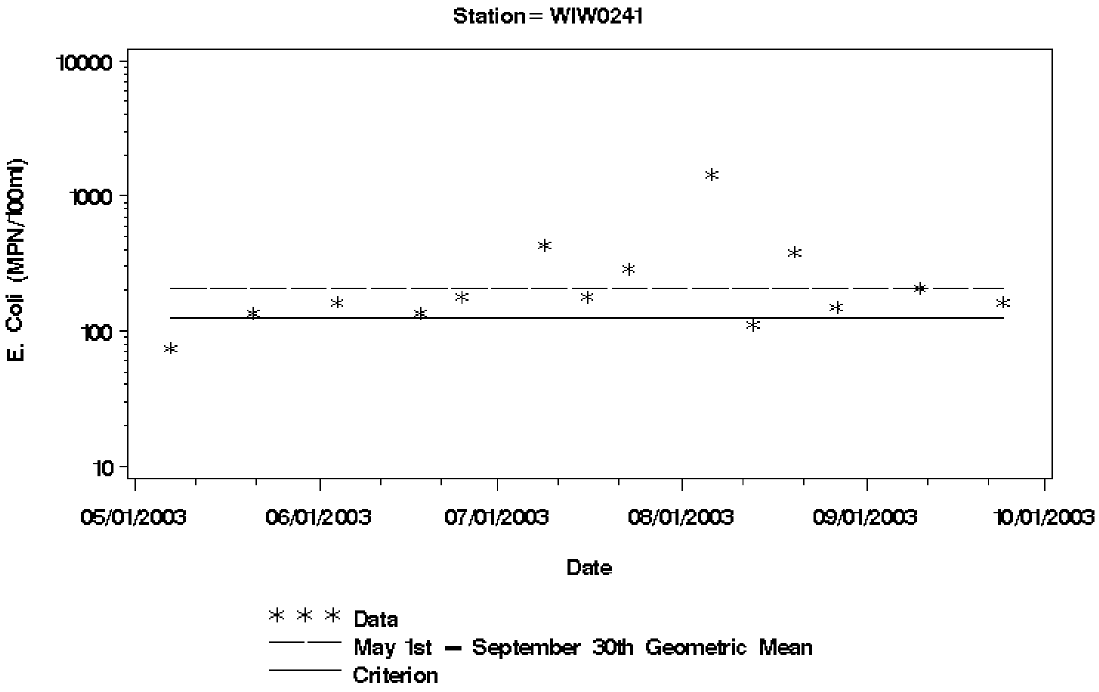


Figure A-24: *E. coli* Concentration vs. Time for Wicomico River Headwaters Monitoring Station WIW0241 (Seasonal Condition)

Appendix B - Flow Analysis

The Wicomico River Headwaters watershed has no active USGS flow gauges. The flow analysis for the development of this TMDL was originally directed towards the development of flow duration curves. As explained in Section 4.2 of the main document, flow duration curves are needed for the conceptual model to divide the daily flow frequency into strata that are representative of hydrologic conditions. For this purpose, flow data from several flow gauges in the Wicomico River watershed (USGS 01486500), Pocomoke River Headwaters watershed (USGS 01484983, 01484981, 01484985) and in the Chicamacomico River watershed (USGS 01490000) were analyzed to determine their possible use in the Wicomico River Headwaters watershed flow analysis.

To accurately estimate flows in a watershed, at least 10 years of continuous gauged flow data is recommended for developing a flow duration curve for a particular site. In addition, as part of the flow duration curve, flow frequency for the monitoring dates are needed to plot the bacteria monitoring data in a daily flow duration curve format. The dates of the bacteria monitoring surveys in the Wicomico River Headwaters sites range from October 10, 2002 to October 22, 2003. The flow gauges analyzed had less than 10 years of flow data and only one of them covers the same date period as the bacteria monitoring data. For these factor among others as explained in Table B-1, it was concluded that none of these gauges were suitable for use in this analysis.

Table B-1: USGS Gauges analyzed for use in the Wicomico River Headwaters Watershed Flow Analysis

USGS Gauge	Gauged flow data period	Description
01486500 Beaverdam Creek near Salisbury, MD	Oct 1, 2000 to Sep 30, 2003	Gauge located downstream of flow-controlled pond (Schumaker Pond). Only three years of available recent data.
01490000 Chicamacomico River near Salem, MD	Oct 1, 2002 to Sep 30, 2004	Similar watershed characteristics but only two years of available recent data.
01484981 North Fork Green Run near Whitesville, DE	June 1, 1994 to June 30, 2003	Channelized Stream, very different watershed characteristics. Data not available after June 30, 2003.
01484983 South Fork Green Run near Whitesville, DE	May 24, 1994 to June 30, 2003	Channelized Stream, very different watershed characteristics. Data not available after June 30, 2003.
01484985 Green Run near Careytown, MD	May 1, 1994 to Sep 30, 2002	Channelized Stream, very different watershed characteristics. Data not available after Sep 30, 2002.

After review of the above listed USGS gauges, a decision was made to estimate average flows in the Wicomico River Headwaters watershed using an alternative methodology. The conceptual model used in previous MDE non-tidal bacteria that was developed to better represent different hydrologic conditions will not be used for the reasons explained above. The TMDL analysis will be based on average flow conditions.

Typical methods for estimating flows at ungauged location include using regional regression equations or a drainage area ratio approach with a gauged basin. The drainage area ratio approach was discarded because, an appropriate flow gauge could not be established.

Previous regression studies for predicting flows in Maryland are by Dillow (1995), Rule (1999), Moglen et. al. (2002) and Versar (2004). All of these studies identify that the most statistically significant watershed characteristic for predicting flow is the watershed area. Results from Versar (2004) indicated, in general for the Coastal Plain Region, that the flow regression equations described more of the variability found in high flows than for low flows and a reasonably accurate description of average flows with mean flow R^2 value of 0.9794 and standard deviation of 0.0714.

Average flows were estimated in the five subwatersheds of the Wicomico River Headwaters using the flow regression equations from Versar's study: "Development of Regional Flow Duration Curves in Maryland, 2004". For comparison purposes, the flow duration curves from two of the above analyzed flow gauges data were plotted against the regression results for Coastal Plain Region gauges. Flow duration curves for the flow gauges and the regression equation results are presented in Figure B-1.

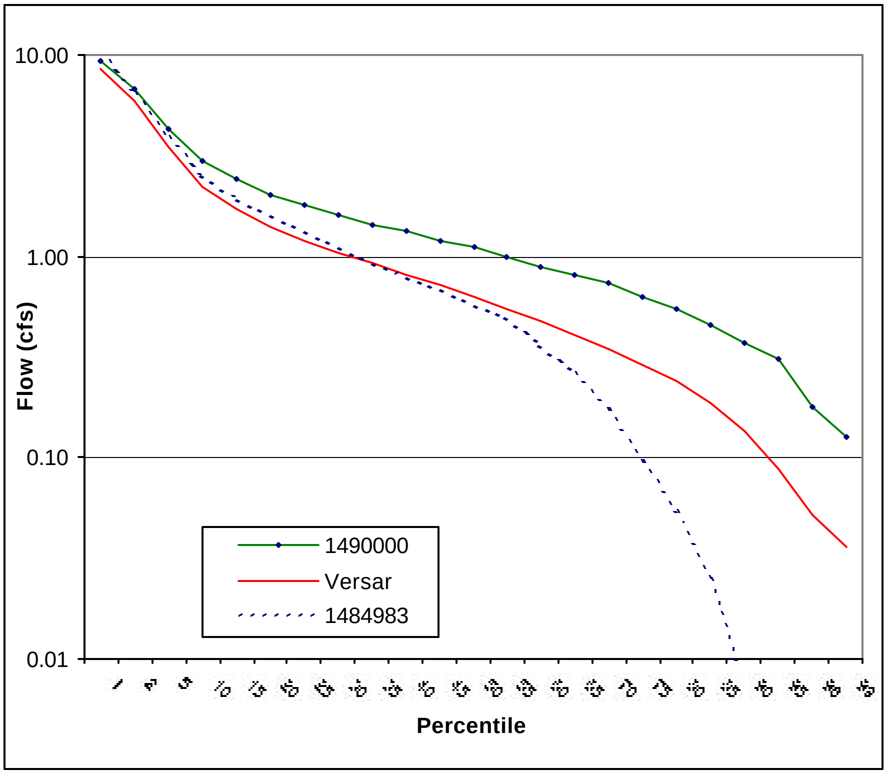


Figure B-1: USGS gauges and Regression Results at Station WIW0241 Flow Duration Curves

Results from Figure B-1 support that there is more variability during low flows when compared with high flows. Average flows are predicted with reasonable accuracy. Average flows were better predicted for the Pocomoke River Headwaters flow gauge than those predicted for the Chicamacomico River gauge. The regression results are a reasonable estimation for average flows in the Wicomico River Headwaters watershed.

An average mean flow for the five subwatersheds of the Wicomico River Headwaters was estimated using Versar's Coastal Plain Group regression equation and parameters as follows:

$$MeanFlow-(cfs)=10^{(\text{int}\,ercept+value\,\log_{10}(Area-inSq.Miles))} \qquad (1)$$

Table B-2: Mean Flow Parameters for Coastal Plain Group Regression Equation

Coastal Plain	Intercept		Variable: Area			Regression Equation	
	Value	SE	Value	SE	R^2	SE	R^2
Mean flow	-0.0194	0.05139	1.04044	0.03769	0.9794	0.0714	0.9794

Table B-3: Mean Flow Regression Equation Results at Monitoring Stations

Station	Area (acres)	Area (miles^2)	Mean Flow (cfs)	Unit Flow (cfs/miles^2)
NLO0003	6,020.5	9.4	9.7	1.047
LPR0028	2,272.0	3.6	3.6	1.007
WIW0241sub	8,382.3	13.1	13.9	1.061
BWB0010	3,803.3	5.9	6.1	1.028
MNC0003	4,136.1	6.5	6.7	1.031

REFERENCES FOR APPENDIX B

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

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

Rule, T. C. 1999.

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

Appendix C - Bacterial Source Tracking Report

Identifying Sources of Fecal Pollution in the Wicomico River Headwaters Watershed, Maryland

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

May 13, 2005

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 Wicomico River Headwaters 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 shipped to the SU BST lab.

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

Bacterial isolates were plated onto tryptic soy agar plates, each containing a different concentration of a given antibiotic. Plates were incubated overnight at 37^{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 table includes the antibiotics and concentrations used for isolates in the Wicomico River Headwaters Watershed analysis.

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

Antibiotic	Concentration (ug ml^{-1})
Amoxicillin	0.625
Cephalothin	10, 15, 30, 50
Chlortetracycline	60, 80, 100
Erythromycin	10, 15, 30, 50
Neomycin	40, 60, 80
Oxytetracycline	20, 40, 60, 80, 100
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, dog, horse, deer, raccoon, rabbit, fox, and goose. A library of patterns of *Enterococcus* isolate responses to the panel of antibiotics was analyzed using the statistical software CART® (Salford Systems, San Diego, CA). The library consisted of response patterns of 1662 *Enterococcus* isolates from the Wicomico Headwaters and Wicomico River Watersheds. The combination of watersheds from which isolates were obtained was chosen after examination of possible library combinations (Figure 1). Possible watersheds libraries were the Wicomico Headwaters (WIC), Wicomico River (WIS), and Nanticoke River (NAN). “All East” watersheds included WIC, WIS, and the NAN. The classification models in Figure C-1 show the sharp increase in percent unknown isolates for Wicomico Headwaters and Wicomico River Watersheds at a cutoff probability > 50%.

Enterococci isolate response patterns were also obtained from bacteria in water samples collected at the nine (9) monitoring stations in the Wicomico River Headwaters basin. Using statistical techniques, these patterns were then compared to those in the combined library to identify the probable source of each water isolate.

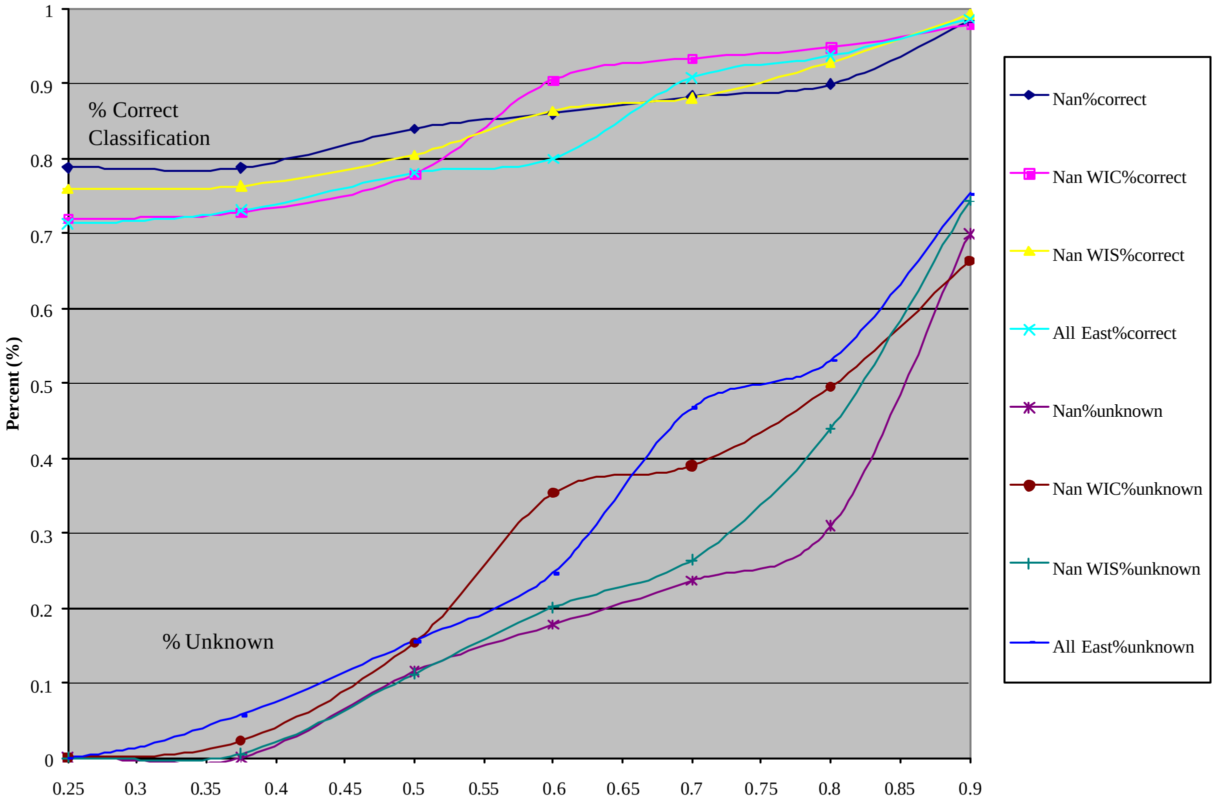


Figure C-1. Classification models for determination of composition of known-source library for identification of Nanticoke River water isolates.

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

---

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

the *stopping* criterion are referred to as *terminal* nodes.[2] The collection of *terminal* nodes defines the classification model. Each *terminal* node is associated with one source, the source that is most populous among the library isolates in the node. Each water sample isolate (*i.e.*, an isolate with an unknown source), based on its antibiotic resistance pattern, is identified with one specific *terminal* node and is assigned the source of the majority of library isolates in that *terminal* node.[3]

We imposed an additional requirement in our classification method for determining the sources of water sample isolates. We interpreted the proportion of the majority source among the library isolates in a *terminal* node as a probability. This proportion is an estimate of the probability that an isolate with unknown source, but with the same antibiotic resistance pattern as the library isolates in the *terminal* node, came from the source of the majority of the library isolates in the *terminal* node. If that probability was less than a specified *acceptable source identification probability*, we did not assign a source to the water sample isolates identified with that *terminal* node. Instead we assigned "Unknown" as the source for that node and "Unknown" for the source of all water sample isolates identified with that node. For the Wicomico River Headwaters 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 combined Wicomico River Headwaters and Wicomico River Watersheds known-source library were grouped into four categories: pet (specifically dog), human, livestock, and wildlife (Table C-2).

---

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

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

Table C-2. Category and number of isolates by watershed, and total numbers of isolates and of unique patterns in the combined known source library.

Category	Potential Sources	Wicomico Headwaters Isolates	Wicomico River Isolates	Total No. Isolates	Unique Patterns
Pet	Dogs	163	76	239	171
Human	Humans	266	183	449	370
Livestock	Horses, Cows Chickens	120	124	244	100
Wildlife	Geese, Raccoon, Deer, Foxes, Rabbits	435	295	730	395
Total		984	678	1662	1036

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 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 for the combined Wicomico Headwaters and Wicomico River libraries used to identify probable sources of Wicomico River Headwaters water isolates.

Cutoff Probability	Percent Unknown	(ARCC) Percent Correct
0.25	0.0%	71.0%
0.375	1.6%	71.6%
0.50	16.2%	76.1%
0.60	22.8%	78.4%
0.70	49.6%	92.3%
0.80	56.5%	95.6%
0.90	68.1%	98.4%

A cutoff probability of 0.50 (50%) was shown to yield an acceptable ARCC of 76%. The percent correct using no cutoff was 71%. Using a cutoff probability of 0.50 (50%), the combined library isolates that were not classified and thus were unknown were removed. The library containing the remaining isolates was then used to test the ability of the combined library to correctly predict the known-source isolates obtained from the Wicomico River Headwaters Watershed. The rates of correction classification for the four categories of sources in the Wicomico Headwaters known-source isolate library are shown in Table C-4 below. The combined library was then used in the statistical prediction of probable sources of bacteria in water samples collected from the Wicomico River Headwaters.

Table C-4. Actual source categories versus predicted categories of Wicomico River Headwaters known-source isolate library using the two-watershed combined 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	**115**	7	1	4	36	16	127	91%
Human	5	**211**	5	9	36	266	230	92%
Livestock	0	3	**84**	6	27	120	93	90%
Wildlife	50	25	82	**218**	60	435	385	58%
Sum	170	246	172	237	159	984	825	

[1] RCC = Number of correctly predicted species category / Total number classified (predicted). Example: One hundred seven (115) Pet correctly predicted / 127 total number classified for Pet = 115/127 = 91% RCC.

RESULTS: WATER

Wicomico River Headwaters Water Samples. Monthly monitoring from the Wicomico River Headwaters monitoring stations was the source of water samples. If weather conditions prevented sampling at a station, a second collection 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 1928 *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.

Table C- 5. Probable host sources of water isolates by category, number of isolates, percent isolates classified at cutoff probabilities of 50%

Category	No.	% Isolates Classified 50% Prob.
Pet	420	21.8%
Human	384	19.9%
Livestock	121	6.3%
Wildlife	801	41.5%
Unknown	202	10.5%
Missing Data	0	
Total w/ Complete Data	1928	
Total	1928	
% Classified		89.5%

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

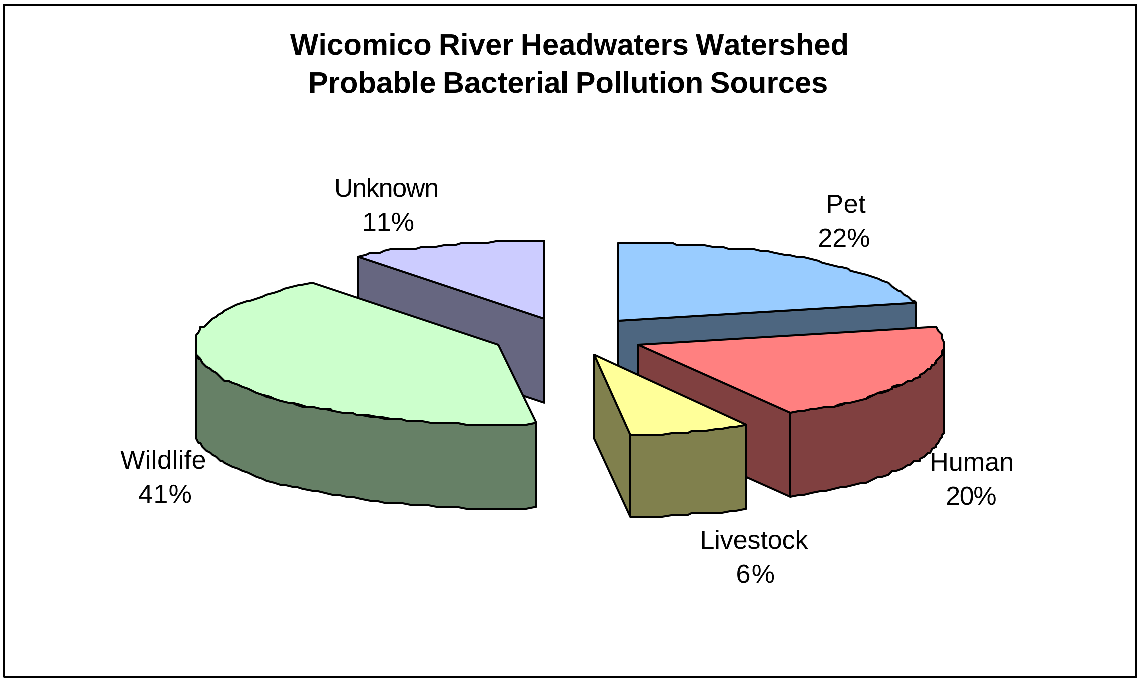


Figure C-2. Wicomico River Headwaters Watershed relative contributions by probable sources of *Enterococci* contamination.

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

Table C-6: Enterococci isolates from water collected and analyzed during the fall, winter, spring, and summer seasons for Wicomico River Headwaters monitoring stations.

Station	Fall	Winter	Spring	Summer	Total
WIW0216	73	48	47	72	240
WIW0241	47	24	71	139	281
LPR0020	67	4	61	95	227
MNC0003	50	48	72	93	263
WIW0226	49	32	69	73	223
WIW0231	71	48	48	92	259
LPR0024	19	33	7	81	140
NLO0003	23	25	11	74	133
LPR0028	24	20	51	67	162
Total	423	282	437	786	1928

Tables C-7 through C-10 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
LPR0020	**11/18/2002**	3	2	10	4	3
LPR0020	**12/04/2002**	4	3	3	11	2
LPR0020	**03/05/2003**	1	2	0	1	0
LPR0020	**04/24/2003**	2	2	0	6	3
LPR0020	**05/07/2003**	5	1	0	16	2
LPR0020	**06/04/2003**	1	0	0	23	0
LPR0020	**06/25/2003**	8	1	7	5	2
LPR0020	**07/09/2003**	3	7	0	13	1
LPR0020	**07/23/2003**	8	3	0	13	0

Station	Date	# Domestic Animals	# Human	# Livestock	# Wildlife	# Unknown
LPR0020	**08/06/2003**	1	2	0	21	0
LPR0020	**09/24/2003**	6	5	2	3	6
LPR0024	**11/18/2002**	0	2	0	15	2
LPR0024	**01/08/2003**	8	4	1	7	4
LPR0024	**03/05/2003**	4	2	1	1	1
LPR0024	**04/24/2003**	3	0	0	0	0
LPR0024	**05/07/2003**	0	0	0	1	2
LPR0024	**06/04/2003**	0	0	0	1	0
LPR0024	**06/25/2003**	8	2	1	9	3
LPR0024	**07/23/2003**	6	5	3	10	0
LPR0024	**08/06/2003**	2	1	0	18	1
LPR0024	**09/10/2003**	1	0	1	9	1
LPR0028	**11/18/2002**	4	3	2	9	6
LPR0028	**01/08/2003**	3	2	1	6	3
LPR0028	**03/05/2003**	0	0	1	3	1
LPR0028	**04/24/2003**	1	1	2	4	4
LPR0028	**05/07/2003**	3	11	2	7	1
LPR0028	**06/04/2003**	7	0	0	8	0
LPR0028	**06/25/2003**	3	15	0	0	3
LPR0028	**07/23/2003**	1	2	1	18	0
LPR0028	**08/06/2003**	6	0	0	18	0
MNC0003	**11/18/2002**	7	1	1	6	9
MNC0003	**12/04/2002**	7	9	0	7	1
MNC0003	**01/08/2003**	1	5	0	10	8
MNC0003	**03/05/2003**	7	0	3	11	3
MNC0003	**04/24/2003**	3	3	2	11	5
MNC0003	**05/07/2003**	3	14	0	7	0

Station	Date	# Domestic Animals	# Human	# Livestock	# Wildlife	# Unknown
MNC0003	**06/04/2003**	7	6	0	11	0
MNC0003	**06/25/2003**	7	9	0	7	0
MNC0003	**07/09/2003**	11	5	1	7	0
MNC0003	**08/06/2003**	13	5	0	5	0
MNC0003	**09/10/2003**	13	1	1	8	0
MNC0003	**10/08/2003**	0	0	0	2	0
NLO0003	**11/18/2002**	3	1	0	13	6
NLO0003	**01/08/2003**	0	2	0	12	6
NLO0003	**03/05/2003**	1	0	0	2	2
NLO0003	**05/07/2003**	0	0	0	7	0
NLO0003	**06/04/2003**	1	0	0	3	0
NLO0003	**06/25/2003**	0	12	2	8	2
NLO0003	**07/09/2003**	2	0	4	0	1
NLO0003	**07/23/2003**	3	14	0	5	0
NLO0003	**08/06/2003**	11	2	0	8	0
WIW0216	**11/18/2002**	6	7	2	5	3
WIW0216	**12/04/2002**	2	3	0	7	11
WIW0216	**01/08/2003**	3	10	0	9	2
WIW0216	**03/05/2003**	3	5	2	11	3
WIW0216	**04/24/2003**	4	1	0	0	2
WIW0216	**05/07/2003**	0	9	1	5	5
WIW0216	**06/04/2003**	6	4	3	7	0
WIW0216	**06/25/2003**	8	5	3	7	1
WIW0216	**07/09/2003**	8	12	0	4	0
WIW0216	**08/06/2003**	5	2	0	17	0
WIW0216	**09/24/2003**	10	1	0	11	0
WIW0216	**10/08/2003**	4	0	0	1	0

Station	Date	# Domestic Animals	# Human	# Livestock	# Wildlife	# Unknown
WIW0226	**11/18/2002**	3	2	4	11	4
WIW0226	**12/04/2002**	3	2	12	0	7
WIW0226	**01/08/2003**	4	1	0	4	8
WIW0226	**03/05/2003**	10	4	0	0	1
WIW0226	**04/24/2003**	1	9	3	8	3
WIW0226	**05/07/2003**	1	5	0	15	1
WIW0226	**06/04/2003**	7	9	1	6	0
WIW0226	**06/25/2003**	12	4	0	8	0
WIW0226	**07/09/2003**	0	0	1	0	0
WIW0226	**08/06/2003**	1	8	0	13	2
WIW0226	**09/10/2003**	8	1	2	13	0
WIW0226	**10/08/2003**	0	0	0	0	1
WIW0231	**11/18/2002**	2	1	1	13	6
WIW0231	**12/04/2002**	11	7	0	0	6
WIW0231	**01/08/2003**	7	4	1	5	7
WIW0231	**03/05/2003**	3	2	9	8	2
WIW0231	**04/24/2003**	11	4	0	9	0
WIW0231	**05/07/2003**	4	8	0	11	1
WIW0231	**06/25/2003**	0	20	0	0	0
WIW0231	**07/09/2003**	4	19	0	1	0
WIW0231	**08/06/2003**	7	2	0	15	0
WIW0231	**09/10/2003**	0	2	0	4	18
WIW0231	**10/08/2003**	2	8	8	5	1
WIW0241	**03/05/2003**	2	4	6	7	5
WIW0241	**04/24/2003**	5	9	2	7	1
WIW0241	**05/07/2003**	8	1	0	15	0
WIW0241	**06/04/2003**	3	0	0	20	0

Station	Date	# Domestic Animals	# Human	# Livestock	# Wildlife	# Unknown
WIW0241	**06/25/2003**	8	1	1	13	0
WIW0241	**07/09/2003**	4	3	0	16	0
WIW0241	**07/23/2003**	3	1	0	19	1
WIW0241	**08/06/2003**	4	7	0	13	0
WIW0241	**08/20/2003**	7	2	0	15	0
WIW0241	**08/27/2003**	3	0	0	18	0
WIW0241	**09/24/2003**	6	4	2	6	5
WIW0241	**10/08/2003**	5	4	5	9	1

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

Station	Date	% Domestic Animals	% Human	% Livestock	% Wildlife	% Unknown
LPR0020	**11/18/2002**	13.6	9.1	45.5	18.2	13.6
LPR0020	**12/04/2002**	17.4	13.0	13.0	47.8	8.7
LPR0020	**03/05/2003**	25.0	50.0	0.0	25.0	0.0
LPR0020	**04/24/2003**	15.4	15.4	0.0	46.2	23.1
LPR0020	**05/07/2003**	20.8	4.2	0.0	66.7	8.3
LPR0020	**06/04/2003**	4.2	0.0	0.0	95.8	0.0
LPR0020	**06/25/2003**	34.8	4.3	30.4	21.7	8.7
LPR0020	**07/09/2003**	12.5	29.2	0.0	54.2	4.2
LPR0020	**07/23/2003**	33.3	12.5	0.0	54.2	0.0
LPR0020	**08/06/2003**	4.2	8.3	0.0	87.5	0.0
LPR0020	**09/24/2003**	27.3	22.7	9.1	13.6	27.3
LPR0024	**11/18/2002**	0.0	10.5	0.0	78.9	10.5
LPR0024	**01/08/2003**	33.3	16.7	4.2	29.2	16.7
LPR0024	**03/05/2003**	44.4	22.2	11.1	11.1	11.1

Station	Date	% Domestic Animals	% Human	% Livestock	% Wildlife	% Unknown
LPR0024	**04/24/2003**	100.0	0.0	0.0	0.0	0.0
LPR0024	**05/07/2003**	0.0	0.0	0.0	33.3	66.7
LPR0024	**06/04/2003**	0.0	0.0	0.0	100.0	0.0
LPR0024	**06/25/2003**	34.8	8.7	4.3	39.1	13.0
LPR0024	**07/23/2003**	25.0	20.8	12.5	41.7	0.0
LPR0024	**08/06/2003**	9.1	4.5	0.0	81.8	4.5
LPR0024	**09/10/2003**	8.3	0.0	8.3	75.0	8.3
LPR0028	**11/18/2002**	16.7	12.5	8.3	37.5	25.0
LPR0028	**01/08/2003**	20.0	13.3	6.7	40.0	20.0
LPR0028	**03/05/2003**	0.0	0.0	20.0	60.0	20.0
LPR0028	**04/24/2003**	8.3	8.3	16.7	33.3	33.3
LPR0028	**05/07/2003**	12.5	45.8	8.3	29.2	4.2
LPR0028	**06/04/2003**	46.7	0.0	0.0	53.3	0.0
LPR0028	**06/25/2003**	14.3	71.4	0.0	0.0	14.3
LPR0028	**07/23/2003**	4.5	9.1	4.5	81.8	0.0
LPR0028	**08/06/2003**	25.0	0.0	0.0	75.0	0.0
MNC0003	**11/18/2002**	29.2	4.2	4.2	25.0	37.5
MNC0003	**12/04/2002**	29.2	37.5	0.0	29.2	4.2
MNC0003	**01/08/2003**	4.2	20.8	0.0	41.7	33.3
MNC0003	**03/05/2003**	29.2	0.0	12.5	45.8	12.5
MNC0003	**04/24/2003**	12.5	12.5	8.3	45.8	20.8
MNC0003	**05/07/2003**	12.5	58.3	0.0	29.2	0.0
MNC0003	**06/04/2003**	29.2	25.0	0.0	45.8	0.0
MNC0003	**06/25/2003**	30.4	39.1	0.0	30.4	0.0
MNC0003	**07/09/2003**	45.8	20.8	4.2	29.2	0.0
MNC0003	**08/06/2003**	56.5	21.7	0.0	21.7	0.0
MNC0003	**09/10/2003**	56.5	4.3	4.3	34.8	0.0

Station	Date	% Domestic Animals	% Human	% Livestock	% Wildlife	% Unknown
MNC0003	**10/08/2003**	0.0	0.0	0.0	100.0	0.0
NLO0003	**11/18/2002**	13.0	4.3	0.0	56.5	26.1
NLO0003	**01/08/2003**	0.0	10.0	0.0	60.0	30.0
NLO0003	**03/05/2003**	20.0	0.0	0.0	40.0	40.0
NLO0003	**05/07/2003**	0.0	0.0	0.0	100.0	0.0
NLO0003	**06/04/2003**	25.0	0.0	0.0	75.0	0.0
NLO0003	**06/25/2003**	0.0	50.0	8.3	33.3	8.3
NLO0003	**07/09/2003**	28.6	0.0	57.1	0.0	14.3
NLO0003	**07/23/2003**	13.6	63.6	0.0	22.7	0.0
NLO0003	**08/06/2003**	52.4	9.5	0.0	38.1	0.0
WIW0216	**11/18/2002**	26.1	30.4	8.7	21.7	13.0
WIW0216	**12/04/2002**	8.7	13.0	0.0	30.4	47.8
WIW0216	**01/08/2003**	12.5	41.7	0.0	37.5	8.3
WIW0216	**03/05/2003**	12.5	20.8	8.3	45.8	12.5
WIW0216	**04/24/2003**	57.1	14.3	0.0	0.0	28.6
WIW0216	**05/07/2003**	0.0	45.0	5.0	25.0	25.0
WIW0216	**06/04/2003**	30.0	20.0	15.0	35.0	0.0
WIW0216	**06/25/2003**	33.3	20.8	12.5	29.2	4.2
WIW0216	**07/09/2003**	33.3	50.0	0.0	16.7	0.0
WIW0216	**08/06/2003**	20.8	8.3	0.0	70.8	0.0
WIW0216	**09/24/2003**	45.5	4.5	0.0	50.0	0.0
WIW0216	**10/08/2003**	80.0	0.0	0.0	20.0	0.0
WIW0226	**11/18/2002**	12.5	8.3	16.7	45.8	16.7
WIW0226	**12/04/2002**	12.5	8.3	50.0	0.0	29.2
WIW0226	**01/08/2003**	23.5	5.9	0.0	23.5	47.1
WIW0226	**03/05/2003**	66.7	26.7	0.0	0.0	6.7
WIW0226	**04/24/2003**	4.2	37.5	12.5	33.3	12.5

Station	Date	% Domestic Animals	% Human	% Livestock	% Wildlife	% Unknown
WIW0226	**05/07/2003**	4.5	22.7	0.0	68.2	4.5
WIW0226	**06/04/2003**	30.4	39.1	4.3	26.1	0.0
WIW0226	**06/25/2003**	50.0	16.7	0.0	33.3	0.0
WIW0226	**07/09/2003**	0.0	0.0	100.0	0.0	0.0
WIW0226	**08/06/2003**	4.2	33.3	0.0	54.2	8.3
WIW0226	**09/10/2003**	33.3	4.2	8.3	54.2	0.0
WIW0226	**10/08/2003**	0.0	0.0	0.0	0.0	100.0
WIW0231	**11/18/2002**	8.7	4.3	4.3	56.5	26.1
WIW0231	**12/04/2002**	45.8	29.2	0.0	0.0	25.0
WIW0231	**01/08/2003**	29.2	16.7	4.2	20.8	29.2
WIW0231	**03/05/2003**	12.5	8.3	37.5	33.3	8.3
WIW0231	**04/24/2003**	45.8	16.7	0.0	37.5	0.0
WIW0231	**05/07/2003**	16.7	33.3	0.0	45.8	4.2
WIW0231	**06/25/2003**	0.0	100.0	0.0	0.0	0.0
WIW0231	**07/09/2003**	16.7	79.2	0.0	4.2	0.0
WIW0231	**08/06/2003**	29.2	8.3	0.0	62.5	0.0
WIW0231	**09/10/2003**	0.0	8.3	0.0	16.7	75.0
WIW0231	**10/08/2003**	8.3	33.3	33.3	20.8	4.2
WIW0241	**03/05/2003**	8.3	16.7	25.0	29.2	20.8
WIW0241	**04/24/2003**	20.8	37.5	8.3	29.2	4.2
WIW0241	**05/07/2003**	33.3	4.2	0.0	62.5	0.0
WIW0241	**06/04/2003**	13.0	0.0	0.0	87.0	0.0
WIW0241	**06/25/2003**	34.8	4.3	4.3	56.5	0.0
WIW0241	**07/09/2003**	17.4	13.0	0.0	69.6	0.0
WIW0241	**07/23/2003**	12.5	4.2	0.0	79.2	4.2
WIW0241	**08/06/2003**	16.7	29.2	0.0	54.2	0.0
WIW0241	**08/20/2003**	29.2	8.3	0.0	62.5	0.0

Station	Date	% Domestic Animals	% Human	% Livestock	% Wildlife	% Unknown
WIW0241	**08/27/2003**	14.3	0.0	0.0	85.7	0.0
WIW0241	**09/24/2003**	26.1	17.4	8.7	26.1	21.7
WIW0241	**10/08/2003**	20.8	16.7	20.8	37.5	4.2

Table C-9: Overall Percentage of Sources per Station

Station	% Domestic Animals	% Human	% Livestock	% Wildlife	% Unknown
LPR0020	17.9	14.5	9.7	48.9	8.9
LPR0024	15.4	19.1	7.4	45.2	13
LPR0028	21.1	8.8	3.5	53.5	13.4
MNC0003	15.1	15	6.6	50.3	13.1
NLO0003	30.4	21.6	3	34	11.2
WIW0216	28.8	22.9	4.4	32.9	11.2
WIW0226	20.5	18.5	16.3	31.5	13.4
WIW0231	22.1	25.9	4.6	29.9	17.7
WIW0241	20.4	13.3	5.1	57	4.3

SUMMARY

The use of ARA was successful for identification of probable bacterial sources in the Wicomico River Headwaters Watershed as evidenced by the RCCs in the library (a range of from a usable 58% for wildlife to highs of 90%, 91, and 92% for livestock, pet, and human, respectively. When water isolates were compared to the library and probable sources predicted, 90% the water isolates were classified by statistical analysis. The largest category of probable sources in the watershed was wildlife (41%). The remaining probable sources included pets (22%), human (20%) and livestock (6%).

REFERENCES FOR APPENDIX C

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

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

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

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

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

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

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

ACKNOWLEDGEMENTS

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