Total Maximum Daily Loads of Fecal Coliform for the Restricted Shellfish Harvesting Area in the Wye River Basin in Queen Anne's and Talbot Counties, Maryland

FINAL

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List of Abbreviations

ARA	Antibiotic Resistance Analysis
BMP	Best Management Practice
BST	Bacteria Source Tracking
CAFO	Confined Animal Feeding Operations
cfs	Cubic Feet per Second
CFR	Code of Federal Regulations
COMAR	Code of Maryland Regulations
CSO	Combined Sewer Overflow
CWA	Clean Water Act
CWP	Center for Watershed Protection
EPA	Environmental Protection Agency
FA	Future Allocation
FDA	U.S. Food and Drug Administration
GIS	Geographic Information System
HEM-3D	VIMS Hydrodynamic Eutrophication Model in 3 Dimensions
km	Kilometer
LA	Load Allocation
M_2	Lunar semi-diurnal tidal constituent
MACS	Maryland Agricultural Cost Share Program
MAR	Multiple-antibiotic-resistance
MASS	Maryland Agricultural Statistics Service
MDE	Maryland Department of the Environment
MDP	Maryland Department of Planning
ml	Milliliter(s)
MOS	Margin of Safety
MPN	Most Probable Number
MRLC	Multi-Resolution Land Cover
MSSCC	Maryland State's Soil Conservation Committee
NOAA	National Oceanic and Atmospheric Administration
NPDES	National Pollutant Discharge Elimination System
NSSP	National Shellfish Sanitation Program
TMDL	Total Maximum Daily Load
USDA	U.S. Department of Agriculture
USGS	United States Geological Survey
VIMS	Virginia Institute of Marine Science
WLA	Wasteload Allocation
WQIA	Water Quality Improvement Act
WQLS	Water Quality Limited Segment
WWTP	Waste Water Treatment Plant

EXECUTIVE SUMMARY

Section 303(d) of the federal Clean Water Act (CWA) and the U.S. Environmental Protection Agency's (EPA) implementing regulations direct each state to identify and list waters, known as water quality limited segments (WQLSs), in which current required controls of a specified substance are inadequate to achieve water quality standards. For each WQLS, the State is to either establish a Total Maximum Daily Load (TMDL) of the specified substance that the waterbody can receive without violating water quality standards, or demonstrate that water quality standards are being met.

The Wye River (basin number 02130503) was first identified on the 1996 303(d) List submitted to U.S. Environmental Protection Agency (EPA) by the Maryland Department of the Environment (MDE). The designated uses in Wye River were impaired by sediments, nutrients, and fecal coliform in tidal portions, with listings of biological impacts in the non-tidal portions added in 2002. On the State's 2004 303(d) List, the fecal coliform listing was clarified by the identification of the Wye River as the specific area of impairment. This document, upon EPA approval, establishes a TMDL for fecal coliform for the Wye River. The nutrient, biological, and sediment impairments within the Wye River basin will be addressed at a future date.

An inverse three-dimensional model was used to estimate current fecal coliform loads and to establish allowable loads for the restricted shellfish harvesting area in the Wye River watershed. The inverse model incorporates influences of freshwater discharge, tidal and density-induced transport, and fecal coliform decay, thereby representing the fate and transport of fecal coliform in the Wye River and its corresponding restricted shellfish harvesting area. The potential sources (human, livestock, pets, and wildlife) are identified by analysis of the Bacteria Source Tracking (BST) collected in the Wye River over a one-year period.

The allowable loads for the restricted shellfish harvesting area were then computed using both the median concentration water quality criterion for shellfish harvesting use of 14 Most Probable Number (MPN)/100ml, and the 90th percentile criterion concentration of 49 MPN/100ml (COMAR 26.08.02.03-3.C). An implicit Margin of Safety (MOS) was incorporated into the analysis to account for uncertainty. The TMDLs developed for the restricted shellfish harvesting area of the Wye River watershed for fecal coliform median load and 90th percentile load are as follows:

Wye River:
The median fecal coliform TMDL = 1.588×10^{10} counts per day
The 90th percentile fecal coliform TMDL = 7.389×10^{10} counts per day

The goal of load allocation is to determine the estimated loads for sources in the watershed while ensuring that the water quality standard can be attained. For the Wye River area, the 90th percentile criterion requires the greatest reduction – about 83% within the watershed. Therefore, the load reduction scenario is developed based on the 90th percentile TMDL, and will result in the load reductions that allow attainment of the water quality standard. Reductions from current baseline conditions are estimated and presented in this report.

Once EPA has approved these TMDLs, MDE will begin an iterative process of implementation, focusing first on those sources that have the greatest impact on water quality while giving consideration to the relative ease of implementation and cost. The source contributions estimated from the BST data results may be used as a tool to target and prioritize initial implementation efforts. Continued monitoring will be undertaken by MDE's Shellfish Certification Division and will be used to assess the effectiveness of the Department's implementation efforts on an ongoing basis.

1.0 INTRODUCTION

Section 303(d)(1)(C) of the federal Clean Water Act (CWA) and the U.S. Environmental Protection Agency's (EPA) implementing regulations direct each state to develop a 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 including a protective margin of safety (MOS) to account for scientific uncertainty. A TMDL reflects the total pollutant loading of the impairing substance a waterbody can receive and still meet water quality standards.

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

Fecal coliform bacteria are found in the intestinal tract of humans and other warm-blooded animals. Fecal coliform may occur in surface waters from point and nonpoint sources. Few fecal coliform are pathogenic; however, the presence of elevated levels of fecal coliform in shellfish waters may indicate recent sources of pollution. Some common waterborne diseases associated with the consumption of raw clams and oysters harvested from polluted water include viral and bacterial gastroenteritis and hepatitis A.

Fecal coliform is an indicator organism used in water quality monitoring in shellfish waters to indicate fresh sources of pollution from human and other animal wastes. When the water quality standard for fecal coliform in shellfish waters is exceeded, waters are closed to shellfish harvesting to protect human health due to the potential risk from consuming raw molluscan shellfish from sewage contaminated waters. The U.S. Food and Drug Administration (FDA), rather than EPA, is responsible for food safety. Water quality criteria for shellfish waters are established under the National Shellfish Sanitation Program (NSSP), a cooperative program that involves states, industry, academic and federal agencies with oversight by FDA. The NSSP continues to use fecal coliform as the indicator organism to assess shellfish harvesting waters. The water quality goal of this TMDL is to reduce high fecal coliform concentrations to levels whereby the designated uses for this restricted shellfish harvesting area will be met.

In both the 1996 and 1998 Maryland 303(d) Lists of Impaired Waterbodies, many 8-digit watersheds were identified as being impaired by fecal coliform, since these waterbodies are closed to shellfish harvesting. Shellfish waters are continuously monitored, and openings and closings occur routinely. The 2004 303(d) List indicates currently restricted shellfish harvesting areas that require TMDLs within an 8-digit watershed.

The Wye River (basin number 02130503) was first identified on the 1996 303(d) List submitted to EPA by the Maryland Department of the Environment (MDE). The designated uses in the Wye River were impaired by sediments, nutrients, and fecal coliform in tidal portions, with listings of biological impacts in the non-tidal portions added in 2002. On the 2004 303(d) List, the fecal coliform listing was clarified by the identification of the Wye River as the specific area of impairment. This document, upon EPA approval, establishes a TMDL for fecal coliform for the Wye River. The basis of the shellfish harvesting area closure was current fecal coliform data from MDE's shellfish monitoring program showing values that exceeded water quality criteria, and therefore resulted in the areas being classified as "restricted" or closed to direct harvest. The criteria include both a median and a 90th percentile. The nutrient, biological, and sediment impairments within the Wye River basin will be addressed at a future date.

2.0 SETTING AND WATER QUALITY DESCRIPTION

2.1 General Setting

The Wye River is located on Maryland's Eastern Shore in Queen Anne's and Talbot Counties, as shown in Figure 2.1.1. The Wye River is composed of Wye East River, Wye Narrows and Wye River mainstem, and has a length of approximately 15 km both from south to north and from southwest to northeast. Its width ranges from 300 to 600 m upstream and approximately 1 km at its mouth, where it flows into Eastern Bay. The Wye River restricted shellfish harvesting area has a drainage area of 50,534.9 acres (204.51 km^2).

The soils in the Wye River watershed range from moderately well-drained silty soils that have a firm silty clay loam to plastic clay subsoil to well-drained and moderately well-drained soils that consist of a sandy clay loam subsoil (U.S. Department of the Agriculture (USDA), 1966). The dominant tide in this region is the lunar semi-diurnal (M_2) tide, with a tidal range of 0.40 m in the restricted portion of the Wye River with a tidal period of 12.42 hours (National Oceanic and Atmospheric Administration (NOAA), 2004). Please refer to Table 2.1.1 for the mean volume and mean water depth of this restricted shellfish harvesting area.

Table 2.1.1: Physical Characteristics of the Wye River Restricted Shellfish Harvesting Area

Restricted Shellfish Harvesting Area	Mean Water Volume in m^3	Mean Water Depth in m
Wye River	24,585,511.3	1.62

The 2000 Maryland Department of Planning (MDP) land use/land cover data show that the watershed can be characterized as rural for the Wye River, with 64% of the area being cropland and another 24% being forest. The land use information for the restricted shellfish harvesting area in the Wye River Basin is shown in Table 2.1.2 and Figure 2.1.2. Residential urban land use identified in Table 2.1.2 includes low-density residential, medium-density residential, and high-density residential. Non-residential urban land use in this table includes commercial, industrial, institutional, extractive, and open urban land.

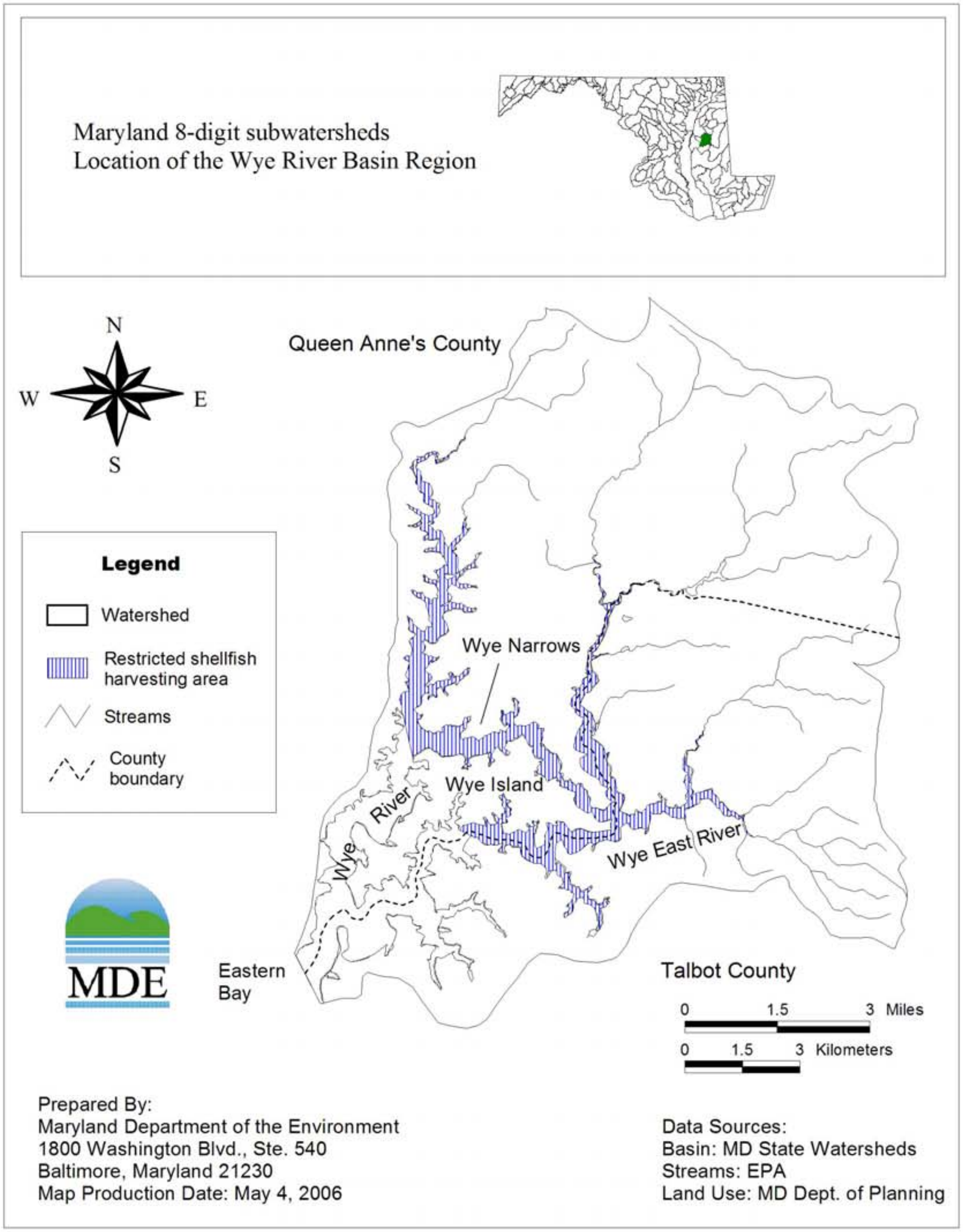

Figure 2.1.1: Location Map of the Wye River Basin

Table 2.1.2: Land Use Percentage Distribution for Wye River

Land Type	Acreage	Percentage
Residential urban	2,866.7	5.67
Non-Residential urban	1,061.9	2.10
Cropland	32,383.2	64.09
Pasture	1,199.6	2.37
Feedlot	266.4	0.53
Forest	12,309.2	24.36
Water	98.1	0.19
Wetlands	302.1	0.60
Barren	25.7	0.05
Transportation	22.0	0.04
Totals	**50,534.9**	**100.00**

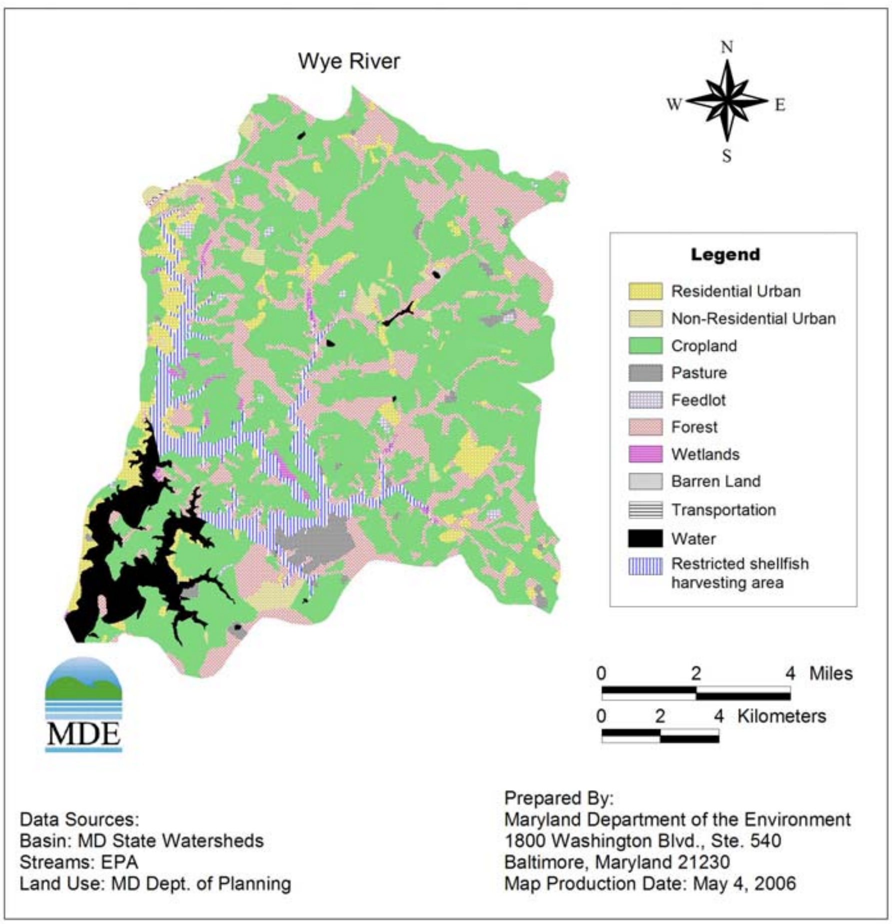

Figure 2.1.2: Land Use in the Wye River Basin

2.2 Water Quality Characterization

MDE's Shellfish Certification Program is responsible for classifying shellfish harvesting waters to ensure oysters and clams are safe for human consumption. As discussed above, MDE adheres to the requirements of the National Shellfish Sanitation Program (NSSP), with oversight by FDA. MDE conducts shoreline surveys and collects routine bacteria water quality samples in the shellfish waters of Maryland to assure that Maryland's shellfish waters are properly classified.

MDE's Shellfish Certification Program monitors shellfish waters throughout Maryland. There are six shellfish monitoring stations in the restricted shellfish harvesting area addressed in this report. The station identification and observations recorded during the period of June 2000 – June 2005, except Station 08-02-026A, are provided in Table 2.2.1 and Figure 2.2.1 through Figure 2.2.7. For Station 08-02-026A, data for the period of 2002 to 2005 were recorded. Tabulations of observed fecal coliform values at the monitoring stations included in this report are provided in Appendix D.

Table 2.2.1: Locations of the Shellfish Monitoring Stations in the restricted shellfish harvesting area of Wye River

Station Location	Shellfish Monitoring Station	Obs. Period	Total Obs.	LATITUDE Deg-min-sec	LONGITUDE Deg-min-sec
Wye River	08-02-013A	2000-2005	90	38 54 33.0	76 10 12.0
Wye River	08-02-014	2000-2005	90	38 54 19.0	76 09 36.0
Wye River	08-02-023	2000-2005	91	38 52 56.0	76 08 51.0
Wye River	08-02-026	2000-2005	91	38 52 50.0	76 07 07.0
Wye River	08-02-026A	2002-2005	67	38 53 08.7	76 06 09.7
Wye River	08-02-304	2000-2005	91	38 54 27.4	76 07 55.1

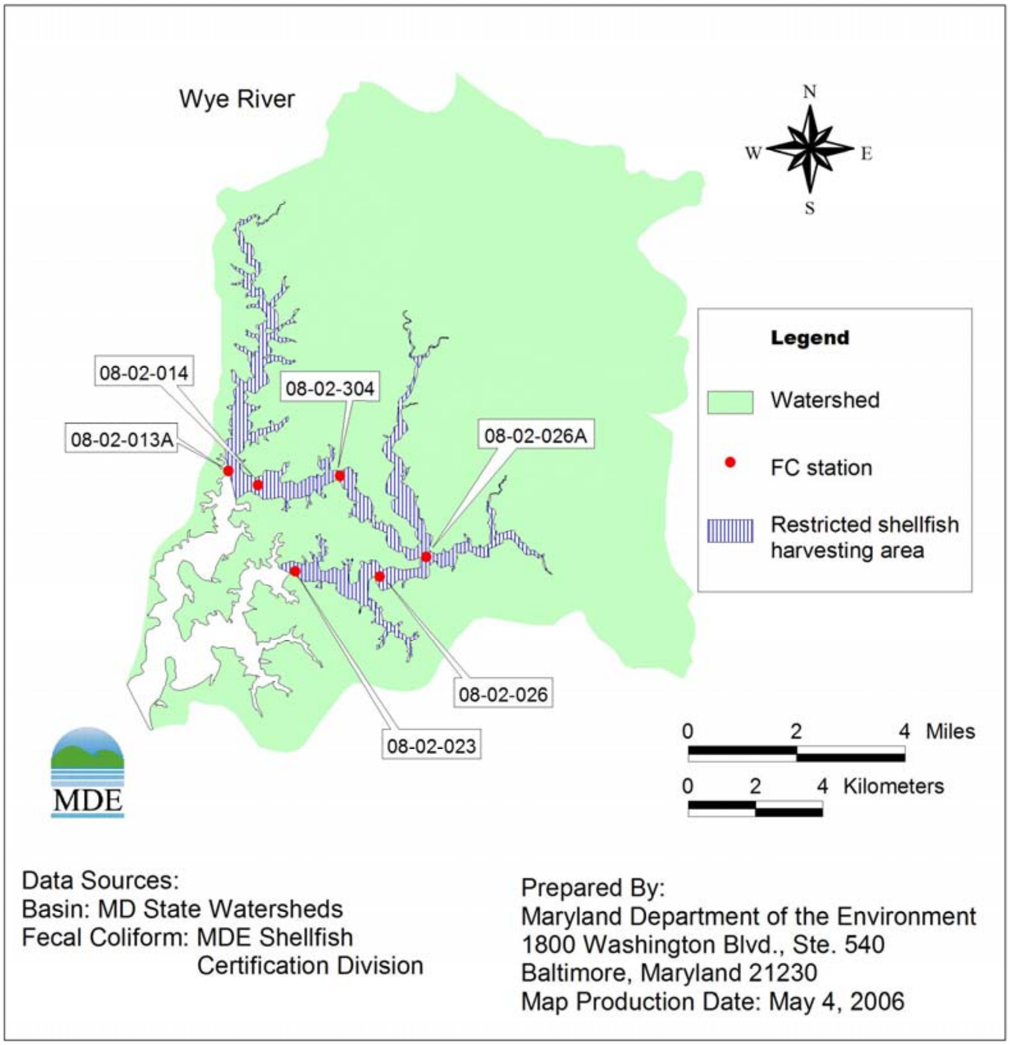

Figure 2.2.1: Shellfish Monitoring Stations in Wye River

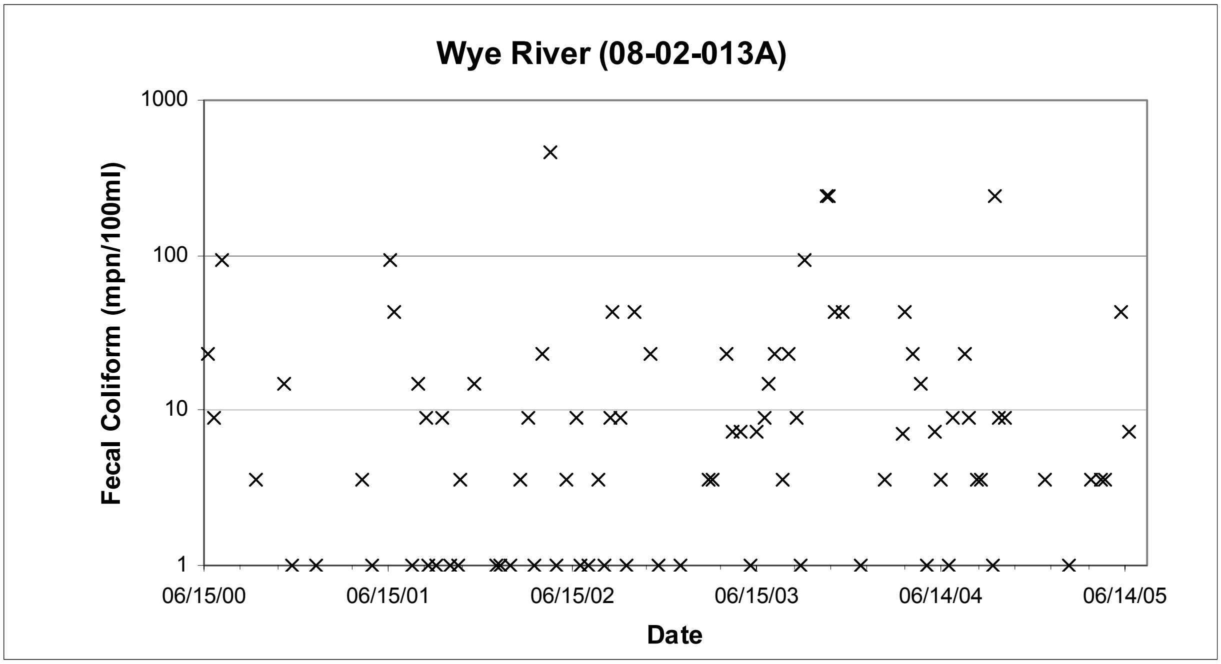

Figure 2.2.2: Observed Fecal Coliform Concentrations at Station 08-02-013A

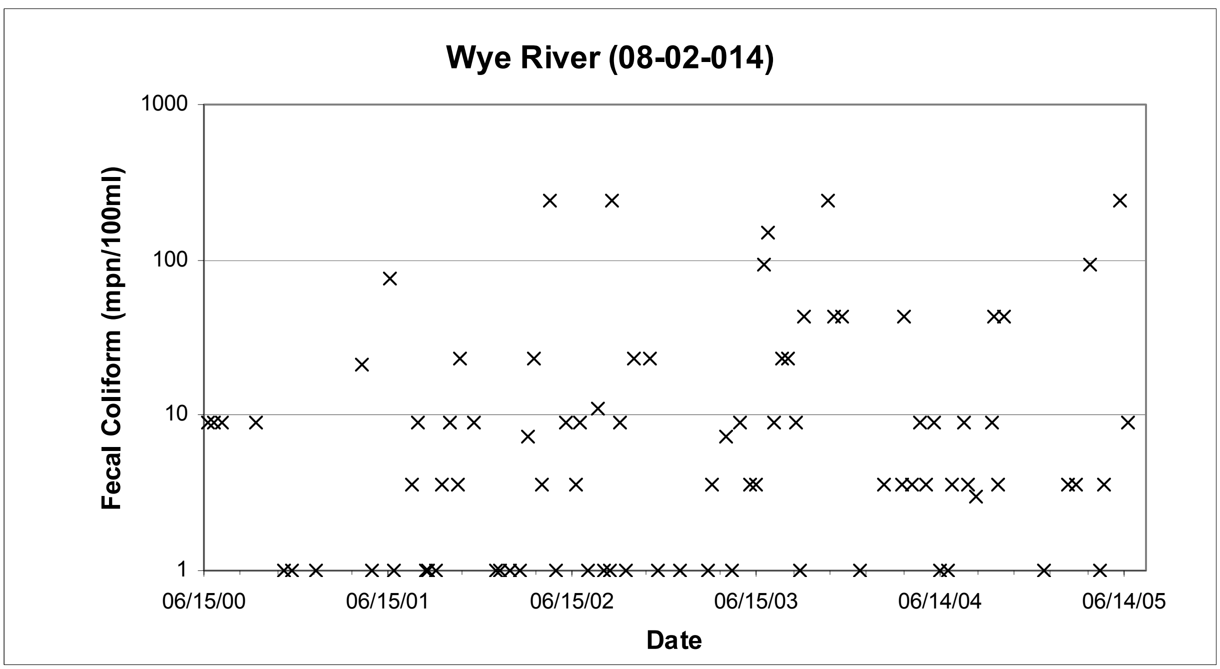

Figure 2.2.3: Observed Fecal Coliform Concentrations at Station 08-02-014

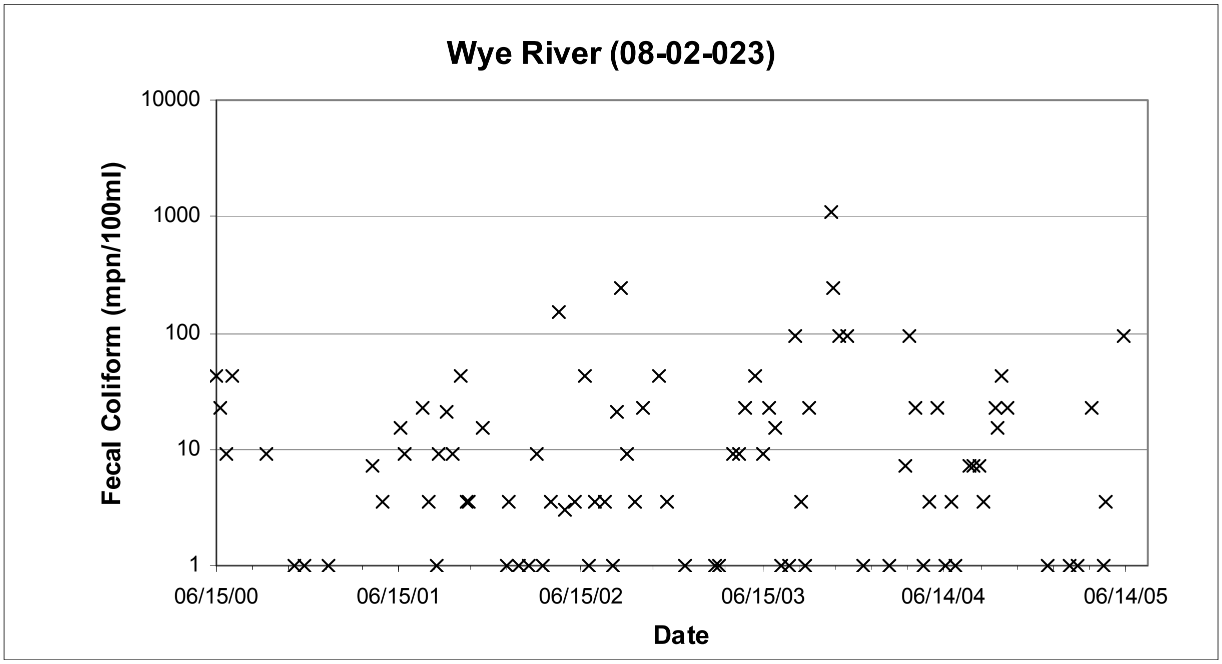

Figure 2.2.4: Observed Fecal Coliform Concentrations at Station 08-02-023

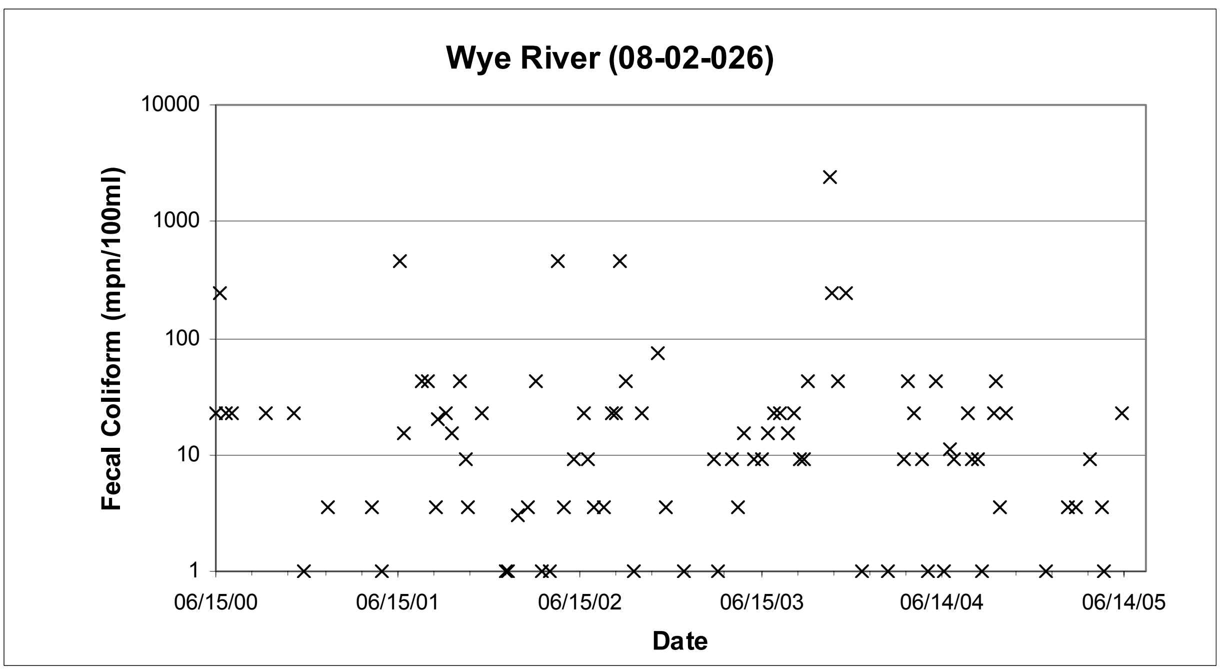

Figure 2.2.5: Observed Fecal Coliform Concentrations at Station 08-02-026

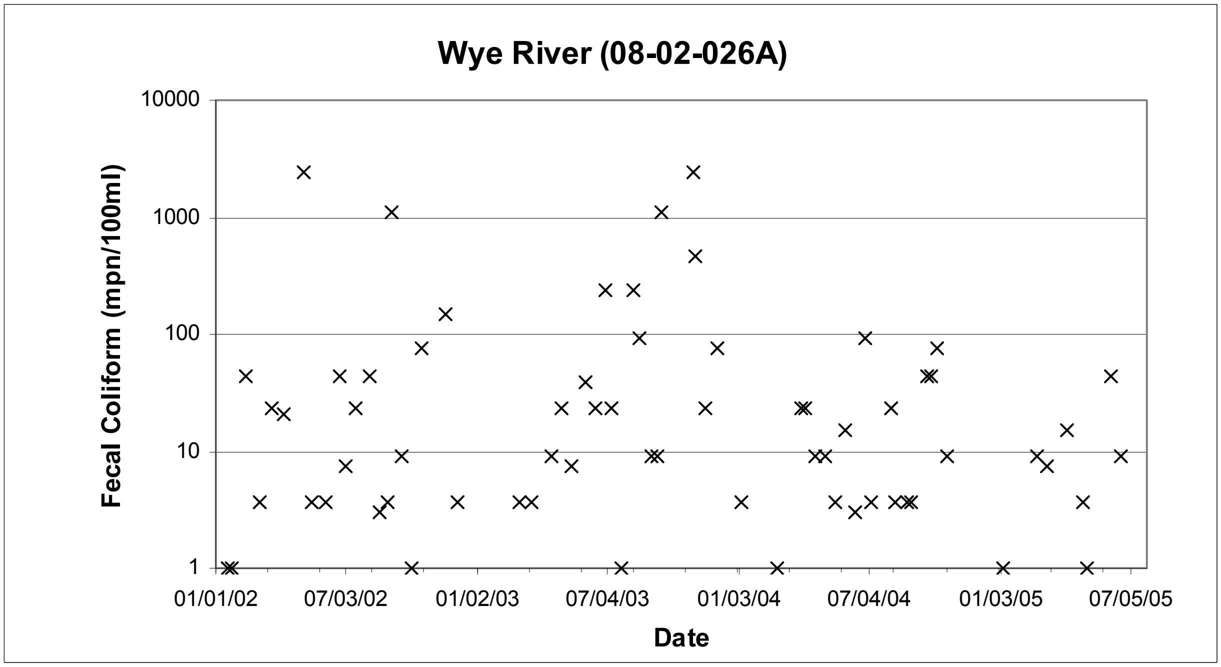

Figure 2.2.6: Observed Fecal Coliform Concentrations at Station 08-02-026A

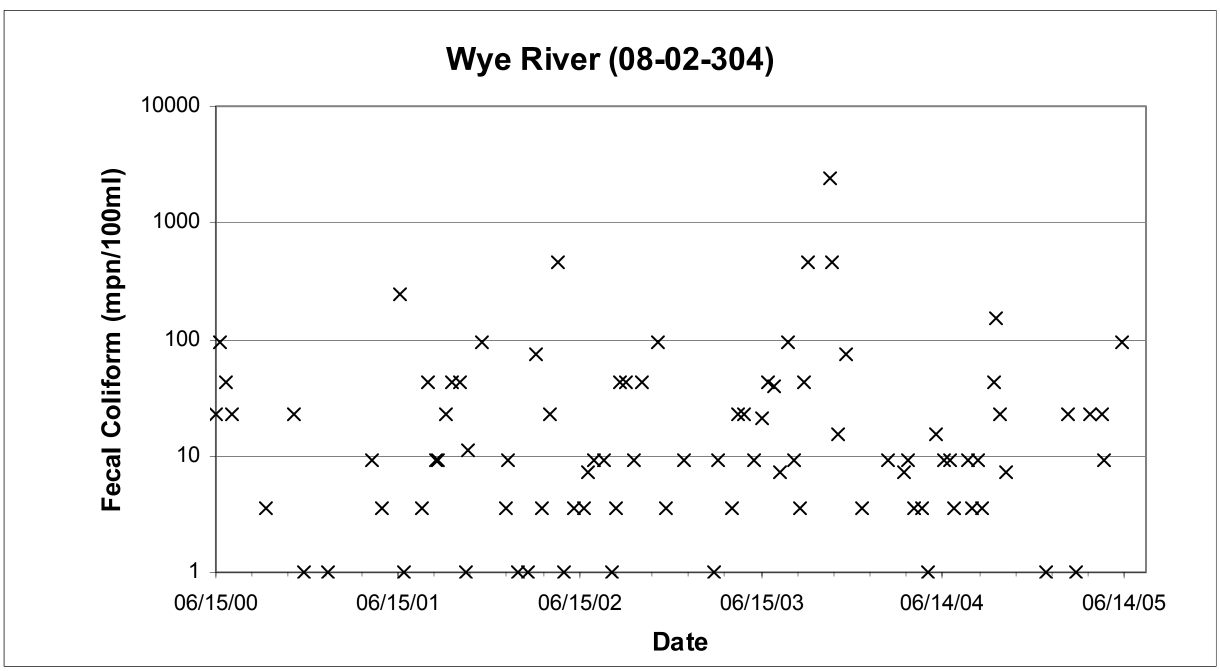

Figure 2.2.7: Observed Fecal Coliform Concentrations at Station 08-02-304

2.3 Water Quality Impairment

The fecal coliform impairment addressed in this analysis was determined with reference to Maryland's Classification of Use II Waters- Support of Estuarine and Marine Aquatic Life and Shellfish Harvesting in the Code of Maryland Regulations (COMAR), Surface Water Quality Criteria 26.08.02.03-3.C(2), which states:

> 2) Classification of Use II Waters for Harvesting.
>
> (a) Approved classification means that the median fecal coliform MPN of at least 30 water sample results taken over a 3-year period to incorporate inter-annual variability does not exceed 14 per 100 milliliters; and:
>
> (i) In areas affected by point source discharges, not more than 10 percent of the samples exceed an MPN of 43 per 100 milliliters for a five tube decimal dilution test or 49 MPN per 100 milliliters for a three tube decimal dilution test; or
>
> (ii) In other areas, the 90th percentile of water sample results does not exceed an MPN of 43 per 100 milliliters for a five tube decimal dilution test or 49 MPN per 100 milliliters for a three tube decimal dilution test.

MDE updated and promulgated shellfish water quality criteria for shellfish waters in June 2004. Although bacteriological criteria for shellfish harvesting waters were unchanged, the update included classification criteria required under the NSSP that were not included previously in COMAR. In 2005, MDE revised the use designations in COMAR as part of the Chesapeake Bay Program revision to reflect living resources-based habitat needs, but did not change the fecal coliform criteria for shellfish harvesting waters or shellfish harvesting use designations.

For this analysis, MDE used routine monitoring data collected during a five-year period between June 2000 and June 2005. Most shellfish harvesting areas have been monitored routinely since before 1950 and, due to an emerging oyster aquaculture industry, there are a few shellfish harvesting areas that have less than five years worth of data. For the purpose of classifying shellfish harvesting areas, a minimum of 30 samples is required. For TMDL development, if fewer than 30 samples are available, all of the most recent data will be used to estimate current loads, and the assimilative capacity will be based on the approved classification requirements of a median of 14 MPN/100 ml and a 90^{th} percentile of less than 49 MPN/100 ml.

In 1996, the Wye River was included on the State's 303(d) List as impaired by fecal coliform. The water quality impairment in the Wye River was assessed as not meeting the 90^{th} percentile at five monitoring stations (note that Maryland uses the 3-tube decimal dilution test for fecal coliform bacteria). Descriptive statistics of the monitoring data and the requirements for the approved classification are shown in Table 2.3.1.

Table 2.3.1: Wye River Fecal Coliform Statistics (data from 2000-2005)

Area Name	Station	Median		90th Percentile	
		Monitoring Data	Criterion	Monitoring Data	Criterion
		MPN/100ml	MPN/100ml	MPN/100ml	MPN/100ml
Wye River	08-02-013A	7.25	14	48.38	49
Wye River	08-02-014	3.60	14	54.63	49
Wye River	08-02-023	7.30	14	57.92	49
Wye River	08-02-026	9.10	14	89.75	49
Wye River	08-02-026A	9.10	14	169.34	49
Wye River	08-02-304	9.10	14	94.91	49

2.4 Source Assessment

<u>**Nonpoint Source Assessment**</u>

Nonpoint sources of fecal coliform do not have one discharge point but occur over the entire length of a stream or waterbody. There are many types of nonpoint sources in watersheds discharging to the restricted shellfish harvesting areas. The possible introductions of fecal coliform to the land surface are through 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 coliform over the land surface and is introduced into surface waters. The deposition of non-human fecal coliform directly to the restricted shellfish waters may occur 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 as well as through pollution from recreational vessel discharges. The potential transport of fecal coliform from land surfaces to restricted shellfish harvesting waters is dictated by the hydrology, soil type, land use, and topography of the watershed.

In order to determine the sources of fecal coliform contribution and reduction needed to achieve water quality criteria and to allocate fecal coliform load among these sources, it is necessary to identify all existing sources. MDE conducted sampling over a one-year period in the Wye River watershed using the Bacteria Source Tracking (BST) method to identify sources of fecal coliform. The nonpoint source assessment was conducted by analyzing BST results to quantify source loadings from humans, livestock, pets, and wildlife.

In the Wye River basin, wildlife contributions, both mammalian and avian, are considered natural conditions and may represent a background level of bacterial loading. Livestock contributions, such as those from mammalian and avian livestock, mainly result from surface runoff. The watershed is predominately cropland and forest. According to land use information, the wildlife and livestock could be the dominant source. Pet contributions usually occur through runoff from streets and land. Human sources mainly result from failure of septic systems. Figure 2.1.2 shows the land use categories and Figure 2.4.1 shows the septic system distribution in the watershed. Most of septic systems are distributed along the eastern side of the Wye River and the upper watershed of the Wye River. Based on the analysis of BST data, wildlife is the predominant bacteria source followed by livestock, pet, and human sources. Table 2.4.1 summarizes the source distribution based on BST data analysis. Detailed results of BST analysis are presented in Appendix B.

Table 2.4.1: Source Distribution Based on BST Data Analysis

Human	Livestock	Wildlife	Pets
11.84 %	23.62 %	60.94 %	3.60 %

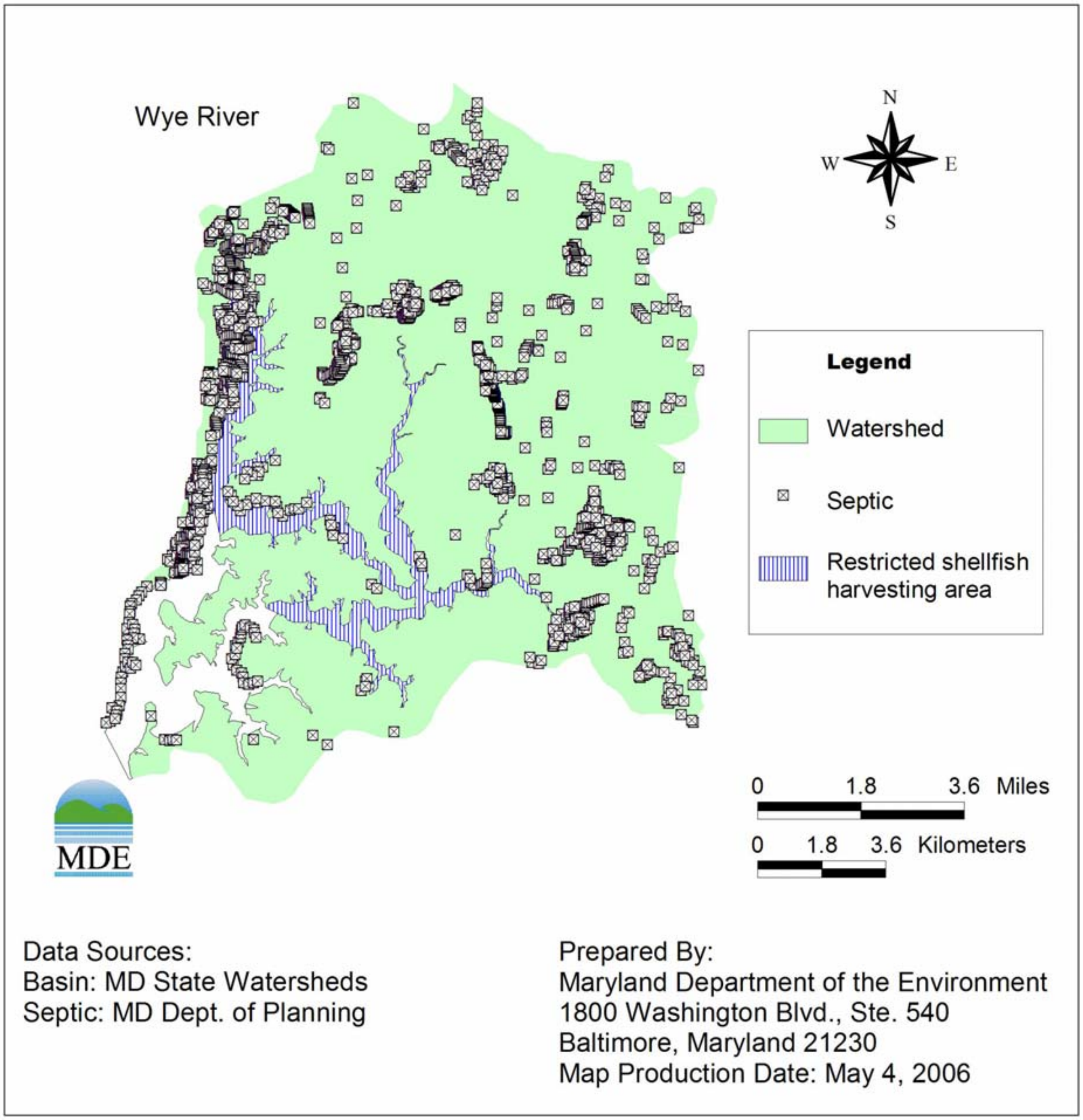

Figure 2.4.1: Distribution of Septic Systems in the Wye River Basin

Point Source Assessment

There are three point source facilities in the reported restricted shellfish harvesting area that have National Pollution Discharge Elimination System (NPDES) permit numbers: MD0000043 (SEW Friel, a vegetable cannery), MD0065170 (Wye Institute, aquarium overflow), and MD0024384 (Chesapeake College). Of these three point sources, only Chesapeake College has a permit regulating the discharge of fecal coliform directly to the Wye River. Its permit specifies limitations of 14 MPN/100ml monthly median fecal coliform concentration with a flow of 0.015

million gallons per day (MGD). The total fecal coliform discharged from this point source is 7.949×10^6 counts per day. The allocation of the permitted load from this point source facility will be addressed in Section 4.8.

3.0 TARGETED WATER QUALITY GOAL

The overall objective of the fecal coliform TMDLs in this document is to establish the maximum loading allowed to ensure attainment of water quality standards in the restricted shellfish harvesting waters in the Wye River. These standards are described fully in Section 2.3, Water Quality Impairment.

4.0 TOTAL MAXIMUM DAILY LOADS AND LOAD ALLOCATION

4.1 Overview

This section documents detailed fecal coliform TMDLs and load allocation development for the restricted shellfish harvesting waters in the Wye River watershed. The required load reduction was determined based on data from June 2000 to June 2005. The TMDLs are presented as counts/day. The second section describes the analysis framework for simulating fecal coliform concentration in restricted shellfish harvesting waters in the Wye River. The third section addresses critical conditions and seasonality. The fourth section presents the TMDL calculations. The fifth section discusses TMDL loading caps. The sixth section presents the load allocations. The margin of safety is discussed in Section 4.7. Finally, the TMDL equation is summarized in Section 4.8.

A TMDL is the total amount of a pollutant that can be assimilated by the receiving water while still achieving water quality criteria, in this case Maryland's water quality criteria for shellfish harvesting waters. A TMDL may be expressed as a "mass per unit time, toxicity, or other appropriate measure" (40 Code of Federal Regulations (CFR) 130.2(i)). It is also important to note that the TMDLs presented herein are not literal daily limits. These loads are based on an averaging period that is defined by the specific water quality criteria for shellfish harvesting waters (*i.e.*, at least 30 samples). The averaging period used for development of these TMDLs requires at least 30 samples and uses a five-year window of data to identify current baseline conditions.

A TMDL is comprised of the sum of individual wasteload allocations (WLAs) for point sources, load allocations (LAs) for nonpoint sources, incorporating natural background levels. The TMDL must include a margin of safety (MOS), either implicitly or explicitly, that accounts for the uncertainty in the relationship between pollutant loads and the quality of the receiving waterbody, and in the scientific and technical understanding of water quality in natural systems. In addition, the TMDL may include a future allocation (FA) when necessary. Conceptually, this definition is denoted by the equation:

$$\text{TMDL} = \text{WLAs} + \text{LAs} + \text{MOS} + (\text{FA, where applicable})$$

4.2 Analysis Framework

In general, tidal waters are exchanged through their connecting boundaries. The tide and amount of freshwater discharged into the restricted shellfish harvesting area are the dominant forces that influence the transport of fecal coliform. The Wye River is a looped tidal river that branches into the Wye East and Wye Rivers approximately 2 miles from the mouth. The Wye East River connects to Wye Narrows near Wye Island. Wye Narrows connects to the Wye River along its eastern side. The current distribution in the system varies as tidal and freshwater discharges change. In order to simulate the transport processes in the Wye River accurately, the 3-dimensional hydrodynamic and eutrophication model (HEM-3D) has been used for this study. The HEM-3D model is a general 3D model for environmental studies. The model simulates density and topographically induced circulation as well as tidal and wind-driven flows, and spatial and temporal distributions of salinity, temperature, and suspended sediment concentration, conservative tracers, eutrophication processes, and fecal coliform. For a detailed model description, the reader is referred to Park *et al*. (1995).

The Wye River is represented by a horizontal network of model grid cells. There are a total of 71 model grid cells in the modeling domain. To better simulate the stratification effect, 3 layers are used in the vertical. For this study, the model was calibrated for the tide and long-term mean salinity distribution. In order to address the standards of median and 90th percentile, an inverse approach has been adopted here to estimate the loads from the watershed. The watershed is divided into 24 subwatersheds. The loads from each subwatershed are discharged into the river from the river's tributaries.

The model was forced by the M_2 constituent of the tide and the mean salinity concentration at the river mouth. The long-term mean freshwater input estimated based on data from United States Geological Survey (USGS) gage station 01492500 was used. The discharges from subwatersheds are estimated based on the ratio of subwatershed area to the total drainage basin of the USGS station. The inverse method is used to estimate the existing load discharged from each subwatershed based on median and 90th percentile data obtained from observations. The model is also used to establish the allowable loads for the river. Detailed modeling procedures are described in Appendix A.

4.3 Critical Condition and Seasonality

EPA's regulations require TMDLs to take into account critical conditions for stream flow, loading, and water quality parameters (40 CFR 130.7 (c)(1)). The intent of this requirement is to ensure that the water quality of the waterbody is protected during times when it is most vulnerable. The critical condition accounts for the hydrologic variation in the watershed over many sampling years, whereas the critical period is the time during which a waterbody is most likely to violate the water quality standard(s).

The 90th percentile concentration is the concentration exceeded only 10% of the time. Since the data used were collected over a five-year period, the critical condition is implicitly included in the value of the 90th percentile. Given the length of the monitoring record used and the limited applicability of best management practices to extreme conditions, the 90th percentile is utilized instead of the absolute maximum.

A comparison of the median values and the 90th percentile values against the water quality criteria determines which represents the more critical condition or higher percent reduction. If the median values dictate the higher reduction, this suggests that, on average, water sample counts are very high with limited variation around the mean. If the 90th percentile criterion requires a higher reduction, this suggests an occurrence of high fecal coliform due to the variation of hydrological conditions.

The seasonal fecal coliform distributions for the six applicable monitoring stations are presented in Appendix C. The results show the seasonal variability of fecal coliform concentrations. High concentrations occur in May and between September and November in the Wye River restricted shellfish harvesting area. The largest standard deviations correspond to the highest variability in concentration for each station. These high concentrations result in a high 90th percentile concentration. The results indicate that exceedances may occur only during a few months of the year.

Similar to the critical condition, seasonality is also implicitly included in the analysis due to the averaging required in the water quality standards. The MDE shellfish-monitoring program uses a systematic random sampling design that was developed to cover inter-annual variability. The monitoring design and the statistical analysis used to evaluate water quality attainment therefore implicitly include the effect of seasonality. By examining the seasonal variability of fecal coliform, the highest fecal coliform concentration often occurs during the few months of the year that correspond to the critical condition. If loads under the critical condition can be controlled, water quality attainment can be achieved.

4.4 TMDL Computation

According to the water quality standard for fecal coliform in shellfish waters, computation of a TMDL requires analyses of both the median and 90th percentile. These analyses are described below.

Routine monitoring data were used to estimate the current loads. These data were analyzed for the median and for the 90th percentile conditions. The restricted shellfish harvesting area in Wye River has six shellfish monitoring stations. As stated above, in order to estimate accurately the load with consideration of available monitoring data, the watershed was segmented into 24 subwatersheds. The load for each subwatershed was discharged into its corresponding receiving water model. The inverse method was used to compute the watershed loads discharged into the river based on the best match of observations and model simulation of fecal coliform concentrations in the river. The total loads are reported in Table 4.4.1 and Table 4.4.2. Detailed results by subwatershed are also listed in Appendix A.

The allowable load is calculated using the water quality criteria of a median of 14 MPN/100ml and a 90th percentile of 49 MPN/100ml. The 3-D model was used to compute the allowable load for each subwatershed by reducing the existing loads from the watershed so that the fecal coliform concentration in the receiving water meets the standards. The total loads discharged into the river are the summation of loads discharged from each subwatershed. For the Wye River, the median standard is met at all six stations. Therefore, no reduction is needed for median loads. The existing median load is used as the allowable load. The load reduction needed for the attainment of the criteria is determined as follows:

$$\textbf{Load Reduction} = \frac{\textbf{Current Load} - \textbf{Allowable Load}}{\textbf{Current Load}} \times 100\,\%$$

The TMDL calculations are presented in Appendix A. The calculated results are listed in Table 4.4.1 and Table 4.4.2.

Table 4.4.1: Median Analysis of Current Load and Estimated Load Reduction

Area	Mean Volume M³	Fecal Coliform Median Standard MPN/100mL	Current Load counts/day	Allowable Load counts/day	Required Percent Reduction (%)
Wye River	66,387,662	14	1.588E+10	1.588E+10*	0.00

* No reduction was required for the median analysis (current load used for the allowable load).

Table 4.4.2: 90th Percentile Analysis of Current Load and Estimated Load Reduction

Area	Mean Volume M³	Fecal Coliform 90th Percentile Standard MPN/100mL	Current Load counts/day	Allowable Load counts/day	Required Percent Reduction (%)
Wye River	66,387,662	49	4.392E+11	7.389E+10	83.18

4.5 TMDL Loading Caps

This section presents the TMDLs for the median and 90th percentile conditions. Seasonal variability is addressed implicitly through the interpretation of the water quality standards. The TMDLs for the restricted shellfish harvesting waters of the Wye River Basin are as follows:

Wye River:
The median load of fecal coliform TMDL = 1.588×10^{10} counts per day
The 90th percentile of fecal coliform TMDL = 7.389×10^{10} counts per day

The greater reduction required when comparing the median and the 90th percentile results (see Table 4.4.1 and Table 4.4.2) was used for the source allocation. In this case, the 90th percentile requires the greater reduction for the area. It is important to note that the TMDLs presented herein are not literal daily limits. These loads are based on an averaging period that is defined by the water quality criteria (*i.e.*, at least 30 samples). The averaging period used for development of these TMDLs is five years.

4.6 Load Allocation

The purpose of this section is to allocate the TMDLs between point (WLA) and nonpoint (LA) sources. As stated above, there are three point source facilities in the reported restricted shellfish harvesting area that have NPDES permit numbers: MD0000043 (SEW Friel, a vegetable cannery), MD0065170 (Wye Institute, aquarium overflow), and MD0024384 (Chesapeake College). Of these three point sources, only Chesapeake College has a permit regulating the discharge of fecal coliform directly to the Wye River. The permitted fecal coliform load from this point source is approximately 7.95×10^{6} counts per day and will be included in the WLA. The remaining loads assimilative capacity will be allocated to the load allocation.

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 the allocations are consistent with the achievement of water quality standards. This load allocation results in load reductions shown in Table 4.6.1 for the restricted shellfish harvesting area of the Wye River watershed.

Table 4.6.1: Load Reductions

Restricted Shellfish Harvesting Area	Required Reduction
Wye River	**83.18%**

Since the load reduction applied to this watershed was based on the 90th percentile water quality standard, it targets only those critical events that occur less frequently. Therefore, the load reduction established is not a literal daily reduction, but rather an indicator that the control of measures for bacterial loads is needed for these more extreme events. Extreme events are often a result of hydrologic variability, land use practices, water recreation uses, or wildlife activities.

4.7 Margin of Safety

A MOS is required as part of a TMDL in recognition of many uncertainties in the understanding and simulation of water quality in natural systems. For example, knowledge is incomplete regarding the exact nature and magnitude of pollutant loads from various sources and the specific impacts of those pollutants on the chemical and biological quality of complex, natural waterbodies. The MOS is intended to account for such uncertainties in a manner that is conservative from the standpoint of environmental protection.

For TMDL development, the MOS needs to be incorporated to account for uncertainty due to model parameter selection. The decay rate is one of the most sensitive parameters in the model. For a given system, the higher the decay rate, the higher the assimilative capacity. The value of the decay rate varies from 0.7 to 3.0 per day in salt water (Mancini, 1978; Thomann and Mueller, 1987). A decay rate of 0.7 per day was used as a conservative estimate in the TMDL calculation. Further literature review supports this assumption as a conservative estimate of the decay rate (MDE, 2004). Therefore the MOS is implicitly included in the calculation.

4.8 Summary of Total Maximum Daily Loads

There is a point source facility (Chesapeake College, NPDES permit number MD0024384) that has a permit regulating the discharge of fecal coliform directly into the Wye River. The permitted fecal coliform load from this point source is approximately 7.95×10^6 counts per day and will be included in the WLA. The remaining loads assimilative capacity will be allocated to the load allocation. The TMDLs are summarized as follows:

The median TMDL (counts per day):

Area	TMDL	=	LA	+	WLA	+	FA	+	MOS
Wye River	**1.59×10^{10}**		**1.59×10^{10}**	+	**7.95×10^{06}**	+	**N/A**	+	**Implicit**

The 90th percentile TMDL (counts per day):

Area	TMDL	=	LA	+	WLA	+	FA	+	MOS
Wye River	**7.39×10^{10}**		**7.39×10^{10}**	+	**7.95×10^{06}**	+	**N/A**	+	**Implicit**

Where:

TMDL = Total Maximum Daily Load
LA = Load Allocation (Nonpoint Source)
WLA = Waste Load Allocation (Point Source)
FA = Future Allocation
MOS = Margin of Safety

5.0 ASSURANCE OF IMPLEMENTATION

This section provides the basis for reasonable assurances that the fecal coliform TMDLs will be achieved and maintained. The appropriate measures to reduce pollution levels in the impaired segments include, where appropriate, the use of better treatment technology or installation of best management practices (BMPs). Details of these methods are to be described in the implementation plan.

In general, MDE intends for the required reductions to be implemented in an iterative process that first addresses those sources with the greatest impact on water quality, with consideration given to ease of implementation and cost. The source contributions estimated from the watershed analysis (see Table 2.4.1) may be used as a tool to target and prioritize initial implementation efforts. 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 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. Additional funding available for local governments includes the State Water Quality Revolving Loan Fund and the Stormwater Pollution Cost Share Program. Details of these programs and additional funding sources can be found at http://www.dnr.state.md.us/bay/services/summaries.html. Property owners can apply for a low interest loan, through MDE, that can be used to improve a failing septic system. It is anticipated that in 2006, there may be funding available to provide improvement to a portion of septic systems in Maryland's designated Critical Areas. Maryland law, § 9-333 of the Environment Article, requires the following types of facilities to have pumpout stations: Existing marinas wishing to expand to a total of 11 or more slips that are capable of berthing vessels that are 22 feet or larger; new marinas with more than 10 slips capable of berthing vessels that are 22 feet or larger; and marinas with 50 or more slips and that berth any vessel over 22 feet in length. Any public or private marina in Maryland is eligible to apply for up to $15,000 in grant funds to install a pumpout station through the Maryland Department of Natural Resources.

Regulatory enforcement of potential bacteria sources may include MDE's routine sanitary surveys of shellfish growing areas, and through National Pollutant Discharge Elimination System (NPDES) permitting activities such as Confined Animal Feeding Operations (CAFOs). Though not directly linked, it is assumed that the nutrient management plans from the Maryland's Water Quality Improvement Act of 1998 (WQIA) will result in some reduction of bacteria from manure application practices.

As part of Maryland's commitment to the NSSP, MDE continues to monitor shellfish waters and classify harvesting areas. Those waters meeting shellfish water quality standards are reclassified as open to harvesting and may serve to track the effectiveness of TMDL implementation and water quality improvements.

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 will not meet water quality standards. However, neither the State of Maryland nor EPA is proposing the elimination of wildlife to allow for the attainment of water quality standards. This is considered to be an impracticable and undesirable action. While managing the overpopulation of wildlife remains an option for State and local stakeholders, the reduction of wildlife or changing a natural background condition is not the intended goal of a TMDL.

Implementation may begin by first managing controllable resources (human, livestock, and pets) and then determining if the TMDL can be achieved. If the total required reduction is still not met, then a reduction may need to be applied to the wildlife source. Given the nonpoint source characteristics of the wildlife contribution, it may be assumed that best management practices applied to controllable sources may also reduce some wildlife sources contributing to the restricted shellfish harvesting area.

Following this first implementation stage, MDE would re-assess the water quality to determine if the designated use is being achieved. If the water quality standards are not attained, then MDE may consider developing either a risk-based adjusted water quality assessment or a Use Attainability Analysis to reflect the presence of naturally high bacteria levels from uncontrollable (natural) sources.

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Appendix A. Model Development

The 3-dimensional hydrodynamic and eutrophication model (HEM-3D) has been used for this study. The HEM-3D model is a general 3D model for environmental studies. The model simulates density and topographically induced circulation as well as tidal and wind-driven flows, and spatial and temporal distributions of salinity, temperature, and suspended sediment concentration, conservative tracers, eutrophication processes, and fecal coliform. The model has been applied for varieties of environmental problems in estuaries (Hamrick, 1992a; Park et al., 1995; Shen et al., 1999). For a detailed discussion of the model theory, readers are referred to Hamrick (1992b).

Figure A-1 is the model grid superimposed on the 24 subwatersheds of the Wye River. The modeling domain consists of 71 grid cells. Because the Wye River is narrow, a horizontal network approach is used to represent the river. To better simulate estuarine circulation, a total of 3 layers are used in the vertical. The fecal coliform is simulated using a conservative tracer with first-order decay. The decay rate varies from 0.7 to 3.0 per day in salt water (Mancini, 1978; Thomann and Mueller, 1987). A decay rate of 0.7 per day was used as a conservative estimate in this TMDL study.

The Wye River is a tidal river. The dominant tidal constituent is M_2. To simulate tide correctly, a calibration of tide was conducted. The model was forced by seven tidal harmonic constituents at the river mouth. The tidal harmonic constituents at the mouth were obtained from the 3-dimensional Chesapeake Bay UnTRIM model developed at the Virginia Institute of Marine Science (VIMS) (Shen et al., in press). Since there are no tide observations available inside the Wye River, the UnTRIM model results obtained from the coarse grid were used as the tidal benchmark for calibrating the HEM-3D model. The HEM-3D model results compare well against results from the coarse grid model and the difference in the range of the dominant M_2 tide is less than 2 cm. Because there are no real-time observation data of stream flow, tide, and wind available in the Wye River, comparison of real-time salinity simulation against the observed salinity cannot be performed. Therefore, the model calibration for the mean condition of salinity distribution was performed to reproduce the averaged salinity distribution at 20 stations along the river. The locations of these stations are shown in Figure A-2. For the mean salinity calibration, the dominant M_2 tide was used as a forcing at the model open boundary. Mean salinity measured at the station nearest the mouth was used as the salinity boundary condition. The quantity of freshwater discharged from each subwatershed was estimated according to the average long-term flow from the USGS gage of 01492500 (Sallie Harris Creek near Carmichael, MD). The flow of each subwatershed was estimated based on the ratio of the subwatershed area to the drainage basin area of the USGS gage. The mean flows used for the model calibration are listed in Table A-1 below for the subwatersheds shown in Figure A-1. A comparison of model results against observations is shown in Figure A-3. It can be seen that the model simulated salinity distribution well in the estuary.

Since the water quality standards for fecal coliform are median and 90th percentile, the modeling tasks are to estimate fecal coliform mean daily loads from the watershed corresponding to the median and 90th percentile, respectively. For a relatively small waterbody, the tidal prism model has been used to estimate the loads based on the observations and water quality standards using the inverse method (or back calculation) (MDE, 2005). For this study, an inverse modeling approach method built on the HEM-3D has been used to estimate fecal coliform loading from the watershed. The purpose of the inverse modeling is to estimate the long-term average daily loads corresponding to the median and 90th percentile concentrations in the waterbody. Therefore, the fecal coliform daily loads from each subwatershed can be considered as constant model parameters. The inverse methods have been used for many environmental problems to estimate point source loads and model parameters (Shen and Kuo, 1996; Sun and Yeh, 1990; Shen, 2006).

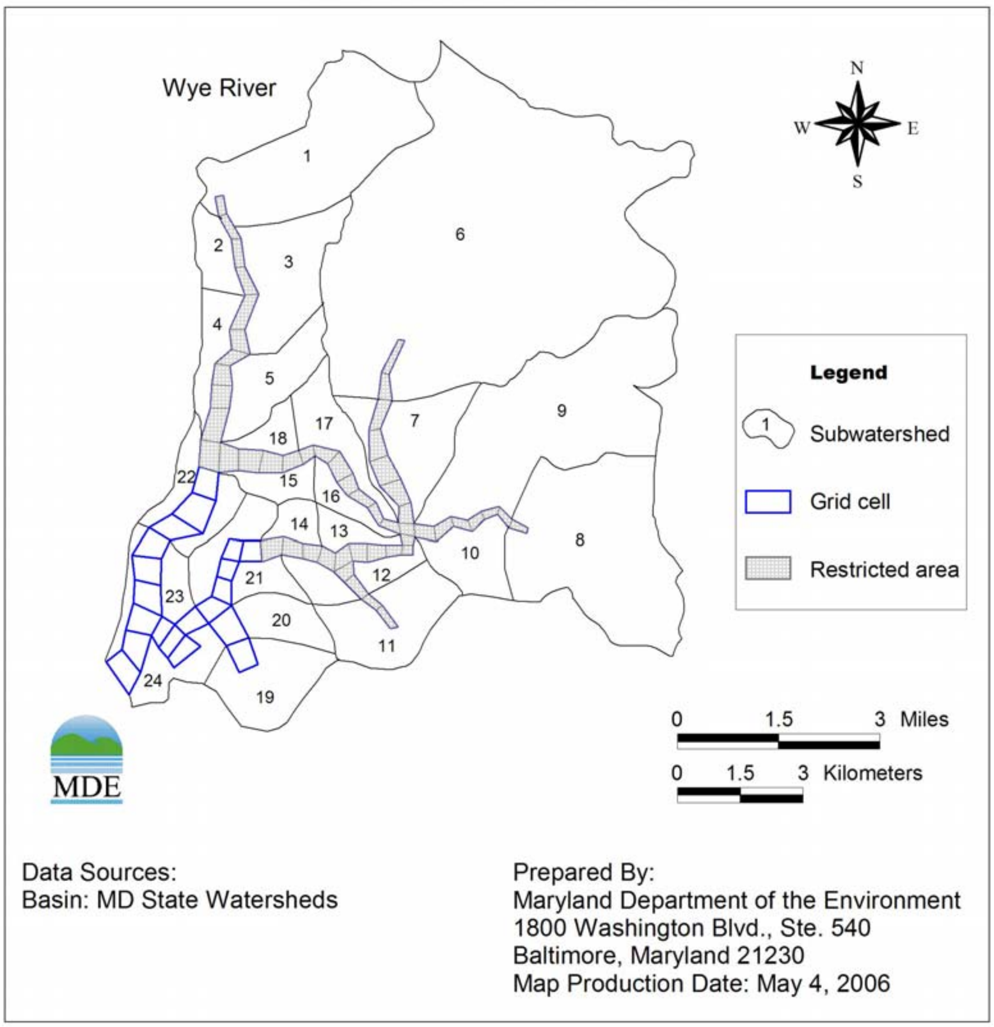


Figure A-1: HEM-3D grid cells and subwatersheds in the Wye River

Table A-1: Drainage areas and estimated mean flows of subwatersheds in the Wye River

Subwatershed	Area (m^2)	Estimated Flow ($m^3 s^{-1}$)
1	17,125,083	0.178206
2	2,337,038	0.024320
3	7,943,742	0.082664
4	2,621,189	0.027277
5	5,057,478	0.052629
6	69,679,035	0.725090
7	7,056,409	0.073430
8	20,903,543	0.217525
9	23,466,291	0.244193
10	4,186,028	0.043561
11	7,030,494	0.073160
12	3,361,013	0.034975
13	1,524,258	0.015858
14	1,304,705	0.013577
15	1,951,585	0.020309
16	1,558,684	0.016220
17	4,105,548	0.042723
18	1,698,517	0.017675
19	6,120,742	0.063693
20	3,005,198	0.031273
21	4,267,040	0.044403
22	2,938,169	0.030575
23	2,599,378	0.027049
24	2,649,003	0.027566

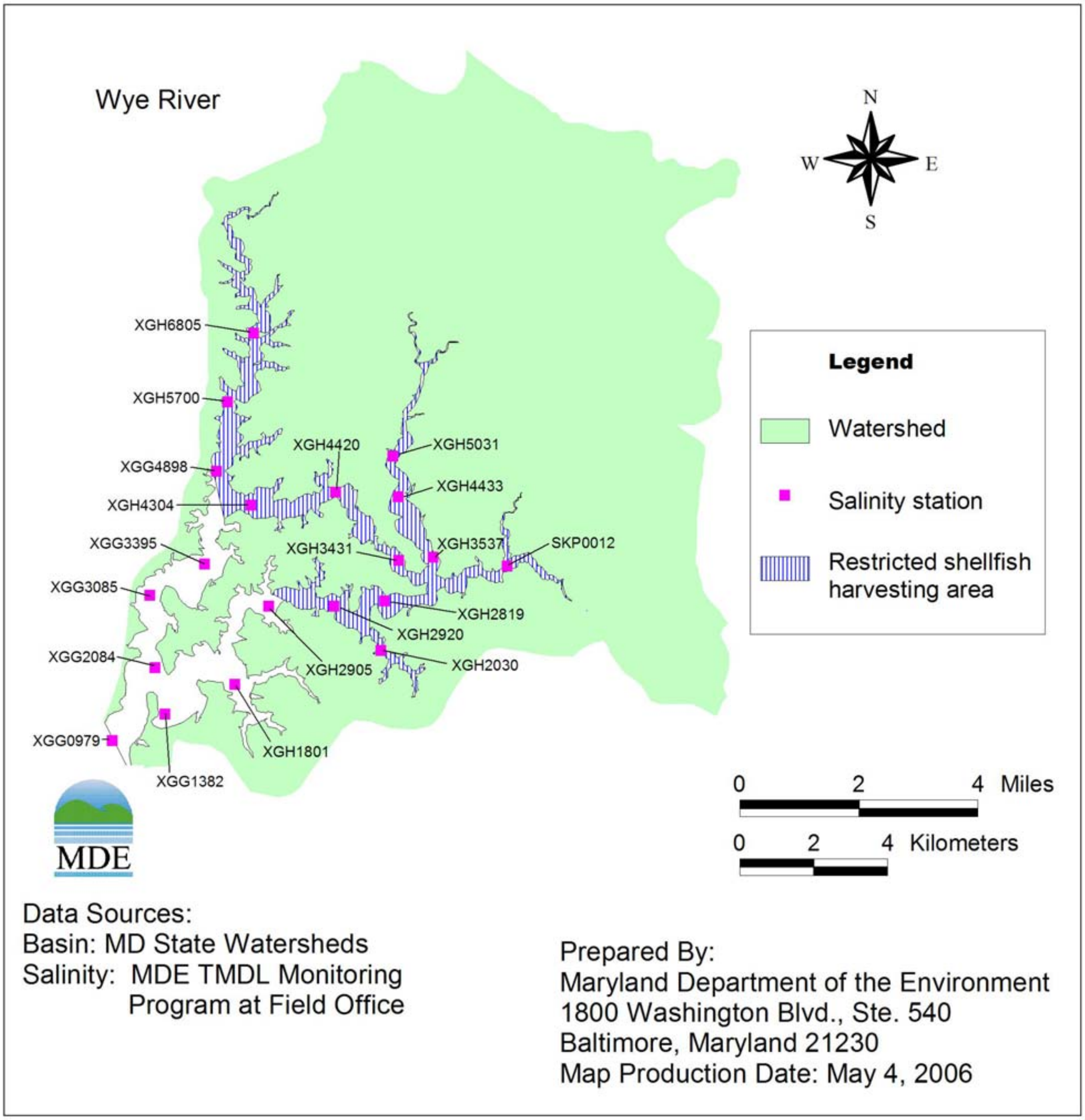
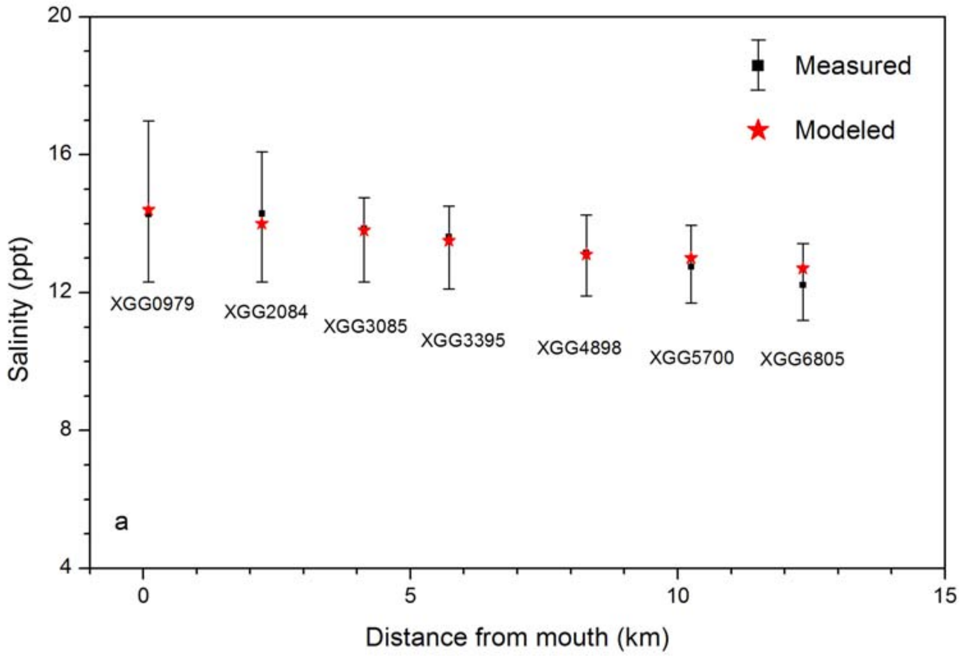


Figure A-2: Salinity stations of the Wye River used in model calibration



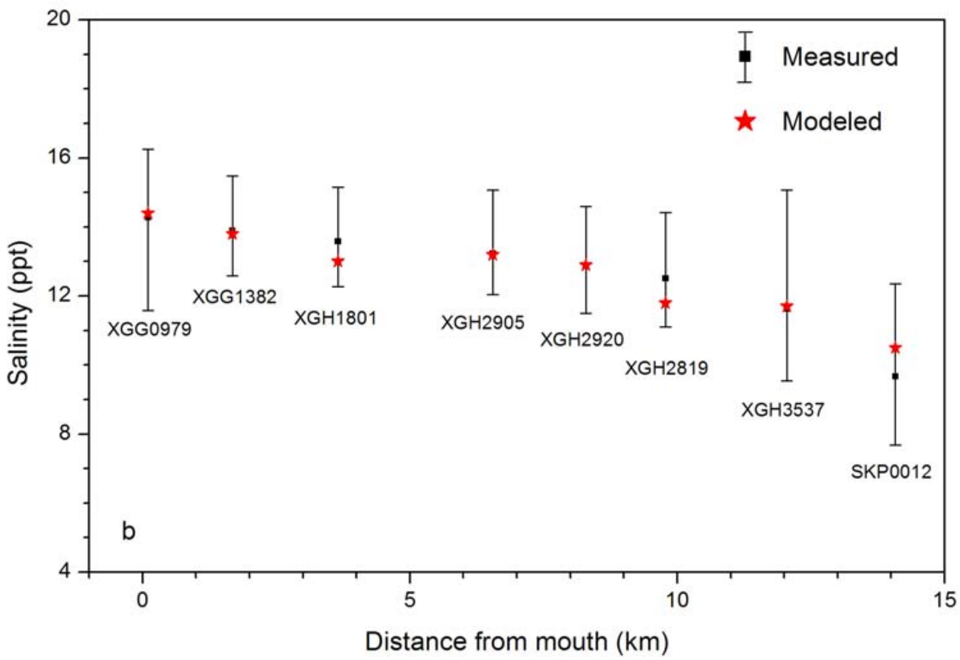


Figure A-3: Comparison of measured and calculated salinities

The problem of loads estimation can be treated as an inverse problem: to find a set of loads such that a defined goal function (or cost function), which measures the data misfit between the model predictions and the observations, becomes minimal. It can be presented as follows:

$$J(\mathbf{C};\boldsymbol{\beta}^*) = \min J(\mathbf{C};\boldsymbol{\beta}) \tag{1}$$

subject to:

$$\boldsymbol{\beta}^* \in \boldsymbol{\beta}_0 \tag{2}$$
$$\mathbf{F} = 0 \tag{3}$$

where $\boldsymbol{J}$ is a goal or cost function; $\boldsymbol{\beta}^* = (\beta_1, \beta_2, ..., \beta_m)$ is the optimal parameter (*i.e.*, loads); $\boldsymbol{\beta_0}$ is an acceptable set of loads. **F** is transport function. Different methods can be used to characterize the noninferior solutions. Choosing a weighted least-square criterion to measure the data misfit, the scalar cost function is then defined as follows:

$$J(\mathbf{C};\boldsymbol{\beta}) = \int_{T_N} \iint_{\Omega} \frac{w}{2}(C(x,z,t) - C^0(x,z,t))^2 \, d\Omega dt \tag{4}$$

where C and C^0 are modeled and measured fecal coliform in the River, w is weights, Ω is the spatial domain in the x- and z- directions, T_N is time later than the last date when the prototype observations are available, and w is the weight. In our case, let $C_m^0(x)$ be the median or 90th percentile obtained from the observations at location (x). If we choose:

$$C_m(x) = \max(C(x,z,t)) \quad for \quad T_0 < t < T_N \tag{5}$$

Equation (1) can be written as:

$$J(\mathbf{C};\boldsymbol{\beta}) = \int_X \frac{w}{2}(C_m(x,t) - C_m^0(x))^2 \, dx \tag{6}$$

The algorithm can be constructed as a sequence of the unconstrained minimization problem. Many authors have studied the solution of the optimization problem extensively. Several different methods can be used to solve the problem including the Gradient method, Conjugate direction method, and the Variational method (Bertsekas, 1995). For this study, the modified Newton method was used to solve the optimization problem (Shen, 2006).

The fecal coliform loads discharged to the River consist of 24 subwatersheds, as shown in Figure A-1. For estimating of existing median loads, the model was forced by an M_2 tide and mean salinity at the mouth. The mean freshwater inflows from the subwatersheds are discharged into the River. A set of initial loads from 24 subwatersheds was estimated and discharged to the River. The initial loads are estimated based on the land use type and drainage sizes. The model was run for 20 days to reach equilibrium and the maximum concentration at the last day was used to calculate the cost function against the observed median along the River. Wye River fecal coliform monitoring stations are shown in Figure A-4, and the fecal coliform concentrations from these stations are shown in Table A-2. The modified Newton method was used to update the loads until the cost function is minimum. For estimating the existing loads for 90th percentile, the same method was used except the existing 90th percentile concentrations were used to minimize the cost function.

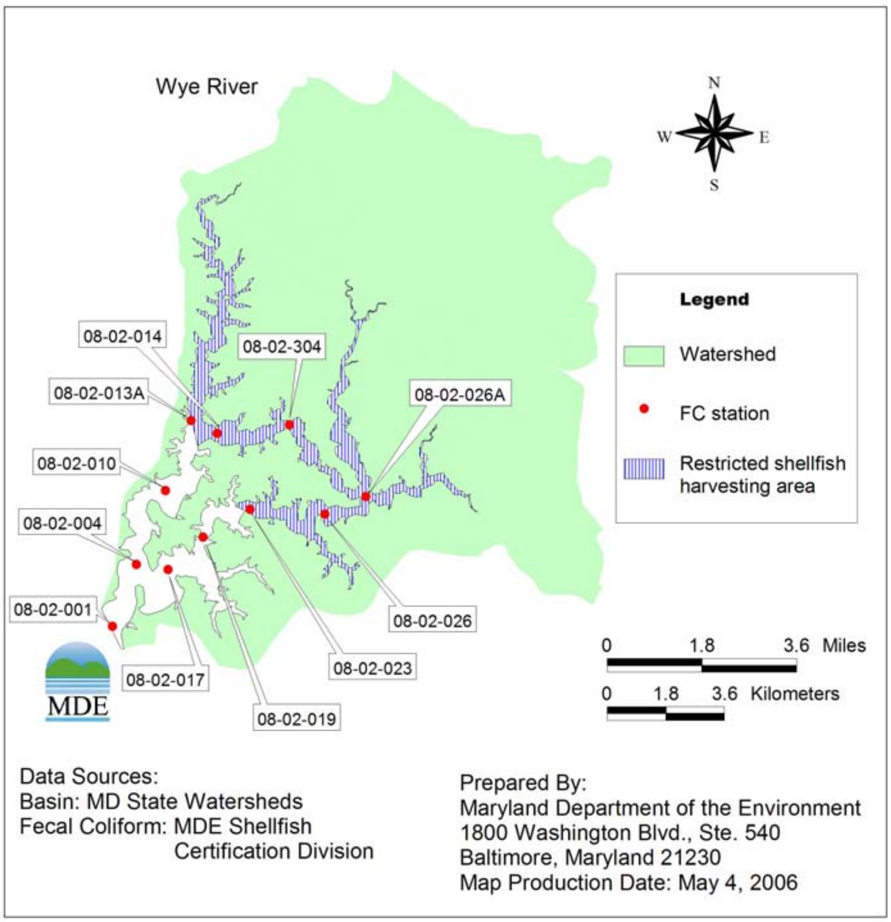


Figure A-4: Locations of Wye River fecal coliform monitoring stations

Table A-2: Wye River Fecal Coliform Statistics (data from 2000-2005)

Area Name	Station	Median		90th Percentile	
		Monitoring Data	Criterion	Monitoring Data	Criterion
		MPN/100ml	MPN/100ml	MPN/100ml	MPN/100ml
Wye River	08-02-001	3.60	14	11.38	49
Wye River	08-02-004	3.60	14	15.81	49
Wye River	08-02-010	3.60	14	26.96	49
Wye River	08-02-013A	7.25	14	48.38	49
Wye River	08-02-014	3.60	14	54.63	49
Wye River	08-02-017	3.60	14	24.55	49
Wye River	08-02-019	9.10	14	42.26	49
Wye River	08-02-023	7.30	14	57.92	49
Wye River	08-02-026	9.10	14	89.75	49
Wye River	08-02-026A	9.10	14	169.34	49
Wye River	08-02-304	9.10	14	94.91	49

Figures A-5 and A-6 show the model results of simulated median and 90th percentile, respectively, along the Rivers. It can be seen that the model results are satisfactory. The existing loads for each subwatershed are listed in Table A-3.

For TMDL calculation, the existing 90th percentile loads were reduced so that the model simulated fecal coliform along the river meet the median and 90th percentile standards, respectively. The resultant loads are the allowable loads for the river. With the use of existing loads and TMDLs, the percentage reduction can be estimated. Since there is no violation of median concentration of fecal coliform, the load reduction is not required. Therefore, the existing median load is used as the TMDL. Comparing the reduction needed for both median and 90th percentile loads, the maximum reductions required for each watershed are used to establish the TMDLs. The existing and allowable loads are listed in Table A-3. Note that the current median loads are used as allowable loads.

Table A-3: TMDL calculation results for each subwatershed

Subwatershed	Median			90th Percentile		
	Allowable Load*	**Current Load**	**Percent Reduction**	**Allowable Load**	**Current Load**	**Percent Reduction**
	Counts/day	**Counts/day**		**Counts/day**	**Counts/day**	
1-5	6.43E+09	6.43E+09	N/A	8.196E+09	1.300E+11	93.69%
6-10	2.22E+09	2.22E+09	N/A	4.022E+09	2.344E+11	98.28%
11-14	9.16E+08	9.16E+08	N/A	9.159E+08	1.134E+10	91.93%
15-18	3.54E+08	3.54E+08	N/A	6.945E+09	9.771E+09	28.92%
19-21, 24	3.71E+09	3.71E+09	N/A	4.450E+10	4.450E+10	0.00%
22-23	2.24E+09	2.24E+09	N/A	9.315E+09	9.315E+09	0.00%
TOTALS	**1.59E+10**	**1.59E+10**	N/A	**7.389E+10**	**4.392E+11**	**83.18%**

* No reduction was required for the median analysis. Here, the current load was used for the allowable load.

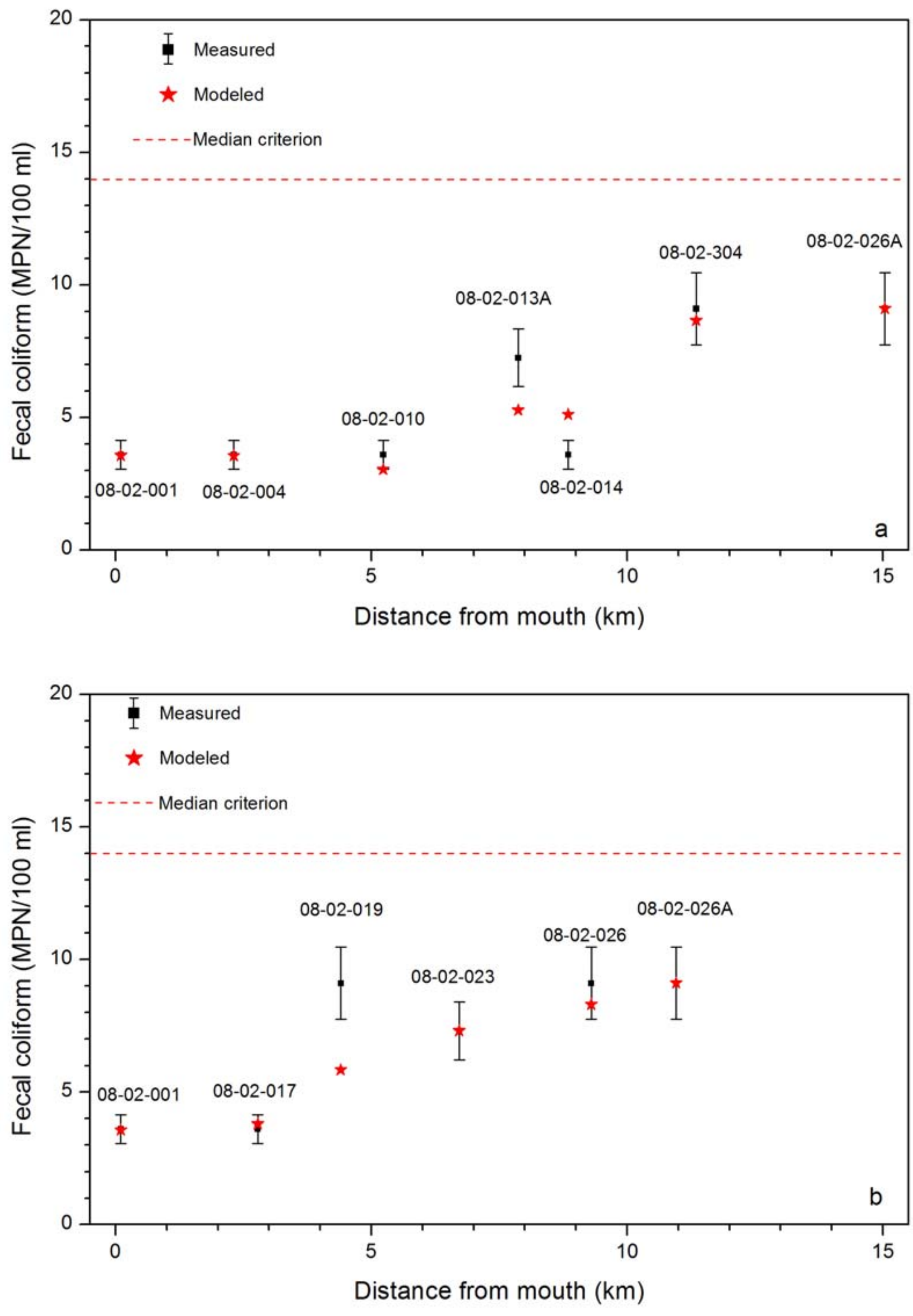
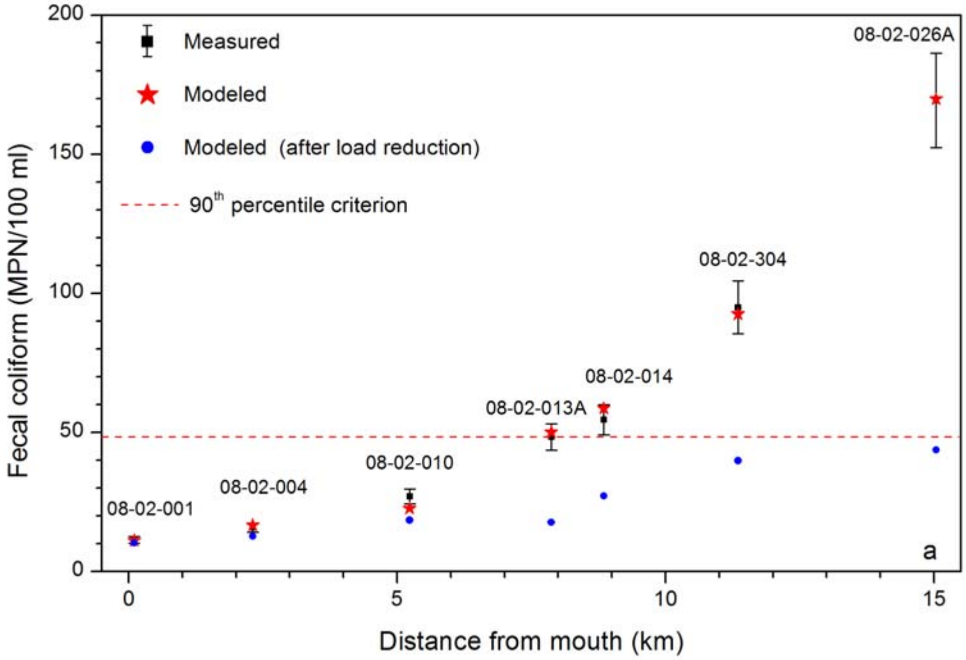


Figure A-5: Measured and modeled fecal coliform for the median criterion



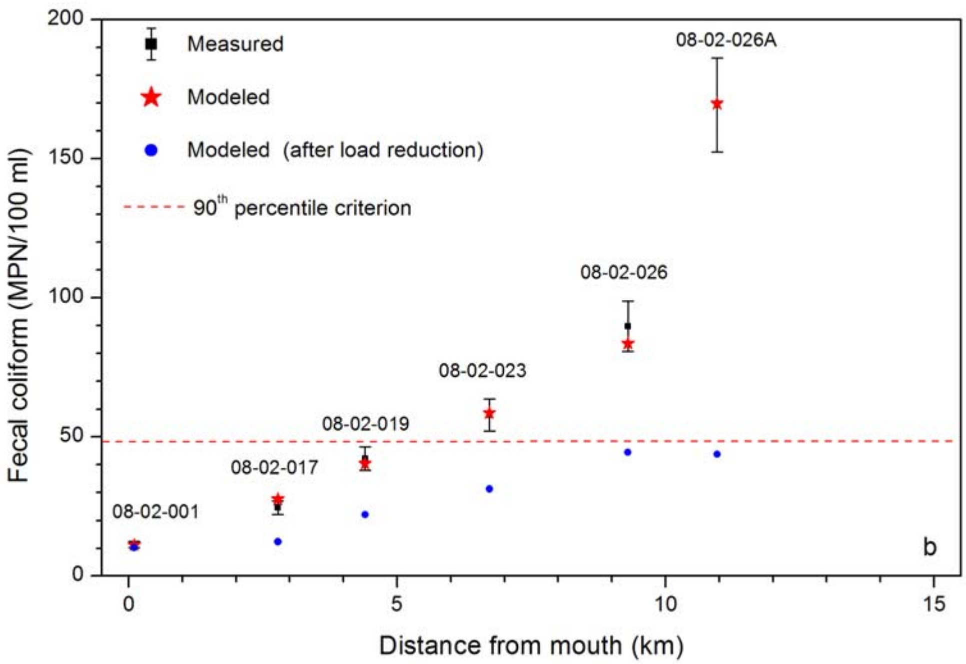


Figure A-6: Measured and modeled fecal coliform for the 90th percentile criterion

By comparing the reductions required for median and 90th percentile, one can see that the 90th percentile requires the largest reduction. Therefore, the reductions required to meet the 90th percentile at each subwatershed are the overall reductions required for the subwatersheds. The allowable loads and required reductions for the watershed are listed in Table A-4.

Table A-4: Load allocation and reduction by subwatershed

Subwatershed	Load Allocation	Required Reduction
1-5	8.196E+09	93.69%
6-10	4.022E+09	98.28%
11-14	9.159E+08	91.93%
15-18	6.945E+09	28.92%
19-21, 24	4.450E+10	0.00%
22-23	9.315E+09	0.00%
TOTALS	**7.389E+10**	**83.18%**

Appendix B. Bacteria Source Tracking

Nonpoint sources of fecal coliform do not have one discharge point but occur over the entire length of a stream or waterbody. There are many types of nonpoint sources in watersheds discharging to the restricted shellfish harvesting areas. The possible introductions of fecal coliform bacteria to the land surface are through 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 coliform over the land surface and discharges to the restricted shellfish harvesting area. The deposition of non-human fecal coliform directly to the restricted shellfish area can also occur when livestock or wildlife have direct access to the waterbody. Nonpoint source contributions to the bacterial levels from human activities generally arise from failing septic systems and their associated drain fields as well as through pollution from recreation vessel discharges. The transport of fecal coliform from the land surface to the restricted shellfish harvesting area is dictated by the hydrology, soil type, land use, and topography of the watershed.

In order to determine the sources of fecal coliform contribution and reduction needed to achieve water quality criteria and to allocate fecal coliform load among these sources, it is necessary to identify all existing sources. The nonpoint source assessment was conducted using the fecal coliform monitoring data (MDE Shellfish Certification Division) and bacteria source tracking to quantify source loadings from humans, livestock, pets, and wildlife.

Bacteria Source Tracking

In order to assess the potential fecal bacteria sources that contribute to the Wye River, seven stations in the Wye River were selected to evaluate the source characterization through a process called Bacteria Source Tracking (BST). BST is used to provide evidence regarding contributions from anthropogenic sources (*i.e.*, human or livestock) as well as background sources, such as wildlife. Twelve months of sampling was conducted from January 2002 through December 2002. To obtain fecal coliform sources from the watershed, the BST analysis that includes Antibiotic Resistance Analysis (ARA) was used to determine the potential sources of fecal coliform discharged into the waterbody. ARA uses fecal streptococcus or *Escherichia coli* (*E. coli*) and patterns of antibiotic resistance for separation of sources. The premise is that human, domestic animal, and wild animal fecal bacteria will have significantly different patterns of resistance to the battery of antibiotics used in this test. Therefore, the ARA is used to estimate the percent loading per source category to the water. There are studies being initiated nationwide to compare the accuracy of the ARA method with other bacterial source tracking approaches.

Statistical software Minitab v.13.1 was used to classify the antibiotic resistance observations for scat isolates into two groups (human vs. nonhuman), three groups (human vs. livestock vs. wildlife) and five groups (human vs. bird vs. dog vs. livestock vs. wildlife). The predictive capability of the resistance profiles to correctly characterize the isolates by group was determined. The false-negative rate, or the percentage of misclassified isolates for a particular source, was determined by subtracting the percentage correctly identified from 100%. The false-positive rate was the percentage of isolates from sources other than human that were misclassified as human. Quadratic discrimination was used rather than linear discriminant analysis, since the proportion correctly classified was uniformly higher for the quadratic. For the full BST report for the Wye River basin, the reader is referred to Frana and Venso (2003).

E. coli concentrations in water samples obtained at seven Wye River sampling stations are shown in Table B-1. Results of September and November sampling were the highest, with monthly means of 187 and 106 colonies/100 ml. The stations with the most E. coli concentrations were 26A, 26, and 304A, respectively.

Table B-1: Number and Means of E. Coli Colonies per 100 ml Water

Month	Station Number							
	13A	**14**	**23**	**26**	**26A**	**304**	**304A**	**Mean**
January	1	4	7	3	11	3	3	5
February	0	1	1	2	11	3	12	4
March	2	11	2	7	7	4	35	10
April	4	6	9	9	9	12	14	9
May	22	14	10	22	87	49	180	55
June	3	3	11	18	13	11	12	10
July	4	6	4	25	8	4	5	8
August	16	12	4	8	15	8	40	15
September	23	26	84	280	833	26	35	187
October	26	10	11	20	51	25	26	24
November	29	34	47	117	330	83	100	106
December	1	2	1	2	4	2	1	2
Mean	11	11	16	43	115	19	39	36

MAR and DNA Libraries

A total of 403 known source multiple-antibiotic-resistance (MAR) patterns were obtained for the MAR Library. A total of 215 DNA fingerprint-banding patterns from known sources were added to Salisbury University's DNA Fingerprinting Library. Table B-2 lists the number and type of species of MAR resistance patterns and DNA fingerprinting patterns.

Table B-2: Number of Isolates for MAR and DNA Patterns

Species	Number of Isolate MAR Patterns	Number of Isolates DNA Patterns
Black Vulture	10	2
Cow	60	38
Deer	62	36
Dog	30	20
Fox	77	16
Goose	14	12
Horse	26	11
Human	64	54
Rabbit	10	4
Raccoon	40	16
Swan	10	6
TOTAL	**403**	**215**

A total of 403 known source isolates from 11 different species were analyzed by discriminant analysis. Percentages of isolates that were resistant to antibiotics were recorded. Resistance is indicated by antibiotic concentration and category (human, livestock, and wildlife). Antibiotics to which isolates were particularly resistant at some concentration included neomycin, oxytetracycline, streptomycin, and tetracycline.

Initially, in the discriminant analysis of the data, a resistance or sensitivity entry was used for each of the isolates at each of the 28 antibiotic-concentration combinations. After rejection of highly correlated or essentially constant antibiotic-concentration combinations, the number of combinations was reduced to six or seven antibiotics and 13 concentrations.

The resistance profile for the 2-way analysis (human vs. nonhuman) correctly identified 333 isolates as to group for a total correct classification of 83%. The false positive rates were 23% and 16% for human and nonhuman, respectively.

Discriminant analysis was also performed on the antibiotic resistance profiles of the known source isolates to group the observations into three categories (human, livestock, and wildlife), with a 79% overall correct prediction rate. The lower percentage for correct classification of human isolates was responsible for the drop in overall prediction ability. The false-positive rates were 37% for human, 16% for livestock, and 19% for wildlife. The false-negative rate for human was 4%, which was much lower than the 23% seen in predicting membership in only 2 groups (human vs. nonhuman).

The overall percent correctly classified dropped to 73% when classifying membership of isolates into five groups (human, livestock, wildlife, dogs, and birds). The proportion correctly identified dropped for the previously analyzed groups, but accurately identified a high 87% of isolates from dogs. The false-positive rate for the human group was 3%, the smallest percentage misclassified as human of the three classification schemes. However, the false-negative rate for the E. coli isolates from human was 42%.

MAR – Water

A total of 1636 isolates of E. coli were obtained from the Wye River water samples. These isolates were analyzed for group membership by discriminant analysis using the 403 known source isolates as the basis for classification. Therefore, the same 13 antibiotic-concentration combinations were used in the analysis. Tables B-3, B-4, and B-5 contain the results of the 2-way, 3-way, and 5-way discriminant analysis. An examination of the three tables indicates that most of the isolates were from nonhuman sources and that no one station stands out as significantly more heavily contaminated by any one group.

Table B-3: Source Allocation – Antibiotic Resistance Analysis – BST 2-Way Analysis

Station	Human	Nonhuman
13A	18.9 %	81.1 %
14	23.8 %	76.2 %
23	18.3 %	81.7 %
26	23.9 %	76.1 %
26A	23.1 %	76.9%
304	21 %	79 %
304A	25.9 %	74.1 %

Table B-4: Source Allocation – Antibiotic Resistance Analysis – BST 3-Way Analysis

Station	Human	Livestock	Wildlife
13A	14 %	28.7 %	57.3 %
14	14.1 %	31.4 %	54.6%
23	11.7 %	32 %	56.3 %
26	13 %	22.3 %	64.8 %
26A	17.1 %	27.5 %	55.4 %
304	12.5 %	22.2 %	65.4 %
304A	16.8 %	30.6 %	52.5 %

Table B-5: Source Allocation – Antibiotic Resistance Analysis – BST 5-Way Analysis

Station	Human	Livestock	Wildlife	Dog	Bird
13A	9.1 %	22.4 %	50.3 %	3.5 %	14.7 %
14	10.8 %	24.9 %	54.6 %	4.3 %	10.3 %
23	9.1 %	27.9 %	56.3 %	5.1 %	11.2 %
26	11.3 %	22.3 %	64.8 %	0.1 %	14.2 %
26A	10.1 %	24.4 %	55.4 %	4.5 %	19.5 %
304	13.6 %	18.3 %	65.4 %	2.3 %	14 %
304A	16.1 %	25.6 %	52.5 %	10.4 %	4.7 %

According to the BST, wildlife is the predominant bacteria source followed by livestock, pet, and human. According to the GIS background information collection, livestock is the predominant bacteria source, followed by wildlife, and then pets and human. Table B-6 summarizes the BST data analysis.

Table B-6: BST Data Analysis

Human	Livestock	Wildlife	Pets
11.84 %	23.62 %	60.94 %	3.60 %

Appendix C. Seasonality Analysis

The Code of Federal Regulations (40 CFR 130.7 (c)(1)) requires that TMDL studies take into account critical conditions for stream flow, loading, and water quality parameters. The EPA also requires that these TMDL studies take into account seasonal variations. The consideration of critical condition and seasonal variation is to account for the hydrologic and source variations. The intent of the requirements is to ensure that the water quality of the water body is protected during the most vulnerable times.

In the Chesapeake Bay region, both fecal coliform sources and delivery vary seasonally due to changes of hydrological conditions and land use practices. The most probable fecal coliform sources result from runoff from agricultural practices and livestock, wildlife, and developed areas. Precipitation and temperature fluctuate seasonally, producing varied stream flow and surface runoff that serve as a delivery mechanism for fecal coliform, as well as seasonal change in vegetation. Vegetation, particularly in pastureland and agriculture buffer zones, is very important for trapping and preventing fecal coliform from entering waters by both decreasing surface runoff and absorbing fecal coliform. Warm-blooded animals, the sources of fecal coliform, are directly or indirectly connected with vegetation productivity via food chain relationships. In temperate forests, for example, wildlife are active during summer and fall due to ample food supply, resulting in large sources of fecal coliform, and the probability of their direct contact with receiving waters is comparatively high during warm seasons. The seasonal variation of fecal coliform concentration in water not only results from activities of wildlife on forestland and wetland, but also is related to agricultural activities. Fecal coliform deposition on the field by livestock can be transported into streams and rivers through surface runoff, and thus tends to increase fecal coliform concentrations during wet seasons. In croplands, fecal coliform discharge is often related to the timing of crop planting and fertilization. Manure application during crop planting may increase the risk of exceeding fecal coliform standards in the receiving water. Such seasonal changes in both the sources and the delivery mechanisms perhaps lead to obvious seasonal patterns for receiving water fecal coliform concentration in the shellfish growing area.

The five-year monthly mean fecal coliform concentration and its standard deviation were calculated for the six monitoring stations used in this report. The results are presented in Figure C-1 through Figure C-6. It is shown that high fecal coliform concentrations occur in the months of May and between September and November in the Wye River. Although seasonal distributions vary from one month to the next, a large standard deviation that corresponds to the high fecal coliform concentration variability at each station suggests that the violation frequently may occur in a few months of the year.

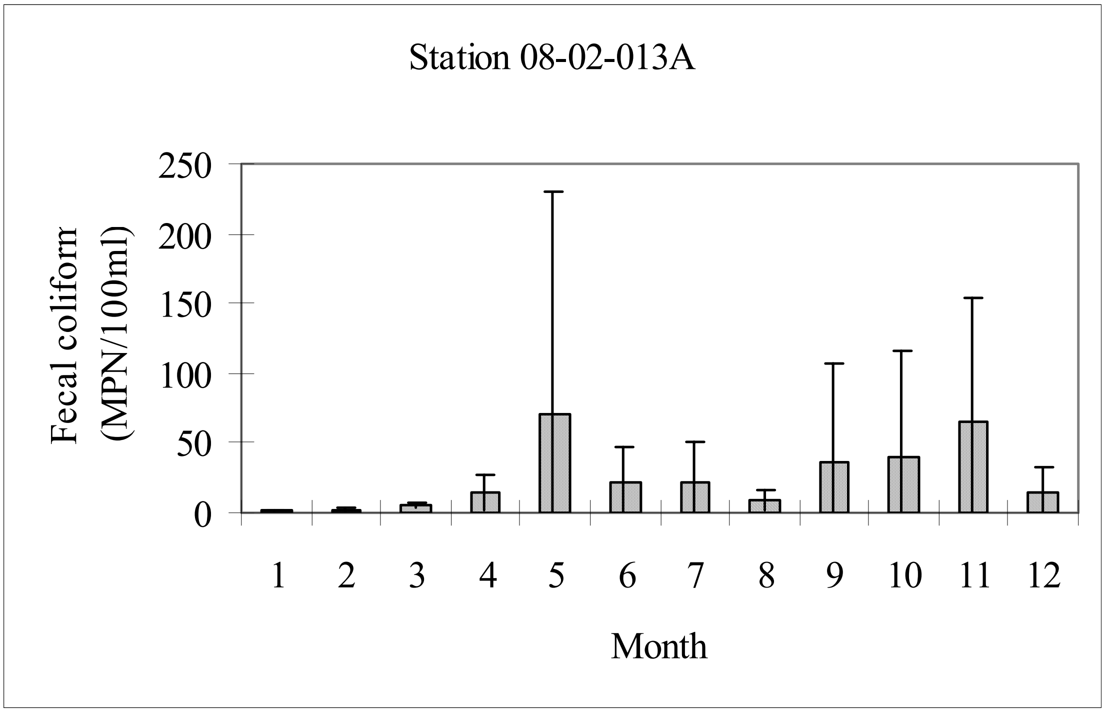


Figure C-1: Seasonality analysis of fecal coliform at Wye River Station 08-02-013A

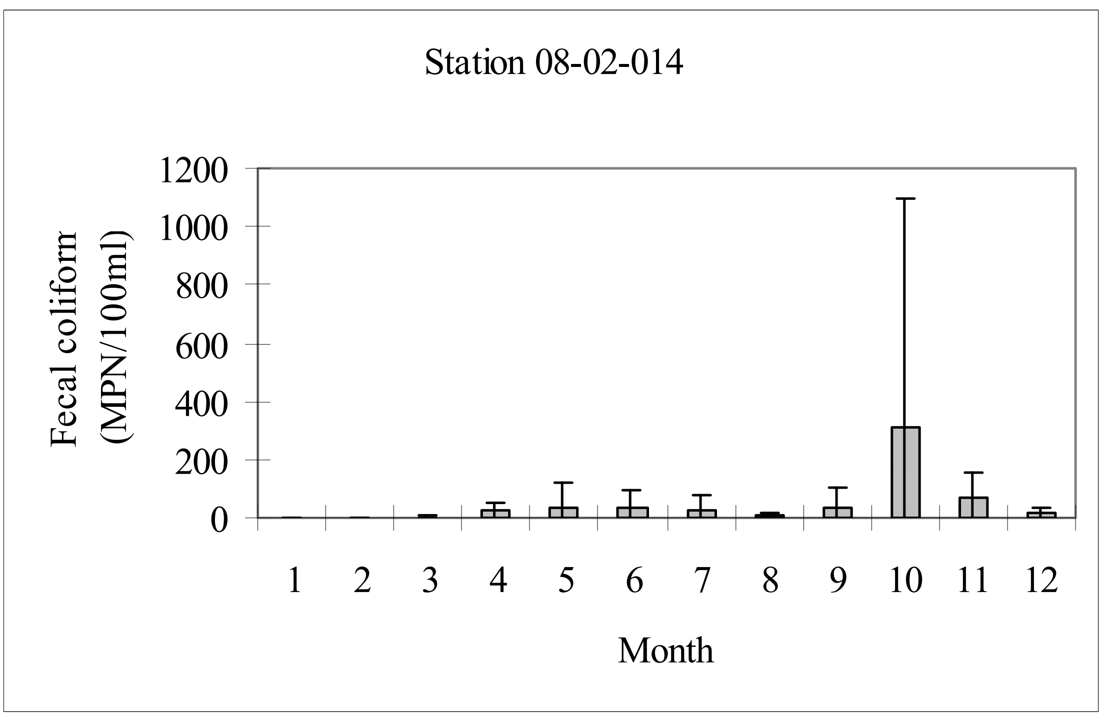


Figure C-2: Seasonality analysis of fecal coliform at Wye River Station 08-02-014

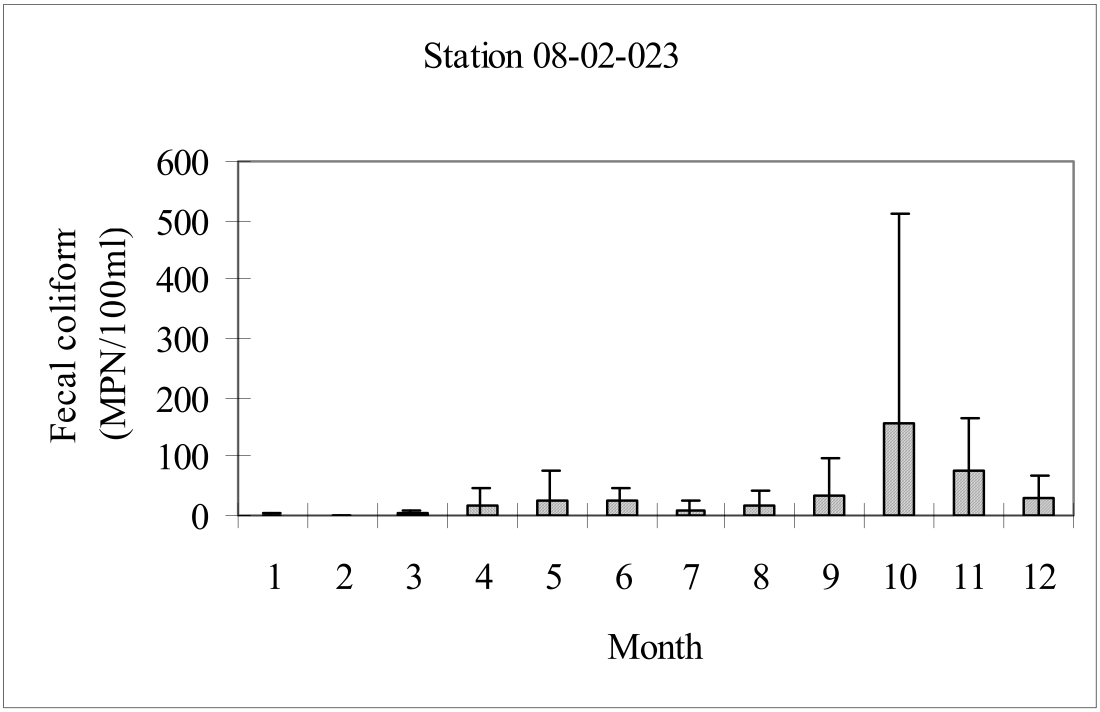


Figure C-3: Seasonality analysis of fecal coliform at Wye River Station 08-02-023

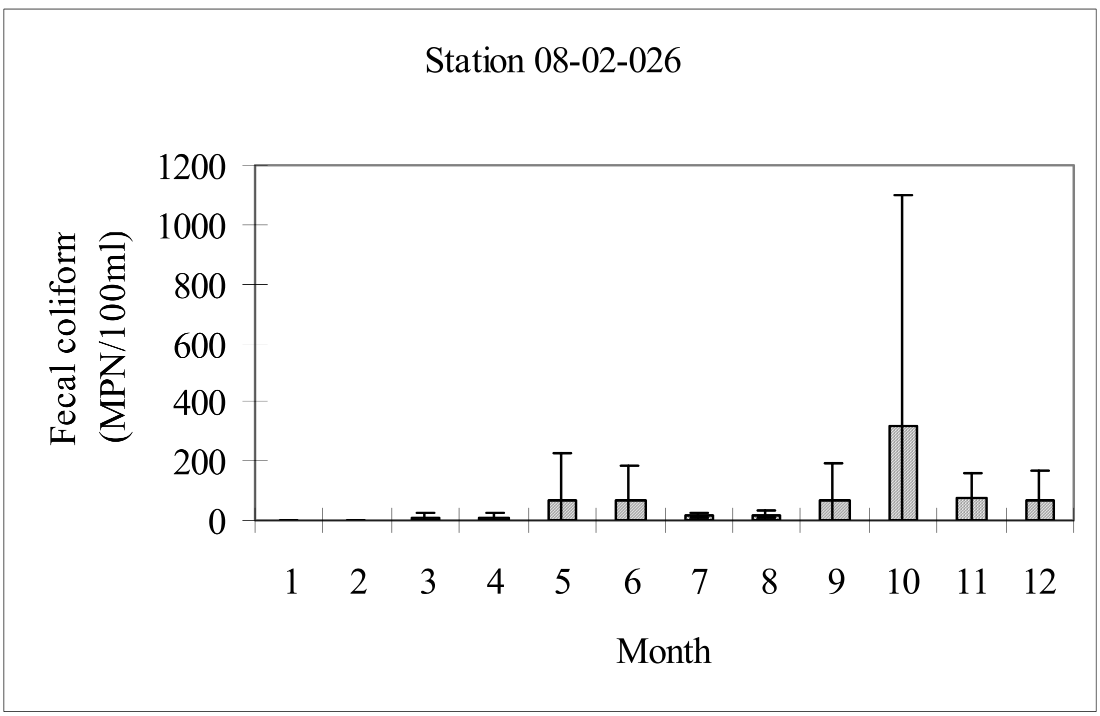


Figure C-4: Seasonality analysis of fecal coliform at Wye River Station 08-02-026

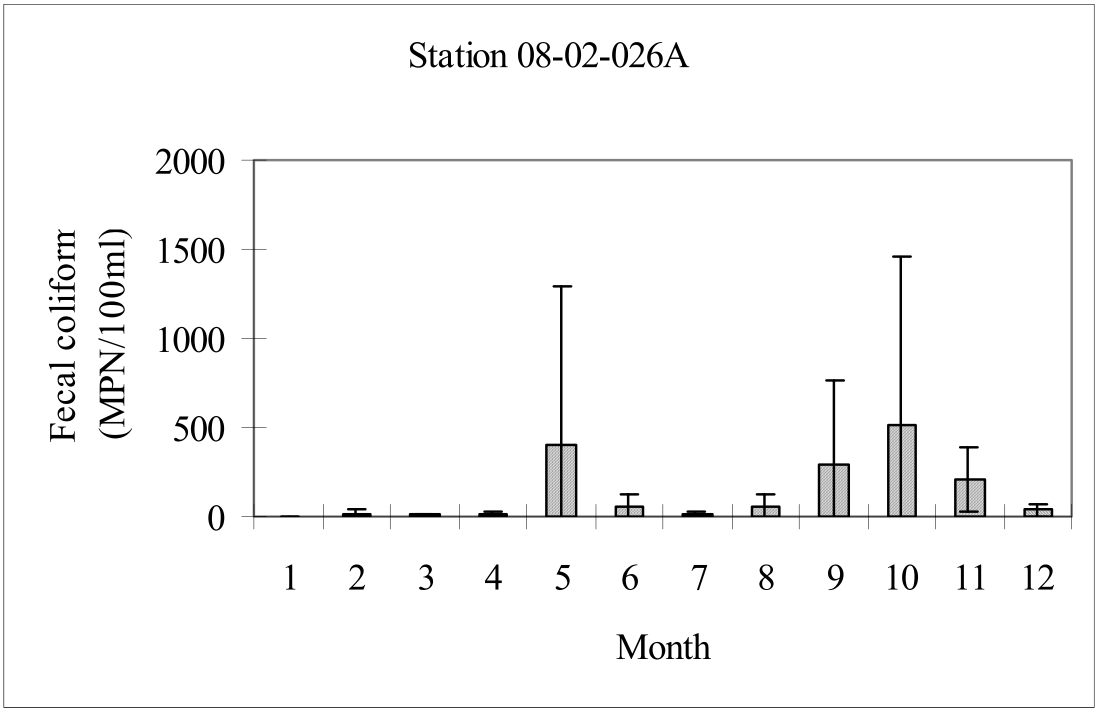


Figure C-5: Seasonality analysis of fecal coliform at Wye River Station 08-02-026A

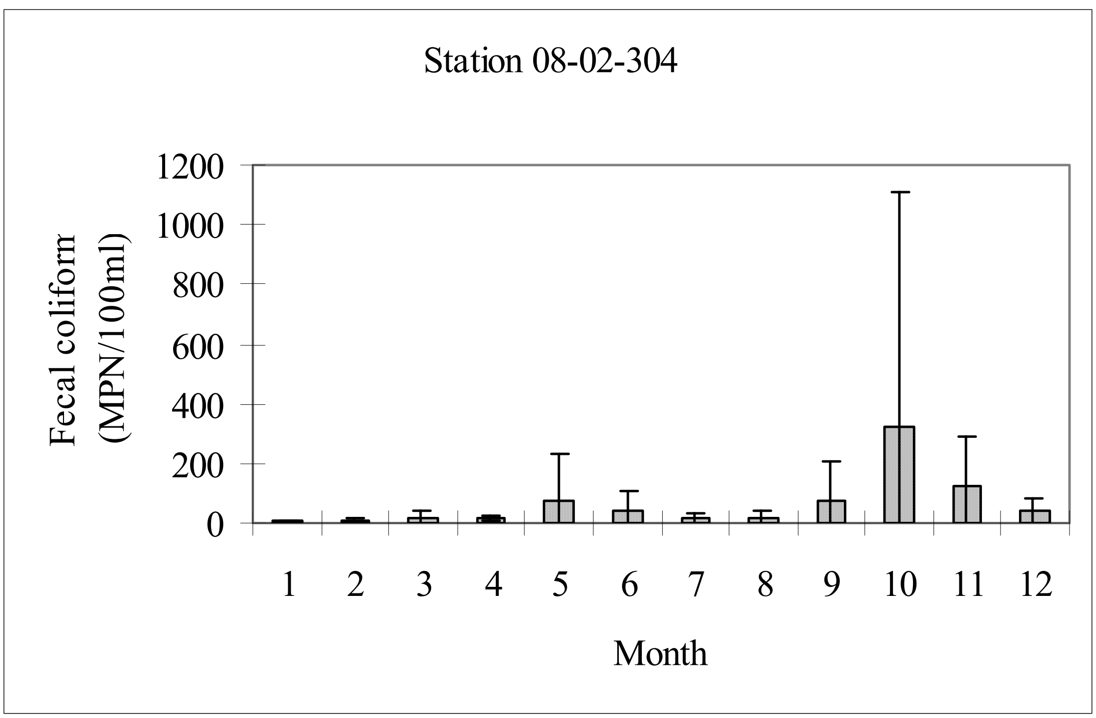


Figure C-6: Seasonality analysis of fecal coliform at Wye River Station 08-02-304

Appendix D. Tabulation of Fecal Coliform Data

This appendix provides a tabulation of fecal coliform values for the monitoring stations of the Wye River of the Wye River Basin in Tables D-1 through D-6. These data are plotted in report Figures 2.2.2 through 2.2.7.

Table D-1: Observed Fecal Coliform data at Wye River Station 08-02-013A

DATE	Fecal Coliform MPN/100 ml	DATE	Fecal Coliform MPN/100 ml
6/13/2000	43	3/11/2003	3.6
6/22/2000	23	3/20/2003	3.6
7/5/2000	9.1	4/17/2003	23
7/19/2000	93	4/30/2003	7.2
9/25/2000	3.6	5/13/2003	7.3
11/20/2000	15	6/2/2003	1
12/7/2000	1	6/16/2003	7.3
1/25/2001	1	6/30/2003	9.1
4/24/2001	3.6	7/10/2003	15
5/16/2001	1	7/22/2003	23
6/18/2001	93	8/7/2003	3.6
6/28/2001	43	8/18/2003	23
8/1/2001	1	9/2/2003	9.1
8/15/2001	15	9/11/2003	1
8/29/2001	9.1	9/16/2003	93
9/4/2001	1	10/30/2003	240
9/18/2001	1	11/3/2003	240
10/1/2001	9.1	11/17/2003	43
10/17/2001	1	12/3/2003	43
10/31/2001	1	1/5/2004	1
11/5/2001	3.6	2/25/2004	3.6
12/3/2001	15	3/29/2004	7
1/17/2002	1	4/5/2004	43
1/23/2002	1	4/19/2004	23
2/12/2002	1	5/3/2004	15
3/4/2002	3.6	5/17/2004	1
3/19/2002	9.1	6/1/2004	7.3
4/1/2002	1	6/15/2004	3.6
4/15/2002	23	6/29/2004	1
5/3/2002	460	7/6/2004	9.1

DATE	Fecal Coliform MPN/100 ml	DATE	Fecal Coliform MPN/100 ml
5/16/2002	1	8/2/2004	23
6/3/2002	3.6	8/10/2004	9.1
6/24/2002	9.1	8/25/2004	3.6
7/1/2002	1	9/1/2004	3.6
7/16/2002	1	9/23/2004	1
8/5/2002	3.6	9/29/2004	240
8/19/2002	1	10/7/2004	9.1
8/29/2002	9.1	10/20/2004	9.1
9/3/2002	43	1/6/2005	3.6
9/17/2002	9.1	2/22/2005	1
10/1/2002	1	4/7/2005	3.6
10/17/2002	43	4/28/2005	3.6
11/18/2002	23	5/5/2005	3.6
12/4/2002	1	6/7/2005	43
1/13/2003	1	6/22/2005	7.3

Table D-2: Observed Fecal Coliform data at Wye River Station 08-02-014

DATE	Fecal Coliform MPN/100 ml	DATE	Fecal Coliform MPN/100 ml
6/13/2000	15	3/11/2003	1
6/22/2000	9.1	3/20/2003	3.6
7/5/2000	9.1	4/17/2003	7.3
7/19/2000	9.1	4/30/2003	1
9/25/2000	9.1	5/13/2003	9.1
11/20/2000	1	6/2/2003	3.6
12/7/2000	1	6/16/2003	3.6
1/25/2001	1	6/30/2003	93
4/24/2001	21	7/10/2003	150
5/16/2001	1	7/22/2003	9.1
6/18/2001	75	8/7/2003	23
6/28/2001	1	8/18/2003	23
8/1/2001	3.6	9/2/2003	9.1
8/15/2001	9.1	9/11/2003	1
8/29/2001	1	9/16/2003	43
9/4/2001	1	10/30/2003	2400
9/18/2001	1	11/3/2003	240
10/1/2001	3.6	11/17/2003	43

DATE	Fecal Coliform MPN/100 ml	DATE	Fecal Coliform MPN/100 ml
10/17/2001	9.1	12/3/2003	43
10/31/2001	3.6	1/5/2004	1
11/5/2001	23	2/25/2004	3.6
12/3/2001	9.1	3/29/2004	3.6
1/17/2002	1	4/5/2004	43
1/23/2002	1	4/19/2004	3.6
2/12/2002	1	5/3/2004	9.1
3/4/2002	1	5/17/2004	3.6
3/19/2002	7.3	6/1/2004	9.1
4/1/2002	23	6/15/2004	1
4/15/2002	3.6	6/29/2004	1
5/3/2002	240	7/6/2004	3.6
5/16/2002	1	8/2/2004	9.1
6/3/2002	9.1	8/10/2004	3.6
6/24/2002	3.6	8/25/2004	3
7/1/2002	9.1	9/23/2004	9.1
7/16/2002	1	9/29/2004	43
8/5/2002	11	10/7/2004	3.6
8/19/2002	1	10/20/2004	43
8/29/2002	1	1/6/2005	1
9/3/2002	240	2/22/2005	3.6
9/17/2002	9.1	3/9/2005	3.6
10/1/2002	1	4/7/2005	93
10/17/2002	23	4/28/2005	1
11/18/2002	23	5/5/2005	3.6
12/4/2002	1	6/7/2005	240
1/13/2003	1	6/22/2005	9.1

Table D-3: Observed Fecal Coliform data at Wye River Station 08-02-023

DATE	Fecal Coliform MPN/100 ml	DATE	Fecal Coliform MPN/100 ml
6/13/2000	43	3/20/2003	1
6/22/2000	23	4/17/2003	9.1
7/5/2000	9.1	4/30/2003	9.1
7/19/2000	43	5/13/2003	23
9/25/2000	9.1	6/2/2003	43
11/20/2000	1	6/16/2003	9.1
12/7/2000	1	6/30/2003	23
1/25/2001	1	7/10/2003	15

DATE	Fecal Coliform MPN/100 ml	DATE	Fecal Coliform MPN/100 ml
4/24/2001	7.3	7/22/2003	1
5/16/2001	3.6	8/7/2003	1
6/18/2001	15	8/18/2003	93
6/28/2001	9.1	9/2/2003	3.6
8/1/2001	23	9/11/2003	1
8/15/2001	3.6	9/16/2003	23
8/29/2001	1	10/30/2003	1100
9/4/2001	9.1	11/3/2003	240
9/18/2001	21	11/17/2003	93
10/1/2001	9.1	12/3/2003	93
10/17/2001	43	1/5/2004	1
10/31/2001	3.6	2/25/2004	1
11/5/2001	3.6	3/29/2004	7.3
12/3/2001	15	4/5/2004	93
1/17/2002	1	4/19/2004	23
1/23/2002	3.6	5/3/2004	1
2/12/2002	1	5/17/2004	3.6
3/4/2002	1	6/1/2004	23
3/19/2002	9.1	6/15/2004	1
4/1/2002	1	6/29/2004	3.6
4/15/2002	3.6	7/6/2004	1
5/3/2002	150	8/2/2004	7.3
5/16/2002	3	8/10/2004	7.2
6/3/2002	3.6	8/25/2004	7.3
6/24/2002	43	9/1/2004	3.6
7/1/2002	1	9/23/2004	23
7/16/2002	3.6	9/29/2004	15
8/5/2002	3.6	10/7/2004	43
8/19/2002	1	10/20/2004	23
8/29/2002	21	1/6/2005	1
9/3/2002	240	2/22/2005	1
9/17/2002	9.1	3/9/2005	1
10/1/2002	3.6	4/7/2005	23
10/17/2002	23	4/28/2005	1
11/18/2002	43	5/5/2005	3.6
12/4/2002	3.6	6/7/2005	93
1/13/2003	1	6/22/2005	9.1
3/11/2003	1		

Table D-4: Observed Fecal Coliform data at Wye River Station 08-02-026

DATE	Fecal Coliform MPN/100 ml	DATE	Fecal Coliform MPN/100 ml
6/13/2000	23	3/20/2003	1
6/22/2000	240	4/17/2003	9.1
7/5/2000	23	4/30/2003	3.6
7/19/2000	23	5/13/2003	15
9/25/2000	23	6/2/2003	9.1
11/20/2000	23	6/16/2003	9.1
12/7/2000	1	6/30/2003	15
1/25/2001	3.6	7/10/2003	23
4/24/2001	3.6	7/22/2003	23
5/16/2001	1	8/7/2003	15
6/18/2001	460	8/18/2003	23
6/28/2001	15	9/2/2003	9.1
8/1/2001	43	9/11/2003	9.1
8/15/2001	43	9/16/2003	43
8/29/2001	3.6	10/30/2003	2400
9/4/2001	20	11/3/2003	240
9/18/2001	23	11/17/2003	43
10/1/2001	15	12/3/2003	240
10/17/2001	43	1/5/2004	1
10/31/2001	9.1	2/25/2004	1
11/5/2001	3.6	3/29/2004	9.1
12/3/2001	23	4/5/2004	43
1/17/2002	1	4/19/2004	23
1/23/2002	1	5/3/2004	9.1
2/12/2002	3	5/17/2004	1
3/4/2002	3.6	6/1/2004	43
3/19/2002	43	6/15/2004	1
4/1/2002	1	6/29/2004	11
4/15/2002	1	7/6/2004	9.1
5/3/2002	460	8/2/2004	23
5/16/2002	3.6	8/10/2004	9.1
6/3/2002	9.1	8/25/2004	9.1
6/24/2002	23	9/1/2004	1
7/1/2002	9.1	9/23/2004	23
7/16/2002	3.6	9/29/2004	43
8/5/2002	3.6	10/7/2004	3.6

DATE	Fecal Coliform MPN/100 ml	DATE	Fecal Coliform MPN/100 ml
8/19/2002	23	10/20/2004	23
8/29/2002	23	1/6/2005	1
9/3/2002	460	2/22/2005	3.6
9/17/2002	43	3/9/2005	3.6
10/1/2002	1	4/7/2005	9.1
10/17/2002	23	4/28/2005	3.6
11/18/2002	75	5/5/2005	1
12/4/2002	3.6	6/7/2005	23
1/13/2003	1	6/22/2005	7.3
3/11/2003	9.1		

Table D-5: Observed Fecal Coliform data at Wye River Station 08-02-026A

DATE	Fecal Coliform MPN/100 ml	DATE	Fecal Coliform MPN/100 ml
1/17/2002	1	9/11/2003	9.1
1/23/2002	1	9/16/2003	1100
2/12/2002	43	10/30/2003	2400
3/4/2002	3.6	11/3/2003	460
3/19/2002	23	11/17/2003	23
4/5/2002	21	12/3/2003	75
5/3/2002	2400	1/5/2004	3.6
5/16/2002	3.6	2/25/2004	1
6/3/2002	3.6	3/29/2004	23
6/24/2002	43	4/5/2004	23
7/1/2002	7.3	4/19/2004	9.1
7/16/2002	23	5/3/2004	9.1
8/5/2002	43	5/17/2004	3.6
8/19/2002	3	6/1/2004	15
8/29/2002	3.6	6/15/2004	3
9/3/2002	1100	6/29/2004	93
9/17/2002	9.1	7/6/2004	3.6
10/1/2002	1	8/2/2004	23
10/17/2002	75	8/10/2004	3.6
11/18/2002	150	8/25/2004	3.6
12/4/2002	3.6	9/1/2004	3.6
3/1/2003	3.6	9/23/2004	43
3/20/2003	3.6	9/29/2004	43
4/17/2003	9.1	10/7/2004	75

DATE	Fecal Coliform MPN/100 ml	DATE	Fecal Coliform MPN/100 ml
4/30/2003	23	10/20/2004	9.1
5/13/2003	7.3	1/6/2005	1
6/2/2003	39	2/22/2005	9.1
6/16/2003	23	3/9/2005	7.3
6/30/2003	240	4/7/2005	15
7/10/2003	23	4/28/2005	3.6
7/22/2003	1	5/5/2005	1
8/7/2003	240	6/7/2005	43
8/18/2003	93	6/22/2005	9.1
9/2/2003	9.1		

Table D-6: Observed Fecal Coliform data at Wye River Station 08-02-304

DATE	Fecal Coliform MPN/100 ml	DATE	Fecal Coliform MPN/100 ml
6/13/2000	23	3/20/2003	9.1
6/22/2000	93	4/17/2003	3.6
7/5/2000	43	4/30/2003	23
7/19/2000	23	5/13/2003	23
9/25/2000	3.6	6/2/2003	9.1
11/20/2000	23	6/16/2003	21
12/7/2000	1	6/30/2003	43
1/25/2001	1	7/10/2003	39
4/24/2001	9.1	7/22/2003	7.3
5/16/2001	3.6	8/7/2003	93
6/18/2001	240	8/18/2003	9.1
6/28/2001	1	9/2/2003	3.6
8/1/2001	3.6	9/11/2003	43
8/15/2001	43	9/16/2003	460
8/29/2001	9.1	10/30/2003	2400
9/4/2001	9.1	11/3/2003	460
9/18/2001	23	11/17/2003	15
10/1/2001	43	12/3/2003	75
10/17/2001	43	1/5/2004	3.6
10/31/2001	1	2/25/2004	9.1
11/5/2001	11	3/29/2004	7.3
12/3/2001	93	4/5/2004	9.1
1/17/2002	3.6	4/19/2004	3.6
1/23/2002	9.1	5/3/2004	3.6

DATE	Fecal Coliform MPN/100 ml	DATE	Fecal Coliform MPN/100 ml
2/12/2002	1	5/17/2004	1
3/4/2002	1	6/1/2004	15
3/19/2002	75	6/15/2004	9.1
4/1/2002	3.6	6/29/2004	9.1
4/15/2002	23	7/6/2004	3.6
5/3/2002	460	8/2/2004	9.1
5/16/2002	1	8/10/2004	3.6
6/3/2002	3.6	8/25/2004	9.1
6/24/2002	3.6	9/1/2004	3.6
7/1/2002	7.3	9/23/2004	43
7/16/2002	9.1	9/29/2004	150
8/5/2002	9.1	10/7/2004	23
8/19/2002	1	10/20/2004	7.3
8/29/2002	3.6	1/6/2005	1
9/3/2002	43	2/22/2005	23
9/17/2002	43	3/9/2005	1
10/1/2002	9.1	4/7/2005	23
10/17/2002	43	4/28/2005	23
11/18/2002	93	5/5/2005	9.1
12/4/2002	3.6	6/7/2005	93
1/13/2003	9.1	6/22/2005	7.3
3/11/2003	1		