Total Maximum Daily Loads of Fecal Coliform in the Restricted Shellfish Harvesting Area of Ellis Bay in the Lower Wicomico River in Wicomico County, Maryland

FINAL

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List of Abbreviations

ARA	Antibiotic Resistance Analysis
BAT	Best Available Technology
BIBI	Benthic Index of Biotic Integrity
BMP	Best Management Practice
BRFAC	Bay Restoration Fund Advisory Committee
BST	Bacteria Source Tracking
CBP	Chesapeake Bay Program
cfs	Cubic Feet per Second
CFR	Code of Federal Regulations
COMAR	Code of Maryland Regulations
CREP	Conservation Reserve Enhancement Program
CWA	Clean Water Act
CWP	Center for Watershed Protection
DNR	Department of Natural Resources
EPA	Environmental Protection Agency
EQIP	Environmental Quality Incentives Program
FDA	Food and Drug Administration
FIBI	Fish Index of Biotic Integrity
GIS	Geographic Information System
LA	Load Allocation
MACS	Maryland Agricultural Cost Share Program
MDA	Maryland Department of Agriculture
MDE	Maryland Department of the Environment
MDP	Maryland Department of Planning
ml	Milliliter(s)
MOS	Margin of Safety
MPN	Most Probable Number
MS4	Municipal Separate Storm Sewer System
NOAA	National Oceanic and Atmospheric Administration
NPDES	National Pollution Discharge Elimination System
NSSP	National Shellfish Sanitation Program
PCBs	Polychlorinated Biphenyls
T^{-1}	Per Tidal Cycle
TMDL	Total Maximum Daily Load
USGS	United States Geological Survey
WLA	Wasteload Allocation
WIP	Watershed Implementation Plan
WQIA	Water Quality Improvement Act
WQLS	Water Quality Limited Segment
WQS	Water Quality Standards
WWTP	Waste Water Treatment Plant

EXECUTIVE SUMMARY

Section 303(d) of the federal Clean Water Act (CWA) and the U.S. Environmental Protection Agency (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 (WQSs). For each WQLS, the State is to either establish a Total Maximum Daily Load (TMDL) for the specified substance that the waterbody can receive without violating WQSs, or demonstrate that WQSs are being met (CFR 2020a). This document, upon approval by the EPA, establishes a TMDL for fecal coliform in the restricted shellfish area of Ellis Bay in the Lower Wicomico River Basin in Maryland.

Maryland WQSs specify that all surface waters of the State shall be protected for water contact recreation, fishing, and protection of aquatic life and wildlife (COMAR 2020a). The Maryland Department of the Environment (MDE) first identified the waterbody of Ellis Bay in lower Wicomico River Mesohaline Chesapeake Bay Segment (Integrated Report Assessment Unit ID: MD-WICMH-Ellis_Bay) on the State's 2014 Integrated Report as impaired by fecal coliform in the restricted shellfish harvesting area (MDE 2018a). The TMDL established herein by MDE will address the fecal coliform listing for the restricted shellfish harvesting area in Ellis Bay. A data solicitation was conducted for this TMDL, and all readily available data have been considered.

Fecal coliform 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 pathogen-bearing 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.

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 the US Food and Drug Administration (FDA). Fecal coliforms are indicator organisms 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 contaminated waters.

The overall objective of the fecal coliform TMDL established in this document is to ensure that the "shellfish harvesting" designated use, which is protective of human health related to the consumption of shellfish from these shellfish harvesting areas, is supported. Field observations collected between 2016 and 2019 and a steady-state tidal prism model were used to estimate the current fecal coliform load based on volume and concentration, and to establish allowable loads for Ellis Bay restricted shellfish harvesting area in the Lower Wicomico River Basin. The tidal prism model incorporates influences of freshwater discharge, tidal transport, and fecal coliform decay, thereby representing the fate and transport of fecal coliform in the restricted shellfish

harvesting area. The loadings from non-point sources (human, livestock, pets, and wildlife) were quantified through bacteria source tracking (BST) analysis using data collected in the Lower Wicomico River basin over a one-year period. The dominant source is from wildlife, which accounts for 56% of the total non-point source, followed by sources from human (25%), livestock (12%) and pets (7%). There are no point sources identified within the drainage area of the restricted shellfish harvesting area of Ellis Bay.

The allowable loads for the restricted shellfish harvesting area were 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 for a three-tube decimal dilution. An implicit Margin of Safety (MOS) was incorporated into the analysis to account for uncertainty.

The goal in setting TMDL allocations is to determine the maximum allowable load for each known source in the watershed that will ensure the attainment of the water quality standard. The TMDL allocation proposed in this document was developed based on the criterion requiring the largest percent reduction - the 90th percentile criterion for Ellis Bay restricted shellfish harvesting area. Reductions by each source category were assigned by first managing controllable sources (human, livestock and pets) and then determining if the TMDL could be achieved. If the total required reduction was not achieved, then the wildlife source was reduced.

Summaries of the baseline loads, TMDL allocations and reductions for the restricted shellfish harvesting area of Ellis Bay in the Lower Wicomico River basin are presented in Table ES-1. It is important to note that the TMDLs presented herein should be applied as a three-year averaging period consistent with the water quality criteria for fecal coliform.

Table ES-1: Summary of Fecal Coliform Baseline Loads, TMDL Allocations, and Load Reductions for the Restricted Shellfish Harvesting Area of Ellis Bay in the Lower Wicomico River

Source	Baseline Load (Counts/day)	Baseline Load (%)	TMDL (Counts/day)	TMDL Reduction (%)
Human	4.05E+11	25%	0	100%
Livestock	1.94E+11	12%	0	100%
Wildlife	9.07E+11	56%	5.99E+11	34%
Pets	1.13E+11	7%	0	100%
Nonpoint Sources	1.62E+12	100%	5.99E+11	63%
Point Sources	NA	NA	NA	NA
MOS	-	-	Implicit	-
Total	1.62E+12	100%	5.99E+11	63%

Federal regulations require that TMDL analysis take into account the impact of critical conditions and seasonality on water quality (CFR 2020b). The intent of these requirements is to ensure that load reductions required by this TMDL, when implemented, will produce water quality conditions supportive of the designated use at all times. The 90^{th}-percentile concentration is the concentration exceeded only 10% of the time. Since data collected during the most recent three-year period was used to calculate the 90^{th} percentile, the critical condition is implicitly included in the value of the 90^{th} percentile. 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's 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.

Once EPA has approved this 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 TMDL reductions to be implemented in an iterative process that first addresses those sources with the largest impact to water quality, with consideration given to cost of implementation. The source contributions estimated from the BST 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 the data will be used to assess the effectiveness of the Department's implementation efforts on an ongoing basis.

1.0 INTRODUCTION

Section 303(d) of the federal Clean Water Act (CWA) and the U.S. Environmental Protection Agency (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 (WQSs). For each WQLS, the State is to either establish a TMDL for the specified substance that the waterbody can receive without violating WQSs, or demonstrate that WQSs are being met (CFR 2020a). This document, upon approval by the EPA, establishes Total Maximum Daily Loads (TMDL) for fecal coliform in the restricted shellfish harvesting areas of Ellis Bay in the Lower Wicomico River in Maryland.

TMDLs are established to determine the maximum pollutant load at which WQSs are met. A WQS 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, protection of aquatic life, fish and shellfish propagation and harvesting. 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.

Maryland WQSs specify that all surface waters of the State shall be protected for water contact recreation, fishing, and protection of aquatic life and wildlife (COMAR 2020a). The Maryland Department of the Environment (MDE) first identified the waterbody of Ellis Bay in Lower Wicomico River Mesohaline Chesapeake Bay Segment (Integrated Report Assessment Unit ID: MD-WICMH-Ellis_Bay) on the State's 2014 Integrated Report as impaired by fecal coliform in the restricted shellfish harvesting area (MDE 2018a). The TMDL established herein by MDE will address the fecal coliform listing for the restricted shellfish harvesting area in Ellis Bay, for which a data solicitation was conducted, and all readily available data have been considered.

Fecal coliform 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 pathogen-bearing 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.

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 the US Food and Drug Administration (FDA). Fecal coliforms are indicator organisms 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 contaminated waters.

2.0 SETTING AND WATER QUALITY DESCRIPTION

2.1 General Setting

Location

Ellis Bay is located at the mouth of Wicomico River in Wicomico County, Maryland. The lower Wicomico River, which drains directly to the Chesapeake Bay, is located on Maryland's eastern shore. Ellis Bay is located within the eponymous Ellis Bay Wildlife Management Area, which was purchased in 1957 by the Maryland Department of Natural Resources (DNR) in order to preserve Chesapeake Bay wetlands, and is still managed by DNR today. Mostly marsh and forested wetland, Ellis Bay is an ideal habitat for ducks, geese, wading birds, and many other wildlife species.

The lower Wicomico River, shown in Figure 1, is located in the Atlantic Coastal Plain Eco-region. The topography of the watershed is generally flat to slightly rolling and the soils are relatively well-drained with a low water table and fairly good percolation. The soils mainly consist of sand, silt, and clay (USDA 2020). It can be characterized as moderate to low runoff. The dominant tide in this region is the lunar semi-diurnal (M_2) tide, with a tidal range of 0.54 m in the nearby National Oceanic and Atmospheric Administration (NOAA) station with a tidal period of 12.42 hours (NOAA 2020). Table 1 presents the mean volume and mean water depth of Ellis Bay restricted shellfish harvesting area. The drainage area of Ellis Bay restricted shellfish harvesting area is about 7,859 acres (Figure 1).

Table 1: Physical Characteristics of Ellis Bay Restricted Shellfish Harvesting Area

Restricted Shellfish Harvesting Area	Mean Water Volume in m^3	Mean Water Depth in m
Ellis Bay	1.69×10^6	0.9

Land Use

Land use data from the Phase 6 Chesapeake Bay Watershed Model was used to estimate the land use information for the drainage area of Ellis Bay restricted shellfish harvesting waters. The land use percentage distribution (excluding the restricted waters) is shown in Table 2 and Figure 2.

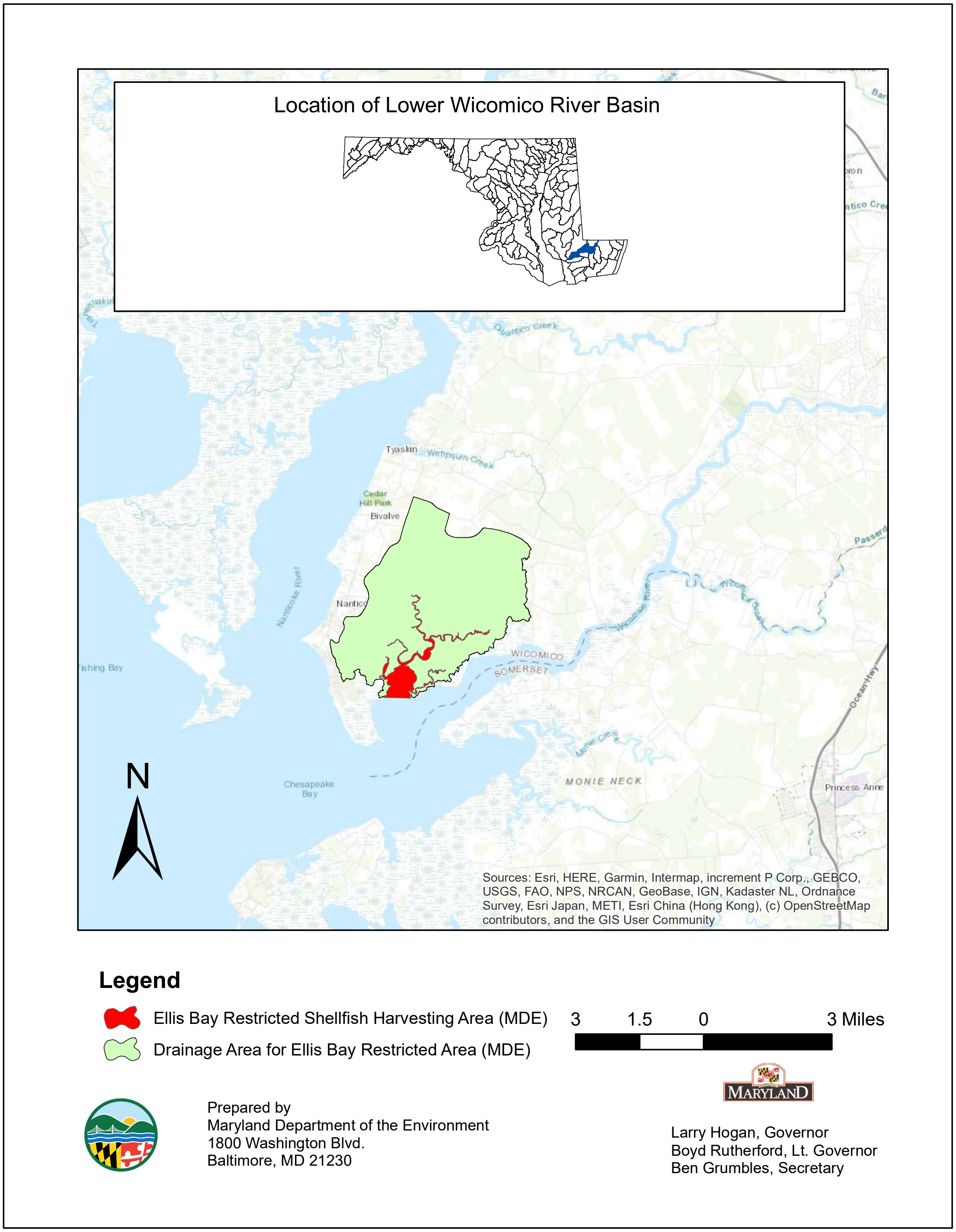

Figure 1: Location Map of Ellis Bay Restricted Shellfish Harvesting Area and the Drainage Area in the Lower Wicomico River Basin

Table 2: Land Use Percentage Distribution for Ellis Bay Watershed

Land Use	Acreage	Percentage
Water/Wetland	3,359	43%
Forest	3,662	47%
Agriculture	623	8%
Urban	215	3%
Totals	7,859	100%

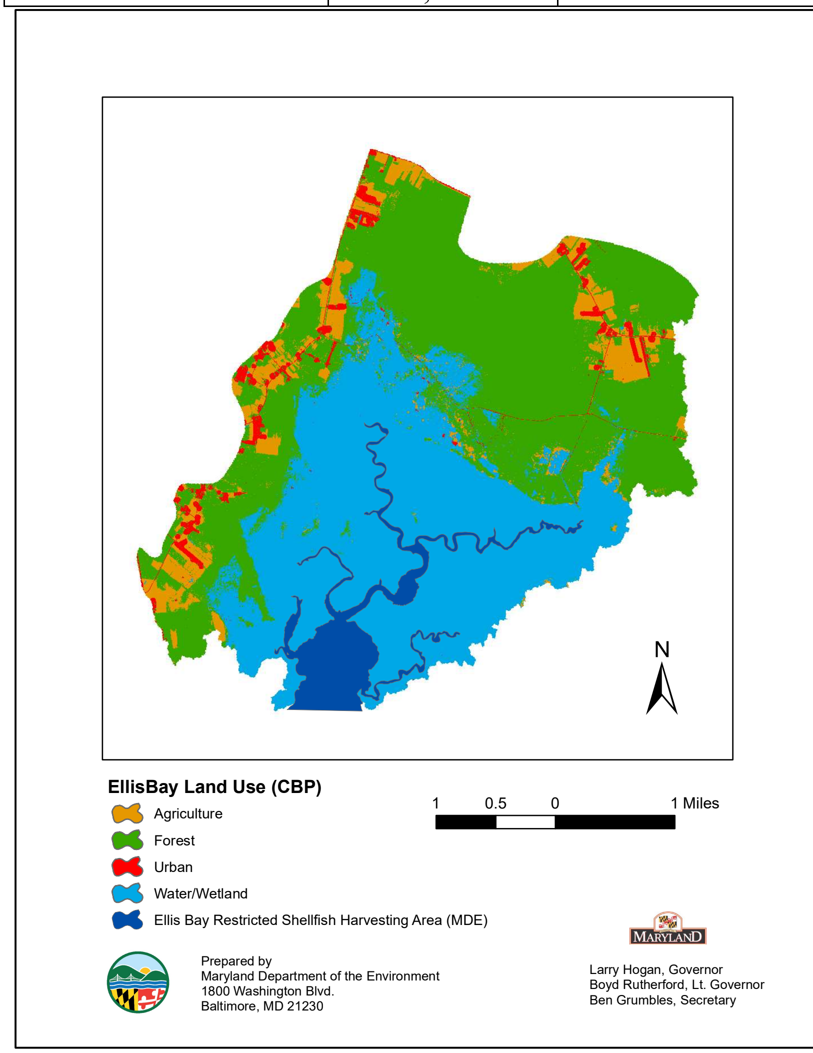

Figure 2: Land Use in the Ellis Bay Drainage Area

2.2 Water Quality Characterization and Impairment

Maryland WQSs specify that all surface waters of the State shall be protected for water contact recreation, fishing, and protection of aquatic life and wildlife (COMAR 2020a). The designated use of the restricted shellfish harvesting areas of Ellis Bay is Use II – *Support of Estuarine and Marine Aquatic Life and Shellfish Harvesting* (COMAR 2020b). There are no "high quality" or Tier II, stream segments (Benthic Index of Biotic Integrity [BIBI] and Fish Index of Biotic Integrity [FIBI] aquatic life assessment scores > 4 [scale 1-5]) located within the drainage area of Ellis Bay restricted shellfish harvesting area (COMAR 2020c).

MDE's Shellfish Certification Program is responsible for classifying shellfish harvesting waters to ensure oysters and clams are safe for human consumption. As discussed in the Introduction, MDE adheres to the requirements of the 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 has monitored shellfish growing regions throughout Maryland. The shellfish monitoring stations in and adjacent to the restricted shellfish harvesting area of Ellis Bay are shown in Figure 3.

The fecal coliform impairments addressed in this document were 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:

> 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 (COMAR 2020d).[1]

[1] Note that Maryland uses the three-tube decimal dilution test for fecal coliform bacteria monitoring purposes.

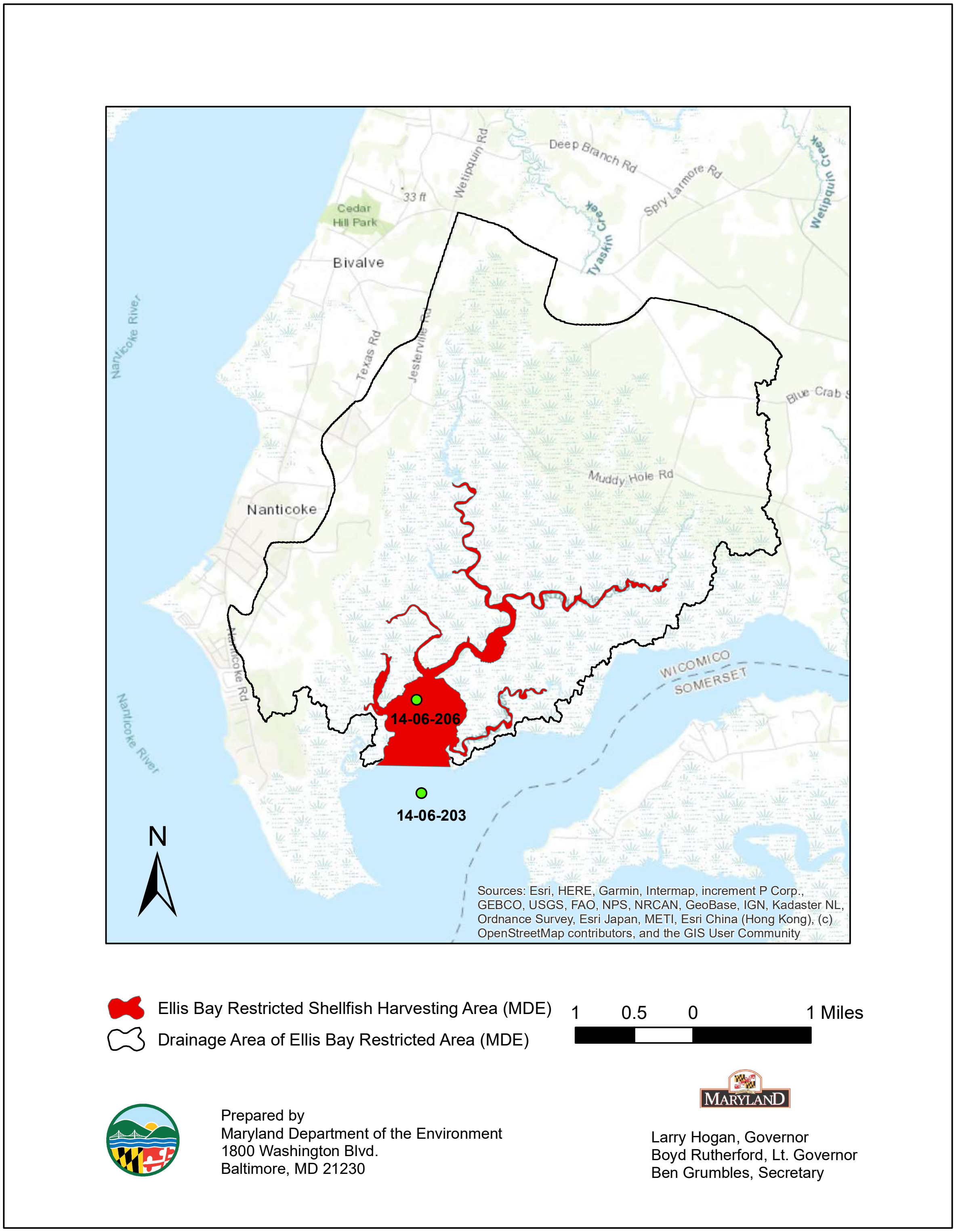

Figure 3: Shellfish Monitoring Stations in and Adjacent to the Ellis Bay Restricted Shellfish Harvesting Area

For this report, the water quality impairment was assessed based on the three-year median and 90th percentile concentrations from November 2016 to November 2019. Descriptive statistics of the monitoring data at the stations for Ellis Bay restricted shellfish harvesting area and the water quality criterion are shown in Table 3. Station 14-06-206 is located inside of the restricted area and fecal coliform data exceed both median and 90th percentile criterion. Station 14-06-203 is located at the boundary and outside of the restricted area and there are no exceedances of fecal coliform criteria at this station.

Table 3: Summary of Fecal Coliform Data for Ellis Bay Restricted Shellfish Harvesting Area from November 2016 to November 2019

Area Name	Station	Median		90th Percentile	
		Monitoring Data	Criterion	Monitoring Data	Criterion
		MPN/100ml	MPN/100ml	MPN/100ml	MPN/100ml
Ellis Bay	14-06-206	15	14	93	49
	14-06-203	7.3	14	43	49

2.3 Source Assessment

Fecal coliform 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. This section provides a summary of the sources that have been identified as contributing fecal coliform loads to the restricted shellfish area of Ellis Bay.

<u>Nonpoint Sources</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 draining to 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. During rain events, surface runoff can transport fecal coliform from the land surface to the restricted shellfish harvesting area. The transport of fecal coliform from land surface to the restricted shellfish harvesting area is dictated by the hydrology, soil type, land use, and topography of the watershed. Deposition of non-human fecal coliform directly to a restricted shellfish harvesting area can 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.

In order to determine the sources of fecal coliform contribution and reduction needed to achieve water quality criteria and to allocate fecal coliform loads among these sources, it is necessary to identify all existing sources. MDE conducted sampling over a one-year period (November 2002 to October 2003) in the shellfish harvesting area of the Lower Wicomico River using a bacteria source tracking (BST) methodology 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. The detailed description of the BST analysis are presented in Appendix B.

In the lower Wicomico 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. Pet contributions usually occur through runoff from streets and land. Human sources mainly result from failure of septic systems and from recreation vessel discharges. Table 4 summarizes the source distribution based on BST data analysis for restricted shellfish harvesting area of the Lower Wicomico River. The detailed description of the BST analysis are presented in Appendix B.

Table 4: Source Distribution Based on BST Data Analysis

Area	Human	Livestock	Wildlife	Pets
Ellis Bay	25%	12%	56%	7%

The BST data analysis includes a statistical comparison of known sources collected in the watershed and compared with unknown source samples collected over the study period. The fecal coliform sources in water samples are unknown until matched with the library of known sources. The dominant source is from wildlife for the restricted shellfish area of Ellis Bay. The source distributions from the BST analysis for the restricted shellfish area of Ellis Bay appear consistent with the land use information of the drainage area, in that the watershed is primarily forest and wetland.

Point Sources

There are no National Pollutant Discharge Elimination System (NPDES)-regulated municipal wastewater treatment plants (WWTPs), industrial process water facilities or regulated stormwater discharges under Phase I or Phase II of the NPDES stormwater program within the drainage area of the Ellis Bay restricted shellfish harvesting areas. Therefore, there are no point source allocations of fecal coliform loads for the drainage area of Ellis Bay restricted shellfish harvesting area.

Source Assessment Summary

From this source assessment, all known point and nonpoint sources of fecal coliform to the restricted area of Ellis Bay in Lower Wicomico River watershed have been identified and characterized. The nonpoint sources of fecal coliform are from four categories: human, livestock, wildlife and pets. There are no point sources with permits regulating the discharge of fecal coliform load identified for the restricted shellfish area. Estimated fecal coliform loads from these nonpoint sources represent the baseline conditions for the drainage area of the restricted shellfish harvesting area of Ellis Bay.

3.0 TARGETED WATER QUALITY GOAL

As described in the Water Quality Characterization and Impairment Sections above, MDE evaluates whether a waterbody is restricted for shellfish harvesting based on both the median fecal coliform concentration of 14 MPN/100ml and 90^{th}-percentile fecal coliform concentration of 49MPN/100ml of at least 30 samples taken over a three-year period. The overall objective of the fecal coliform TMDLs established in this document is to meet these criteria, ensuring that the "shellfish harvesting" designated use, which is protective of human health related to the consumption of shellfish, is supported.

The achievement of this water quality endpoint will ensure that all other designated uses will be supported relating to indicator bacteria. Maryland's criteria relating to recreational use in marine waters is based on enterococcus counts rather than the fecal coliform counts used in this TMDL. That criteria, a median value of 35 counts per 100 mL, originated from a 1986 Recreational Water Quality Criteria document from EPA, which related it to a fecal coliform concentration of 200 counts per 100 mL (USEPA 2012). The endpoints of this TMDL, a median value of 14 MPN/100 mL and a 90^{th}-percentile concentration of 49 MPN/100 mL, are well below that concentration. This indicates that by achieving the TMDL endpoints for shellfish harvesting, that the recreational designated use for Ellis Bay will be achieved as well.

4.0 TOTAL MAXIMUM DAILY LOADS AND LOAD ALLOCATION

4.1 Overview

A TMDL is the total amount of an impairing substance that a waterbody can receive and still meet WQSs. The TMDL may be expressed as a mass per unit time, toxicity, or other appropriate measure and should be presented in terms of wasteload allocations (WLAs) for point sources, load allocations (LAs) for nonpoint sources, and either an implicit or explicit margin of safety (MOS) (CFR 2017a):

$$\text{TMDL} = \text{WLAs} + \text{LAs} + \text{MOS} \qquad \text{(Equation 5.1)}$$

This section describes how the fecal coliform TMDL and the corresponding LAs and WLAs have been developed for the drainage area of the restricted shellfish harvesting area Ellis Bay in the lower Wicomico River.

4.2 Analysis Framework

In general, tidal waters are exchanged through their connecting boundaries. The tide and amount of freshwater discharge into the embayment are the dominant influences on the transport of fecal coliform. The methodology used assumes that freshwater input, tidal range, and the first-order decay of fecal coliform are all constant. The TMDL is calculated based on the steady state tidal prism model. Compared to the volumetric method (USEPA Shellfish Workshop 2002), the steady state tidal prism model provides improvements incorporating the influences of tidal induced transport, freshwater, and decay of fecal coliform in the embayment. A detailed description of the model is presented in Appendix A.

Using the steady state tidal prism model, the loads can be estimated according to the equation as follows (see also Appendix A):

$$L = \left[C\left(Q_b + kV\right) - Q_0 C_0\right] \times Cf \qquad \text{(Equation 5.2)}$$

where:
L = fecal coliform load (counts per day)
C = fecal coliform concentration (MPN /100ml) of embayment
Q_b = the quantity of mixed water that leaves the embayment on the ebb tide that did not enter the embayment on the previous flood tide (m^3 per tidal cycle)
k = the fecal coliform decay rate (per tidal cycle)
V = the mean volume of the embayment (m^3)
Q_0 = the quantity of water that enters the embayment on the flood tide through the ocean boundary that did not flow out of the embayment on the previous ebb tide (m^3 per tidal cycle)
C_0 = the fecal coliform concentration (MPN/100ml) at the oceanside boundary
Cf = the unit conversion factor

Q_b and Q_0 are estimated based on the steady state condition as follows:

$Q_b = Q_0 + Q_f$

where Q_f is mean freshwater discharge during the tidal cycle

$Q_0 = \beta Q_T$

where β is an exchange ratio and Q_T is the total ocean water entering the bay on the flood tide, which is calculated based on tidal range. The dominant tide in this region is the lunar semi-diurnal (M_2) tide with a tidal period of 12.42 hours; therefore, the M_2 tide is used for the representative tidal cycle. In general, the exchange ratio varies from 0.3 to 0.7, based on the previous model tests in Virginia coastal embayments (Kuo et al., 1998; Shen, Wang, and Sisson 2002). The observed salinity data were also used to estimate the exchange ratio. The estimated values range from 0.3-0.8; therefore, a value of 0.5 is used for the exchange ratio.

The stream flow used for the estimation of Q_f was based on the flows of the United States Geological Survey (USGS) gage # 01486000, located in Manokin Branch near Princess Anne, Somerset County, MD. For the restricted shellfish harvesting area, the average of three-year (November 2016 to November 2019) monthly average flow for this USGS gage (i.e., 5.78 cfs) was adjusted by the ratio of the Ellis Bay drainage area to that of the gage's drainage basin (3,072 acres) to derive estimates of the long-term flows. The estimated freshwater flow to the restricted shellfish harvesting area of Ellis Bay is 15.65 cfs.

Baseline Conditions

The most recent three-year (November 2016 to November 2019) median and 90^{th}-percentile fecal coliform concentrations in the restricted shellfish harvesting area were used to characterize the current (baseline) conditions. The monitoring station (14-06-203) is located outside of the restricted shellfish harvesting area and the fecal coliform concentration of this station was used as the boundary condition. Baseline concentrations for median and 90^{th} percentile scenarios and the estimated baseline loads from the drainage area of the restricted shellfish harvesting area of Ellis Bay are presented in Table 5.

Table 5: Baseline Loads Estimated for Ellis Bay Restricted Shellfish Harvesting Area

Median Scenario					
Restricted Shellfish Harvesting Area	**Embayment Median Fecal Coliform Concentration (MPN/100mL)**	**Boundary Median Fecal Coliform Concentration. (MPN/100mL)**	**Decay Rate Per Tidal Cycle**	**Estimated Residence Time (day)**	**Baseline Load (Counts/day)**
Ellis Bay	15.0	7.3	0.36	1.7	2.58E+11
90th Percentile Scenario					
Restricted Shellfish Harvesting Area	**Embayment 90th percentile Fecal Coliform Concentration (MPN/100mL)**	**Boundary 90th percentile Fecal Coliform Concentration. (MPN/100mL)**	**Decay Rate Per Tidal Cycle**	**Estimated Residence Time (day)**	**Baseline Load (Counts/day)**
Ellis Bay	93.0	43.0	0.36	1.7	1.62E+12

TMDL Scenarios

TMDL scenarios were run to estimate the maximum allowable loads the restricted shellfish harvesting areas could receive from the responding drainage area in order to assure that the fecal coliform concentrations within the embayment would not exceed the criteria. The allowable loads are calculated using the water quality criteria for the fecal coliform concentrations both in the embayment and at the boundary. The estimated allowable loads from the drainage area of the restricted shellfish harvesting area of Ellis Bay are presented in Table 6.

Table 6: Allowable Loads Estimated for Ellis Bay Restricted Shellfish Harvesting Area

Median Scenario					
Restricted Shellfish Harvesting Area	**Embayment Median Fecal Coliform Concentration (MPN/100mL)**	**Boundary Median Fecal Coliform Concentration. (MPN/100mL)**	**Decay Rate Per Tidal Cycle**	**Estimated Residence Time (day)**	**Allowable Load (Counts/day)**
Ellis Bay	14.0	14.0	0.36	1.7	1.71E+11
90th Percentile Scenario					
Restricted Shellfish Harvesting Area	**Embayment 90th percentile Fecal Coliform Concentration (MPN/100mL)**	**Boundary 90th percentile Fecal Coliform Concentration. (MPN/100mL)**	**Decay Rate Per Tidal Cycle**	**Estimated Residence Time (day)**	**Allowable Load (Counts/day)**
Ellis Bay	49.0	49.0	0.36	1.7	5.99E+11

4.3 Critical Condition and Seasonality

Federal regulations require TMDL analysis to take into account the impact of critical conditions and seasonality on water quality (CFR 2020a). The intent of this requirement is to ensure that water quality is protected when it is most vulnerable.

The 90th percentile concentration is the concentration exceeded only 10% of the time. Since data collected during the most recent three-year period was used to calculate the 90th percentile, the critical condition during that period is implicitly included in the value of the 90th percentile.

Similar to the critical condition, seasonality is also implicitly included in the analysis due to the duration of the period for which water quality standards are assessed. The monitoring design and the statistical analysis used to evaluate water quality attainment therefore implicitly include the effect of seasonality.

4.4 TMDL Allocations and Reductions

All TMDLs need to be presented as a sum of WLAs for point sources and LAs for nonpoint source loads generated within the assessment unit, and if applicable, LAs for the natural background, tributary, and adjacent segment loads (CFR 2020b). The State reserves the right to revise these allocations provided the revisions are consistent with achieving WQSs. The allocations described in this section summarize the fecal coliform TMDLs established to meet the shellfish harvesting designated use in the restricted shellfish harvesting area of Ellis Bay in the Lower Wicomico River Basin.

In Section 4.2, the baseline loads and allowable loads were estimated for the Ellis Bay restricted shellfish harvesting area. The load reductions needed for the attainment of the criteria for median and 90th percentile scenarios are determined by subtracting the allowable loads from the current loads and the results are listed in Table 7.

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

Table 7: Load Reductions for the Restricted Shellfish Harvesting Area

Median Scenario			
Restricted Shellfish Harvesting Area	**Current Load (Counts/day)**	**Allowable Load (Counts/day)**	**Required Reduction (%)**
Ellis Bay	2.58E+11	1.71E+11	34%
90th Percentile Scenario			
Restricted Shellfish Harvesting Area	**Current Load (Counts/day)**	**Allowable Load (Counts/day)**	**Required Reduction (%)**
Ellis Bay	1.62E+12	5.99E+11	63%

When comparing the median and the 90th percentile scenario results, the loads from scenario with greater percent load reductions were chosen as the final TMDL loads and used for source allocation. For the Ellis Bay restricted shellfish harvesting area, the 90th percentile scenario requires greater percent reduction to meet the criterion, therefore, the 90th percentile scenario results are chosen as the final TMDLs and will be used for source allocation for the restricted shellfish harvesting area. It is also important to note that the TMDLs presented herein should be applied as a three year averaging period with a minimum of 30 samples collected consistent with the water quality standard.

As stated in Section 4.2, there are no point sources with permits regulating the discharge of fecal coliform to the Ellis Bay restricted shellfish harvesting area, therefore there is no WLA allocation and all the loads will be allocated to the nonpoint sources.

According to Section 2.3, there are four nonpoint source categories of fecal coliform: human, livestock, wildlife and pets. Table 4 presents the percentage distribution from each category for the Ellis Bay restricted area. Source reductions were assigned by first managing controllable sources (human, livestock and pets) and then determining if the TMDL could be achieved. If the total required reduction was not achieved, then the wildlife source was reduced. Based on these assumptions, the source allocation for the watershed for each of the major source categories is estimated. Results are presented in Table 8.

Table 8: Load Allocations and Reductions by Sources

Ellis Bay				
Source	**Baseline Load (Counts/day)**	**Baseline Load (%)**	**TMDL (Counts/day)**	**TMDL Reduction (%)**
Human	4.05E+11	25%	0	100%
Livestock	1.94E+11	12%	0	100%
Wildlife	9.07E+11	56%	5.99E+11	34%
Pets	1.13E+11	7%	0	100%
Nonpoint Sources	1.62E+12	100%	5.99E+11	63%
Point Sources	NA	NA	NA	NA
Total	1.62E+12	100%	5.99E+11	63%

4.5 Margin of Safety

All TMDLs must include a MOS to account for the lack of knowledge and the many uncertainties in the understanding and simulation of water quality parameters in natural systems (*i.e.*, the relationship between modeled loads and water quality). The MOS is intended to account for such uncertainties in a manner that is conservative from the standpoint of environmental protection. 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 water bodies.

Based on previous analysis (VIMS 2004), it was determined that the most sensitive parameter used in this modeling is the decay rate. 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.

4.6 TMDL Summary

Table 9 summarizes the fecal coliform baseline loads, TMDL allocations and load reductions for the restricted shellfish harvesting area of Ellis Bay in the Lower Wicomico River basin.

Table 9: Summary of Fecal Coliform Baseline Loads, TMDL Allocations, Associated Percent Reductions for the Restricted Shellfish Harvesting Area of Ellis Bay in the Lower Wicomico River Basin

Ellis Bay				
Source	**Baseline Load (Counts/day)**	**Baseline Load (%)**	**TMDL (Counts/day)**	**TMDL Reduction (%)**
Human	4.05E+11	25%	0	100%
Livestock	1.94E+11	12%	0	100%
Wildlife	9.07E+11	56%	5.99E+11	34%
Pets	1.13E+11	7%	0	100%
Nonpoint Sources	1.62E+12	100%	5.99E+11	63%
Point Sources	NA	NA	NA	NA
MOS	-	-	Implicit	-
Total	1.62E+12	100%	5.99E+11	63%

5.0 ASSURANCE OF IMPLEMENTATION

This section provides the basis for reasonable assurances that the fecal coliform TMDL 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.

MDE anticipates that reductions in fecal coliform levels will be driven by a variety of state and county-level programs. These include programs that are intended to address bacteria sources directly, and ones that are intended to address other water quality concerns, such as nutrients, but that also impact bacteria loads. For example, practices put in place as part of a strategy for meeting the 2010 Chesapeake Bay TMDLs for nutrients and sediment, may also manage fecal coliform. Many of these practices are described in Maryland's 2019 Phase III Watershed Implementation Plan (WIP) for the Chesapeake Bay TMDL.

Existing Funding and Regulatory Framework

As of June 2019, the Bay Restoration Fund had funded over 10,000 upgrades of septic systems to Best Available Technology (BAT) across the state, with nearly 6,500 of these upgrades occurring in Maryland's Critical Areas, defined as land within 1,000 feet of the State's tidal waters (BRFAC 2020). Although BAT upgrades are designed to reduce nitrogen loadings, the fund prioritizes grants for failing septic systems in the Critical Areas. Failing systems are more likely to discharge fecal coliform to the environment, and fecal coliform discharged close to tidal waters have a greater likelihood of being delivered into those waters. Replacing a failing system with a properly functioning one, BAT or otherwise, reduces the likelihood of fecal coliform loadings to shellfish harvesting areas. The Bay Restoration Fund can also pay for projects that connect households to municipal sewers, another approach for addressing failing septic systems.

Agricultural practices can reduce potential livestock sources of bacteria within watersheds. Many of these practices are being implemented as part of the WIP, and funded through federal and state cost-share programs. Stream protection, either using fencing or remote watering holes away from streams, prevents direct animal contact with surface waters. These measures can be funded through Maryland Department Agriculture's (MDA's) Maryland Agricultural Water Quality Cost-Share (MACS) Program and USDA's Environmental Quality Incentives Program (EQIP). These programs also fund animal waste management systems—structures at animal feeding operations that enable waste to be handled and stored correctly and which control runoff. Riparian forest and grass buffers, linear strips of vegetation between fields and streams that can filter pollutants, can receive funding through MACS and USDA's Conservation Reserve Enhancement Program (CREP). Other funding mechanisms, such as the Chesapeake Bay Targeted Watersheds Grant Program can be used for pasture management. At a county level, Soil Conservation Districts promote soil and water quality programs, by providing technical expertise to farmers related to agricultural best management practices (BMPs).

Implementation of the 2010 Chesapeake Bay TMDL for nitrogen, phosphorus and sediment could also provide some reductions in fecal coliform. While the objectives of the two efforts differ, with the 2010 Bay TMDLs focused on nitrogen, phosphorus and sediment while this TMDL targets fecal coliform, many of the reductions achieved through implementation activities for the Bay TMDL could result in progress toward both goals.

In addition, funding for practices to address polluted runoff could be provided through grants from Maryland's Bay Restoration Fund or its Chesapeake and Atlantic Coastal Bays Trust Fund. While stormwater management practices are not typically designed to reduce bacteria loads, those that direct runoff from impervious surfaces into the groundwater would have that beneficial effect.

In terms of bacteria pollution from watercraft, Maryland law 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 (Maryland 1996). 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.

Portions of Wicomico County have a municipal separate storm sewer system (MS4) permit regulating stormwater. As part of the permit, there is a requirement for an education program for pet waste removal (MDE 2018b). It is anticipated that the program would be developed County-wide. An example of an outreach program is MDE's 2013 media campaign called, 'Scoop the Poop' This program included a social media campaign, tables set up at various festivals getting the information to the public and a website (MDE 2013). More information for this campaign, can be found at:
https://mde.maryland.gov/programs/Marylander/outreach/pages/scoop%20the%20poop.aspx

Regulatory enforcement of potential bacteria sources would be covered by MDE's routine sanitary surveys of shellfish growing areas and NPDES permitting activities. Also, although not directly linked, it is assumed that the nutrient management plans from the Water Quality Improvement Act of 1998 (WQIA) will result in some reduction of bacteria from manure application practices.

As part of Maryland's commitment to the NSSP, MDE's Shellfish Certification Program continues to monitor shellfish waters and classify shellfish harvesting areas as restricted, approved, or conditionally approved. A major component of MDE's responsibilities under the Shellfish Certification Program is to identify potential pollution sources and correct or eliminate them. Waters meeting shellfish water quality standards are reclassified as approved or conditionally approved harvesting areas. The removal of shellfish harvesting restrictions may serve as a tracking tool measuring water quality improvements. However, when performing such analyses, it is important to understand that, per FDA/NSSP requirements, areas located near point sources are expected to remain restricted. Existence of such restrictions does not necessarily mean that the area is not meeting water quality standards.

Implementation and Wildlife Sources

It is expected that, due to significant wildlife bacteria contribution, some waterbodies will not be able to meet water quality standards even after all anthropogenic sources are controlled. 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 the changing of a natural background condition is not the intended goal of a TMDL.

MDE envisions an iterative approach to TMDL implementation, which first addresses the controllable sources (i.e., human, livestock, and pets), especially those that have the largest impacts on water quality and create the greatest risks to human health, with consideration given to ease the cost of implementation. It is expected that the best management practices applied to controllable sources may also result in reduction of some wildlife sources. Following the initial implementation stage, MDE expects to re-assess the water quality to determine if the designated use is being attained. If the water quality standards are not attained, other sources may need to be controlled. However, if the required controls go beyond maximum practical reductions, MDE might 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: Method Used to Estimate Fecal Coliform Load

A steady-state Tidal Prism Model was used to estimate the fecal coliform loads from the drainage area of the restricted shellfish harvesting area of Ellis Bay in the Lower Wicomico River basin. A detailed description of the tidal flushing model is presented in this section. It is assumed that a single volume can represent a water body, and that the pollutant is well mixed in the water body system, as shown in Figure A-1.

The mass balance of water can be written as follows (Guo and Lordi 2000):

$$\frac{dV}{dT} = (Q_0 - Q_b + Q_f) \tag{1}$$

where Q_0 is the quantity of water that enters the embayment on the flood tide through the ocean boundary that did not flow out of the embayment on the previous ebb tide (m^3T^{-1}); Q_b is the quantity of mixed water that leaves the bay on the ebb tide that did not enter the bay on the previous flood tide (m^3 per tidal cycle); Q_f is total freshwater input over the tidal cycle (m^3); V is the volume of the bay (m^3); T is the dominant tidal period (hours).

It is further assumed that Q_0 is the pure ocean water that did not flow out of the embayment on the previous ebb tide, and that Q_b is the embayment water that did not enter into the system on the previous flood tide. The mass balance for the fecal coliform can then be written as follows:

$$\frac{dVC}{dT} = Q_0 C_0 - Q_b C + L_f + L_l - kVC \tag{2}$$

where L_f is the loading from upstream; L_l is the additional loading from the local area within the tidal cycle, k is the fecal coliform decay rate (or a damped parameter for the net loss of fecal coliform), C is fecal coliform concentration in the embayment, and C_0 is the fecal coliform concentration from outside the embayment.

In a steady-state condition, the mass balance equations for the water and the fecal coliform concentration can be written as follows:

$$Q_b = Q_0 + Q_f \tag{3}$$

$$Q_b C + kVC = Q_0 C_0 + L_f + L_l \tag{4}$$

The fecal coliform concentration in the embayment can be calculated as follows:

$$C = \frac{Q_0 C_0 + L_f + L_l}{Q_b + kV} \tag{5}$$

From Equation (4), assuming $L_f + L_l = Load_t$ and letting C_c be the criterion of fecal coliform in the embayment, the loading capacity can be estimated as:

$$Load_T = C_c(Q_b + kV) - Q_0 C_0 \qquad (6)$$

The daily load can be estimated based on the dominant tidal period in the area. For the Chesapeake Bay the dominant tide is lunar semi-diurnal (M_2) tide with a tidal period of 12.42 hours. If fecal coliform concentration is in MPN/100ml, the daily load (counts day^{-1}) can be estimated as:

$$Load = Load_T \times \frac{24}{12.42} \times 10000 \qquad (7)$$

In practice, one may not know Q_0 *a priori*. Instead, one is given the tidal range of the tidal embayment. From that, Q_T, the total ocean water entering the bay on the flood tide, can be calculated. From this, Q_0, the volume of new ocean water entering the embayment on the flood tide can be determined by the use of the ocean tidal exchange ratio β as:

$$Q_0 = \beta Q_T \qquad (8)$$

where β is the exchange ratio and Q_T is the total ocean water entering the bay on the flood tide. The exchange ratio can be estimated from salinity data (Fischer et al., 1979):

$$\beta = \frac{S_f - S_e}{S_0 - S_e} \qquad (9)$$

where S_f is the average salinity of ocean water entering the bay on the flood tide, S_e is the average salinity of the bay water leaving the bay, and S_0 is the salinity at the ocean side. The numerical value of β is usually smaller than 1, and it represents the fraction of new ocean water entering the embayment. Once Q_0 is known, then Q_b can be calculated from equation (3).

The residence time, T_L, is an estimate of time required to replace the existing pollutant concentration in a system; it can be calculated as follows:

$$T_L = \frac{V_b}{Q_b} \qquad (10)$$

where V_b is mean volume of the embayment. From the definition, the denominator can either be Q_T or Q_b. However, using Q_T assumes that the ocean water enters into the embayment during the flood tide is 100% new, whereas using Q_b takes into consideration that a portion of water is not entirely new. It can be shown that the latter is more realistic. If Q_b is used in the residence time calculation, it will result in a longer time scale than if Q_T is used (Ketchum 1951; Guo and Lordi 2000).

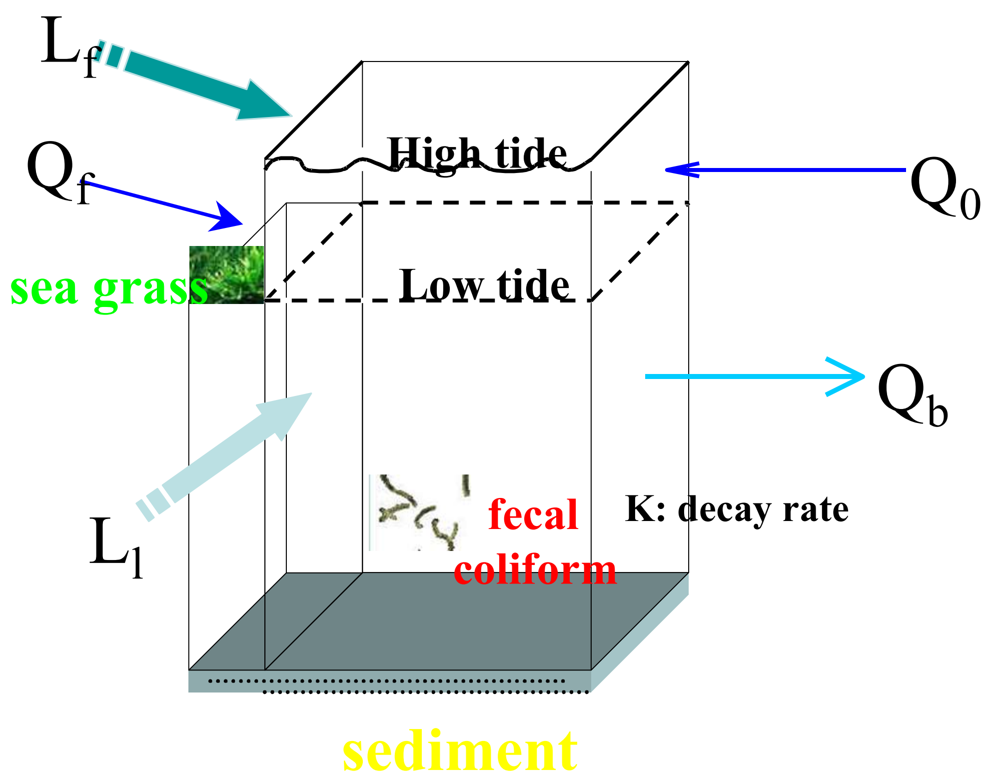

Figure A-1: The schematic diagram for the tidal prism model

The detailed calculations for the current loads and allowable loads from both median and 90th percentile scenarios for the drainage areas of the restricted shellfish harvesting area of Ellis Bay in the lower Wicomico River basin are listed below and the parameters needed for the load calculation are also presented.

A Tidal Prism Model Calculation for Ellis Bay

Median Scenario: The most recent three-year fecal coliform median concentration is used. The median load calculation is illustrated as follows:

V = Mean volume of the embayment = 1691992(m^3)
k = Fecal coliform decay rate =0.36(T^{-1})
Q_f = Freshwater discharge
= $15.65 \times 0.0283 \times 86400 \times 12.42 \div 24 = 19808(m^3T^{-1})$
Q_0 = 501791 (m^3T^{-1})
Q_b = 521599 ($m^3 T^{-1}$)
C_c = water quality criterion = 14 MPN/100ml
C = current fecal coliform 3-year median concentration = 15.0 (MPN/100ml)
C_0 = fecal coliform 3-year median outside of the embayment = 7.3 (MPN/100ml)
T =tidal cycle =12.42 hours
Cf = the unit conversion factor

For allowable load calculation, C_c is used as fecal coliform concentration (i.e., 14 MPN/100ml). The fecal coliform concentration at the outside of the embayment uses 14 MPN/100ml as well. The allowable load is calculated as follows:

Allowable Load =
$$Load = [C_c \times (Q_b + kV) - Q_0 \times C_0] \times Cf$$
$$= [14\times (521599 + 0.36\times 1691992) - 501791\times 14] \times (24\div 12.42)\times 10000$$
$$=1.712\times 10^{11}$$

For the current load estimation, the most recent three-year median fecal coliform median concentration is used for the calculation. The current load is calculated as follows:

Current Load =
$$Load = [C \times (Q_b + kV) - Q_0 \times C_0] \times Cf$$
$$[15\times (521599 + 0.36\times 1691992) - 501791\times 7.3] \times (24\div 12.42)\times 10000$$
$$=2.581\times 10^{11}$$

The load reduction is estimated as follows:

Load Reduction = [(Current Load – Allowable Load)/Current Load] *100 = 33.7%

A Tidal Prism Model Calculation for Ellis Bay (con't.)

90^{th} percentile Scenario: The most recent three-year fecal coliform 90^{th} percentile concentration is used. The 90^{th} percentile load calculation is illustrated as follows:

V = Mean volume of the embayment = 1691992(m^3)
k = Fecal coliform decay rate =0.36(T^{-1})
Q_f = Freshwater discharge
= 15.65 × 0.0283×86400×12.42÷24 = 19808 (m^3T^{-1})
Q_0 = 501791 (m^3T^{-1})
Q_b = 521599 ($m^3 T^{-1}$)
C_c = water quality criterion = 49 MPN/100ml
C = current fecal coliform 3-year 90^{th} percentile concentration = 93.0 (MPN/100ml)
C_0 = fecal coliform 3-year 90^{th} percentile at the outside of the embayment
= 43.0 (MPN/100ml)
T = tidal cycle =12.42 hours
Cf = the unit conversion factor

For allowable calculation, C_c is used as fecal coliform concentration (i.e., 49 MPN/100ml). The fecal coliform concentration at the outside of the embayment uses 49 MPN/100ml as well. The allowable load is calculated as follows:

Allowable Load =
$$Load = [C_c \times (Q_b + kV) - Q_0 \times C_0] \times Cf$$
$$= [49\times (521599 + 0.36\times 1691992) - 501791\times 49] \times (24 \div 12.42) \times 10000$$
$$= 5.991\times 10^{11}$$

For the current load estimation, the most recent 3-year 90^{th} percentile fecal coliform concentration is used for the calculation. The current load is calculated as follows:

Current condition =
$$Load = [C \times (Q_b + kV) - Q_0 \times C_0] \times Cf$$
$$= [93.0\times (521599 + 0.36\times 1691992) - 501791\times 43.0] \times (24 \div 12.42) \times 10000$$
$$= 1.622\times 10^{12}$$

The load reduction is estimated as follows:

Load Reduction = [(Current Load – Allowable Load)/Current Load] * 100 = 63.1%

Appendix B. Bacteria Source Tracking

Nonpoint sources of fecal coliform do not have one discharge point and may occur over the entire length of a stream or waterbody. The possible introductions of fecal coliform bacteria from non-human activities 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 to surface waters. Nonpoint source contributions to the bacteria levels from human activities generally arise from failing septic systems and their associated drain fields and from potential discharge from recreation vessel discharges. The transport of fecal coliform from land surface to shellfish harvesting areas is dictated by the hydrology, soil type, land use, and topography of the watershed.

In order to determine the significant sources of fecal coliform and reduction needed to achieve water quality criteria among these sources, it is necessary to identify all existing sources. The nonpoint source assessment was conducted using the fecal coliform monitoring data (provided by MDE Shellfish Certification Program) and bacteria source tracking analysis 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 Lower Wicomico River, four routine monitoring stations in the Lower Wicomico 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. Sampling was conducted over a twelve-month period from November 2002 through October 2003. Antibiotic Resistance Analysis (ARA) was the chosen BST method used to determine the potential sources of fecal coliform in the Lower Wicomico River. ARA uses enterococci or *Escherichia coli* (*E. coli*) and patterns of antibiotic resistance to identify sources. The premise is that the antibiotic resistance of bacteria isolated from different hosts can be discerned based upon differences in the selective pressure of microbial populations found in the gastrointestinal tract of those hosts (humans, livestock, pets, wildlife) (Wiggins 1996). Bacteria isolated from the fecal material of wildlife would be expected to have a much lower level of resistance to antibiotics than bacteria 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. Enterococci isolates were obtained from known source present in the watershed. For the Wicomico River, these sources included human, cat, dog, chicken, horse, cow, deer, rabbit, fox, and goose. Bacterial isolates collected from water samples are then tested and their resistance results are recorded. Based upon a comparison of resistance patterns of water and known library isolates, a statistical analysis can predict the likely host source of the water isolates (Hagedorn 1999; Wiggins 1999).

A tree classification method, [1]CART®, was applied 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 in order to maximize a specified index of homogeneity for isolate sources within each of the nodes. In subsequent steps, the same process is applied to each resulting node until a *stopping* criterion is satisfied. Nodes where an additional split would lead to only an insignificant increase in the *homogeneity index* relative to the *stopping* criterion are referred to as *terminal* nodes.[2] The collection of *terminal* nodes defines the classification model. Each *terminal* node is associated with one source, the source that is most populous among the library isolates in the node. Each water sample isolate (*i.e.*, an isolate with an unknown source), based on its antibiotic resistance pattern, is identified with one specific *terminal* node and is assigned the source of the majority of library isolates in that *terminal* node.[3] The full BST report for the Wicomico River basin is located in Frana and Venso (2006) Appendix B.

Results

Water samples were collected mostly from the 4 stations in the Lower Wicomico River. If weather conditions prevented sampling at a station, a second collection(s) in a later month was performed. The maximum number of enterococci isolates per water sample was 24, although the number of isolates that actually grew was sometimes fewer than 24. A total of 1015 enterococci isolates were analyzed by statistical analysis. Table B-1 below shows the BST results by category, the number of isolates and percent isolates classified at the 0.50 (50%) cutoff probability, as well as the percent classified overall. The seasonal distribution of water isolates from samples collected at each sampling station is shown below in Table B-2. According to the ARA, wildlife is the predominant bacteria source followed by human. Twenty percent (20%) of the water isolates were from unknown (unclassified) probable sources.

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

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

[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 B-1: Probable Host Sources of Water Isolates by Category, Number of Isolates, and Percent Isolates Classified at Cutoff Probabilities of 50%

Category	Count	Percent	Percent Without Unknowns
Pet	60	5.9%	7.4%
Human	204	20.1%	25.2%
Livestock	96	9.5%	11.9%
Wildlife	448	44.1%	55.5%
Unknown*	207	20.4%	
Total w/ Complete Data	1015		
Total	1015		
% Classified		79.6%	

* Unknown means that the library of known sources failed to classify for isolates from water samples collected

Table B-2: Number of Enterococci Isolates from Water Collected and Analyzed by Season

Station	Fall	Winter	Spring	Summer	Total
14-06-007	69	59	70	72	270
14-06-007T	68	57	71	48	244
14-06-008	71	69	67	48	255
14-06-211	70	38	67	71	246

Appendix C. Fecal Coliform Monitoring Data

The fecal coliform monitoring data used to develop the fecal coliform TMDLs for the restricted shellfish harvesting areas of Ellis Bay is provided in Table C-1.

Table C-1: Observed Fecal Coliform Data in Ellis Bay

Station	Date	Fecal Coliform MPN/100 ml	Station	Date	Fecal Coliform MPN/100 ml
14-06-206	11/28/2016	3.6	14-06-203	11/28/2016	9.1
14-06-206	12/13/2016	15	14-06-203	12/13/2016	9.1
14-06-206	1/12/2017	3.6	14-06-203	1/12/2017	9.1
14-06-206	2/15/2017	7.3	14-06-203	2/15/2017	3.6
14-06-206	4/11/2017	39	14-06-203	4/11/2017	15
14-06-206	5/17/2017	23	14-06-203	5/17/2017	1
14-06-206	7/13/2017	43	14-06-203	7/13/2017	1
14-06-206	8/23/2017	93	14-06-203	8/23/2017	93
14-06-206	9/5/2017	150	14-06-203	9/5/2017	23
14-06-206	10/19/2017	23	14-06-203	10/19/2017	23
14-06-206	11/20/2017	7.3	14-06-203	11/20/2017	3.6
14-06-206	2/21/2018	1	14-06-203	2/21/2018	1
14-06-206	4/9/2018	1	14-06-203	4/9/2018	1
14-06-206	5/9/2018	9.1	14-06-203	5/9/2018	1
14-06-206	6/12/2018	150	14-06-203	6/12/2018	39
14-06-206	6/26/2018	150	14-06-203	6/26/2018	1
14-06-206	7/17/2018	43	14-06-203	7/17/2018	15
14-06-206	8/20/2018	3.6	14-06-203	8/20/2018	3.6
14-06-206	9/12/2018	23	14-06-203	9/12/2018	43
14-06-206	11/30/2018	9.1	14-06-203	11/30/2018	9.1
14-06-206	12/11/2018	9.1	14-06-203	12/11/2018	1
14-06-206	1/29/2019	9.1	14-06-203	1/29/2019	1
14-06-206	2/26/2019	3.6	14-06-203	2/26/2019	1
14-06-206	4/10/2019	93	14-06-203	4/10/2019	43
14-06-206	4/29/2019	7.3	14-06-203	4/29/2019	7.3
14-06-206	5/13/2019	1	14-06-203	5/13/2019	1
14-06-206	6/19/2019	43	14-06-203	6/19/2019	43
14-06-206	7/22/2019	43	14-06-203	7/22/2019	9.1
14-06-206	8/19/2019	43	14-06-203	8/19/2019	23
14-06-206	10/22/2019	75	14-06-203	10/22/2019	1
14-06-206	11/18/2019	3.6	14-06-203	11/18/2019	1