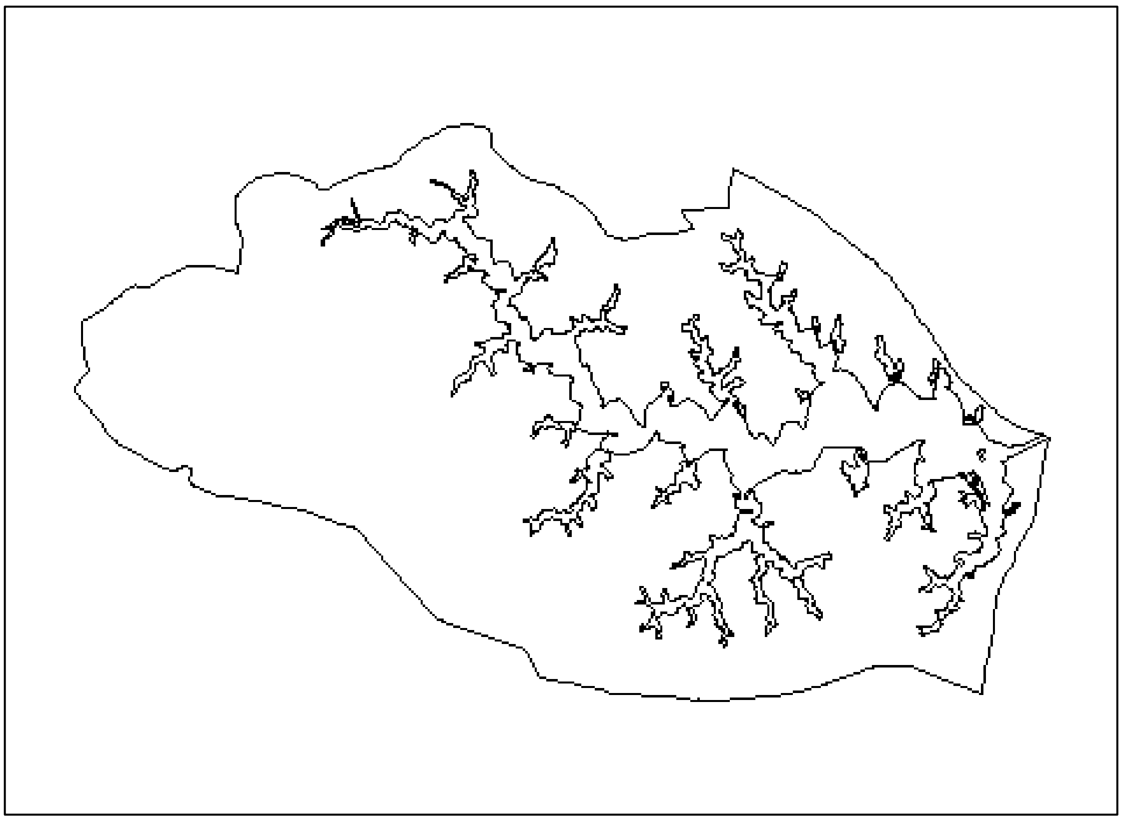
Little Wicomico River Watershed TMDL for Three Shellfish Areas Listed Due to Bacteria Contamination



July, 2003
Virginia Department of Environmental Quality
Richmond, Virginia

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EXECUTIVE SUMMARY

Fecal Coliform Impairment

Four areas of the Little Wicomico River, a rural 1,281 acre, tidal watershed located in Northumberland County, Virginia were placed on the Commonwealth of Virginia's 1998 Section 303(d) List of Impaired Waters because of violations of the fecal coliform bacteria water quality standard for shellfish waters. Of these Four areas, all of which are tributary to the Potomac River, was dropped from the impaired waters list because it no longer violated the water quality standard. This area is represented by station 9w in growing area 10, condemnation area 105. Therefore, a Total Maximum Daily Load (TMDL) allocation was no longer considered necessary for this segment. An additional larger segment was reduced in size of impaired area, this reduced area is addressed in this report resulted from removing station 10-16-180 from consideration. The three segments listed are referenced in this document by the growing area number, shellfish area condemnation number and downstream most water quality station number (e.g. 10-9x-180). These TMDLs focus on fecal coliform impairments that are the organism of concern identified in the water quality standard. Based upon exceedances of this standard recorded at Virginia Department of Health, Division of Shellfish Sanitation (VDH-DSS) monitoring stations, these segments of this estuary do not support the harvest for consumption of shellfish indigenous to these waters. Shellfish may be harvested by permit for transport to unimpaired waters cleanse themselves prior to harvest for consumption

The applicable state standard specifies that the number of fecal coliform bacteria shall not exceed a maximum allowable level of q geometric mean of 14 most probable number (MPN) per 100 milliliters (ml) or a 90th percentile geometric mean value of 49 MPN/100ml, whichever is more stringent (Virginia Water Quality Standard 9-VAC 25-260-5). In TMDL development, the 90th percentile 14 MPN/100 ml was used, since it represented the more stringent standard.

Sources of Fecal Coliform

Potential sources of fecal coliform in the watershed include, but may not be limited to, non-point source contributions, as there are no permitted point source discharges in the watershed. Non-point sources include wildlife; grazing livestock; land application of bio-solids; recreation vessel discharges; failed, malfunctioning, and non-operational septic systems, and uncontrolled discharges (straight pipes conveying gray water from kitchen and laundry areas of private homes, etc.).

Water Quality Modeling

Because the volume of the individual condemned segments and overall watershed were small, land use pattern not complex, and the absence of large point sources a simplified volumetric modeling approach was utilized. This approach has received the approval of the U.S. Environmental protection Agency for use in such non-complex tidal watersheds. In establishing the existing and allocation conditions, seasonal variations in hydrology, climatic conditions, and source contributions were evaluated prior to selecting the simplified model.

EXECUTIVE SUMMARY

Determination of Existing Loadings

To assist in partitioning the loads from the diverse sources within the watershed water quality samples of fecal coliforms were collected for one year and evaluated using an antibiotic resistance analysis in a process called bacterial source tracking. These samples were compared to a reference library of fecal samples from known sources. The resulting data was used to assign portions of the load with in the watershed to wildlife, birds, humans, pets or livestock. The results of this analysis indicated that fecal coliforms of probable human origin were the dominant source in the watershed and that birds and wildlife were secondary contributors that may dominate in some months. This bacterial source tracking (BST) eliminates the need for developing inventories of livestock populations, and utilization of highly subjective wildlife, bird and pet population estimates.
The presence of a large signature attributable to one component is sufficient to establish potential directions for remediation under a future implementation plan.

Load Allocation Scenarios

The next step in the TMDL process was to utilize to determine the appropriate the water quality standard to be applied. This was set as the 90th percentile standard because the data established that the segments were meeting the geometric mean standard. Calculated results of the model for each segment was used to establish the existing load in the system. The load necessary to meet water quality standards was calculated in a similar fashion using the water quality standard criterion in place of the ambient water quality value. The difference between these two numbers represents the necessary level of reduction in each segment.

Finally the results of the BST developed for each segment was used to partition the load allocation that would meet water quality standards according to source. The results of the model, the BST source partitioning and the reductions necessary for each segment are shown below and on the following page.

REDUCTION BASED UPON 90TH PERCENTILE STANDARD

AREA 180 STATION 10-9X	BST Result % of total load	Actual Load (cfu)*	Load Allocation (cfu)*	Reduction needed
Total	100%	7.89E+10	8.59E+10	0%
Bird	16%	1.26E+10	1.26E+10	0%
Wildlife	10%	7.89E+09	7.89E+09	0%
Human	62%	4.89E+10	4.89E+10	0%
Pets	3%	2.37E+09	2.37E+09	0%
Livestock	9%	7.10E+09	7.10E+09	0%

EXECUTIVE SUMMARY

REDUCTION BASED UPON 90TH PERCENTILE STANDARD

AREA 180-A STATION 10-13.5Z	BST Result % of total load	Actual Load (cfu)*	Load Allocation (cfu)*	Reduction needed
Total	100%	8.84E+10	7.47E+10	15%
Bird	23%	2.03E+10	2.03E+10	0%
Wildlife	9%	7.96E+09	7.96E+09	0%
Human	51%	4.51E+10	3.14E+10	30%
Pets	7%	6.19E+09	6.19E+09	0%
Livestock	10%	8.84E+09	8.84E+09	0%

AREA 180 – B STATION 10-19	BST Result % of total load	Actual Load (cfu)*	Load Allocation (cfu)*	Reduction needed
Total	100%	2.56E+11	1.94E+11	24%
Bird	16%	4.10E+10	4.10E+10	0%
Wildlife	10%	2.56E+10	2.56E+10	0%
Human	62%	1.59E+11	1.00E+11	37%
Pets	3%	7.68E+09	7.68E+09	0%
Livestock	9%	2.05E+10	2.05E+10	0%

AREA 180 - B STATION 10-20	BST Result % of total load	Actual Load (cfu)*	Load Allocation (cfu)*	Reduction needed
Total	100%	3.04E+11	1.94E+11	37%
Bird	16%	4.86E+10	4.86E+10	0%
Wildlife	10%	3.04E+10	3.04E+10	0%
Human	62%	1.88E+11	8.20E+10	56%
Pets	3%	9.12E+09	9.12E+09	0%
Livestock	9%	2.43E+10	2.43E+10	0%

Margin of Safety

In order to account for uncertainty in modeled output, a margin of safety (MOS) was incorporated into the TMDL development process by making very conservative choices. A margin of safety can be incorporated implicitly in the model through the use of conservative estimates of model parameters, or explicitly as an additional load reduction requirement. Individual errors in model inputs, such as data used for developing model parameters or data used for calibration, may affect the load allocations in a positive or a negative way. The purpose of the MOS is to avoid an overall bias toward load allocations that are too large for meeting the water quality target. An implicit MOS was used in the development of this TMDL through selection of a high protective level of water quality standard, utilization of entire segment volumes for model calculations, averaging extreme high and low values to ensure that the more protective condition with the largest available data set was addressed and emphasizing watershed based implementation measures.

Recommendations for TMDL Implementation

The goal of this TMDL was to develop an allocation plan that can be met during the implementation phase. Virginia's 1997 Water Quality Monitoring, Information and Restoration Act states in section 62.1-44.19.7 that the "Board shall develop and implement a plan to achieve fully supporting status for impaired waters".

The TMDL developed for the Little Wicomico River impairments provides allocation scenarios that will be a starting point for developing implementation strategies. Modeling shows that meeting the average water quality condition in this tidal water body will ensure that water quality standards are met. The model shows that elimination of the human fecal component alone is sufficient to ensure that water quality standards will be well within the acceptable standard.

Additional monitoring aimed at targeting these reductions is critical to implementation development. Bacterial source tracking to identify more localized sources of contamination and an improved understanding of the episodic, or potentially seasonal bird driven impairment area will contribute greatly to the implementation effort. Once established, continued monitoring will aid in tracking success toward meeting water quality milestones.

Also critical to the implementation process is public participation. Non-point loading to the system is the critical factor in addressing the problem. These sources cannot be addressed without public understanding of and support for the implementation process. Stakeholder input will be critical from the onset of the implementation process in order to develop an implementation plan that will be truly effective.

Public Participation

During development of the TMDL for the Little Wicomico River, public involvement was encouraged through three meetings. Two stakeholder meetings and a formal public meeting were held over the course of 2 years.

A basic description of the TMDL process and the agencies involved was presented at the first stakeholder meeting and again at the public meeting. The second stakeholder meeting was held to discuss the source assessment input, bacterial source tracking, and model results. The final model simulations and the TMDL load allocations were presented during the public meeting. Public understanding of and involvement in the TMDL process was encouraged. Input from these meetings was utilized in the development of the TMDL and improved confidence in the allocation scenarios and TMDL process.

1.0 Introduction

This document details the development of bacteria TMDLs for 3 segments of the Little Wicomico River in Northumberland County, Virginia that are listed as impaired on Virginia's 303(d) Total Maximum Daily Load Priority List. The TMDL is one step in a multi-step process that include a very high level of public participation in order to facilitate the correction of water quality issues which can affect public health and the health of aquatic life.

1.1 Listing of Water Bodies Under the Clean Water Act

Section 303(d) of the Clean Water Act and EPA's Water Quality Planning and Management Regulations (40 CFR Part 130) require states to develop Total Maximum Daily Loads (TMDLs) for water bodies which are exceeding water quality standards. TMDLs represent the total pollutant loading that a water body can receive without violating water quality standards. Water quality standards are numeric or narrative limits on pollutants that are developed to ensure the protection of human health and of aquatic life. The TMDL process establishes the allowable loading of pollutants for a water body based on the relationship between pollution sources and in-stream water quality conditions. By following the TMDL process, states can establish water quality based controls to reduce pollution from both point and non-point sources to restore and maintain the quality of their water resources (EPA, 1991).

Waters that are determined to be impaired can be free flowing streams, lakes and tidal waters, anywhere in Virginia. Bacteria violations are believed to be the most common cause for the impairments. In Virginia, we have identified a need to develop 644 TMDLs by 2010. Of these approximately 230+ are shellfish water closures due to an excessive levels of fecal coliform bacteria. Among these shellfish areas, several areas within the Little Wicomico River have been regulated under Virginia Department of Health, Division of Shellfish Sanitation (VDH-DSS) notice number 145 as restricted harvest areas because the data showed excessive levels of bacteria in these waters. These waters were classified as impaired on the state's 303(d) list of impaired waters and require a TMDL.

1.2 Overview of the TMDL Development Process

The TMDL study for these waters is the first part of a three-step process aimed at restoring water quality. This study is designed to tell us how much of pollutant input needs to be reduced in order to achieve water quality standards. The second step in the process is the development of an implementation plan that identifies which specific control measures are necessary to achieve those reductions, their timing for implementation and at what cost. The implementation plan will also outline potential funding sources. The third step will be the actual implementation process. Implementation will typically occur in stages that allow a review of progress in reducing pollutant input and to make any identified changes to pollutant control measures.

Agencies of the Commonwealth, including the Department of Environmental Quality (DEQ), the VDH-DSS and the Department of Conservation and Recreation (DCR) have worked together with state universities, the U.S. Geological Survey and the U.S. Environmental Protection Agency to develop an appropriate methodology for TMDLs in impaired shellfish waters.

This method utilizes bacteria source tracking (BST) to determine what the sources of fecal coliform in the water are. It has been shown that BST can provide reliable information to identify and target sources of bacterial pollution. In addition to the BST, the TMDL will be developed using VDH-DSS monthly monitoring and sanitary shoreline surveys. The results for this technology as applied to the Little Wicomico River is described in section 5.0. Finally, to assist with the analysis and development of the TMDLs for these rivers and other impaired water bodies in Northumberland County, the Department of Environmental Quality has contracted with the Virginia Institute of Marine Science for further technical assistance.

The TMDL development process also must account for seasonal and annual variations in precipitation, flow, land-use, and pollutant contributions. Such an approach ensures that TMDLs, when implemented, do not result in violations under a wide variety of scenarios that affect bacteria loading.

1.3 Classification of Virginia's Shellfish Growing Areas

The Virginia Department of Health, Division of Shellfish Sanitation (DSS) is responsible for classifying and ensuring the health for human consumption of Virginia's shellfish resources. The VDH-DSS collects monthly samples at over 2,000 stations in the shellfish growing areas of Virginia. They determine if the data show that the water quality standard is met on an annual basis though more frequent consideration is possible. If the water quality standards are exceeded, the shellfish area is closed for the harvest of shellfish that go directly to market. These areas that exceed the water quality standard and are closed for the direct marketing of shellfish are eligible for harvest of shellfish under permit from the Virginia Marine Resources Commission and VDH-DSS. The permit establishes controls that in part require shellfish be allowed to depurate for 15 days in clean growing areas or specially designed licensed on shore facilities. DSS follows the requirements of the National Shellfish Sanitation Program (NSSP), which is regulated by the U.S. Food and Drug Administration. The NSSP classification specifies the use of a shoreline survey completed by DSS as its primary tool for classifying shellfish growing waters. Fecal coliform concentrations in water samples collected in the immediate vicinity of the shellfish beds function to verify the findings of the shoreline survey, and to define the border between approved and condemned (unapproved) waters.

DSS develops the shoreline survey to locate as many sources of pollution as possible within the watersheds of shellfish growing areas. This is accomplished through a property-by-property inspection of the onsite sanitary waste disposal facilities of most properties on un-sewered sections of watersheds, and investigations other sources of pollution such as wastewater treatment plants (WTP), marinas, livestock operations, landfills, etc. The information is compiled into a written report with a map showing the location of the sources of real or potential pollution found, and sends it to the various state agencies that are responsible for regulating these concerns and the city or county.

Once an onsite problem is identified local health departments (LHDs), or other state or local agency may play a major role in the process by obtaining correction of the onsite sanitary waste disposal problems. Most of the DSS effort is focused on locating fecal contamination, and in this manner facilitating the prevention of significant amounts of human pathogens from getting into shellfish waters. In addition to the shoreline survey, the NSSP requires that DSS collect seawater samples in the growing areas as part of the classification procedure. States must use the most recent 30 samples, collected randomly with respect to weather (scheduled one month in advance), to classify a station. The two part standard for fecal coliforms in waters for direct shellfish harvest to market is a geometric mean no greater than 14 MPN fecal coliforms/100 ml and an estimated 90th percentile no greater than 49 MPN/100ml. Exceeding either number requires closure of that station.

When a shellfish growing area is restricted (condemned), shellfish cannot be harvested for human consumption. Shellfish from restricted areas can be moved under a permit during warm weather (when shellfish predictably will feed) to approved waters for 15 days to cleanse themselves. After this "depuration period" the shellfish can be marketed.

2.0 Applicable Water Quality Standard

According to Virginia Water Quality Standards (9 VAC 25-260-5), the term *"water quality standards means provisions of state or federal law which consist of a designated use or uses for the waters of the Commonwealth and water quality criteria for such waters based upon such uses. Water quality standards are to protect the public health or welfare, enhance the quality of water and serve the purposes of the State Water Control Law (§62.1-44.2 et seq. of the Code of Virginia) and the federal Clean Water Act (33 USC §1251 et seq.)."*
As stated above, Virginia water quality standards consist of a designated use or uses and a water quality criteria. These two parts of the applicable water quality standard are presented in the sections that follow.

2.1 Designated Uses

According to Virginia Water Quality Standards (9 VAC 25-260-10A), *"all state waters are designated for the following uses: recreational uses (e.g., swimming and boating); the propagation and growth of a balanced indigenous population of aquatic life, including game fish, which might be reasonably expected to inhabit them; wildlife; and the production of edible and marketable natural resources (e.g., fish and shellfish)."*

2.2 Applicable Water Quality Criteria

For a shellfish supporting water body to be in compliance with Virginia's bacteria standards for primary contact recreational use, VADEQ specifies the following criteria (9 VAC 25-260-160): *"In all open ocean or estuarine waters capable of propagating shellfish or in specific areas where public or leased private shellfish beds are present, and including those waters on which condemnation or restriction classifications are established by the State Department of Health the following criteria for fecal coliform bacteria shall apply; The geometric mean fecal coliform value for a sampling station shall not exceed an MPN (most probable number) of 14 per 100 milliliters. The 90th percentile shall not exceed an MPN of 43 for a 5 tube, 3 dilution test or 49 for a 3 tube, 3 dilution test"*

3.0 Description of Watershed Water Quality Characterization

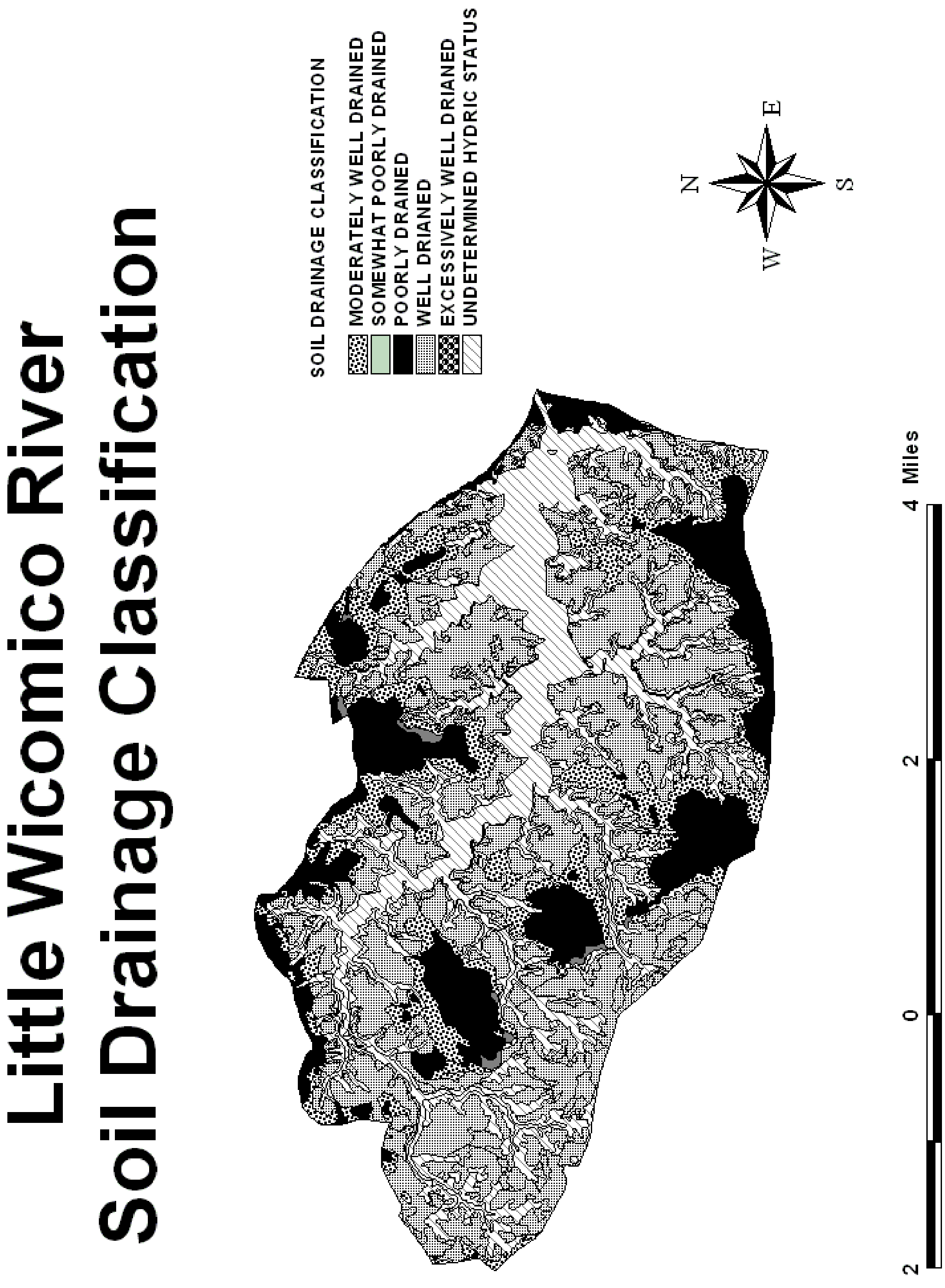
3.1 Physical Environment

The Little Wicomico River watershed is located entirely within and along the northwestern corner of Northumberland County in Virginia's Coastal Plain Physiographic Province and the Coastal Lowland sub-province. The Coastal Lowland sub-province is characterized by flat, low relief regions along the major rivers and Chesapeake Bay. Elevations range from 0' to 60' above mean sea level. A topographic map of the Little Wicomico River Watershed is shown in Figure 3-1. The Virginia Coastal plain is underlain by deep tertiary and cretaceous formations of marine and deltaic sands and clay, overlain by Yorktown and Eastover formations of marine sand and clay, this is topped by quaternary formations that are comprised of silts, sands and clays of principally fluvial and estuarine origin. The foregoing layers rest atop the igneous and metamorphic rock base formation. Near surface soils in the watershed range from poorly drained to well drained with the well-drained and moderately well drained soils dominating the tributary branches. The poorly drained soils occupy the northern and southern periphery of the watershed. The soil drainage classification is shown in Figure 3-2. The Little Wicomico River watershed drains northeast to the Potomac River and Chesapeake Bay at Smith Point. As such it is subject to the ebb and flow of the tide. The Little Wicomico River flows northeast from its headwaters bordering State Route 360 to the south and Route 640 and Route 646 at Blundon Corner to the west, and Route 644 from Gonyon to Ophelia to the northwest and north. It enters the Potomac River at the southeastern edge of the mouth of the river.

Figure 3-1

The drainage area of the Little Wicomico River watershed is approximately 1,281 acres. The nearest two climate stations are located in Warsaw Virginia approximately 35 miles west of the study area, and Tangier Island 20 miles east-southeast. The average annual rainfall as recorded at Warsaw, Virginia is 43 inches and at Tangier Island 38 inches. Tables 3.1 and 3.2 presented below provide summaries of climate data for the Warsaw and Tangier Island, Virginia weather stations (SRCC 2002).

Figure 3-2


Little Wicomico River
Soil Drainage Classification
SOIL DRAINAGE CLASSIFICATION
MODERATELY WELL DRAINED
SOMEWHAT POORLY DRAINED
POORLY DRAINED
WELL DRIANED
EXCESSIVELY WELL DRIANED
UNDETERMINED HYDRIC STATUS
N
E
S
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2
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2
4 Miles

Table 3-1. Climate Summary for Warsaw, Virginia													
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Annual**
Average Max. Temperature (F)	46.4	49.8	58.3	69.3	77.5	85.0	88.7	86.9	81.1	70.6	60.1	49.7	68.6
Average Min. Temperature (F)	27.3	29.3	35.9	44.7	53.9	62.3	66.9	65.3	58.7	47.3	38.7	30.7	46.8
Average Total Precipitation (in.)	3.22	2.84	3.83	2.97	4.04	3.52	4.53	4.29	4.07	3.33	3.18	3.11	42.94
Average Total Snow Fall(in.)	5.7	5.0	2.4	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.5	2.6	16.3

Table 3-2. Climate Summary for Tangier Island, Virginia													
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Annual**
Average Max. Temperature (F)	44.3	46.5	54.2	65.0	74.6	82.6	87.0	85.7	80.6	69.9	59.2	49.6	66.6
Average Min. Temperature (F)	30.5	31.6	37.7	47.1	56.4	65.1	70.9	69.8	64.5	53.7	43.6	35.1	50.5
Average Total Precipitation (in.)	2.86	2.89	4.16	2.61	3.03	2.79	3.90	3.81	3.17	2.92	2.78	2.93	37.86
Average Total Snow Fall(in.)	2.8	1.8	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	6.3

** Source: Southeast Regional Climate Center, sercc@dnr.state.sc.us*

Land use in the Little Wicomico River watershed exhibits little development and remains primarily rural. Forest and agriculture are the dominant land use types. The land use by category within the watershed is illustrated in Figure 3-2 and enumerated in Table 3-3.

3.2 Little Wicomico River Estuary Water Quality Impairment by Condemnation Area

Segments of the Little Wicomico River were listed as impaired on Virginia's 1998 303(d) Total Maximum Daily Load Priority List and Report (VADEQ, 1998) due to violations of the State's water quality standard for fecal coliform bacteria in shellfish supporting waters. Department of Health, Division of Shellfish Sanitation, Notice and Description of Shellfish Condemnation Numbers 105, Little Wicomico River, and number 180 Little Wicomico River: Bridge Creek, lists and describes three condemnation areas in the Little Wicomico River and its tributaries. This list was amended in 2002 to remove of two areas from consideration for TMDL.

Table 3-3. Land use in the Little Wicomico River watershed

Land Use Category	Area (acres)	Area (%)
Transitional	10	>1
Forest	805	63
Wetland	96	8
Bare sand/rock/clay	12	1
Agricultural land (Pasture/Hay/Row crops)	272	21
Commercial/Industrial/Transportation	57	4
Residential	14	1
Open Water	15	1
Total	1,281	100

Source: Virginia National Land Cover Data (NLCD) Version 05-27-99

The four segments on the 303 (d) impaired waters list as of the date of this report are shown in Table 3-4.

The Little Wicomico River water quality monitoring network consists of 27 water quality monitoring stations. These stations are monitored by the DSS annually for fecal bacteria and the status of closure areas is re-evaluated. The network of water quality monitoring stations for the Little Wicomico River estuary is shown in Figure 3-4. Of these 27 monitoring stations, a subset of six stations representing the downstream limit of the existing shellfish closure areas identified in the closure notice were selected for a pilot study to facilitate the development of TMDL's for these segments. The purpose of this TMDL study was to perform bacterial source tracking at these stations on a monthly basis from September of 2001 through August of 2002. Detailed companion water quality data for the BST data for each TMDL station in the pilot study is found in the Appendix A.

Figure 3-3

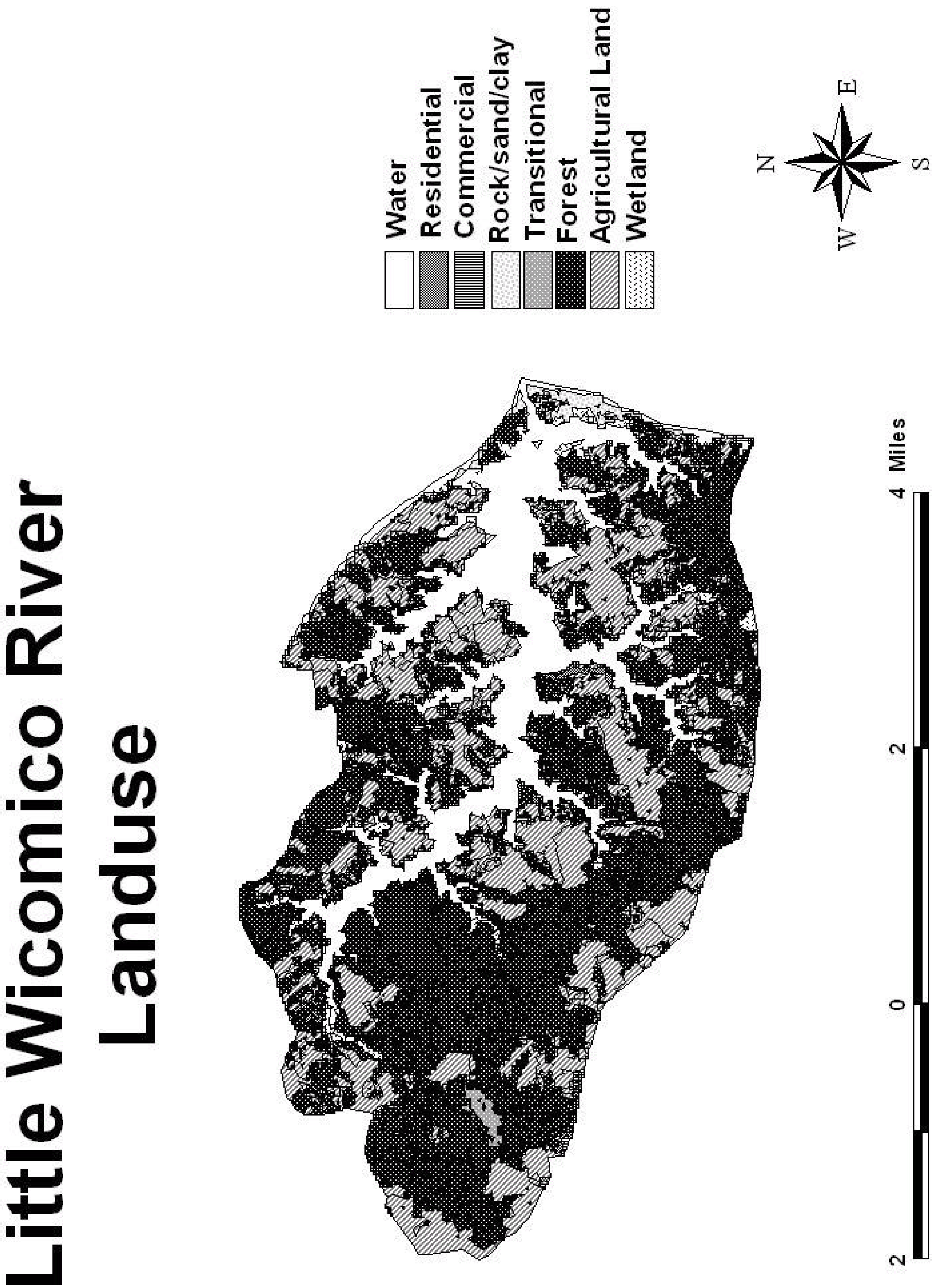

Little Wicomico River
Landuse
Water
Residential
Commercial
Rock/sand/clay
Transitional
Forest
Agricultural Land
Wetland
N
E
S
W
2
0
2
4 Miles

Figure 3-5 shows the location of the TMDL study stations and their adjacent condemnation areas. Of these stations, numbers 10-16 and 10-9W represent shellfish areas which were removed from the impaired waters list because they met or currently meet water quality standards and do not require TMDLs. Table 3.4 shows the water quality data for 30 months ending with the special study period.

The data for the study areas for the last 30 monthly sampling events which covers the period from February 2000 through August 2002 are graphically represented in Figure 3-6. These data show that three of the four stations representing the condemnation areas that are the subject of this TMDL report, do not meet the established 90th Percentile standard of 49 MPN/100ml, and two stations do not meet the geometric mean standard of 14 MPN/100ml. One station, station 9X representing the DSS closure area 105-E, Bridge Creek, appears to meet both of the standards for the period of this report. This station remained in compliance as of December of 2002 and is scheduled to be removed from the condemnation area list and will be de-listed from the 303(d)/305(b) impaired waters list. Two other stations not the subject of this report because they were not closed during the study period (9w, 16) show that they do not meet the 30 month average test. These segments will be addressed in future TMDLs. Overall temporal trends in the data for the period of record are shown in Figure 3-7 for each of the study stations. Stations that consistently exceed the standard of 14 MPN per 100ml are readily visible.

Table 3-4. Little Wicomico River Estuary Bacterial Water Quality Data Summary For The 30 Month Period From Feb. and the Study Period from Feb 2000 – Aug. 2002

Closure Area /Station	90th percentile	Water Quality Standard	Station Meets Standard ?	Geometric Mean	Geometric Mean Standard	Station Meets Standard ?	Current Condem-nation
AREA 180 STATION 9X	45	49 MPN /100ml	yes	7.6	14 MPN /100ml	yes	yes
AREA 105-A STATION 13.5Z	58.0		yes	7.7		yes	yes
AREA 105– B STATION 19	64.9		no	26.3		no	yes
AREA 105 – B STATION 20	76.8		no	17.3		no	yes

Stations 19 and 20 had the highest peak values for fecal coliforms at the 90th percentile of 101.8 MPN/100ml and 64.9 MPN/100m respectively. Both stations also exceeded the geometric mean standard of 14MPN/100ml as well. The data make it evident that the controlling condition for the bacterial levels is at the 90th percentile for all of the shellfish condemnation TMDL's in the Little Wicomico River watershed. Efforts undertaken to address this standard in the watershed have a high probability of ensuring that the geometric mean standard would also be met and would provide an adequate margin of safety.

4.0 Assessment of Bacteria Sources

There are several methods that are utilized to determine the potential sources of bacteria to the system. Chief among these are:

1. VADEQ Point Source Inventory to determine permitted point sources such as sewage treatment plants;
2. DSS Shoreline Survey to determine principal non-point sources such as failing septic systems and farm based non-point source operations; and,
3. bacterial source tracking to quantify source loadings from humans, livestock, and wildlife.

All of these are utilized in this report.

4.1 Point Source Contributions

There are no known permitted point source contributions to the Little Wicomico River watershed. However the shoreline survey did identify several direct discharges to the watershed from laundry/kitchen facilities and potential stormwater sources as illustrated in Figure 4-1.

4.2 Non-Point Source Contributions

The shoreline survey is conducted by direct observation of direct and indirect discharges to the watershed from human activities. Such discharges include storm water systems, failing septic systems, waste water treatment plants, livestock yards and pastures, as well as surface runoff from lawns and undeveloped landscapes. Figure 4-1 shows the results of the DSS sanitary shoreline survey dated February 1997 for the Little Wicomico River watershed. The most significant areas of direct and indirect sources of pollution are identified. The textual portion of the complete survey is provided in the Appendix B which also includes the condemnation area notices developed by VDH.

There are many avenues of non-point source pollution into watersheds. Some of these contributions such as those contributed by wildlife, both mammalian and avian, are natural conditions and may represent a background level of bacterial loading. Other contributions such as those contributed by mammalian livestock and avian livestock result from runoff from pastureland, concentrated animal feeding operations, or livestock yards. Pet contributions usually occur through street and land runoff into tributary streams. Non-Point source

Figure 3-4

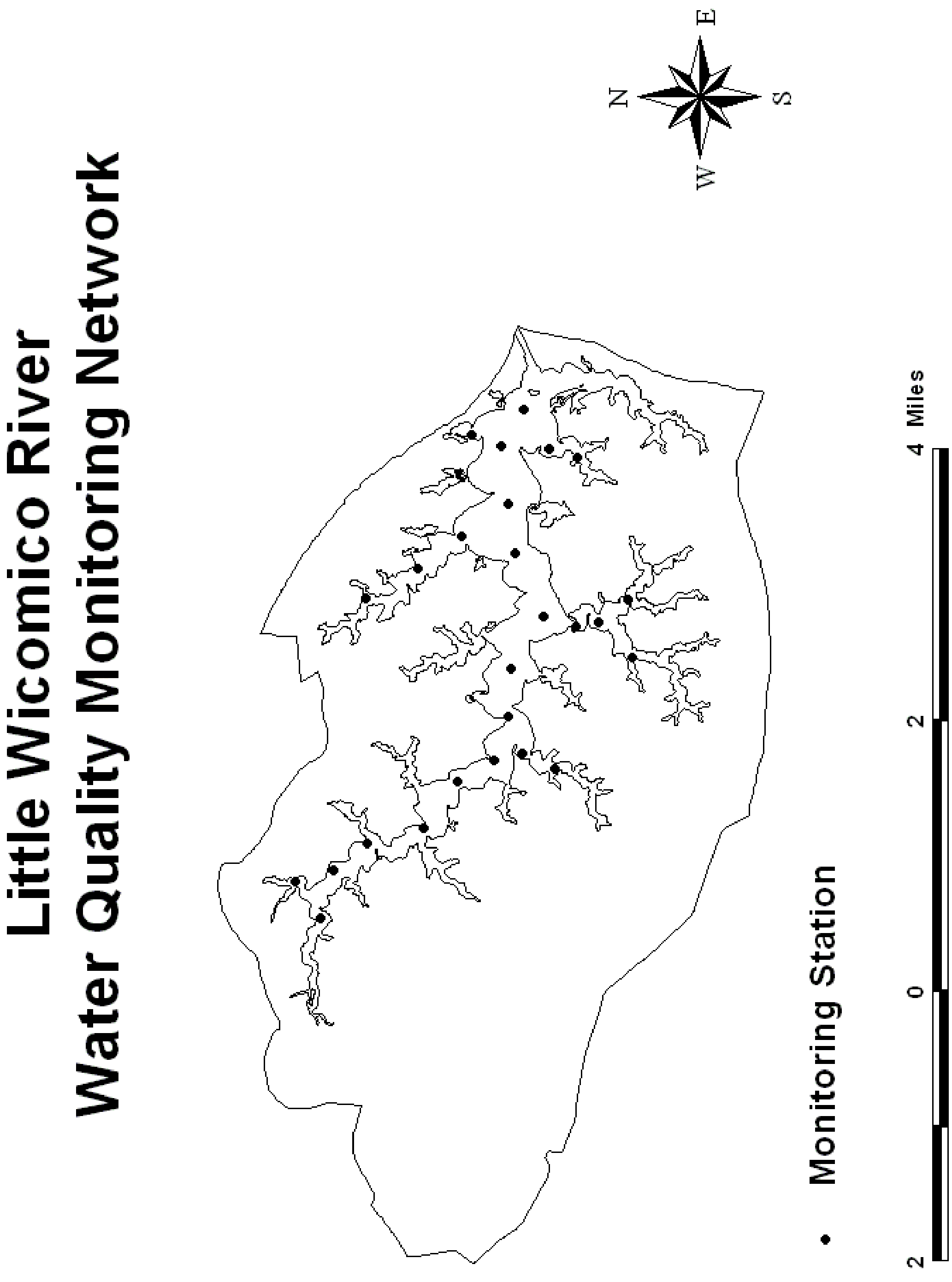
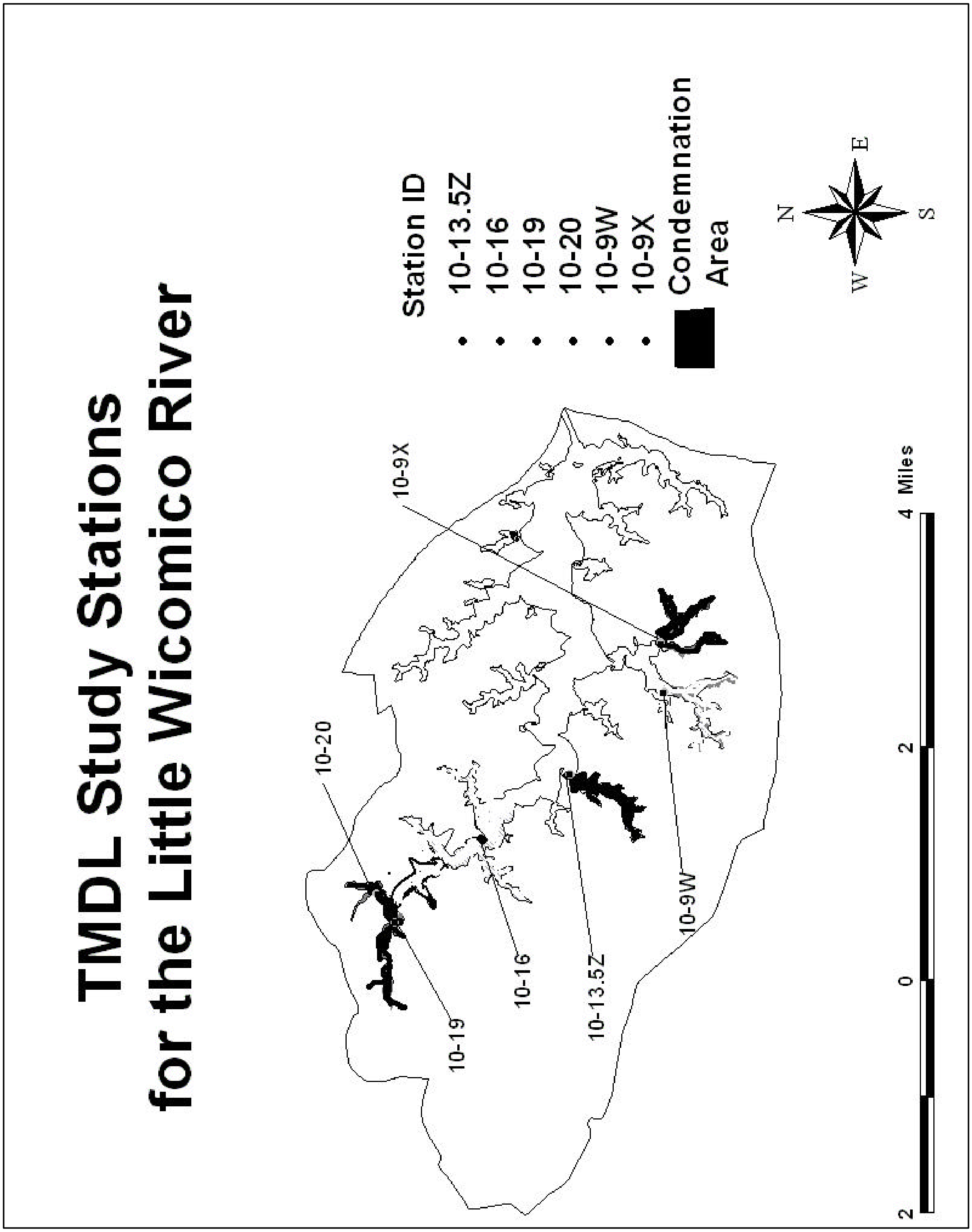

Little Wicomico River
Water Quality Monitoring Network
N
E
S
W
Monitoring Station
2
0
2
4 Miles

Figure 3-5



TMDL Study Stations
for the Little Wicomico River
Station ID
10-13.5Z
10-16
10-19
10-20
10-9W
10-9X
Condemnation Area
10-20
10-19
10-16
10-13.5Z
10-9W
10-9X
N
E
S
W
2
0
2
4 Miles

Figure 3-6

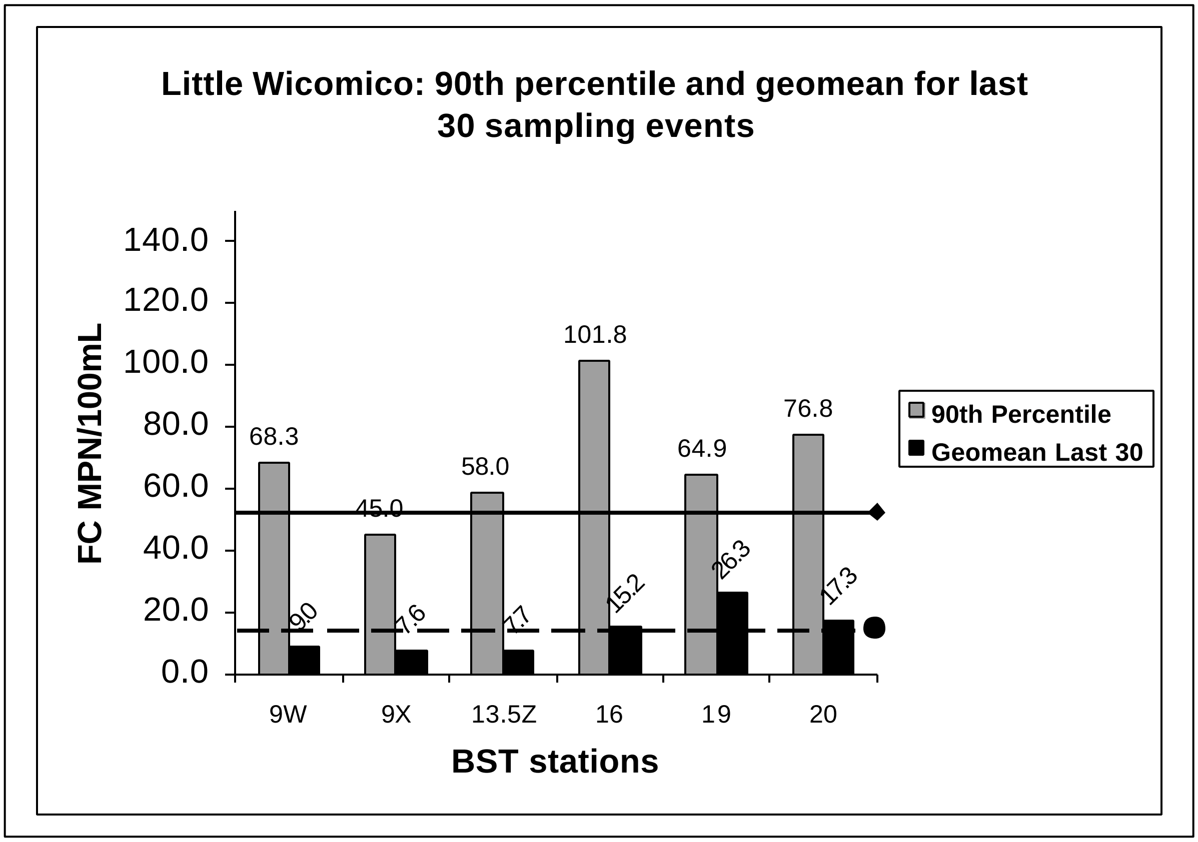
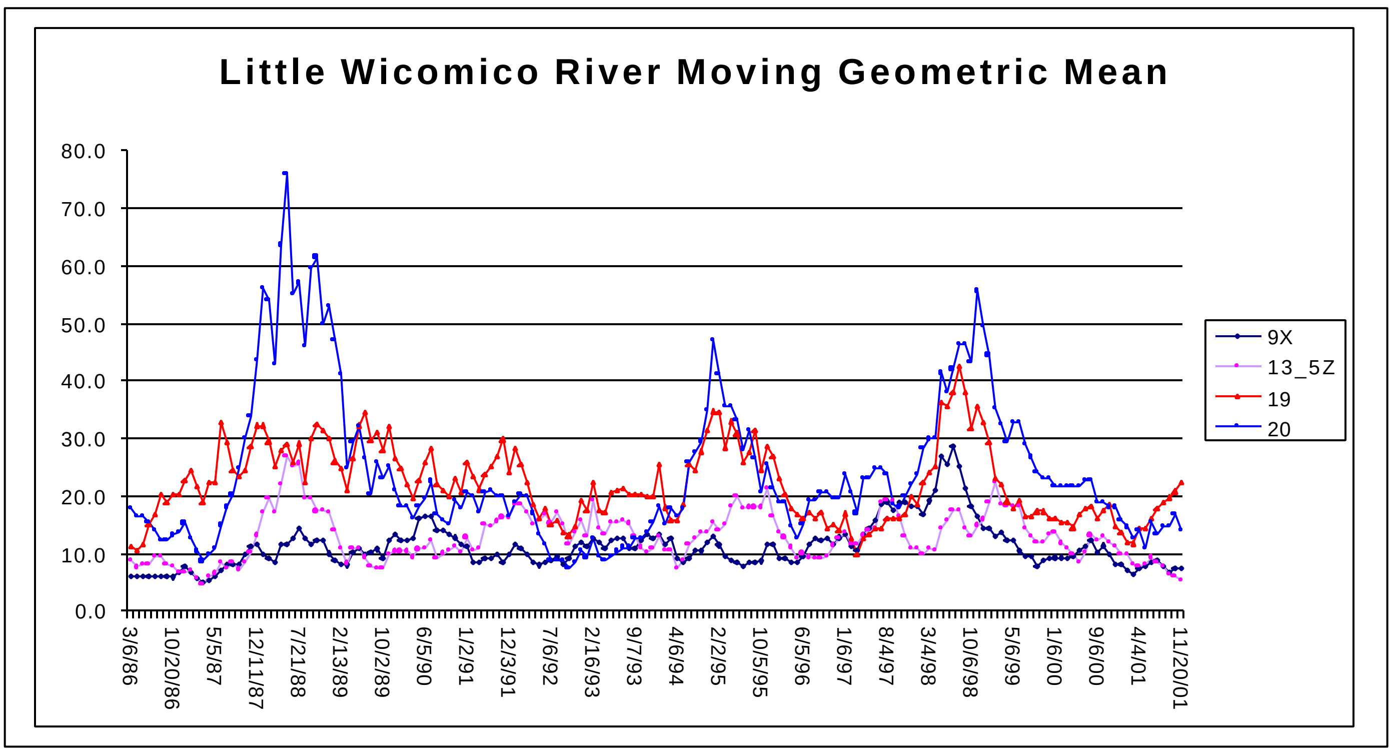


Figure 3-7



contributions to the bacterial levels in the Little Wicomico River from human activities generally arise from failing septic systems and associated drain fields, combined sewer outfalls and rarely exfiltration from sewer systems. In the Little Wicomico River watershed there is no municipal sewer system and therefore no combined outfalls. Homes in this watershed utilize septic systems and drain fields for waste treatment. It is therefore a high probability that human loading is due to failures in septic waste treatment systems, or through potential pollution from recreation vessel discharges.

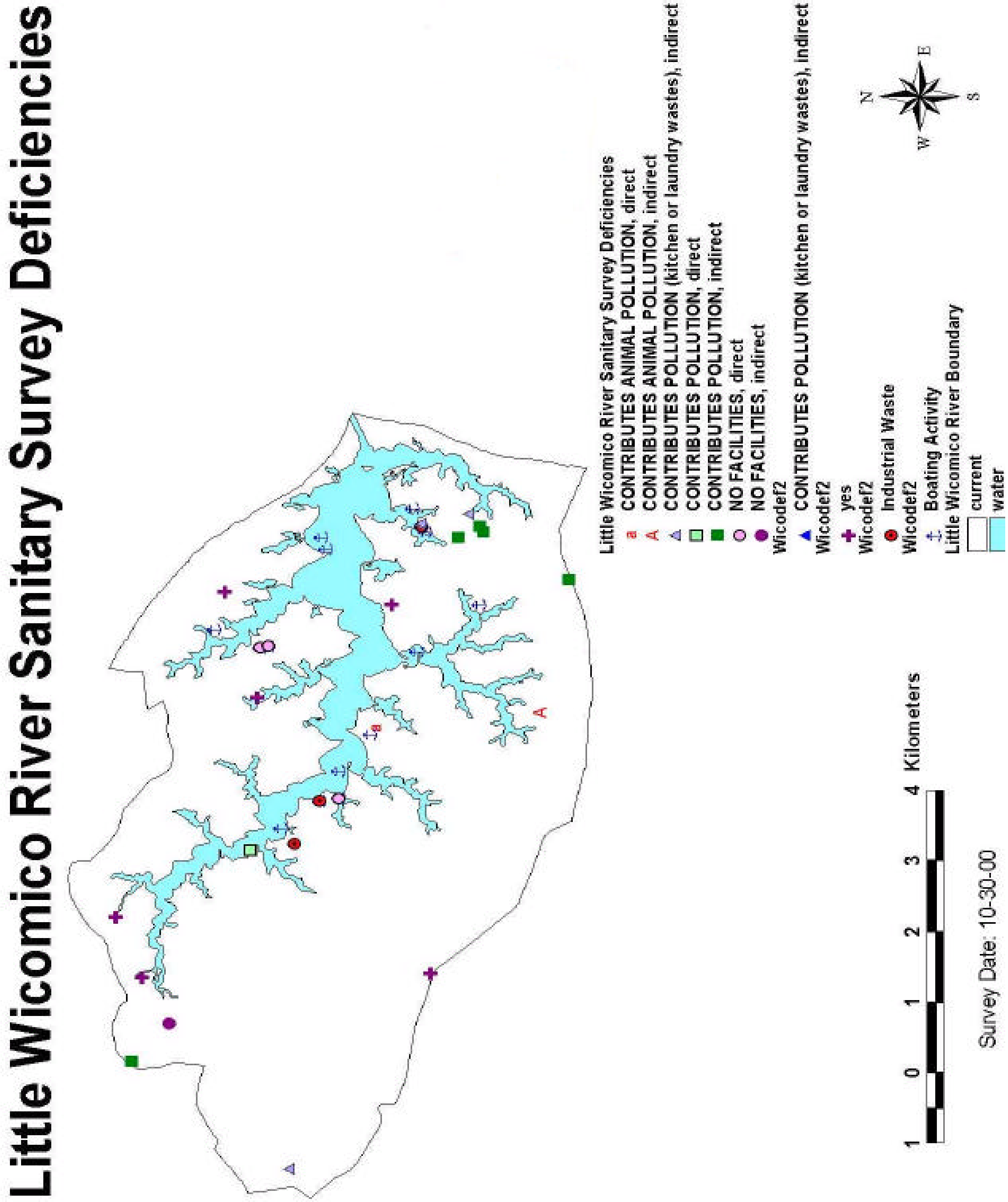
4.3 Bacterial Source Tracking

The six stations, four of which are the subject of this TMDL, that were selected as a pilot study were also evaluated for source characterization through a process called Bacterial Source Tracking or, BST. Twelve months of sampling was conducted from September 2001 through August 2002 to obtain the necessary fecal coliform isolates. The pilot study BST analysis uses the Antibiotic Resistance Approach (ARA), to determine the sources of fecal coliform to the water-body. 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. There are studies being initiated around the country to compare the accuracy of the ARA method with other bacterial source tracking approaches. The ARA determines the percent loading per source category to the water. The five major source categories are human, pets, livestock, wildlife, and birds. Figure 3-5 shows the pilot study stations that are also the BST monitoring stations for the Little Wicomico River watershed. The full BST report for the Little Wicomico River is located in the Appendix A.

The data developed for the Little Wicomico river watershed show that the dominant contribution in virtually all of the closure areas is overwhelmingly human in origin. Second to human, avian contributions, primarily from naturally occurring populations of waterfowl and wildlife are the largest components of the bacterial loading to the system. The monthly data by closure area is shown in on the following pages both in graphical and tabular form.

These data clearly show that human contributions to the Little Wicomico River and its tributaries are dominant in all of the established shellfish closure areas**.** The presence of bacteria of human origin in any water body is a source of concern from a public health perspective for both shellfish consumption and recreational use. Having determined both the in stream bacterial concentrations and the source contributions to the Little Wicomico River Shellfish closure areas, as well as their potentially contributions from non-point sources, a TMDL for each of these areas can be developed. The BST data pertinent to the four stations in this TMDL report are summarized in parts a through d of this section.

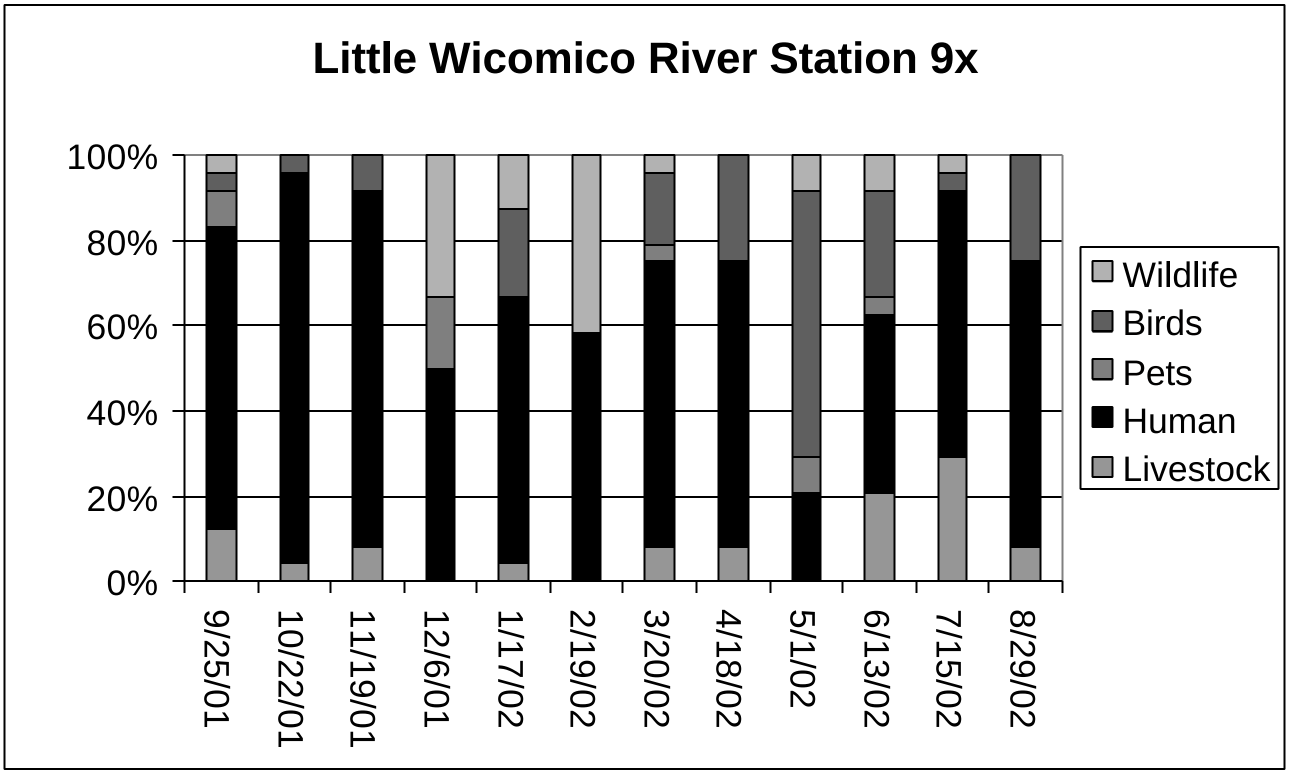
Figure 4-1


Little Wicomico River Sanitary Survey Deficiencies
Little Wicomico River Sanitary Survey Deficiencies
CONTRIBUTES ANIMAL POLLUTION, direct
CONTRIBUTES ANIMAL POLLUTION, indirect
CONTRIBUTES POLLUTION (kitchen or laundry wastes), indirect
CONTRIBUTES POLLUTION, direct
CONTRIBUTES POLLUTION, indirect
NO FACILITIES, direct
NO FACILITIES, indirect
Wicodef2
CONTRIBUTES POLLUTION (kitchen or laundry wastes), indirect
Wicodef2
yes
Wicodef2
Industrial Waste
Wicodef2
Boating Activity
Little Wicomico River Boundary
current
water
N
E
S
W
1 0 1 2 3 4 Kilometers
Survey Date: 10-30-00

a). Little Wicomico River Station 9x - DSS Closure Area 180, Bridge Creek "Jamie"

As shown in Figure 4-2 and its associated table, the dominant source contributing to the bacterial levels of Bridge Creek is human in origin in all months of the study period except May. Birds were the predominant source during May. Due to the short duration of the current BST sampling effort under the special study, it is unknown whether this seasonal effect is persistent from year to year. For this reason considerations of seasonal limits await further data development.

Figure 4-2

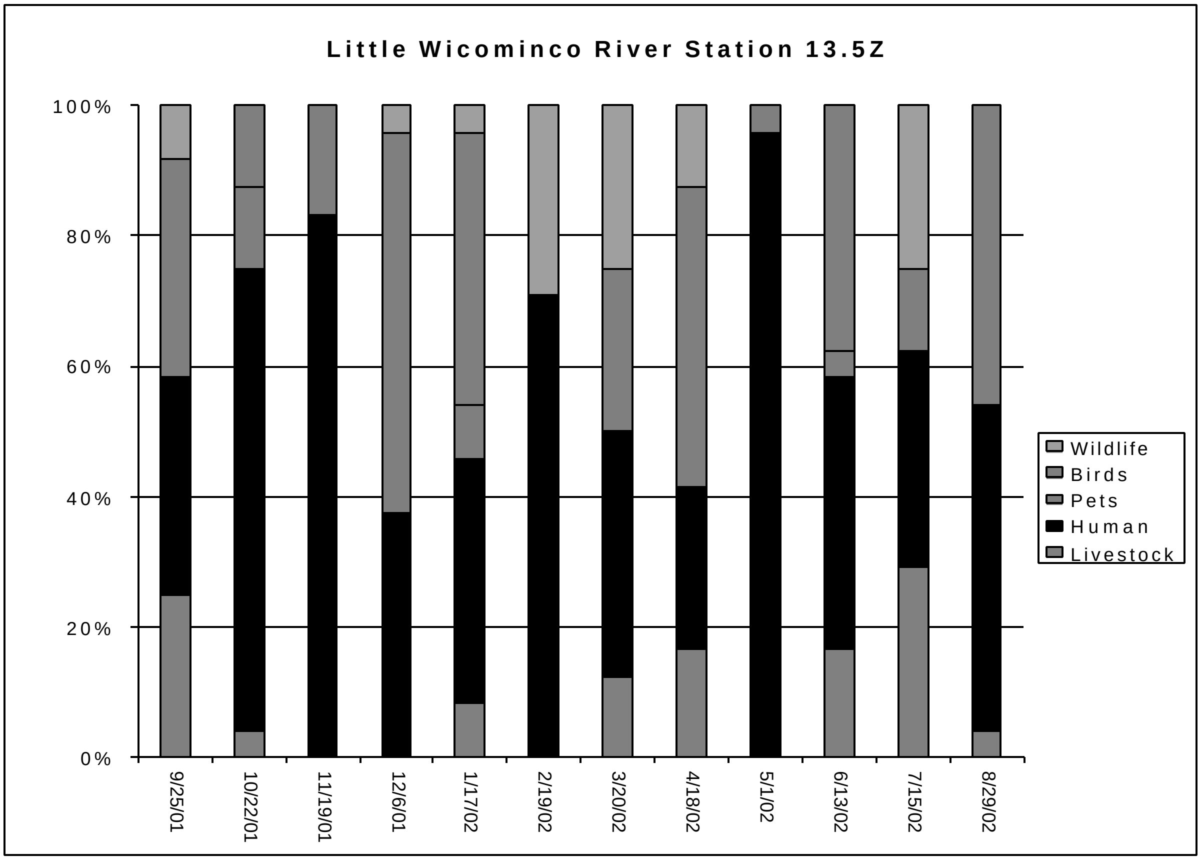


DATE	BACTERIA MPN/100ML	BIRDS	HUMAN	LIVESTOCK	PETS	WILDLIFE
9/25/01	43	4.17	70.83	12.5	8.33	4.17
10/22/01	2.9	4.17	91.67	4.17	0	0
11/19/01	9.1	8.33	83.33	8.33	0	0
12/6/01	2.9	0	50	0	16.67	33.33
1/17/02	3.6	20.83	62.5	4.17	0	12.5
2/19/02	2.9	0	58.33	0	0	41.67
3/20/02	15	16.67	66.67	8.33	4.17	4.17
4/18/02	9.1	25	66.67	8.33	0	0
5/1/02	15	62.5	20.83	0	8.33	8.33
6/13/02	9.1	25	41.67	20.83	4.17	8.33
7/15/02	3.6	4.17	62.5	29.17	0	4.17
8/29/02	9.1	25	66.67	8.33	0	0
Average	**10.44**	**16.32**	**61.81**	**8.68**	**3.47**	**9.72**

b). Little Wicomico River Station 13.5z - DSS Closure Area 105 A. Cod Creek.

Figure 4-3 and its associated table, shows that the dominant source contributing to the bacterial levels of Cod Creek is human in origin in all months of the study period except December, January and April. Birds were the dominant sources during December while birds were dominant in January and April.

Figure 4-3

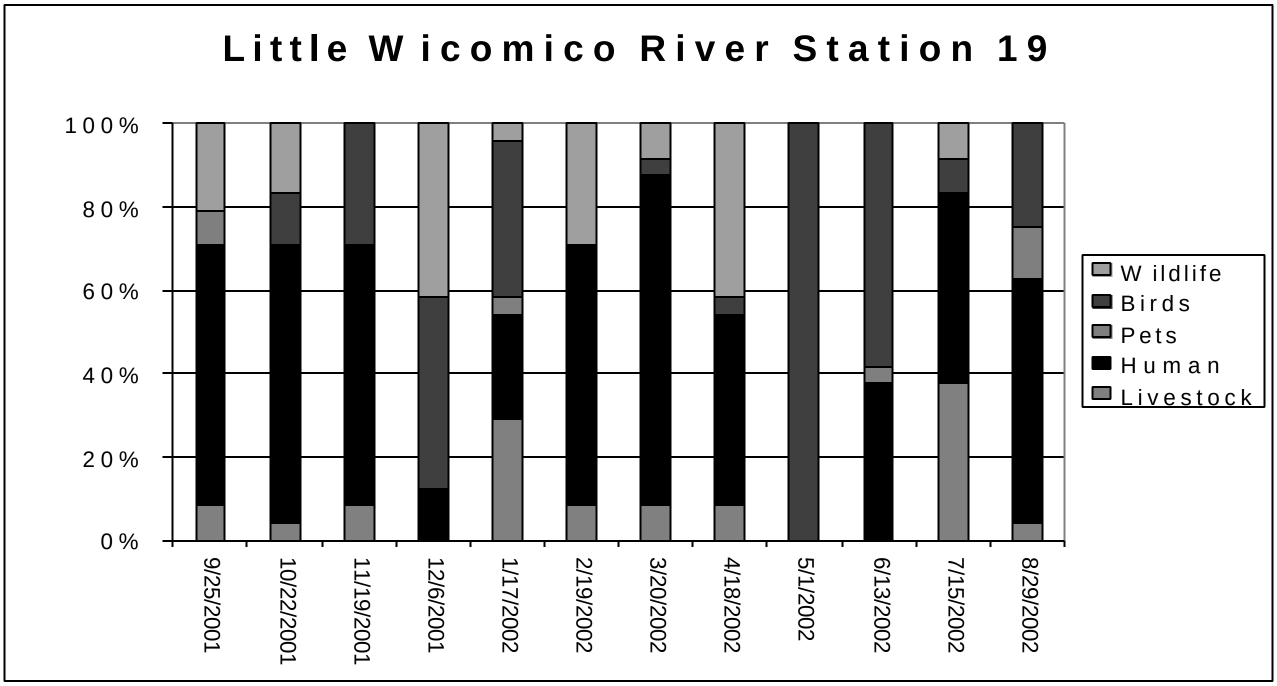


DATE	BACTERIA MPN/100ML	BIRDS	HUMAN	LIVESTOCK	PETS	WILDLIFE
9/25/01	43	33.33	33.33	25	0	8.33
10/22/01	2.9	12.5	70.83	4.17	12.5	0
11/19/01	3.6	16.67	83.33	0	0	0
12/6/01	43	0	37.5	0	58.33	4.17
1/17/02	3.6	41.67	37.5	8.33	8.33	4.17
2/19/02	3.6	0	70.83	0	0	29.17
3/20/02	43	25	37.5	12.5	0	25
4/18/02	15	45.83	25	16.67	0	12.5
5/1/02	9.1	4.17	95.83	0	0	0
6/13/02	3.6	37.5	41.67	16.67	4.17	0
7/15/02	9.1	12.5	33.33	29.17	0	25
8/29/02	2.9	45.83	50	4.17	0	0
13.14	**15.2**	**22.92**	**51.39**	**9.72**	**6.94**	**9.03**

c). Little Wicomico River Station 19 - DSS Closure Area 105 B. Cod Creek.

Figure 4-4 and its associated table, show that the dominant source contributing to the bacterial levels of Cod Creek is human in origin in all months of the study period except December, January, May and June. Birds were dominant those months. Due to the short duration of the current BST sampling effort under the special study, it is unknown whether this seasonal effect is persistent from year to year. For this reason considerations of seasonal limits await further data development.

Figure 4-4

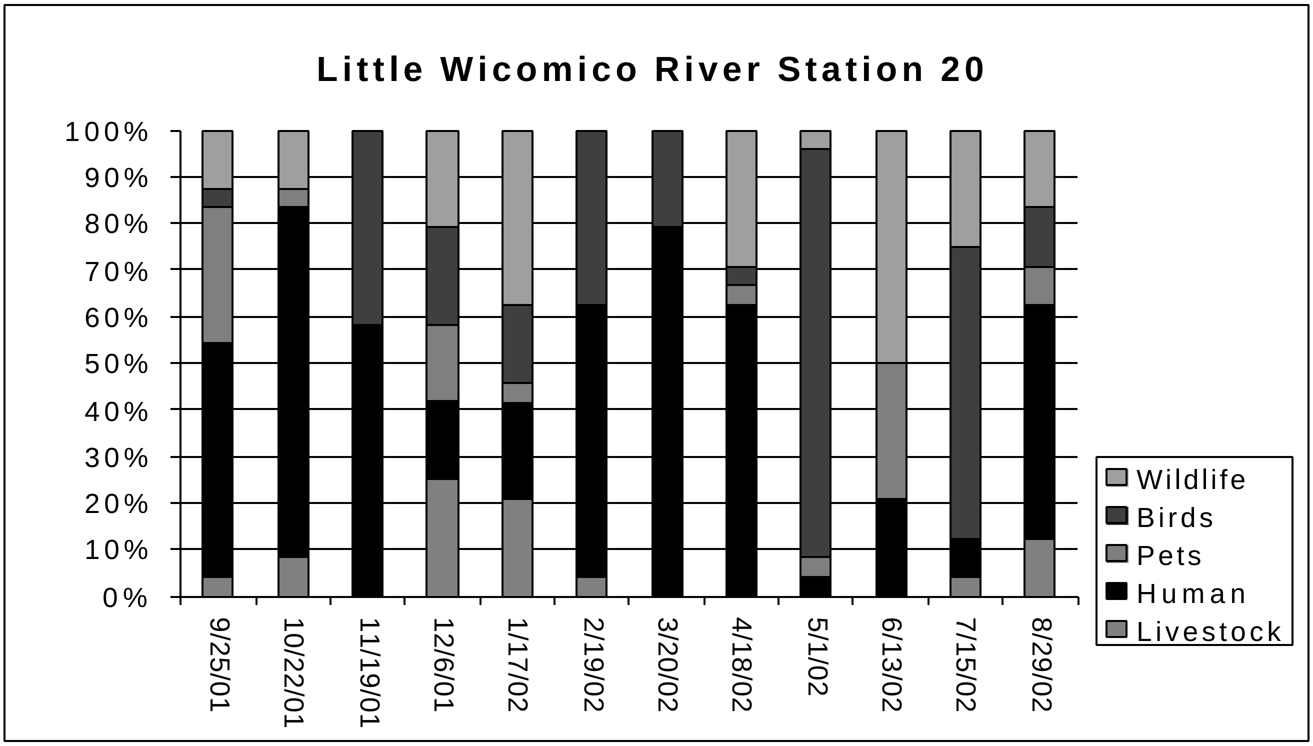


DATE	BACTERIA MPN/100ML	BIRDS%	HUMAN%	LIVESTOCK %	PETS%	WILDLIFE%
9/25/01	43	0	62.5	8.33	8.33	20.83
10/22/01	39	12.5	66.67	4.17	0	16.67
11/19/01	43	29.17	62.5	8.33	0	0
12/6/01	93	45.83	12.5	0	0	41.67
1/17/02	2.9	37.5	25	29.17	4.17	4.17
2/19/02	93	0	62.5	8.33	0	29.17
3/20/02	240	4.17	79.17	8.33	0	8.33
4/18/02	75	4.17	45.83	8.33	0	41.67
5/1/02	43	100	0	0	0	0
6/13/02	93	58.33	37.5	0	4.17	0
7/15/02	150	8.33	45.83	37.5	0	8.33
8/29/02	23	25	58.33	4.17	12.5	0
Average	**78.16**	**27.08**	**46.53**	**9.72**	**2.43**	**14.24**

d). Little Wicomico River Station 20 - DSS Closure Area 105 B. Cod Creek.

Figure 4-4 and its associated table, shows that the dominant source contributing to the bacterial levels of Cod Creek is human in origin in all months of the study period except December, may and June. Birds and wildlife where predominant sources during May and July. Due to the short duration of the current BST sampling effort under the special study, it is unknown whether this seasonal effect is persistent from year to year. For this reason considerations of seasonal limits await further data development.

Figure 4-5



DATE	BACTERIA MPN/100ML	BIRDS	HUMAN	LIVESTOCK	PETS	WILDLIFE
9/25/01	93	4.17	50	4.17	29.17	12.5
10/22/01	3.6	0	75	8.33	4.17	12.5
11/19/01	43	41.57	58.33	0	0	0
12/6/01	23	20.83	16.67	25	16.67	20.83
1/17/02	3.6	16.67	20.83	20.83	4.17	37.5
2/19/02	3.6	37.5	58.33	4.17	0	0
3/20/02	93	20.83	79.17	0	0	0
4/18/02	93	4.17	62.5	0	4.17	29.17
5/1/02	15	87.5	4.17	0	4.17	4.17
6/13/02	240	0	20.83	0	29.17	50
7/15/02	9.1	62.5	8.33	4.17	0	25
8/29/02	43	12.5	50	12.5	8.33	16.67
Average	**55.24**	**25.69**	**42.01**	**6.6**	**8.34**	**17.36**

5.0 TMDL Development

5.1 Simplified Modeling Approach(Tidal Volumetric Model):

Personnel from EPA, Virginia DEQ, Virginia Department of Conservation and Recreation (DCR), Maryland Department of the Environment (MDE), Virginia DSS, Virginia Institute of Marine Sciences (VIMS), United States Geological Survey, Virginia Polytechnic University, James Madison University, and Tetra Tech composed the shellfish TMDL workgroup and developed a procedure for developing TMDLs using either a simplified approach to the development of the TMDL. The goal of the procedure is to use bacteriological source tracking (BST) data to determine the sources of fecal coliform violations and the load reductions needed to attain the applicable criteria. The Little Wicomico River watershed meets the criteria for using the simple modeling approach because it has a small drainage area, land use is not complex, and there are no complex point source contributions.

5.2 The TMDL Calculation:

The most recent 30-months of data have been reviewed to determine the loading to the water body. The approach insures compliance with the 90^{th} percentile and geometric mean criteria. The geometric mean loading is based on the most recent 30-month geometric mean of fecal coliform. The load is also quantified for the 90^{th} percentile of the 30-month grouping.

5.2.1. Geometric Mean Analysis:

The geometric mean load is determined by multiplying the geometric mean concentration based on the most recent 30 month period of record by the volume of the water. The acceptable load is then determined by multiplying the geometric mean criteria by the volume of the water. The load reductions needed for the attainment of the geometric mean are then determined by subtracting the acceptable load from the geometric mean load. The detailed geometric mean determinations for each of the TMDL's by monitoring station is included in the Appendix. Calculations of the geometric mean loads and allowable geometric mean loads are shown in Table 5-1.

Example : (Geometric Mean Value MPN/100ml) x (volume) = Existing Load

(Criteria Value 14 MPN/100ml) x (volume) = Allowable Load

Existing Load – Allowable Load = Load Reduction

5.2.2. 90^{th} Percentile Analysis:

The 90^{th} percentile load is determined by multiplying the 90^{th} percentile concentration, based on the most recent 30 month period of record, by the volume of the water. The acceptable load us determined by multiplying the 90^{th} percentile criteria by the volume of the water. The load reductions needed for the attainment of the 90^{th} percentile criteria are determined by subtracting the acceptable load from the 90^{th} percentile load. This is shown in Table 5-2. The more stringent criteria between the two methods is be used for the TMDL.

Table 5-1 Geometric Mean Calculations for the Little Wicomico River TMDL's

CLOSURE ID AND STATION NUMBER	GEO – METRI C MEAN	SEGMENT VOLUME (CUBIC METERS)	VOLUME X GEO – MEAN	VOLUME X CRITERIA (14MPN/100ML)	REQUIRED REDUCTION IN PERCENT
180 STATION 10-9X	7.6	175,387.31	1.34E+10	2.46E+10	0%
180-A STATION 10-13.5Z	7.7	152,369.11	1.17E+10	2.13E+10	0%
180 – B STATION 10-19	26.3	395,208.17	1.04E+11	5.53E+10	87.8%
180 – B STATION 10-20	17.3	Using same volume as STA 19	6.85E+10	5.53E+10	23.8%

A comparison of the geometric mean data and the 90th percentile data for the last 30 months shows that the 90th percentile data is the more critical condition. In essence the 90th percentile criteria is that criteria most frequently exceeded and it is reductions in these bacterial loadings that will yield water quality improvements which address the water quality standard. Therefore the 90th percentile loading is combined with the results of the bacterial source tracking (BST) to allocate source contributions and establish load reduction targets among the various contributing sources.

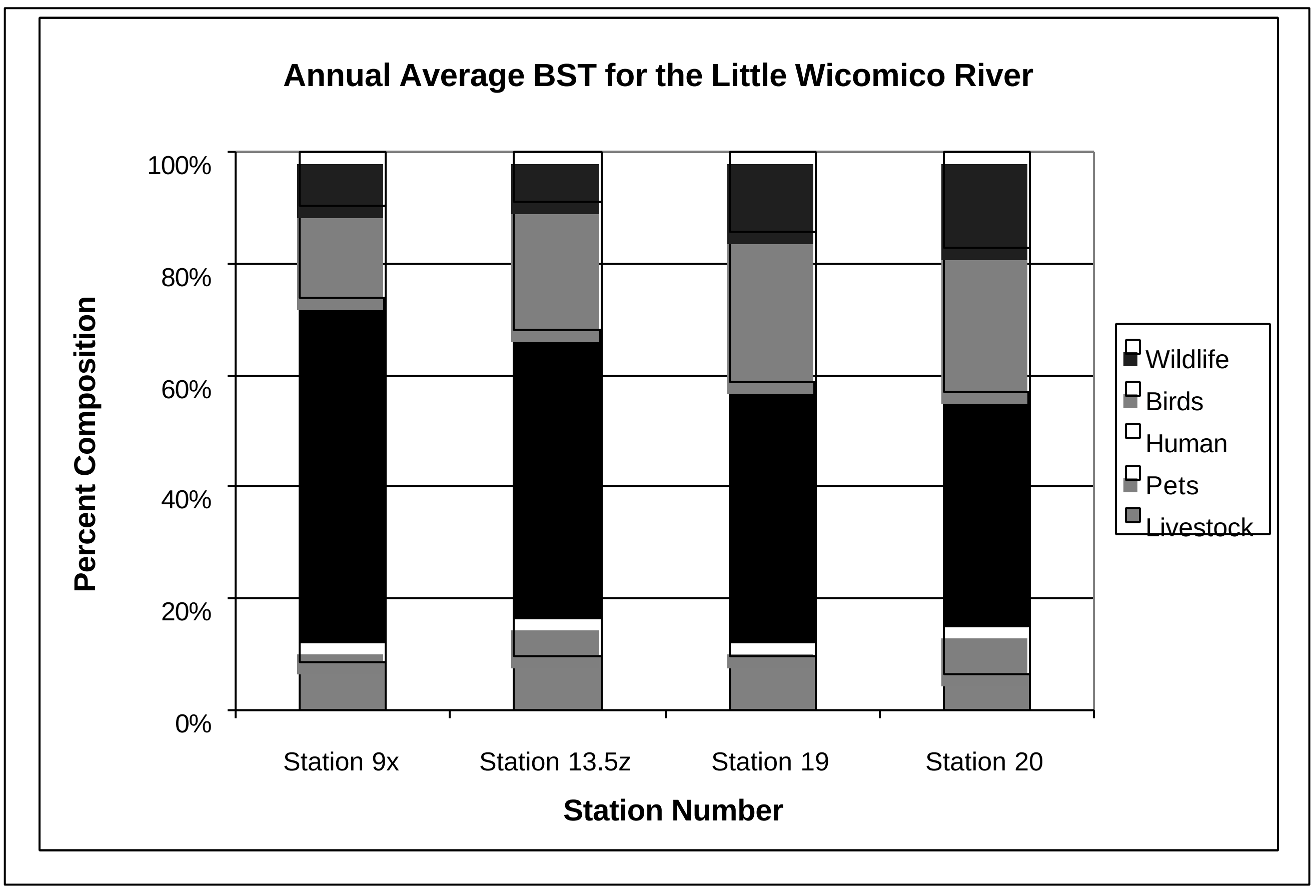
Table 5-2. 90th Percentile Calculations for the Little Wicomico River TMDL's

CLOSURE ID AND STATION NUMBER	90TH PERCENT. VALUE (MPN/100 ML)	SEGMENT VOLUME (CUBIC METERS)	VOLUME X 90TH PERCENT. VALUE = ACTUAL LOAD	VOLUME X 90TH CRITERIA (49MPN/100ML) = LOAD ALLOCATION	REQUIRED LOAD REDUCTION IN PERCENT
105 STATION 10-9X	45.0	175,387.31	7.89E+10	8.59E+10	0%
180 A STATION 10-13.5Z	58.0	152,369.11	8.84E+10	7.47E+10	15%
180 – B STATION 10-19	64.9	395,208.17	2.64E+11	1.94E+11	24.22%
180 – B STATION 10-20	76.8	Using same volume as STA 19	3.04E+11	1.94E+11	36.18%

5.2.3. BST Data:

The BST data determines the percent loading for each of the major source categories and is used to determine where load reductions are needed. Since there are 12 BST samples for each TMDL, the percent loading per source may be averaged over the 12 month period if there are no seasonal differences between sources. If seasonal differences between the sources are established seasonal averaging may be employed to group seasons and evaluate loading by season. The percent loading by source is multiplied by the total geometric mean, or 90^{th} percentile load, to determine the load by source. Whether geometric mean or 90^{th} percentile load is used is based upon which one is determined to be the controlling loading condition. The percent reduction needed to attain the water quality standard or criteria are allocated to each source category. This is shown in Table 5-3 and serves to fulfill the TMDL requirements by insuring that the criteria is attained. Additionally it ensures that all sources and loadings are identified and quantified via the BST and mathematical calculations, season variability is addressed, and critical conditions are identified. The annual average BST results for all stations are shown in Table 5-1.

Figure 5-1



5.3. Consideration of Critical Conditions

EPA regulations at 40 CFR 130.7 (c)(1) require TMDLs to take into account critical conditions for stream flow, loading, and water quality parameters. The intent of this requirement is to ensure that the water quality of the Little Wicomico river and its tributaries are protected during times when they are most vulnerable.

Critical conditions are important because they describe the factors that combine to cause a violation of water quality standards and will help in identifying the actions that may have to be undertaken to meet water quality standards. The sources of bacteria for the Little Wicomico River estuary are a mixture of dry and wet weather driven sources. TMDL development utilized the volumetric load determination approach the results of which are summarized in Table 5.4. Therefore, addressing the critical conditions for this watershed is implicit in the TMDL development. There was very little seasonality in BST results and in the measured concentrations of fecal coliforms. This justifies an averaging approach to load allocation in the water shed. A margin of safety is implicit in this approach as it is developed to target the highest level of non-compliance with the water quality standard, assumes no flushing, and has a conservative assimilation capacity.

Table 5-3 REDUCTION BASED UPON 90TH PERCENTILE STANDARD

AREA 180 STATION 10-9X	BST Result % of total load	Actual Load (cfu)*	Load Allocation (cfu)*	Reduction needed
Total	100%	7.89E+10	8.59E+10	0%
Bird	16%	1.26E+10	1.26E+10	0%
Wildlife	10%	7.89E+09	7.89E+09	0%
Human	62%	4.89E+10	4.89E+10	0%
Pets	3%	2.37E+09	2.37E+09	0%
Livestock	9%	7.10E+09	7.10E+09	0%

AREA 180-A STATION 10-13.5Z	BST Result % of total load	Actual Load (cfu)*	Load Allocation (cfu)*	Reduction needed
Total	100%	8.84E+10	7.47E+10	15%
Bird	23%	2.03E+10	2.03E+10	0%
Wildlife	9%	7.96E+09	7.96E+09	0%
Human	51%	4.51E+10	3.14E+10	30%
Pets	7%	6.19E+09	6.19E+09	0%
Livestock	10%	8.84E+09	8.84E+09	0%

AREA 180 – B STATION 10-19	BST Result % of total load	Actual Load (cfu)*	Load Allocation (cfu)*	Reduction needed
Total	100%	2.56E+11	1.94E+11	24%
Bird	16%	4.10E+10	4.10E+10	0%
Wildlife	10%	2.56E+10	2.56E+10	0%
Human	62%	1.59E+11	1.00E+11	37%
Pets	3%	7.68E+09	7.68E+09	0%
Livestock	9%	2.05E+10	2.05E+10	0%

Table 5-3 REDUCTION BASED UPON 90TH PERCENTILE STANDARD (CONTINUED)

AREA 180 - B STATION 10-20	BST Result % of total load	Actual Load (cfu)*	Load Allocation (cfu)*	Reduction needed
Total	**100%**	**3.04E+11**	**1.94E+11**	**37%**
Bird	**16%**	**4.86E+10**	**4.86E+10**	**0%**
Wildlife	**10%**	**3.04E+10**	**3.04E+10**	**0%**
Human	**62%**	**1.88E+11**	**8.20E+10**	**56%**
Pets	**3%**	**9.12E+09**	**9.12E+09**	**0%**
Livestock	**9%**	**2.43E+10**	**2.43E+10**	**0%**

5.4. Consideration of Seasonal Variations

This TMDL development utilized the volumetric load determination approach the results of which are summarized in Table 5.4. Addressing the critical conditions for this watershed is implicit in this methodology as used in this TMDL development in part because the entire volume of the impaired segment is utilized rather than only a portion of the water column. Because there was no readily discernible seasonality in BST results and in the measured concentrations of fecal coliforms, an averaging approach to load allocation in the watershed was considered justified. Additionally, a margin of safety is implied in this approach as it is developed to target the highest level at which the ambient levels exceed the water quality standard, assumes no flushing, and has a conservative assimilation capacity. Further BST and water quality monitoring station location, monitoring frequency; among other factors do not allow precision in determining actual sources. Therefore emphasis on the watershed, rather than individual segments, ensures that potential sources are addressed in a comprehensive manner regardless of their position in the landscape and proximity to an affected area. This approach is conservative in that it considers all potential sources, does not differentiate the runoff of bacterial pollutants from up-slope areas from those in the near shore, and assumes that all contributions to the segments in system are not exported by tide or current.

Table 5.4 TMDL Summary for Six Closures in the Little Wicomico River Watershed

Water Body / Closure ID	Pollutant Identified	TMDL c.f.u.*	Waste Load Allocation c.f.u.*	Load Allocation c.f.u.*	Margin of Safety
Little Wicomico River 180 Station 9X	Fecal Coliform	8.59 E+10	0	8.59 E+10	Implicit
Little Wicomico River 180-A Station 13.5Z	Fecal Coliform	7.47 E+11	0	7.47 E+11	Implicit
Little Wicomico River 180-B Station 19	Fecal Coliform	1.94 E+11	0	1.94 E+11	Implicit
Little Wicomico River 180-B Station 20	Fecal Coliform	1.94 E+11	0	1.94 E+11	Implicit

* c.f.u. = colony forming units of bacteria

6.0 Implementation

The goal of the TMDL program is to establish a three-step path that will lead to attainment of water quality standards. The first step in the process is to develop TMDLs that will result in meeting water quality standards. This report represents the culmination of that effort for the bacterial impairments for segments located on the on the Little Wicomico River. The second step is to develop a TMDL implementation plan. The final step is to implement the TMDL implementation plan, and to monitor stream water quality to determine if water quality standards are being attained.

Once a TMDL has been approved by EPA, measures must be taken to reduce pollution levels in the impaired segments. These measures, which can include where appropriate the use of better treatment technology or the installation of best management practices (BMPs), that are implemented in an iterative process that is described along with specific BMPs in the implementation plan. The process for developing an implementation plan has been described in the recent "TMDL Implementation Plan Guidance Manual", published in July 2003 and is available upon request from the DEQ and DCR TMDL project staff or at http://www.deq.state.va.us/tmdl/implans/ipguide.pdf With successful completion of implementation plans, Virginia will be well on the way to restoring impaired waters and enhancing the value of this important resource. Additionally, development of an approved implementation plan will improve a locality's chances for obtaining financial and technical assistance during implementation.

6.1 Staged Implementation

In general, Virginia intends for the required reductions to be implemented in an iterative process that first addresses those sources with the largest impact on water quality. The iterative implementation of BMPs in the watershed has several benefits:

1. It enables tracking of water quality improvements following BMP implementation through follow-up stream monitoring;
2. It provides a measure of quality control, given the uncertainties inherent in computer simulation modeling;
3. It provides a mechanism for developing public support through periodic updates on BMP implementation and water quality improvements;
4. It helps ensure that the most cost effective practices are implemented first; and
5. It allows for the evaluation of the adequacy of the TMDL in achieving water quality standards.

Watershed stakeholders will have opportunity to participate in the development of the TMDL implementation plan. Specific goals for BMP implementation will be established as part of the implementation plan development

6.2 Link to ongoing Restoration Efforts

Implementation of this TMDL will contribute to on-going water quality improvement efforts aimed at restoring water quality in the Chesapeake Bay. A tributary strategy has been developed for the Potomac River Embayments. Up-to-date information on tributary strategy development can be found at http://www.snr.state.va.us/Initiatives/TributaryStrategies/shenandoah.cfm.

6.3 Reasonable Assurance for Implementation

6.3.1 Follow-Up Monitoring

VDH-DSS will continue sampling at the established bacteriological monitoring stations in accordance with its shellfish monitoring program. VADEQ will continue to use data from these monitoring stations and related ambient monitoring stations to evaluate improvements in the bacterial community and the effectiveness of TMDL implementation in attainment of the general water quality standard.

6.3.2. Regulatory Framework

While section 303(d) of the Clean Water Act and current EPA regulations do not require the development of TMDL implementation plans as part of the TMDL process, they do require reasonable assurance that the load and wasteload allocations can and will be implemented. Additionally, Virginia's 1997 Water Quality Monitoring, Information and Restoration Act (the "Act") directs the State Water Control Board to "develop and implement a plan to achieve fully supporting status for impaired waters" (Section 62.1-44.19.7). The Act also establishes that the implementation plan shall include the date of expected achievement of water quality objectives, measurable goals, corrective actions necessary and the associated costs, benefits and environmental impacts of addressing the impairments. EPA outlines the minimum elements of an approvable implementation plan in its'

1999 "Guidance for Water Quality-Based Decisions: The TMDL Process." The listed elements include; implementation actions/management measures, timelines, legal or regulatory controls, time required to attain water quality standards, monitoring plans and milestones for attaining water quality standards.

Once developed, DEQ intends to incorporate the TMDL implementation plan into the appropriate Water Quality Management Plan (WQMP), in accordance with the Clean Water Act's Section 303(e). In response to a Memorandum of Understanding (MOU) between EPA and DEQ, DEQ also submitted a draft Continuous Planning Process to EPA in which DEQ commits to regularly updating the WQMPs. Thus, the WQMPs will be, among other things, the repository for all TMDLs and TMDL implementation plans developed within a river basin.

6.3.3. Implementation Funding Sources

One potential source of funding for TMDL implementation is Section 319 of the Clean Water Act. Section 319 funding is a major source of funds for Virginia's Non-point Source Management Program. Other funding sources for implementation include the U.S. Department of Agriculture's Conservation Reserve Enhancement and Environmental Quality Incentive Programs, the Virginia State Revolving Loan Program, and the Virginia Water Quality Improvement Fund. The TMDL Implementation Plan Guidance Manual contains additional information on funding sources, as well as government agencies that might support implementation efforts and suggestions for integrating TMDL implementation with other watershed planning efforts.

6.3.4 Addressing Wildlife Contributions

In some waters for which TMDLs have been developed, water quality modeling indicates that even after removal of all of the sources of bacteria (other than wildlife), the stream will not attain standards under all flow regimes at all times. This is not the case for the Little Wicomico River. **However, neither the Commonwealth of Virginia, nor EPA are proposing the elimination of wildlife to allow for the attainment of water quality standards.** This is obviously an impractical and wholly undesirable action. While managing over-populations of wildlife remains as an option to local stakeholders, the reduction of wildlife or changing a natural background condition is not the intended goal of a TMDL.

Based on the above, EPA and Virginia have developed a TMDL strategy to address the wildlife issue. The first step in this strategy is to develop a reduction goal such as presented in Table 5.4. The pollutant reductions for the interim goal are applied only to controllable, anthropogenic sources identified in the TMDL, setting aside any control strategies for wildlife. During the first implementation phase all controllable sources would be reduced to the maximum extent practicable using the staged approach outlined above. Following completion of the first phase, DEQ would re-assess water quality in the stream to determine if the water quality standard is attained. This effort will also evaluate if the modeling assumptions were correct. If water quality standards are not being met, a UAA may be initiated to reflect the presence of naturally high bacteria levels due to uncontrollable sources. In some cases, the effort may never have to go to the second phase because the water quality standard exceedances attributed to wildlife in the model are very small and infrequent and fall within the margin of error.

7.0. Public Participation

The development of the Little Wicomico River TMDL would not have been possible without public participation. Two stakeholder meetings were held at the Northumberland County Library in Heathsville, Virginia on February 2002 and again on May 2003 to discuss the process for TMDL development and the source assessment results. Sixteen 16 people representing state and local government, private citizens and academic research institutions attended each meeting. A list of the organizations present is provided in Appendix C. Copies of the presentation materials were available for public distribution and subsequently posted on the VADEQ web page.

The formal notice of the public meeting was printed in the Virginia Register on June 30, 2003. Notices were also published in two local area newspapers. The public meeting was held at the Northumberland County Courthouse on July 22, 2003. Members of the County Board of Supervisors, concerned citizens, affected state agencies attended. A formal presentation of the results contained in this TMDL report was made by DEQ staff and public comment solicited. A copy of the presentation was made available at the meeting and on he DEQ website. The attendance list for the public meeting and the questions asked by the participants is shown in Appendix C. There followed a 30-day public comment period and no written comments were received. Generally, the public comment garnered at the meeting focused on the issue of how the implementation of the TMDL could be funded. Secondary issues, such as potential bacterial sources, changes in use designation, and concerns over the regulatory consequences of not meeting the TMDL load allocation. A summary of these questions and the answers by DEQ staff is also found in Appendix C.

References

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GLOSSARY

Note: All entries in italics are taken from USEPA (1998).

303(d). A section of the Clean Water Act of 1972 requiring states to identify and list water bodies that do not meet the states' water quality standards.

Allocations. That portion of a receiving water's loading capacity attributed to one of its existing or future pollution sources (nonpoint or point) or to natural background sources. (A wasteload allocation [WLA] is that portion of the loading capacity allocated to an existing or future point source, and a load allocation [LA] is that portion allocated to an existing or future nonpoint source or to natural background levels. Load allocations are best estimates of the loading, which can range from reasonably accurate estimates to gross allotments, depending on the availability of data and appropriate techniques for predicting loading.)

Ambient water quality. Natural concentration of water quality constituents prior to mixing of either point or nonpoint source load of contaminants. Reference ambient concentration is used to indicate the concentration of a chemical that will not cause adverse impact on human health.

Anthropogenic. Pertains to the [environmental] influence of human activities.

Bacteria. Single-celled microorganisms. Bacteria of the coliform group are considered the primary indicators of fecal contamination and are often used to assess water quality.

Bacterial source tracking (BST). A collection of scientific methods used to track sources of fecal contamination.

Best management practices (BMPs). Methods, measures, or practices determined to be reasonable and cost-effective means for a landowner to meet certain, generally nonpoint source, pollution control needs. BMPs include structural and nonstructural controls and operation and maintenance procedures.

Clean Water Act (CWA). The Clean Water Act (formerly referred to as the Federal Water Pollution Control Act or Federal Water Pollution Control Act Amendments of 1972), Public Law 92-500, as amended by Public Law 96-483 and Public Law 97-117, 33 U.S.C. 1251 et seq. The Clean Water Act (CWA) contains a number of provisions to restore and maintain the quality of the nation's water resources. One of these provisions is section 303(d), which establishes the TMDL program.

Concentration. Amount of a substance or material in a given unit volume of solution; usually measured in milligrams per liter (mg/L) or parts per million (ppm).

Contamination. The act of polluting or making impure; any indication of chemical, sediment, or biological impurities.

Cost-share program. A program that allocates project funds to pay a percentage of the cost of constructing or implementing a best management practice. The remainder of the costs is paid by the producer(s).

Critical condition. The critical condition can be thought of as the "worst case" scenario of environmental conditions in the waterbody in which the loading expressed in the TMDL for the pollutant of concern will continue to meet water quality standards. Critical conditions are the combination of environmental factors (e.g., flow, temperature, etc.) that results in attaining and maintaining the water quality criterion and has an acceptably low frequency of occurrence.

Designated uses. Those uses specified in water quality standards for each waterbody or segment whether or not they are being attained.

Domestic wastewater. Also called sanitary wastewater, consists of wastewater discharged from residences and from commercial, institutional, and similar facilities.

Drainage basin. A part of a land area enclosed by a topographic divide from which direct surface runoff from precipitation normally drains by gravity into a receiving water. Also referred to as a watershed, river basin, or hydrologic unit.

Existing use. Use actually attained in the waterbody on or after November 28, 1975, whether or not it is included in the water quality standards (40 CFR 131.3).
Fecal Coliform. Indicator organisms (organisms indicating presence of pathogens) associated with the digestive tract.
Geometric mean. A measure of the central tendency of a data set that minimizes the effects of extreme values.
GIS. Geographic Information System. A system of hardware, software, data, people, organizations and institutional arrangements for collecting, storing, analyzing and disseminating information about areas of the earth. (Dueker and Kjerne, 1989)
Infiltration capacity. The capacity of a soil to allow water to infiltrate into or through it during a storm.
Interflow. Runoff that travels just below the surface of the soil.
Loading, Load, Loading rate. The total amount of material (pollutants) entering the system from one or multiple sources; measured as a rate in weight per unit time.
Load allocation (LA). The portion of a receiving waters loading capacity attributed either to one of its existing or future nonpoint sources of pollution or to natural background sources. Load allocations are best estimates of the loading, which can range from reasonably accurate estimates to gross allotments, depending on the availability of data and appropriate techniques for predicting the loading. Wherever possible, natural and nonpoint source loads should be distinguished (40 CFR 130.2(g)).
Loading capacity (LC). The greatest amount of loading a water can receive without violating water quality standards.
Margin of safety (MOS). A required component of the TMDL that accounts for the uncertainty about the relationship between the pollutant loads and the quality of the receiving waterbody (CWA section 303(d)(1)©). The MOS is normally incorporated into the conservative assumptions used to develop TMDLs (generally within the calculations or models) and approved by EPA either individually or in state/EPA agreements. If the MOS needs to be larger than that which is allowed through the conservative assumptions, additional MOS can be added as a separate component of the TMDL (in this case, quantitatively, a TMDL = LC = WLA + LA + MOS).
Mean. The sum of the values in a data set divided by the number of values in the data set.
Monitoring. Periodic or continuous surveillance or testing to determine the level of compliance with statutory requirements and/or pollutant levels in various media or in humans, plants, and animals.
Narrative criteria. Nonquantitative guidelines that describe the desired water quality goals.
Non-point source. Pollution that originates from multiple sources over a relatively large area. Non-point sources can be divided into source activities related to either land or water use including failing septic tanks, improper animal-keeping practices, forest practices, and urban and rural runoff.
Numeric targets. A measurable value determined for the pollutant of concern, which, if achieved, is expected to result in the attainment of water quality standards in the listed waterbody.
Point source. Pollutant loads discharged at a specific location from pipes, outfalls, and conveyance channels from either municipal wastewater treatment plants or industrial waste treatment facilities. Point sources can also include pollutant loads contributed by tributaries to the main receiving water stream or river.
Pollutant. Dredged spoil, solid waste, incinerator residue, sewage, garbage, sewage sludge, munitions, chemical wastes, biological materials, radioactive materials, heat, wrecked or discarded equipment, rock, sand, cellar dirt, and industrial, municipal, and agricultural waste discharged into water. (CWA section 502(6)).
Pollution. Generally, the presence of matter or energy whose nature, location, or quantity produces undesired environmental effects. Under the Clean Water Act, for example, the term is defined as the man-made or man-induced alteration of the physical, biological, chemical, and radiological integrity of water.

Privately owned treatment works. Any device or system that is (a) used to treat wastes from any facility whose operator is not the operator of the treatment works and (b) not a publicly owned treatment works.
Public comment period. The time allowed for the public to express its views and concerns regarding action by EPA or states (e.g., a Federal Register notice of a proposed rule-making, a public notice of a draft permit, or a Notice of Intent to Deny).
Publicly owned treatment works (POTW). Any device or system used in the treatment (including recycling and reclamation) of municipal sewage or industrial wastes of a liquid nature that is owned by a state or municipality. This definition includes sewers, pipes, or other conveyances only if they convey wastewater to a POTW providing treatment.
Raw sewage. Untreated municipal sewage.
Receiving waters. Creeks, streams, rivers, lakes, estuaries, ground-water formations, or other bodies of water into which surface water and/or treated or untreated waste are discharged, either naturally or in man-made systems.
Riparian areas. Areas bordering streams, lakes, rivers, and other watercourses. These areas have high water tables and support plants that require saturated soils during all or part of the year. Riparian areas include both wetland and upland zones.
Riparian zone. The border or banks of a stream. Although this term is sometimes used interchangeably with floodplain, the riparian zone is generally regarded as relatively narrow compared to a floodplain. The duration of flooding is generally much shorter, and the timing less predictable, in a riparian zone than in a river floodplain.
Runoff. That part of precipitation, snowmelt, or irrigation water that runs off the land into streams or other surface water. It can carry pollutants from the air and land into receiving waters.
Septic system. An on-site system designed to treat and dispose of domestic sewage. A typical septic system consists of a tank that receives waste from a residence or business and a drain field or subsurface absorption system consisting of a series of percolation lines for the disposal of the liquid effluent. Solids (sludge) that remain after decomposition by bacteria in the tank must be pumped out periodically.
Sewer. A channel or conduit that carries wastewater and storm water runoff from the source to a treatment plant or receiving stream. Sanitary sewers carry household, industrial, and commercial waste. Storm sewers carry runoff from rain or snow. Combined sewers handle both.
Slope. The degree of inclination to the horizontal. Usually expressed as a ratio, such as 1:25 or 1 on 25, indicating one unit vertical rise in 25 units of horizontal distance, or in a decimal fraction (0.04), degrees (2 degrees 18 minutes), or percent (4 percent).
Stakeholder. Any person with a vested interest in the TMDL development.
Surface area. The area of the surface of a waterbody; best measured by planimetry or the use of a geographic information system.
Surface runoff. Precipitation, snowmelt, or irrigation water in excess of what can infiltrate the soil surface and be stored in small surface depressions; a major transporter of nonpoint source pollutants.
Surface water. All water naturally open to the atmosphere (rivers, lakes, reservoirs, ponds, streams, impoundments, seas, estuaries, etc.) and all springs, wells, or other collectors directly influenced by surface water.
Topography. The physical features of a geographic surface area including relative elevations and the positions of natural and man-made features.

Total Maximum Daily Load (TMDL). The sum of the individual wasteload allocations (WLAs) for point sources, load allocations (LAs) for nonpoint sources and natural background, plus a margin of safety (MOS). TMDLs can be expressed in terms of mass per time, toxicity, or other appropriate measures that relate to a state's water quality standard.
VADEQ. Virginia Department of Environmental Quality.
VDH. Virginia Department of Health.
Virginia Pollutant Discharge Elimination System (NPDES). The national program for issuing, modifying, revoking and re-issuing, terminating, monitoring, and enforcing permits, and imposing and enforcing pretreatment requirements, under sections 307, 402, 318, and 405 of the Clean Water Act.
Wasteload allocation (WLA). The portion of a receiving waters' loading capacity that is allocated to one of its existing or future point sources of pollution. WLAs constitute a type of water quality-based effluent limitation (40 CFR 130.2(h)).
Wastewater. Usually refers to effluent from a sewage treatment plant. See also **Domestic wastewater**.
Wastewater treatment. Chemical, biological, and mechanical procedures applied to an industrial or municipal discharge or to any other sources of contaminated water to remove, reduce, or neutralize contaminants.
Water quality. The biological, chemical, and physical conditions of a waterbody. It is a measure of a waterbody's ability to support beneficial uses.
Water quality criteria. Levels of water quality expected to render a body of water suitable for its designated use, composed of numeric and narrative criteria. Numeric criteria are scientifically derived ambient concentrations developed by EPA or states for various pollutants of concern to protect human health and aquatic life. Narrative criteria are statements that describe the desired water quality goal. Criteria are based on specific levels of pollutants that would make the water harmful if used for drinking, swimming, farming, fish production, or industrial processes.
Water quality standard. Law or regulation that consists of the beneficial designated use or uses of a waterbody, the numeric and narrative water quality criteria that are necessary to protect the use or uses of that particular waterbody, and an antidegradation statement.
Watershed. A drainage area or basin in which all land and water areas drain or flow toward a central collector such as a stream, river, or lake at a lower elevation.
WQIA. Water Quality Improvement Act.

APPENDIX A

1) Non-point Sources of Fecal Contamination in the Coan River and Little Wicomico River Watersheds Final Report presented to the Virginia Department of Environmental Quality

2) Non-point Sources of Fecal Contamination in the Coan River and Little Wicomico River Watersheds Addendum to Final Report presented to the Virginia Department of Environmental Quality

Non-point Sources of Fecal Contamination in the Coan River and Little Wicomico River Watersheds
Final Report presented to
The Virginia Department of Environmental Quality

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1. Summary

Sources of fecal contamination were determined for the Coan River and Little Wicomico River Watersheds using the antibiotic resistance analysis (ARA) procedure. ARA is a bacterial source tracking (BST) method that involves development of a known source library (*Escherichia coli* or *E. coli* for this project) and then classifying *E. coli* isolates from water (unknown sources) to determine their origin. The identification of sources is accomplished by using the statistical method of logistic regression analysis to classify each isolate recovered from water samples by comparing its ARA patterns with the ARA patterns of isolates in the known source library.

For ARA, 1,248 *E. coli* isolates were collected from known sources in the two watersheds and included birds (shorebirds and waterfowl), humans, livestock (cattle and horses), pets (dogs), and wildlife (deer, raccoon, muskrat). The rates of correct classification (RCC) ranged from 84.7% for birds to 62.1% for dogs, and the average rate of correct classification (ARCC) for the entire library was 71.9%.

Water samples were collected monthly at stations along the Coan River (108 samples from nine stations) and Little Wicomico River (72 samples from six stations) over a 12-month period from September 2001 through August 2002 (collections made by employees of the Virginia Department of Health, Division of Shellfish Sanitation, VDH-DSS). *Escherichia coli* populations were measured to evaluate the quantity of fecal material in the water (by Dr. Howard Kator, VIMS), and the *E. coli* isolates from the bacterial enumerations were then profiled by ARA (24 isolates per sample) to determine origin. Monitoring results indicated that 42.2% of the samples from both rivers exceeded the Virginia shellfish standard for fecal pollution. Source tracking results demonstrated that human-derived pollution was pervasive and dominant in both rivers, followed by birds as the major sources that contributed to fecal pollution in the Coan and Little Wicomico watersheds. Wildlife, pets, and livestock were minor contributors in comparison to humans and birds.

2. Introduction

Methods to identify sources of fecal pollution are important because fecal contamination of water is still a widespread problem in the United States (U.S. EPA 1986 and 1997). In Virginia, roughly one third of over 78,000 km of streams and rivers have been adequately monitored, and to date 3,486 km of streams and 253 km^2 of estuaries (of those monitored) are listed as impaired (Friends of the Rivers of Virginia, 2001; Virginia Department of Environmental Quality 2001). The leading cause of impairments (over 60%) is violation of the fecal coliform standard, and non-point agriculture is the most widely suspected (but unproven) source. The situation in Virginia where large numbers of impairments are due to fecal pollution is typical of many states (U.S. EPA 1999a and 1999b).

There have been numerous recent reports on methodologies that have the potential to differentiate between human and non-human sources of fecal pollution in water. These methodologies include antibiotic resistance analysis or ARA (Bower 2001; Harwood *et al*. 2000; Wiggins 1996; Wiggins *et al*. 1999), multiple antibiotic resistance profiles (Parveen *et al*. 1997), ribotyping (Carson *et al*. 2001; Hartel *et al*. 1999; Parveen *et al*. 1999), pulsed-field gel electrophoresis (Simmons *et al*. 1995; Simmons and Herbein 1998), ribosomal genetic markers in *Bacteroides-Prevotella* (Bernhard and Field 2000a and 2000b), repetitive DNA sequences (Dombeck *et al*.

2000), source-specific phages (Hsu *et al*. 1995), and sorbitol-fermenting bifidiobacteria (Rhodes and Kator 1999). While none of these methods are entirely new, their use in identifying sources of fecal pollution in water represents a novel application (Hagedorn *et al*. 1999). None of these methods have yet emerged as the best ones, and there is a clear lack of comparative multi-year studies to determine the relative strengths and weaknesses of each (although three such studies are now in progress).

The Total Maximum Daily Load (TMDL) program (section 303, the Clean Water Act, U.S. EPA 1986) requires the establishment of source load allocations and inclusion of seasonal variation in determining allowable pollution loads (McKenzie 1998; McClellan *et al*. 2000). Accurate identification of sources of fecal pollution for load allocations during studies that include seasonal variation will require source classification of substantial numbers of isolates over multi-month time frames to determine proportionality of sources. Fecal contamination in natural waterways can lead to several problems, including higher incidences of pathogens (Sinton *et al.* 1993), and increased nutrient levels that lead to algal blooms and deoxygenation of waterways (as is currently the case in the Chesapeake Bay, Alliance for Chesapeake Bay, 1993). Fecal contamination in waterways has consistently been demonstrated by the
presence of indicator organisms such as fecal coliforms or enterococci. However, differentiation of the sources of fecal contamination in waters receiving mixed agricultural and human waste is more difficult. Knowledge of the source of fecal contamination is important because humans are more susceptible to infections by pathogens found in human feces (Sinton *et al.* 1993). Once the source is identified, steps can be taken to control the influx of fecal pollution.

Antibiotic resistant bacteria can develop in animals and humans as a result of treatment with antibiotics. This is the basic premise of antibiotic resistance analysis (ARA), which uses enterococci and *E. coli* as indicator organisms in identification of sources of fecal contamination (Wiggins, 1996). *E. coli* is a species of gram-negative, bacterial rods that ferment lactose and are able to grow at 44.5°C, and is used because it is the regulatory indicator organism for shellfish waters. In the ARA approach, strains of *E. coli* are isolated from known fecal sources and grown on plates containing various concentrations of different antibiotics. The resulting antibiotic resistance patterns of each isolate are then analyzed using logistic regression analysis, a multivariate statistical method. The results are pooled to form a “known library” of antibiotic resistance patterns from different fecal sources. Resistance patterns of isolates from water samples are then compared with this known library to determine the source(s) of fecal pollution in that waterway (Graves *et al.* 2002; Wiggins *et al.* 1999).

In this report, ARA and *E. coli* counts were used to draw conclusions about the source(s) of fecal contamination in the watersheds of the Coan and Little Wicomico Rivers. Both rivers are located in Virginia’s Northern Neck, Northumberland County. The Coan River empties into the lower Potomac River and the Little Wicomico enters into the Chesapeake Bay at Smith Point, where the Potomac River and Chesapeake Bay converge. Both rivers are polluted with fecal matter, and contain shellfish beds that have been closed because of high levels of fecal coliforms. Based on sanitary surveys, the major potential sources of fecal contamination in the two watersheds that needed to be included in the known source library were birds, humans, livestock, pets and wildlife sources.

3. Materials and Methods

A. Sample Collection:

Fecal and water samples were collected by VDH-DSS personnel as part of their regular monitoring program. Additional fecal samples were also collected by Howard Kator (VIMS), Charles Hagedorn, and Cheryl Szeles (VT) during the course of numerous trips to the watersheds. All fecal samples and water samples were delivered to Howard Kator's laboratory at VIMS within 6 hours of collection. The samples were filtered, and the numbers of *E. coli* present was determined using modified m-TEC agar. Filter plates were then shipped to VPI by overnight delivery. Nine sites were sampled in the Coan River watershed (Figure 1), and 6 sites were sampled in the Little Wicomico River watershed (Figure 2) over a 12-month period from September 2001 through August 2002. The goal was to test 24 isolates from each sample, resulting in a confidence level of 95%. Because of low counts, fewer than 24 isolates were analyzed for some samples. To determine the effects of overnight storage on classification, duplicate sets of water samples were collected from the Coan River stations in July and August. One set was filtered within 6 hours, and the other set was refrigerated overnight before filtering (24 hr).

B. Isolation of *E. coli*:

Isolated colonies were selected (24 for unknown samples, and 10-12 for known samples) and transferred to 96-microwell plates containing 0.2 ml of Colilert broth. The microwell plates were incubated at 37°C for 24 hours. MUG-negative isolates (no fluorescence under ultraviolet light) were not analyzed (Whitlock *et al.* 2002).

C. Antibiotics:

Isolates from the 96-microwell plate were transferred to antibiotic-containing Trypticase Soy agar (TSA) plates using a sterile 48-prong replica-plater. Various concentrations of 7 antibiotics were used (28 concentrations total, Graves *et al.* 2002). The isolates were also replica-plated to two TSA plates that did not contain antibiotics as controls. All TSA plates were incubated at 37°C for 24-48 hours. After incubation, the growth of each isolate on each concentration of each antibiotic was determined by comparison to the control plates, and the resulting antibiotic resistance patterns were entered into an Excel spreadsheet ("1" equaling growth and "0" equaling no growth).

D. Statistical Analysis:

The results from resistance testing of the known isolates were entered into the SAS statistical program (JMP Statistical Software, ver. 5.0) where they were analyzed by Logistic Regression using the NOMINAL LOGISTIC procedure, which produces a classification table. The average rate of correct classification (ARCC) is the average rate that known isolates were correctly classified, and was determined by averaging the percentages of correctly classified isolates for each source. For this analysis, all resistance patterns from known sources were kept in the library. The isolates from each water sample were then classified using this library. Logistic Regression identifies the most likely source for each isolate and displays the probability that each isolate belongs to the source that it is classified as.

When multiple regression methods are used to analyze relatively small data sets, random groupings (artificial clustering) based on stochastic processes rather than true relationships can occur. Such artificial classification limits the usefulness of small libraries, and should not exceed the purely random distribution of 20% for the five source categories used in this project. One way to ensure that a library is large enough to avoid this random grouping phenomenon is to randomly assign the isolates to source categories as the library is being constructed. When analysis of the library is carried out, the ARCC for the randomly assigned data set should approximate the probability that an isolate would be assigned to a source category by chance. Whitlock *et al.* (2002) reported ARCCs of 27.9% and 28.9% for two randomly generated data sets and, with four source categories, the probability that any one isolate would be assigned to one of the categories by chance was 25%. The low random ARCCs demonstrated that negligible random groupings occurred when analysis was performed on the relatively large library (2,398 isolates) used in their study.

To measure the representativeness of the library (i.e., how well it represents the diversity of patterns present in the sources in the watershed), all of the isolates from each known sample were successively removed from the library, and then classified based on the library containing the remaining isolates. The ARCC of these removed isolates was then calculated. This "jackknife" method estimates how well "new" isolates would be classified by the library. If there is a large difference between the ARCCs of these two methods, it suggests that the library is not representative of the sources in the watershed. The Minimum Detectable Percentage (MDP) for this library was calculated by determining the mean of the expected frequencies of misclassification (the average percentages of other source types that were misclassified as that type) and adding the value of 4 times the standard deviation of the mean (Whitlock *et al.* 2002). This value is a conservative estimate of the minimum percentage of a source that can be detected in a water sample. Thus, if a source is found at levels above the MDP, it can be reasonably assumed that this is not the result of misclassification of other sources, and therefore is present in the watershed.

4. Results

A. Classification of Known Isolates (Library Composition).

A total of 1,248 isolates were tested from the five sources and the isolates collected from these known fecal sources were analyzed using Logistic Regression (Table 1).

Table 1. Classification of 1,248 isolates of *E. coli* from known sources collected in the Coan River and Little Wicomico River watersheds. Correctly classified isolates (%) are shown in bold and the ARCC for the library was 71.9%. Each grid contains the number of isolates (top number) and the % classified (lower number).

Source	Bird	Human	Livestock	Pets	Wildlife	Totals
Bird	305 **84.72**	24 7.38	22 9.21	3 2.78	34 15.74	388
Human	24 6.67	243 **74.77**	29 12.13	31 28.70	19 8.80	346
Livestock	8 2.22	27 8.31	173 **72.38**	4 3.70	11 5.09	223
Pets	0 0.00	9 2.77	3 1.26	67 **62.04**	10 4.63	89
Wildlife	23 6.39	22 6.77	12 5.02	3 2.78	142 **65.74**	202
Totals	360	325	239	108	216	1248

One hundred and forty known source samples were collected during the course of the project: 42 bird scat samples (shore birds and waterfowl) yielded 360 isolates, 36 human samples (from septic tank pump-out trucks and single dwelling septic tanks) provided 325 isolates, 25 cattle and horse scat samples provided 239 isolates, 14 dog scat samples yielded 108 isolates, and 23 wildlife scat samples (deer, raccoon, muskrat) provided 216 isolates. The average rate of correct classification (ARCC) of the library was 71.9%, which was well above the background random classification level of 20% (based on 5 sources). The rates of correct classification (RCC) were 84.7% for birds, 74.8% for human, 72.4% for livestock, 62.1% for dogs, and 65.7% for wildlife (Table 1).

When the library was classified using a two-way split of human vs. non-human, the ARCC was 80.2% (68.3% RCC for human and 92% RCC for non-human). When the library was classified using a four-way split (combining birds with wildlife), the ARCC was 76.3% (71.0% RCC for human, 76.5% RCC for livestock, 76.8% for pets, and 81.2% RCC for wildlife + birds). The highest RCC for the human category (74.8%) was obtained with the five-way split (Table 1), and this was the reason that the five-way split was then used to classify *E. coli* isolates from water samples.

When the library was analyzed for artificial clustering, the ARCC was 24.8%, only 4.8% higher than random distribution of 20%, indicating that the library was of sufficient size to be used to classify unknown source isolates from water samples (Table 2). The random classifications (artificial clustering) were 22.2% for birds, 24.8% for human, 18.4% for livestock, 28.1% for dogs, and 29.6% for wildlife (Table 2). The largest artificial clusters were obtained with pets and wildlife, and indicated that these would be the sources where more isolates were needed if the library was to be expanded.

Table 2. Classification of 1,248 isolates of *E. coli* randomly assigned to source Categories. Artificial classifications (%) are shown in bold and the ARCC for the library was 24.8%. Each grid contains the number of isolates (top number) and the % classified (lower number).

Source	Birds	Human	Livestock	Pets	Wildlife	Totals
Birds	57 **22.89**	52 20.80	41 16.40	42 16.87	38 15.20	230
Human	51 20.48	62 **24.80**	49 19.60	42 16.87	51 20.40	255
Livestock	30 12.05	35 14.00	46 **18.40**	46 18.47	40 16.00	197
Pets	48 19.28	51 20.40	59 23.60	70 **28.11**	47 18.80	275
Wildlife	63 25.30	50 20.00	55 22.00	49 19.68	74 **29.60**	291
Totals	249	250	250	249	250	1248

The library contained 565 duplicate isolates and 683 unique patterns. When the unique patterns were analyzed using jackknife analysis of individual isolates (performed by Dr. Bruce Wiggins, JMU), the ARCC was 72% (the ARCC of the library was 71.9%, Table 1). This equivalence in classification success indicates that this known source library is representative of the two watersheds. When the library was analyzed using jackknife analysis of individual samples instead of isolates, the ARCC was 64%, only 7.9% lower than the ARCC of

71.9% for the library (also demonstrating equivalence in classification success). Based on the jackknife analysis, the Minimum Detectable Percentage (MDP) for the Coan and Little Wicomico library was calculated. The mean expected frequency of misclassification (EFM) of this library is 6% ± 3% SD. Multiplying the SD of 3 by four (equals 12) and adding this to the mean EFM (6%) results in a MDP of 18%. Multiplying the SD by three results in a level of confidence at the 99.9% level, so four is used as an additional measure to obtain a conservative estimate. The MDP, as proposed by Whitlock *et al.* (2000), reflects the amount of misclassification that occurs for a particular library, and is a conservative estimate of the lower limit for considering a source to be a significant contributor to a watershed.

B. Analysis of Coan River Samples:

i. *E. coli* enumerations. During the study period, 108 samples were collected from 9 sampling stations on the Coan River. The numbers of *E. coli* in these samples, and the total amount of rainfall in the 3 days previous to the sampling are shown in Table 3, listed by sample site and collection date. Forty-six of the samples (42.6%) had levels of *E. coli* that were above the Virginia standard of 14 *E. coli* /100 ml. Five of the nine stations (C-16, C-20, C-27, C-37.5Z, C-38) had consistently high fecal counts, with the geometric mean of the 12 monthly samples exceeding the Virginia standard. Over the nine stations, the months with the highest fecal counts were the April, August, September, and November samples. There appeared to be no correlation with rainfall in the fecal counts, as the April sample had only 0.01 inch and the November sample had none in the preceding three days, while the August sample had 0.62 inch and the September sample had 1.0 inch in the preceding 3 days.

ii. Classification with ARA. Based on the Coan and Wicomico known source library, the 108 samples were classified by source. The results are shown in Table 4, listed by sample site and collection date. There was a strong human signature at all nine sampling stations and the percent of isolates classified as human averaged, over 12 months, above 50% for all stations except C-24 (46.5%). The 12-month averages for the percent human signature were 81.6% for C-7, 74.7% for C-15, 63.2% for C-20, and ranged from 52.1% to 59.0% for the remaining five stations (Table 4). When comparing the five known source categories, human and bird sources were the most common with 95 and 33, respectively, of the 108 samples having percentages that exceeded the Minimum Detectable Percentage (MDP) of 18%. Livestock, pets, and wildlife signatures exceeded the MDP 15, 4, and 12 times, respectively, and are minor contributors to pollution in the Coan River compared to humans and birds.

There was very little seasonality in the results when comparing the wet and dry season averages for the human signature (Table 4). The human signature was higher in the wet season than the dry season for five of the stations, but the percent human isolates (averaged over all stations) for the wet season (69.0%) and the dry season (57.7%) were fairly close (and well above the MDP of 18%). For the 95 samples where the human signature exceeded the MDP, it was dominant in 70 of them, and was dominant in 12/12 samples for C-7 and 10/12 samples for C-15. On a per station basis, the 12-month average human signature exceeded the MDP at all 9 stations. Bird isolates were higher in the dry season than during the wet season for eight of the nine stations, but the seasonal averages over the year were 5.4% for the wet season and 16.6% for the dry season (both below the MDP of 18%). The seasonal average bird signature exceeded the MDP at four stations during the dry season (C-16, C-20, C-24, and C-27) and did not exceed the MDP at any stations during the wet season. On a per station basis, the 12-month average bird signature exceeded the MDP at 5 of the 9 stations (C-16, C-20, C-24, C-27, and C-37.5Z). The bird signature exceeded the MDP 33 times and in 15 of these samples the bird signature was dominant (for example, 91.7% for May at C-16), and the high bird signatures were concentrated in the months of May, June, August, September, November, and December.

As with birds, the dry and wet season annual averages for livestock, pets, and wildlife were all below the MDP of 18% (Table 4). The pet signature did not exceed the MDP for any season at any site while the wildlife signature was barely above the MDP during the wet season at stations C-16 and C-20 (18.1% for both) and during the dry season at station C-37.5Z (29.2%). The pet signature exceeded the MDP just four times and was only dominant once (62.5% for June at C-16). There were twelve samples where wildlife exceeded the MDP, and only three samples where the wildlife signature was dominant (54.2% for March at C-16, 45.8% for December at C-38, and 41.7% for July at C-37.5Z). The livestock signature did not exceed the MDP at any station during the wet season, but exceeded the MDP during the dry season at six stations (C-15, C-16, C-24, C-27, C-33, and C-37.5Z), but was the dominant signature just five times.

In summary, the human signature was predominant and was slightly higher in the wet season than the dry season. Birds were second in importance to humans, and both birds and livestock were both more abundant in the dry season than the wet season, while the pets and wildlife signatures were essentially negligible. Averaging the percent classifications over all sources and samples provides an obvious ranking of the five sources: humans (61%), birds (17%), livestock (8%), wildlife (7%), and pets (3.0%).

iii. Comparison of 6-hour and 24-hour samples. The July and August Coan River samples that were held overnight before processing were analyzed and compared to the 6-hour samples (Table 4A). For the monitoring results, there was excellent agreement between the July (p=.99) and August (p=.45) 6 and 24 hr samples. For source tracking results, the source averages differed from each other by no more than 11% (well below the MDP). These results indicate that were no major differences between samples held for six versus 24 hours.

C. Analysis of Little Wicomico River Samples:

i. *E. coli* enumerations. During the study period, 72 samples were collected from 6 sampling stations on the Little Wicomico River. The numbers of *E. coli* in these samples, and the total amount of rainfall in the 3 days previous to the sampling are shown in Table 5, listed by sample site and collection date. Thirty of the samples (41.7%) had levels of *E. coli* that were above the Virginia standard of 14 *E. coli* /100 ml. Three of the six stations (W-16, W-19, and W-20) had consistently high fecal counts, with the geometric mean of the 12 monthly samples exceeding the Virginia standard. Over the six stations, the months with the highest fecal counts were the March, April, and September samples. There appeared to be no correlation with rainfall in the fecal counts, as the March sample had 0.70 inch of rain, the April sample had only 0.12 inch, and the September sample had 1.45 inch in the preceding three days.

ii. Classification with ARA. Based on the Coan and Wicomico known source library, the 72 samples were classified by source. The results are shown in Table 6, listed by sample site and collection date. There was a strong human signature at all six sampling stations and the average percent of isolates classified as human, over 12 months, ranged from 42.0% (W-20) to 61.8% (W-9X). When comparing the five known source categories, human and bird sources were the most common with 64 and 30, respectively, of the 72 samples having percentages that exceeded the Minimum Detectable Percentage (MDP) of 18%. Livestock, pets, and wildlife signatures exceeded the MDP 11, 5, and 18 times, respectively, and are minor contributors to pollution in the Little Wicomico River compared to humans and birds.

There was very little seasonality in the results when comparing the wet and dry season averages for the human signature (Table 6). The human signature was higher in the wet season than the dry season for five of the stations, but the percent human isolates (averaged over all stations) for the wet season (61.6%) and the dry season (53.9%) were fairly close (and well above the MDP of 18%). For the 64 samples where the human signature exceeded the MDP, it was dominant in 46 of them, and was dominant in 11/12 samples for W-9X and 10/12 samples for W-9W. On a per station basis, the 12-month average human signature exceeded the MDP at all 6 stations. Bird isolates were also very similar for both the wet and dry seasons and the seasonal averages over the year were 16.9% for the wet season and 15.5% for the dry season (both below the MDP of 18%). The seasonal average bird signature exceeded the MDP at two stations during both the wet and dry seasons (W-13.5Z and W-20). On a per station basis, the 12-month average bird signature exceeded the MDP at all 4 of 6 stations. The bird signature exceeded the MDP for 30 samples and was dominant in 12 of those (for example, 100% for May at W-19) and the high bird signatures were concentrated in the months of April, May, June, August, November, December, and January.

As with birds, the dry and wet season annual averages for livestock, pets, and wildlife were all below the MDP of 18% (Table 6). The pet signature did not exceed the MDP for any season at any site while the wildlife signature was barely above the MDP during the wet season at stations W-9X and W-13.5Z (19.5% for both) and during the dry season at station W-20 (18.1%). The pet signature exceeded the MDP just five times and was only dominant twice (41.2% for December at W-9W and 58.3% for December at W-13.5Z). There were 18 samples where wildlife exceeded the MDP, and only three samples where the wildlife signature was dominant (62.5% for May at W-9W, and 37.5% for January and 50.0% for June at W-20). The livestock signature did not exceed the MDP at any station during the wet season, but exceeded the MDP during the dry season at two stations (W-13.5Z and W-16), but was the dominant signature just once (25.0% for December at W-20).

In summary, the results for the Little Wicomico River were similar to those for the Coan River in that the human signature was predominant and was slightly higher in the wet season than the dry season. The bird signature was spread more evenly over all the wet and dry seasons for the Little Wicomico samples and livestock was more abundant in the dry season than the wet season, although the livestock, pets, and wildlife signatures were essentially negligible. Averaging the percent classifications over all sources and samples provides an obvious ranking of the five sources: human (53%), birds (22%), wildlife (11%), livestock (8%), and pets (5%).

5. Discussion

These results clearly show that humans and birds are the major sources of pollution for both the Coan River and Little Wicomico River watersheds. All the sites had samples that contained percentages of both human and bird sources that were at or above the minimum detectable level. Combining the results of both rivers, humans accounted for 57.4% of the samples that were above the MDP while birds accounted for 22.7%. The contributions of wildlife, livestock, and pets were lower, at 10.8%, 9.3%, and 3.2%, respectively. This pattern of sources is consistent with the land use of these watersheds, which contain numerous marinas, older homes adjacent to waterfronts, areas of development, large but fluctuating bird populations, and substantial undeveloped areas. Comparing the Little Wicomico to the Coan River, the human signature was a smaller, and the bird and wildlife signatures were a larger (in the Little Wicomico), but the trends of a dominant human signature followed by birds in importance was the same.

Numerous trips around both watersheds and inspections of the rivers on boat trips with DSS personnel provided visual evidence that supports most of the results presented in this study. Such trips readily demonstrated the large populations of shorebirds and seasonal migrations of waterfowl that impact both watersheds. There is little evidence that pets or livestock would be a major contributor as dogs were rarely seen and livestock areas are well away from the sampling stations. The presence of a human signature was not a surprise due to the numbers of older homes adjacent to waterfront property in some parts of both watersheds, and the occurrence of a occasional pit privies located close to the water. However, the pervasive and large human signature over all sites was the major surprise of this study and indicates that substantial subsurface pollution for human sources is occurring (and must be widely distributed by tidal influences). The only other option is that the results, based on ARA, are not accurate. Currents and movement of water in both rivers due to rising and falling tides are substantial and lend credence to the possibility that the human signature is being distributed and mixed throughout the river embayments from wherever the sources of human pollution might be.

A. Limitations of this study.

There were no major limitations to this study although it is likely that there are additional patterns of potential sources not represented in the library. This concern is the same for all library-based methods and the two rivers were sufficiently close that one library could be made for both and the library passed the statistical tests that are used to determine the necessary size for a library and assessments of how representative it is. A moderately high threshold of 18% was set to ensure that chances of misidentification of sources was small. One limitation with a few samples is that only a small number of isolates were tested. Because of low fecal counts, some samples had very low isolate numbers, and the percentages that result from these low numbers are not precise (i.e., a given source in a sample with just 2 isolates can be only 0%, 50%, or 100%). Caution should be exercised in using the percentage values for these samples. One additional concern is the use of *E. coli* as the test organism. There is now some evidence from the source tracking community that *E. coli* may not be as effective as the enterococci for source tracking purposes, and there are questions about the genetic stability of *E. coli* that raise issues regarding the validity of results obtained with it. However, the ARCC obtained with the library in this study (71.9%) is in the upper range of those reported in the literature and is certainly high enough to be useful for watershed projects. Also, it should be possible to test some of the newer antibiotics that have only been approved for human therapy and see if these offer better distinctions between human and non-human sources of *E. coli*. Finally, it must be kept in mind that all BST methods, including ARA, are still being developed, and there are no "standard methods" yet for any BST procedure. There are many variables that determine the sources of fecal bacteria in water, and most of them are poorly understood.

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Figure 1. Sampling stations in the Coan River watershed. Map courtesy of Howard Kator and Julie Herman from VIMS.

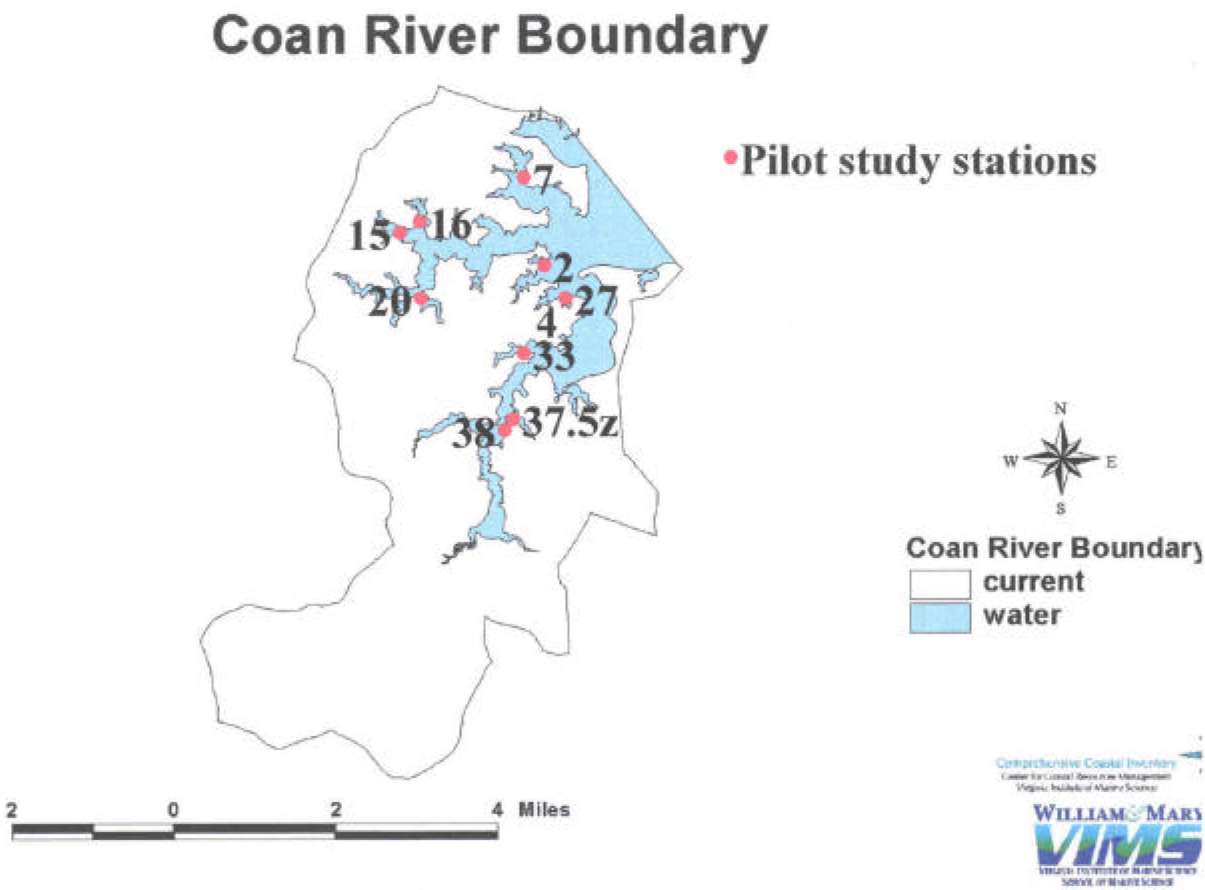

Figure 2. Sampling stations in the Little Wicomico River watershed. Map courtesy of Howard Kator and Julie Herman from VIMS.

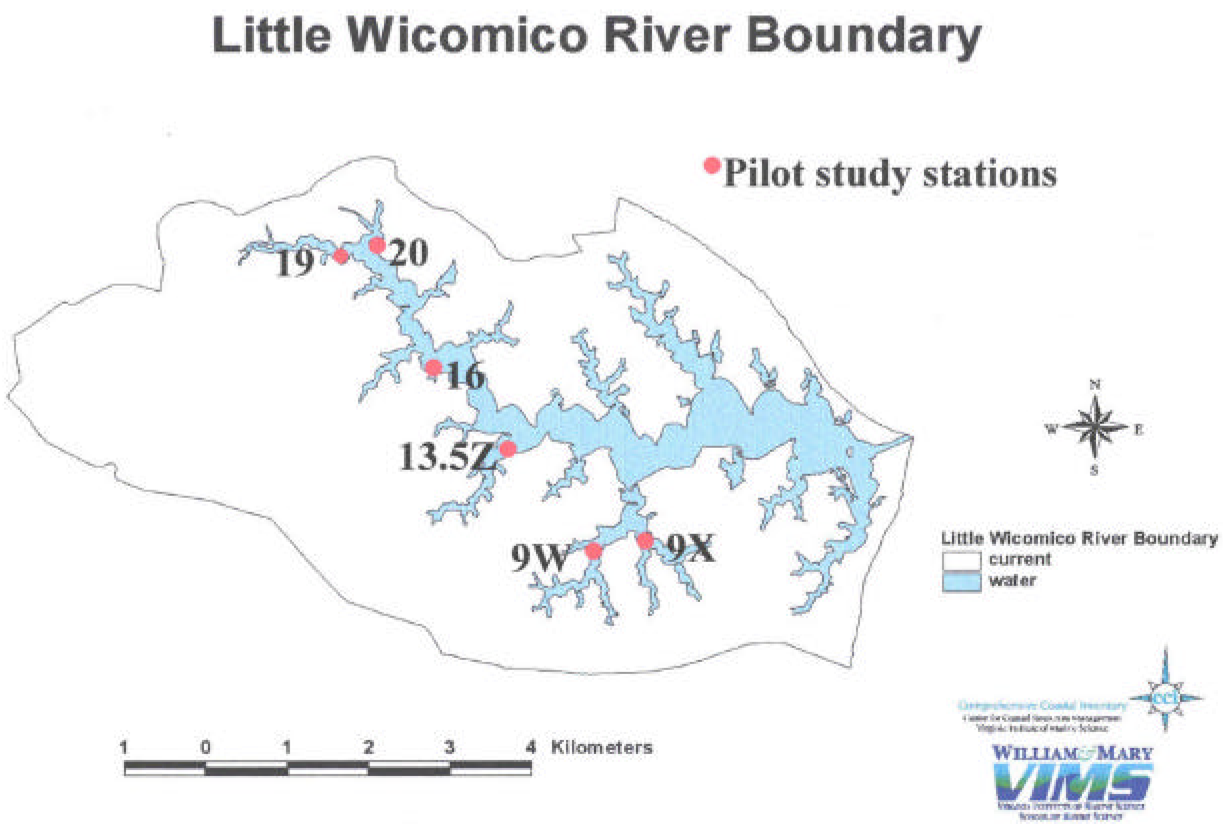

Table 3. Numbers of *E coli* isolates in the Coan River watershed with rainfall 3 days prior to collection.

A. Samples collected at DSS station C-7

DATE	Sample #	No. isolates/100 ml	Rain (inches)
9/25/01	C1	18	1.00
10/22/01	C10	4	0.00
11/19/01	C19	16	0.00
12/6/01	C28	5	0.02
1/17/02	C37	1	0.31
2/19/02	C46	0	0.00
3/20/02	C55	8	0.81
4/18/02	C64	9	0.01
5/1/02	C73	2	0.90
6/13/02	C82	1	0.00
7/15/02	C91	1	0.49
8/29/02	C100	10	0.62

B. Samples collected at DSS station C-15

DATE	Sample #	No. isolates/100 ml	Rain (inches)
9/25/01	C1	13	1.00
10/22/01	C10	4	0.00
11/19/01	C19	4	0.00
12/6/01	C28	10	0.02
1/17/02	C37	2	0.31
2/19/02	C46	0	0.00
3/20/02	C55	15	0.81
4/18/02	C64	12	0.01
5/1/02	C73	1	0.90
6/13/02	C82	2	0.00
7/15/02	C91	3	0.49
8/29/02	C100	8	0.62

C. Sample collected at DSS station C-16

DATE	Sample #	No. isolates/100 ml	Rain (inches)
9/25/01	C3	26	1.00
10/22/01	C12	12	0.00
11/19/01	C21	11	0.00
12/6/01	C30	16	0.02
1/17/02	C39	1	0.31
2/19/02	C48	1	0.00
3/20/02	C57	14	0.81
4/18/02	C66	48	0.01
5/1/02	C75	2	0.90
6/13/02	C84	18	0.00
7/15/02	C93	18	0.49
8/29/02	C102	26	0.62

D. Sample collected at DSS station C-20

DATE	Sample #	No. isolates/100 ml	Rain (inches)
9/25/01	C4	38	1.00
10/22/01	C13	18	0.00
11/19/01	C22	44	0.00
12/6/01	C31	17	0.02
1/17/02	C40	3	0.31
2/19/02	C49	0	0.00
3/20/02	C58	70	0.81
4/18/02	C67	17	0.01
5/1/02	C76	3	0.90
6/13/02	C85	7	0.00
7/15/02	C94	4	0.49
8/29/02	C103	12	0.62

E. Sample collected at DSS station C-24

DATE	Sample #	No. isolates/100 ml	Rain (inches)
9/25/01	C5	25	1.00
10/22/01	C14	3	0.00
11/19/01	C23	18	0.00
12/6/01	C32	5	0.02
1/17/02	C41	4	0.31
2/19/02	C50	0	0.00
3/20/02	C59	20	0.81
4/18/02	C68	4	0.01
5/1/02	C77	5	0.90
6/13/02	C86	8	0.00
7/15/02	C95	5	0.49
8/29/02	C104	27	0.62

F. Sample collected at DSS station C-27

DATE	Sample #	No. isolates/100 ml	Rain (inches)
9/25/01	C6	23	1.00
10/22/01	C15	2	0.00
11/19/01	C24	30	0.00
12/6/01	C33	14	0.02
1/17/02	C42	1	0.31
2/19/02	C51	0	0.00
3/20/02	C60	9	0.81
4/18/02	C69	23	0.01
5/1/02	C78	11	0.90
6/13/02	C87	113	0.00
7/15/02	C96	18	0.49
8/29/02	C105	48	0.62

G. Sample collected at DSS station C-33

DATE	Sample #	No. isolates/100 ml	Rain (inches)
9/25/01	C7	43	1.00
10/22/01	C16	15	0.00
11/19/01	C25	26	0.00
12/6/01	C34	12	0.02
1/17/02	C43	2	0.31
2/19/02	C52	0	0.00
3/20/02	C61	2	0.81
4/18/02	C70	10	0.01
5/1/02	C79	3	0.90
6/13/02	C88	4	0.00
7/15/02	C97	2	0.49
8/29/02	C106	28	0.62

H. Sample collected at DSS station C-37.5Z

DATE	Sample #	No. isolates/100 ml	Rain (inches)
9/25/01	C8	33	1.00
10/22/01	C17	21	0.00
11/19/01	C26	85	0.00
12/6/01	C35	18	0.02
1/17/02	C44	27	0.31
2/19/02	C53	0	0.00
3/20/02	C62	12	0.81
4/18/02	C71	59	0.01
5/1/02	C80	6	0.90
6/13/02	C89	21	0.00
7/15/02	C98	25	0.49
8/29/02	C107	58	0.62

I. Sample collected at DSS station C-38

DATE	Sample #	No. isolates/100 ml	Rain (inches)
9/25/01	C9	20	1.00
10/22/01	C18	9	0.00
11/19/01	C27	56	0.00
12/6/01	C36	11	0.02
1/17/02	C45	2	0.31
2/19/02	C54*	0	0.00
3/20/02	C63	23	0.81
4/18/02	C72	49	0.01
5/1/02	C81	4	0.90
6/13/02	C90	3	0.00
7/15/02	C99	4	0.49
8/29/02	C108	26	0.62

Table 4. Source Tracking percentages of *E. coli* in the Coan River watershed with seasonality results.

A. Samples collected at DSS station C-7

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	C1	0.00	95.83	0.00	4.17	0.00
10/22/01	C10	0.00	95.83	0.00	0.00	4.17
11/19/01	C19	4.17	79.17	16.67	0.00	0.00
12/6/01	C28	16.67	70.83	12.50	0.00	0.00
1/17/02	C37	20.83	70.83	0.00	0.00	8.33
2/19/02	C46	4.17	95.83	0.00	0.00	0.00
3/20/02	C55	4.17	87.50	0.00	0.00	8.33
4/18/02	C64	4.17	91.67	0.00	0.00	4.17
5/1/02	C73	12.50	37.50	29.17	0.00	20.83
6/13/02	C82	0.00	91.67	0.00	0.00	8.33
7/15/02	C91	4.17	95.83	0.00	0.00	0.00
8/29/02	C100	4.17	66.67	16.67	4.17	8.33
	SUM	75.02	979.16	75.01	8.34	62.49
	AVERAGE	6.25	81.60	6.25	0.70	5.21
WET PERIOD						
JAN/FEB/MARCH		29.17	254.16	0.00	0.00	16.66
FOR SEASON		9.72	84.72	0.00	0.00	5.55
DRY PERIOD						
JULY/AUGUST/SEPT		8.34	258.33	16.67	8.34	8.33
FOR SEASON		2.78	86.11	5.56	2.78	2.78

B. Samples collected at DSS station C-15

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	C2	0.00	95.83	0.00	4.17	0.00
10/22/01	C11	0.00	95.83	0.00	0.00	4.17
11/19/01	C20	25.00	45.83	29.17	0.00	0.00
12/6/01	C29	8.33	83.33	8.34	0.00	0.00
1/17/02	C38	0.00	95.83	0.00	0.00	4.17
2/19/02	C46	4.17	95.83	0.00	0.00	0.00
3/20/02	C56	4.17	91.67	0.00	0.00	4.17
4/18/02	C65	4.17	87.50	0.00	0.00	8.33
5/1/02	C74	58.33	25.00	4.17	0.00	12.50
6/13/02	C83	16.67	70.83	0.00	4.17	8.33
7/15/02	C92	0.00	83.33	4.17	0.00	12.50
8/29/02	C101	12.50	25.00	50.00	8.33	4.17
	SUM	133.34	895.81	95.85	16.67	58.34
	AVERAGE	11.11	74.65	7.99	1.39	4.86
WET PERIOD						
JAN/FEB/MARCH		8.34	283.33	0.00	0.00	8.34
FOR SEASON		2.78	94.44	0.00	0.00	2.78
DRY PERIOD						
JULY/AUGUST/SEPT		12.50	204.16	54.17	12.50	16.67
FOR SEASON		4.17	68.05	18.06	4.17	5.56

C. Samples Collected from DSS Station C-16

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	C3	83.33	8.33	0	0	8.33
10/22/01	C12	8.33	75	4.17	0	12.5
11/19/01	C21	12.5	79.17	8.33	0	0
12/6/01	C30	16.67	70.83	12.5	0	0
1/17/02	C39	29.17	62.5	8.33	0	0
2/19/02	C48	4.17	95.83	0	0	0
3/20/02	C57	0	41.67	4.17	0	54.17
4/18/02	C66	0	83.33	0	0	16.67
5/1/02	C75	54.17	33.33	0	0	12.5
6/13/02	C84	4.17	8.33	0	62.5	25
7/15/02	C93	0	45.83	54.17	0	0
8/29/02	C102	41.67	50	8.33	0	0
	SUM	254.18	654.15	100.00	62.50	129.17
	AVERAGE	21.18	54.51	8.33	5.21	10.76
WET PERIOD						
JAN/FEB/MARCH		33.34	200.00	12.50	0.00	54.17
FOR SEASON		11.11	66.67	4.17	0.00	18.06
DRY PERIOD						
JULY/AUGUST/SEPT		125.00	104.16	62.50	0.00	8.33
FOR SEASON		41.67	34.72	20.83	0.00	2.78

D. Samples Collected from DSS Station C-20

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	C4	83.33	12.50	0.00	0.00	4.17
10/22/01	C13	8.33	91.67	0.00	0.00	0.00
11/19/01	C22	12.50	75.00	12.50	0.00	0.00
12/6/01	C31	20.83	54.17	25.00	0.00	0.00
1/17/02	C40	20.83	58.33	4.17	0.00	16.67
2/19/02	C49	0.00	100.00	0.00	0.00	0.00
3/20/02	C58	0.00	62.50	0.00	0.00	37.50
4/18/02	C67	0.00	91.67	0.00	0.00	8.33
5/1/02	C76	29.17	58.33	4.17	4.17	4.17
6/13/02	C85	25.00	8.33	4.17	37.50	25.00
7/15/02	C94	12.50	75.00	8.33	0.00	4.17
8/29/02	C103	25.00	70.83	4.17	0.00	0.00
	SUM	237.49	758.33	62.51	41.67	100.01
	AVERAGE	19.79	63.19	5.21	3.47	8.33
WET PERIOD						
JAN/FEB/MARCH		20.83	220.83	4.17	0.00	54.17
FOR SEASON		6.94	73.61	1.39	0.00	18.06
DRY PERIOD						
JULY/AUGUST/SEPT		120.83	158.33	12.50	0.00	8.34
FOR SEASON		40.28	52.78	4.17	0.00	2.78

E. Samples Collected from DSS Station C-24

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	C5	54.17	25.00	0.00	4.17	16.67
10/22/01	C14	0.00	95.83	0.00	0.00	4.17
11/19/01	C23	37.50	58.33	4.17	0.00	0.00
12/6/01	C32	16.67	62.50	16.67	4.17	0.00
1/17/02	C41	33.33	50.00	4.17	12.50	0.00
2/19/02	C50	0.00	0.00	0.00	0.00	0.00
3/20/02	C59	0.00	45.83	16.67	16.67	20.83
4/18/02	C68	4.17	66.67	0.00	0.00	29.17
5/1/02	C77	91.67	0.00	0.00	8.33	0.00
6/13/02	C86	66.67	4.17	20.83	4.17	4.17
7/15/02	C95	4.17	66.67	29.17	0.00	0.00
8/29/02	C104	4.17	83.33	12.50	0.00	0.00
	SUM	312.52	558.33	104.18	50.01	75.01
	AVERAGE	26.04	46.53	8.68	4.17	6.25
WET PERIOD						
JAN/FEB/MARCH		33.33	95.83	20.84	29.17	20.83
FOR SEASON		11.11	31.94	6.95	9.72	6.94
DRY PERIOD						
JULY/AUGUST/SEPT		62.51	175.00	41.67	4.17	16.67
FOR SEASON		20.84	58.33	13.89	1.39	5.56

F. Samples Collected from DSS Station C-27

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	C6	25.00	25.00	8.33	25.00	16.67
10/22/01	C15	0.00	87.50	4.17	0.00	8.33
11/19/01	C24	54.17	33.33	8.33	4.17	0.00
12/6/01	C33	4.17	79.17	8.33	4.17	4.17
1/17/02	C42	12.50	62.50	16.67	4.17	4.17
2/19/02	C51	0.00	0.00	0.00	0.00	0.00
3/20/02	C60	8.33	75.00	8.33	0.00	8.33
4/18/02	C69	0.00	91.67	4.17	0.00	4.17
5/1/02	C78	95.83	4.17	0.00	0.00	0.00
6/13/02	C87	12.50	79.17	0.00	4.17	4.17
7/15/02	C96	41.67	20.83	25.00	4.17	8.33
8/29/02	C105	0.00	66.67	33.33	0.00	0.00
	SUM	254.17	625.01	116.66	45.85	58.34
	AVERAGE	21.18	52.08	9.72	3.82	4.86
WET PERIOD						
JAN/FEB/MARCH		20.83	137.50	25.00	4.17	12.50
FOR SEASON		6.94	45.83	8.33	1.39	4.17
DRY PERIOD						
JULY/AUGUST/SEPT		66.67	112.50	66.66	29.17	25.00
FOR SEASON		22.22	37.50	22.22	9.72	8.33

G. Samples Collected from DSS Station C-33

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	C7	0.00	75.00	4.17	4.17	16.67
10/22/01	C16	4.17	75.00	4.17	0.00	16.67
11/19/01	C25	25.00	37.50	29.17	4.17	4.17
12/6/01	C34	12.50	62.50	20.83	4.17	0.00
1/17/02	C43	0.00	91.67	0.00	4.17	4.17
2/19/02	C52	0.00	0.00	0.00	0.00	0.00
3/20/02	C61	0.00	87.50	8.33	0.00	4.17
4/18/02	C70	8.33	75.00	4.17	0.00	12.50
5/1/02	C79	50.00	25.00	4.17	0.00	20.83
6/13/02	C88	41.67	54.17	0.00	4.17	0.00
7/15/02	C97	0.00	95.83	0.00	0.00	4.17
8/29/02	C106	8.33	29.17	54.17	0.00	8.33
	SUM	150.00	708.34	129.18	20.85	91.68
	AVERAGE	12.50	59.03	10.77	1.74	7.64
WET PERIOD						
JAN/FEB/MARCH		0.00	179.17	8.33	4.17	8.34
FOR SEASON		0.00	59.72	2.78	1.39	2.78
DRY PERIOD						
JULY/AUGUST/SEPT		8.33	200.00	58.34	4.17	29.17
FOR SEASON		2.78	66.67	19.45	1.39	9.72

H. Samples Collected from DSS Station C-37.5Z

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	C8	4.17	62.50	12.50	4.17	16.67
10/22/01	C17	8.33	83.33	0.00	0.00	8.33
11/19/01	C26	50.00	41.67	8.33	0.00	0.00
12/6/01	C35	45.83	37.50	12.50	4.17	0.00
1/17/02	C44	0.00	100.00	0.00	0.00	0.00
2/19/02	C53	0.00	95.83	0.00	0.00	4.17
3/20/02	C62	0.00	91.67	0.00	0.00	8.33
4/18/02	C71	0.00	91.67	0.00	0.00	8.33
5/1/02	C80	41.67	4.17	41.67	0.00	12.50
6/13/02	C89	62.50	37.50	0.00	0.00	0.00
7/15/02	C98	0.00	37.50	20.83	0.00	41.67
8/29/02	C107	20.83	16.67	33.33	0.00	29.17
	SUM	233.33	700.01	129.16	8.34	129.17
	AVERAGE	19.44	58.33	10.76	0.70	10.76
WET PERIOD						
JAN/FEB/MARCH		0.00	287.50	0.00	0.00	12.50
FOR SEASON		0.00	95.83	0.00	0.00	4.17
DRY PERIOD						
JULY/AUGUST/SEPT		25.00	116.67	66.66	4.17	87.51
FOR SEASON		8.33	38.89	22.22	1.39	29.17

I. Samples Collected from DSS Station C-38

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	C9	4.17	75.00	8.33	12.50	0.00
10/22/01	C18	4.17	66.67	0.00	8.33	20.83
11/19/01	C27	33.33	66.67	0.00	0.00	0.00
12/6/01	C36	0.00	25.00	0.00	29.17	45.83
1/17/02	C45	0.00	95.83	4.17	0.00	0.00
2/19/02	C54*	0.00	0.00	0.00	0.00	0.00
3/20/02	C63	0.00	100.00	0.00	0.00	0.00
4/18/02	C72	75.00	8.33	12.50	0.00	4.17
5/1/02	C81	54.17	25.00	16.67	0.00	4.17
6/13/02	C90	0.00	100.00	0.00	0.00	0.00
7/15/02	C99	4.17	62.50	12.50	0.00	20.83
8/29/02	C108	12.50	62.50	16.67	4.17	4.17
	* No isolates					
	SUM	187.51	687.50	70.84	54.17	100.00
	AVERAGE	15.63	57.29	5.90	4.51	8.33
WET PERIOD						
JAN/FEB/MARCH		0.00	195.83	4.17	0.00	0.00
FOR SEASON		0.00	65.28	1.39	0.00	0.00
DRY PERIOD						
JULY/AUGUST/SEPT		20.84	200.00	37.50	16.67	25.00
FOR SEASON		6.95	66.67	12.50	5.56	8.33

Table 4A. Samples collected on July 15, 2002 (top table), and August 23, 2002 (bottom table). The 24 hour holding times are shown below the 6 hour samples. Paired test results were 0.99 for the July samples and 0.45 for the August samples.

Site #	Sample#	# of isolates	% Bird	% Human	%Livestock	%Pets	%Wildlife
C-7	C91	0.7	4.17	95.83	0.00	0.00	0.00
C-7	C91 (24)	0.3	8.33	91.67	0.00	0.00	0.00
C-15	C92	1.3	0.00	83.33	4.17	0.00	12.50
C-15	C92(24)	2	0.00	95.83	4.17	0.00	0.00
C-16	C93	4.7	0.00	45.83	54.17	0.00	0.00
C-16	C93(24)	6	4.17	20.83	70.83	0.00	4.17
C-20	C94	1	12.50	75.00	8.33	0.00	4.17
C-20	C94(24)	2.3	8.33	79.17	4.17	0.00	8.33
C-24	C95	2.7	4.17	66.67	29.17	0.00	0.00
C-24	C95(24)	2.3	25.00	50.00	16.67	0.00	8.33
C-27	C96	8.7	41.67	20.83	25.00	4.17	8.33
C-27	C96(24)	7	50.00	16.67	0.00	12.50	20.83
C-33	C97	2.7	0.00	95.83	0.00	0.00	4.17
C-33	C97(24)	1	4.17	87.50	4.17	0.00	4.17
C-37.5Z	C98	4.7	0.00	37.50	20.83	0.00	41.67
C-37.5Z	C98(24)	5	12.50	0.00	29.17	0.00	58.33
C-38	C99	2	4.17	62.50	12.50	0.00	20.83
C-38	C99(24)	2.7	4.17	62.50	25.00	0.00	8.33
Average	6hr	3.17	7.41	64.81	17.13	0.46	10.19
	24hr	3.18	12.96	56.02	17.13	1.39	12.50

Site #	Sample#	# of isolates	% Bird	% Human	%Livestock	%Pets	%Wildlife
C-7	C100	10	4.17	66.67	16.67	4.17	8.33
C-7	C100 (24)	11.7	12.50	25.00	45.83	0.00	16.67
C-15	C101	8	0.00	58.33	20.83	4.17	16.67
C-15	C101(24)	8	12.50	25.00	50.00	8.33	4.17
C-16	C102	25.3	4.17	33.33	41.67	4.17	16.67
C-16	C102(24)	16.7	41.67	50.00	8.33	0.00	0.00
C-20	C103	12.3	4.17	45.83	37.50	4.17	8.33
C-20	C103(24)	12.7	25.00	70.83	4.17	0.00	0.00
C-24	C104	27.3	4.17	37.50	4.17	0.00	54.17
C-24	C104(24)	25.3	4.17	83.33	12.50	0.00	0.00
C-27	C105	48	4.17	41.67	29.17	8.33	16.67
C-27	C105(24)	48	0.00	66.67	33.33	0.00	0.00
C-33	C106	28	0.00	41.67	25.00	16.67	16.67
C-33	C106(24)	27	8.33	29.17	54.17	0.00	8.33
C-37.5Z	C107	58	0.00	70.83	8.33	4.17	16.67
C-37.5Z	C107(24)	55.3	20.83	16.67	33.33	0.00	29.17
C-38	C108	26.3	12.50	62.50	16.67	4.17	4.17
C-38	C108(24)	17	8.33	50.00	33.33	0.00	8.33
Average	6 hr	27.02	3.71	50.93	22.22	5.56	17.59
	24 hr	24.63	14.81	46.30	30.55	0.93	7.41

Table 5. Numbers of *E coli* isolates in the Little Wicomico watershed with rainfall 3 days prior to collection.

A. Samples Collected from DSS Station W-9W

DATE	Sample #	No. isolates/100 ml	Rain (inches)
9/25/01	W1	9	1.45
10/22/01	W7	9	0.00
11/19/01	W13	6	0.00
12/6/01	W19	20	0.02
1/17/02	W25	1	0.31
2/19/02	W31	0	0.00
3/20/02	W37	54	0.70
4/18/02	W43	16	0.12
5/1/02	W49	5	0.90
6/13/02	W55	3	0.00
7/15/02	W61	3	0.49
8/29/02	W67	3	0.06

B. Samples Collected from DSS Station W-9X

DATE	Sample #	No. isolates/100 ml	Rain (inches)
9/25/01	W2	12	1.45
10/22/01	W8	3	0.00
11/19/01	W14	2	0.00
12/6/01	W20	6	0.02
1/17/02	W26	1	0.31
2/19/02	W32	0	0.00
3/20/02	W38	18	0.70
4/18/02	W44	13	0.12
5/1/02	W50	7	0.90
6/13/02	W56	2	0.00
7/15/02	W62	2	0.49
8/29/02	W68	2	0.06

C. Samples Collected from DSS Station W-13.5Z

DATE	Sample #	No. isolates/100 ml	Rain (inches)
9/25/01	W3	30	1.45
10/22/01	W9	3	0.00
11/19/01	W15	3	0.00
12/6/01	W21	11	0.02
1/17/02	W27	2	0.31
2/19/02	W33	1	0.00
3/20/02	W39	58	0.70
4/18/02	W45	25	0.12
5/1/02	W51	9	0.90
6/13/02	W57	2	0.00
7/15/02	W63	4	0.49
8/29/02	W69	3	0.06

D. Samples Collected from DSS Station W-16

DATE	Sample #	No. isolates/100 ml	Rain (inches)
9/25/01	W4	32	1.45
10/22/01	W10	9	0.00
11/19/01	W16	5	0.00
12/6/01	W22	18	0.02
1/17/02	W28	1	0.31
2/19/02	W34	1	0.00
3/20/02	W40	71	0.70
4/18/02	W46	39	0.12
5/1/02	W52	46	0.90
6/13/02	W58	6	0.00
7/15/02	W64	7	0.49
8/29/02	W70	17	0.06

E. Samples Collected from DSS Station W-19

DATE	Sample #	No. isolates/100 ml	Rain (inches)
9/25/01	W5	77	1.45
10/22/01	W11	22	0.00
11/19/01	W17	23	0.00
12/6/01	W23	20	0.02
1/17/02	W29	2	0.31
2/19/02	W35	13	0.00
3/20/02	W41	53	0.70
4/18/02	W47	37	0.12
5/1/02	W53	17	0.90
6/13/02	W59	26	0.00
7/15/02	W65	33	0.49
8/29/02	W71	13	0.06

F. Samples Collected from DSS Station W-20

DATE	Sample #	No. isolates/100 ml	Rain (inches)
9/25/01	W6	55	1.45
10/22/01	W12	13	0.00
11/19/01	W18	36	0.00
12/6/01	W24	18	0.02
1/17/02	W30	3	0.31
2/19/02	W36	2	0.00
3/20/02	W42	60	0.70
4/18/02	W48	62	0.12
5/1/02	W54	37	0.90
6/13/02	W60	59	0.00
7/15/02	W66	8	0.49
8/29/02	W72	19	0.06

Table 6. Source Tracking percentages of *E. coli* in the Little Wicomico River watershed with seasonality results.

A. Samples Collected from DSS Station W-9W

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	W1	4.17	75.00	4.17	16.67	0.00
10/22/01	W7	0.00	75.00	0.00	0.00	25.00
11/19/01	W13	29.17	54.17	12.50	4.17	0.00
12/6/01	W19	8.33	8.33	0.00	41.67	41.67
1/17/02	W25	25.00	70.83	0.00	0.00	4.17
2/19/02	W31	12.50	87.50	0.00	0.00	0.00
3/20/02	W37	12.50	67.50	0.00	0.00	0.00
4/18/02	W43	33.33	62.50	4.17	0.00	0.00
5/1/02	W49	33.33	0.00	4.17	0.00	62.50
6/13/02	W55	0.00	100.00	0.00	0.00	0.00
7/15/02	W61	0.00	58.33	29.17	0.00	12.50
8/29/02	W67	0.00	79.17	12.50	4.17	4.17
	SUM	158.33	738.33	66.68	66.68	150.01
	AVERAGE	13.19	61.53	5.56	5.56	12.50
WET PERIOD						
JAN/FEB/MARCH		50.00	225.83	0.00	0.00	4.17
FOR SEASON		16.67	75.28	0.00	0.00	1.39
DRY PERIOD						
JULY/AUGUST/SEPT		4.17	212.50	45.84	20.84	16.67
FOR SEASON		1.39	70.83	15.28	6.95	5.56

B. Samples Collected from DSS Station W-9X

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	W2	4.17	70.83	12.50	8.33	4.17
10/22/01	W8	4.17	91.67	4.17	0.00	0.00
11/19/01	W14	8.33	83.33	8.33	0.00	0.00
12/6/01	W20	0.00	50.00	0.00	16.67	33.33
1/17/02	W26	20.83	62.50	4.17	0.00	12.50
2/19/02	W32	0.00	58.33	0.00	0.00	41.67
3/20/02	W38	16.67	66.67	8.33	4.17	4.17
4/18/02	W44	25.00	66.67	8.33	0.00	0.00
5/1/02	W50	62.50	20.83	0.00	8.33	8.33
6/13/02	W56	25.00	41.67	20.83	4.17	8.33
7/15/02	W62	4.17	62.50	29.17	0.00	4.17
8/29/02	W68	25.00	66.67	8.33	0.00	0.00
	SUM	195.84	741.67	104.16	41.67	116.67
	AVERAGE	16.32	61.81	8.68	3.47	9.72
WET PERIOD						
JAN/FEB/MARCH		37.50	187.50	12.50	4.17	58.34
FOR SEASON		12.50	62.50	4.17	1.39	19.45
DRY PERIOD						
JULY/AUGUST/SEPT		33.34	200.00	50.00	8.33	8.34
FOR SEASON		11.11	66.67	16.67	2.78	2.78

C. Samples Collected from DSS Station W-13.5Z

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	W3	33.33	33.33	25.00	0.00	8.33
10/22/01	W9	12.50	70.83	4.17	12.50	0.00
11/19/01	W15	16.67	83.33	0.00	0.00	0.00
12/6/01	W21	0.00	37.50	0.00	58.33	4.17
1/17/02	W27	41.67	37.50	8.33	8.33	4.17
2/19/02	W33	0.00	70.83	0.00	0.00	29.17
3/20/02	W39	25.00	37.50	12.50	0.00	25.00
4/18/02	W45	45.83	25.00	16.67	0.00	12.50
5/1/02	W51	4.17	95.83	0.00	0.00	0.00
6/13/02	W57	37.50	41.67	16.67	4.17	0.00
7/15/02	W63	12.50	33.33	29.17	0.00	25.00
8/29/02	W69	45.83	50.00	4.17	0.00	0.00
	SUM	275.00	616.65	116.68	83.33	108.34
	AVERAGE	22.92	51.39	9.72	6.94	9.03
WET PERIOD						
JAN/FEB/MARCH		66.67	145.83	20.83	8.33	58.34
FOR SEASON		22.22	48.61	6.94	2.78	19.45
DRY PERIOD						
JULY/AUGUST/SEPT		91.66	116.66	58.34	0.00	33.33
FOR SEASON		30.55	38.89	19.45	0.00	11.11

D. Samples Collected from DSS Station W-16

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	W4	8.33	50.00	12.50	16.67	12.50
10/22/01	W10	16.67	66.67	16.67	0.00	0.00
11/19/01	W16	50.00	45.83	4.17	0.00	0.00
12/6/01	W22	79.17	4.17	4.17	0.00	12.50
1/17/02	W28	16.67	83.33	0.00	0.00	0.00
2/19/02	W34	0.00	91.67	8.33	0.00	0.00
3/20/02	W40	16.67	50.00	0.00	20.83	12.50
4/18/02	W46	50.00	4.17	25.00	0.00	20.83
5/1/02	W52	0.00	95.83	0.00	0.00	4.17
6/13/02	W58	33.33	62.50	4.17	0.00	0.00
7/15/02	W64	8.33	66.67	12.50	4.17	8.33
8/29/02	W70	20.83	50.00	29.17	0.00	0.00
	SUM	300.00	670.84	116.68	41.67	70.83
	AVERAGE	25.00	55.90	9.72	3.47	5.90
WET PERIOD						
JAN/FEB/MARCH		33.34	225.00	8.33	20.83	12.50
FOR SEASON		11.11	75.00	2.78	6.94	4.17
DRY PERIOD						
JULY/AUGUST/SEPT		37.49	166.67	54.17	20.84	20.83
FOR SEASON		12.50	55.56	18.06	6.95	6.94

E. Samples Collected from DSS Station W-19

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	W5	0.00	62.50	8.33	8.33	20.83
10/22/01	W11	12.50	66.67	4.17	0.00	16.67
11/19/01	W17	29.17	62.50	8.33	0.00	0.00
12/6/01	W23	45.83	12.50	0.00	0.00	41.67
1/17/02	W29	37.50	25.00	29.17	4.17	4.17
2/19/02	W35	0.00	62.50	8.33	0.00	29.17
3/20/02	W41	4.17	79.17	8.33	0.00	8.33
4/18/02	W47	4.17	45.83	8.33	0.00	41.67
5/1/02	W53	100.00	0.00	0.00	0.00	0.00
6/13/02	W59	58.33	37.50	0.00	4.17	0.00
7/15/02	W65	8.33	45.83	37.50	0.00	8.33
8/29/02	W71	25.00	58.33	4.17	12.50	0.00
	SUM	325.00	558.33	116.66	29.17	170.84
	AVERAGE	27.08	46.53	9.72	2.43	14.24
WET PERIOD						
JAN/FEB/MARCH		41.67	166.67	45.83	4.17	41.67
FOR SEASON		13.89	55.56	15.28	1.39	13.89
DRY PERIOD						
JULY/AUGUST/SEPT		33.33	166.66	50.00	20.83	29.16
FOR SEASON		11.11	55.55	16.67	6.94	9.72

F. Samples Collected from DSS Station W-20

DATE	Sample #	Birds	Human	Livestock	Pets	Wildlife
9/25/01	W6	4.17	50.00	4.17	29.17	12.50
10/22/01	W12	0.00	75.00	8.33	4.17	12.50
11/19/01	W18	41.57	58.33	0.00	0.00	0.00
12/6/01	W24	20.83	16.67	25.00	16.67	20.83
1/17/02	W30	16.67	20.83	20.83	4.17	37.50
2/19/02	W36	37.50	58.33	4.17	0.00	0.00
3/20/02	W42	20.83	79.17	0.00	0.00	0.00
4/18/02	W48	4.17	62.50	0.00	4.17	29.17
5/1/02	W54	87.50	4.17	0.00	4.17	4.17
6/13/02	W60	0.00	20.83	0.00	29.17	50.00
7/15/02	W66	62.50	8.33	4.17	0.00	25.00
8/29/02	W72	12.50	50.00	12.50	8.33	16.67
	SUM	308.20	504.20	79.20	100.00	208.30
	AVERAGE	25.70	42.00	6.60	8.30	17.40
WET PERIOD						
JAN/FEB/MARCH		75.00	158.30	25.00	4.20	37.50
FOR SEASON		25.00	52.80	8.30	1.40	12.50
DRY PERIOD						
JULY/AUGUST/SEPT		79.20	108.30	20.80	37.50	54.20
FOR SEASON		26.40	36.10	6.90	12.50	18.10

APPENDIX A

2) Non-point Sources of Fecal Contamination in the Coan River and Little Wicomico River Watersheds Addendum to Final Report presented to The Virginia Department of Environmental Quality

Non-point Sources of Fecal Contamination in the Coan River and Little Wicomico River Watersheds

Addendum to Final Report presented to

The Virginia Department of Environmental Quality

Charles Hagedorn, Professor

and

Cheryl Szeles, Graduate Research Assistant

Department of Crop and Soil Environmental Sciences

Virginia Polytechnic Institute and State University

Blacksburg, VA 24061-0404

April 23, 2003

Revision of Results Based on Isolate Probabilities

Two approaches have emerged in source tracking regarding analyzing the patterns of isolates from water samples (unknown sources) against libraries of known sources. The first approach has been widely used with phenotypic source tracking methods (such as ARA) and is based on an ecological perspective where known source isolates in the library are statistically evaluated and sorted into groups or clusters that are source dependent. The goodness-of-fit of these source-derived clusters is reflected in the rates of correct classification (RCC) for each source in the library, and RCCs are averaged to produce the average rate of correct classification (ARCC). Isolates from water samples are then patterned and their patterns are placed into the source-dependent cluster that they most closely resemble. The higher the ARCC of the entire library, the greater the confidence that the water isolates are correctly classified. This was the approach used in the DEQ-DSS project and was described in the final report. The ARCC of the library for the Coan and Little Wicomico Rivers was 71.9%, in the upper range of ARCCs reported in the literature.

The second approach has been widely used with molecular source tracking methods (such as ribotyping) and is based on a clinical perspective where known source isolates in the library are not statistically evaluated and sorted into any types of groups or clusters. Isolates from water samples are patterned and their patterns are compared against every isolate in the library and are identified based on the known source isolate that they most closely resemble. Since no statistical evaluation of the library is involved, most publications using this clinical approach only include those water isolates that resemble a known source isolate at a given probability level, usually 80% or above. Those with matching probabilities below 80% are placed in an unknown or no-match category. In published reports to date, anywhere from one-third to two-thirds of the isolates from water samples are commonly placed in the no-match category.

There have been many debates over molecular and phenotypic techniques in the bacterial source-tracking community when examining the validity of research studies. One criticism that has been directed at ARA is that phenotypic methods are inaccurate when compared to molecular methods and if individual isolates were evaluated at an 80% probability or greater, most if not all of the unknown isolates would be lost due to low probabilities. Simmons *et al.* (2002) performed a molecular BST technique, pulsed-field gel electrophoresis (PFGE), on the Four Mile Run Watershed (Arlington County, Va. They rejected 49% of *E. coli* unknown source isolates (averaged over all sampling sites) when using an 80% probability with PFGE and obtained a match with 278 of 539 total isolates. Samadpour and Chechowitz (1995) performed ribotyping on 589 *E. coli* isolates in Little Soos Creek (King County, Wa) and found that the unknown source isolates had 171 different ribotype profiles, and 67% of these profiles could not be matched by the known source library.
In Tables 1 and 2 (below) the average corrected percentages are shown, and these percentages were isolates where their individual probabilities were greater than 80%. The percentages found in parentheses are actually the old percentages as described in the final report and did not include any cutoff level for isolate probabilities.

In Table 1 the percentages of water isolates placed in the no-match category ranged from 44.7% for station C-17 to 79.2% for station C-33. An average of 53% of all the unknown isolates were not matched and were rejected using an 80% probability for the Coan River Watershed. In Table 2 the percentages of water isolates placed in the no-match category ranged from 43.5% for station W-9X to 55.8% for station W-20. An average of 52% of all the unknown isolates were not matched and were rejected using an 80% probability for the Little Wicomico Watershed. Like Simmons *et al.* (2002) with PFGE, about 50% of the unknown isolates were lost using an 80% probability cutoff.

Table 1. Coan River Corrected Percentage of Sources and Unknown Percentage

	AVERAGE CORRECT PERCENTAGES OLD % in ( )					
DSS STATION	BIRD	HUMAN	LIVESTOCK	PETS	WILDLIFE	UNKNOWN %
C-7	9.8 (6.3)	35.8 (81.6)	1.7 (6.3)	0.0 (0.7)	0.8 (5.2)	51.9
C-15	11.5 (11.1)	33.3 (74.7)	5.9 (8.0)	0.0 (1.4)	2.5 (4.9)	46.8
C-16	26.4 (21.2)	21.9 (54.5)	3.5 (8.3)	0.0 (5.2)	3.5 (10.8)	44.7
C-20	12.9 (19.8)	31.2 (63.2)	1.9 (5.2)	0.0 (3.5)	3.4 (8.3)	50.6
C-24	19.5 (26.0)	28.7 (46.5)	0.5 (8.7)	0.7 (4.2)	0.4 (6.3)	50.2
C-27	12.5 (21.2)	24.9 (45.8)	1.8 (11.1)	0.4 (3.5)	0.4 (5.6)	60.0
C-33	5.9 (9.4)	10.7 (58.0)	3.0 (12.2)	0.8 (2.8)	0.4 (9.4)	79.2
C-37.5Z	9.2 (14.2)	34.8 (57.3)	6.1 (13.9)	0.0 (1.4)	5.0 (13.2)	44.9
C-38	10.0 (25.7)	32.3 (50.7)	5.5 (6.3)	1.6 (5.6)	3.9 (8.7)	46.7

Table 2. Little Wicomico Corrected Percentage of Sources and Unknown Percentage

DSS STATION	AVERAGE CORRECT PERCENTAGES OLD % in ( )					
	BIRD	HUMAN	LIVESTOCK	PETS	WILDLIFE	UNKNOWN %
W-9W	9.2 (13.2)	28.9 (49.0)	4.0 (5.6)	1.9 (8.0)	4.5 (12.8)	51.5
W-9X	17.4 (16.3)	24.6 (64.2)	4.1 (7.6)	1.6 (3.1)	8.8 (10.8)	43.5
W-13.5Z	13.5 (20.1)	18.6 (53.8)	4.5 (9.4)	4.4 (6.9)	10.5 (9.7)	48.5
W-16	20.3 (22.2)	21.4 (54.9)	2.6 (10.1)	0.0 (4.2)	0.4 (8.7)	55.3
W-19	14.5 (22.2)	23.9 (45.8)	2.5 (9.7)	0.8 (2.4)	3.4 (19.1)	54.9
W-20	17.1 (25.7)	19.8 (42.0)	1.8 (6.6)	1.4 (8.3)	4.1 (17.4)	55.8

Conclusions

The phenotypic method employed in our study (ARA) and the molecular methods included in the references provided the same results when using the 80% probability level, and the dominant sources of human followed by birds were not changed (from the previously submitted final report) when the no-match isolates were removed from all categories in Tables 1 and 2. Therefore, the argument that ARA is less accurate than molecular methods must be rejected, as applying the 80% level to the results from the Coan and Little Wicomico Rivers produced the same type of results as those reported for molecular methods.

The more important issue is why so many isolates are placed in the unknown category when using an 80% cutoff. This is clearly related to the representativeness of the known source library, and the loss of isolates means that the library does not have appropriate patterns to match against them (no-match). There are two approaches to consider; the first is to test libraries for representativeness. Such testing is now commonplace with phenotypic method but has not been widely used with molecular methods. Until this is done, it will not be possible to make decisions regarding the usefulness of known source libraries. The second approach is to use a lower percent cutoff as there is nothing justifiable about 80% other than precedent. For example, if a library was divided into five source categories, the probability of an unknown source isolate being placed in any one of the five categories is 20%. It could be argued that any isolate probabilities above 20% could be used. In a recent source tracking review from Dr. Joan Rose’s laboratory at the

University of South Florida (Scott *et al*. 2002), the authors examined the existing literature on all methods and concluded that any isolate probabilities above 50% should be useful. If the isolates form the Coan and Little Wicomico rivers were evaluated at the 50% level (instead of 80%), most of the isolates would be removed from the unknown category and the results would appear very much like those submitted in the final report.

References

Samadpour, M. and Chechowitz, N. (1995) Little Soos Creek Microbial Source Tracking: a Survey. *University of Washington Department of Environmental Health*, Seattle, WA.
Scott, T. M., J. B. Rose, T. M. Jenkins, S. R. Farrah, and J. Lukasik. (2002) Microbial Source Tracking: Current Methodology and Future Directions. *Applied and Environmental Microbiology* 68:5796-5803.
Simmons, G. M., Jr., Waye, D.F., and Herbein, S.A. (2002) Estimating Nonpoint Source Fecal Coliform Sources Using DNA Profile Analysis, *Advance in Water Monitoring Research*, pg. 143-167

APPENDIX B

1) **SANITARY SHORELINE SURVEY RESULTS**

2) **NOTICE AND DESCRIPTION OF SHELLFISH AREA CONDEMNATION NUMBER 105 LITTLE WICOMICO RIVER EFFECTIVE 10 JUNE 1997**

COMMONWEALTH of VIRGINIA

Department of Health

E. ANNE PETERSON, M.D., M.P.H.
STATE HEALTH COMMISSIONER

P O BOX 2448
RICHMOND, VA 23218

TDD 1-800-828-1120

LITTLE WICOMICO RIVER

Northumberland County

Shoreline Sanitary Survey

Date: October 30, 2000

Survey Period: January 14 - August 2, 2000

Total Number of Properties Surveyed: 1152

Surveyed By: D. B. Geeson, J. M. Smither and R. M. Thomas

SECTION A: GENERAL

This survey area extends from Reference Point 10 off State Route 739 (extended to the Potomac River) to Reference point 11 off State Route 652 (extended to the Chesapeake Bay), including the Potomac River and Chesapeake Bay shorelines between these two points, Little Wicomico River (Ellyson Creek, Bridgemans Back Creek, Spences Creek, Spring Cove, Sawmill Cove, Hansons Cove, Willis Creek, Sloop Creek, Cod Creek, Back Creek, Bridge Creek, Horse Pond, Sharps Creek, Slough Creek, Rock Hole), and all of their tributaries.

The topography in this area varies in elevations from 5' or less along the shoreline to a maximum of 100' near the western edge of the survey boundary. The population is mostly sparse with moderate concentrations in the various residential subdivisions in the area. The economy is primarily based on service-oriented businesses, the seafood industry, recreation and agriculture.

Meteorological data indicated that .70" of rain fell January 14-31, 1.21" February 1-29, 2.46" March 1-31, 2.71" April 1-30, 3.00" May 1-31, 4.72" June 1-30, 11.24" July 1-31, and .06" August 1-2 for a total of 26.10" for the survey period.

Current restrictions on shellfish harvesting are Condemned Shellfish Area #105, Little Wicomico River, revised 17 May 1999 and Condemned Shellfish Area #180, Little Wicomico River: Bridge Creek, revised 13 April 2000. Copies of the current condemnation notices and maps are attached to the back of this report.

There were a few houses off State Route 652 in Beverlyville that hooked into the Reedville sewerage system. Those houses are marked on the accompanying map. The processing waste lagoon at Huff and Puff Pet Foods, which was listed as an industrial waste facility (property #364) in the previous report, is no longer being used. This facility is currently owned by Pride of Virginia Seafood and is used as a warehouse. E. F. Lewis and Sons Seafood, also an industrial waste facility (property #455) in the previous report, no longer has an active VPDES discharge permit. The business has stopped processing soft crabs. They currently operate under the name of Lewis Seafood. There were two campgrounds (K.O.A. and Smith Point) in the area that were found to be in satisfactory condition at time of inspection. Smith Point Campground had a modest increase of 12 additional sites.

Information in this report is gathered by and primarily for the use of the Division of Shellfish (DSS), Virginia Department of Health, in order to fulfill its responsibilities of shellfish growing area supervision and classification. However, the data is made available to various agencies participating in shellfish program coordinated activities and other interested parties.

Report copies are provided to the local health department for corrective action of deficiencies listed on the summary page in Sections B.2. and B.3. and the Department of Environmental Quality (DEQ) for possible action at the properties listed on the summary page in Section C.1. The Division of Soil and Water Conservation is provided information on possible sources of animal pollution found in Section E.

This report lists only those properties that have a sanitary deficiency or other environmental significance. ***"DIRECT"*** indicates that the significant activity or deficiency has a direct impact on shellfish waters. Individual field forms with full information on properties listed in this report are on file in the Richmond office of DSS and are available for reference until superceded by a subsequent resurvey of the area.

SECTION B: SEWAGE POLLUTION SOURCES

SEWAGE TREATMENT WORKS

-None

ONSITE SEWAGE DEFICIENCIES

5. NO FACILITIES, *DIRECT - F.* [redacted]. Owner: [redacted]. Private boat docking facility for fish handling business. No contact. Sanitary Notice issued 10-27-00 to field #C116.

6. NO FACILITIES, *DIRECT* - [redacted]. Business- fish processing facility. 6 employees. Using a commercial privy. Sanitary Notice issued 8-21-00 to field #C119.

10. CONTRIBUTES POLLUTION, *DIRECT* - [redacted]. Dwelling- yellow vinyl siding and frame 2 story. 1 person. Effluent erupting from drainfield onto ground surface 50' from the Little Wicomico River. Sanitary Notice issued 6-23-00 to field #C258.

13. NO FACILITIES - [redacted]. Dwelling- white frame 1 story. 1 person. Using a commercial privy. Sanitary Notice issued 8-2-00 to field #C353.

14. CONTRIBUTES POLLUTION (Kitchen or Laundry Wastes) - [redacted]. Dwelling- white cement block 1'/~ story cottage with black trim. 1 person. Laundry wastes discharge through a 2" PVC pipe onto ground surface. Grease trap effluent seeping around lid onto ground surface. Sanitary Notice issued 3-24-00 to field #B11.

18. NO FACILITIES, *DIRECT* - [redacted]. Owner: [redacted]. Business- fish retailer. 3 employees. Sanitary Notice issued 10-27-00 to field #B126.

22. CONTRIBUTES POLLUTION - [redacted], [redacted]. Dwelling- white frame 1 story. 2 persons. Unapproved plywood lid over septic tank. Effluent erupting from septic tank onto ground surface. Sanitary Notice issued 4-28-00 to field #A297.

27. CONTRIBUTES POLLUTION (Kitchen or Laundry Wastes) - [redacted], [redacted]. Dwelling- white "Trotwood" house trailer. No contact. Kitchen wastes draining onto ground surface through a 2" plastic pipe. Sanitary Notice issued 7-21-00 to field #A377.

28. CONTRIBUTES POLLUTION (Kitchen or Laundry Wastes) - [redacted], [redacted]. Dwelling- tan "Prowler" house trailer with a brown stripe. No contact. Kitchen wastes draining onto ground surface through a 2" plastic pipe. Sanitary Notice issued 7-21-00 to field #A376.

30. CONTRIBUTES POLLUTION - Location: [redacted]. Owner: [redacted]. Dwelling- white vinyl siding 1 story with black shutters. No contact. Effluent erupting onto ground surface from a cracked lid on septic tank. Sanitary Notice issued 3-10-00 to field #A45.

31. CONTRIBUTES POLLUTION - Occupant [redacted]. Dwelling white frame 2 story with black shutters. No contact. Effluent erupting from septic tank into a shallow trench leading to roadside ditch; and

CONTRIBUTES POLLUTION (Kitchen or Laundry Wastes) - Grease trap has an unapproved plywood lid. Sanitary Notice issued 3-10-00 to field #A44.

32. CONTRIBUTES POLLUTION - [redacted]. Dwelling- gray frame 1 story with red trim and door. No contact. Clean-out cap broken, exposing contents in sewer line. Sanitary Notice issued 3-10-00 to field #A41.

33. CONTRIBUTES POLLUTION (Kitchen or Laundry Wastes) - [redacted]. Dwelling- white frame 1'/a story with black trim and awnings. 3 persons. Laundry wastes discharge through a 1 " black plastic pipe onto ground surface. Sanitary Notice issued 3-3-00 to field #A34.

35. CONTRIBUTES POLLUTION [redacted]. Dwelling- white frame 1 story with white trim. No contact. Pit privy undermined, allowing waste to seep onto ground surface. Sanitary Notice issued 7-7-00 to field #A313.

POTENTIAL POLLUTION

1. [redacted]. Dwelling- white frame 1'/2 story with asbestos shingles. No contact. Observed on-site were 7 junked vehicles scattered throughout property.

7. [redacted]. Dwelling- brown frame 1 story. No contact. Observed on-site were 4 junked vehicles scattered throughout property.

11. [redacted] Dwelling- white house trailer with red trim. No contact. Observed on-site were 3 junked vehicles, assorted household trash and appliances scattered throughout property.

12. [redacted] 22473. Dwelling frame 1 story. No contact. Observed on-site were 2 cars, 1 bus, numerous tires, and various automobile parts scattered throughout property.

15. [redacted]. Business- agricultural. No contact. Observed on-site were eight tank trailers parked in an old barrow pit. Contents unknown. No evidence of leakage or discharge at time of inspection.

25. Location: [redacted]. Dwelling- blue cement block 1 story with white trim. No contact. A 4" caste iron pipe of undetermined origin was observed exiting waterfront side of house into the Little Wicomico River. No evidence of discharge at time of inspection.

SECTION C: **NON-SEWAGE POLLUTION SOURCES**

INDUSTRIAL WASTES

16. *DIRECT* - [redacted]. Business- commercial crustacean processing plant (VA-70C). 66 employees. Processing and washdown wastes from crabmeat picking and packing rooms, refrigeration rooms, and wastes from retort cookers discharge into the Little Wicomico River. Hand basins discharge into the septic system. Has VAG Permit #VAG524018 from DEQ. Permit expires 7-24-01.

17. *DIRECT* - [redacted]. Business- commercial shellfish processing plant (VA-309SP). 30 employees. Washdown and blower tank wastes discharge into the little Wicomico River. Hand basins discharge into the septic system. Has VAG Permit #VAG524034 from DEQ. Permit expires 7-24-01.

26. *DIRECT* - [redacted]. Business- commercial marina. No contact. Observed on-site were 3 x 600 gallon fuel tanks inside a 4' cinder block berm 50' from Slough Creek. One bottom cinder block was removed for the pipes from the fuel tanks to exit to the fuel pumps on the dock. Also present at time of survey were 3 sunken boats with the inboard engines still intact at the end of the dock.

SOLID WASTE DUMPSITES

-None

SECTION D: BOATING ACTIVITY

MARINAS

5. [redacted]. Private boat docking facility for fish handling business. 6 slips/moorings. Present at time of survey were 2 work boats under 26' and 5 work boats over 26'. Boating services include fuel, electricity, and water. Containers are available for solid waste collection. There are no sanitary facilities, boat holding tank pump-out facilities, or portable toilet dump station facilities available at this location.

6. [redacted]. Business: fish processing facility. 6 employees. 10 slips/moorings available. Present at time of survey were 5 work boats over 26' and 2 work boats under 26'. The only boating service provided was fuel. Containers were available for solid waste collection. There was 1 unisex commercial privy available. Sewage disposal is by pump and haul. There were no portable toilet dump station facilities provided at this location. Owner has an exemption to the requirement to provided boat holding tank pump-out facilities.

7. [redacted]. Commercial marina. 1 person. 41 slips/moorings available. Present at time of survey were 13 pleasure boats under 26', and 24 pleasure boats over 26'. Boating services provided were electricity and water. Containers were available for solid waste collection. Sanitary facilities provided were 1 privy for men and 1 privy for women. There were no portable toilet dump station facilities provided at this location. Owner has an exemption to the requirement to provide boat holding tank pump-out facilities.

8. [redacted]. Commercial marina and boat repair facility. 3 employees. 19 slips/moorings available. Present at time of survey were 17 pleasure boats over 26', 1 work boat under 26', and 1 work boat over 26'. Boating services provided were water, electricity, repair, and in-out ramp. Containers were provided for solid waste collection. There was 1 unisex vault privy available. Sewage disposal is by pump and haul. Boat holding tank pump-out facilities and portable toilet dump station facilities are provided at this location.

19. [redacted]: [redacted] Private marina. No contact. 25 slips/moorings available. Present at time of survey were 8 pleasure boats under 26'. Boating services provided were water, electricity, and an in-out ramp. Containers were not available for solid waste collection. Sanitary facilities provided were 1. commode, 1 urinal, 2 lavatories and 1 shower for men; and 2 commodes, 2 lavatories and 1 shower for women. Sewage disposal is to a septic tank with drainfield, which appeared to be in satisfactory condition at the time of inspection. Portable toilet dump station facilities were provided. Owner has an exemption to the requirement to provide boat holding tank pump-out facilities at this location.

[redacted] ~,~-r~~`~ oo r~.~. aa~.~ ~o

[redacted]. Commercial marina. No contact. 26 slips/moorings available. Present at the time of survey were 7 pleasure boats and 1 work boat under 26' and 17 pleasure boats over 26'. Boating services provided are water, electricity, and fuel. Containers were available for solid waste collection. Sanitary facilities provided are 1 commode, 1 urinal, 1 lavatory, and Z showers for men; and 2 commodes, 1 lavatory, and 2 showers for women. Sewage disposal is to a septic tank with drainfield, which appeared to be in satisfactory condition at the time of inspection. There were no portable toilet dump station facilities or boat holding tank pump-out facilities provided at this location.

34. [redacted]. Commercial marina. 3 employees. 119 slips/moorings/25 dry storage spaces available. Present at time of survey were 6 pleasure boats under 26' and 1 work boat and 31 pleasure boats over 26' in wet storage; and in dry storage there was 1 pleasure boat under 26'. Boating services provided were water, electricity, fuel, repair and an in-out ramp. Containers were available for solid waste collection. Sanitary facilities provided were 2 commodes, 1 urinal, 4 lavatories and 1 shower for men; and 3 commodes, 3 lavatories and 1 shower for women. Sewage disposal is to a septic tank with drainfield, which appeared to be in satisfactory condition at time of inspection. Portable toilet dump station facilities and boat holding tank pump-out facilities were provided at this location.

OTHER PLACES WHERE BOATS ARE MOORED

4. [redacted]. Private boat docking facility. No contact. 4 slips/moorings available. Present at time of survey were 4 work boats over 26'. There were no boating services, containers for solid waste collection, sanitary facilities, boat holding tank pump-out facilities or portable toilet dump station facilities available at this location.

17. [redacted]. Commercial seafood processing plant. 3 employees. 5 slips/moorings available. There were no boats present at time of survey. The only boating service available was electricity. There were no containers provided for solid wastes collection. Sanitary facilities provided were 1 pit privy for men and 1 pit privy for women. Privies appeared to be in satisfactory condition at time of inspection. There were no boat holding tank pump-out facilities or portable toilet dump station facilities available at this location.

18. [redacted], [redacted]. Commercial fish dock. 2 employees. 6 slips/moorings available. Present at time of survey were 2 work boats under 26' and 4 work boats over 26'. Boating services provided were water and electricity. There were no solid waste containers, sanitary facilities, boat holding tank pump-out facilities or portable dump station facilities available at this location.

23. [redacted]. Private pier. No contact. 8 slips/moorings available. Present at the time of survey was 1 pleasure boat under 26'. The only boating service provided was an in-out ramp. Containers were not available for solid waste collection. Owners have an exemption to the requirement to provide onshore sanitary facilities, boat holding tank pump-out facilities and portable toilet dump station facilities at this location.

24. [redacted]: [redacted]. Private pier and boat hull repair facility. No contact. 4 slips/moorings available. Present at the time of survey was 1 work boat under 28'. Boating services provided were electricity and repair. Containers were not available for solid waste collection. There was 1 unisex privy on-site that appeared to be in satisfactory condition at time of inspection. Portable toilet dump station facilities were available. There were no boat holding tank pump-out facilities provided at this location.

29. [redacted] . Private pier and campground. 3 employees. 30 slips/moorings available. Present at the time of survey were 2 pleasure boats under 26' and 2 work boats over 26'. Boating services provided were electricity, water, and an in-out ramp. Containers were available for solid waste collection. Sanitary facilities provided were 4 commodes, 2 urinals, 6 lavatories and 3 showers for men; and 6 commodes, 6 lavatories and 3 showers for women. Sewage disposal is to a septic tank with drainfield, which appeared to be in satisfactory condition at time of inspection. Portable toilet dump station facilities and boat holding tank pump-out facility were available at this location.

UNDER SURVEILLANCE

2. [redacted]. [redacted] Private boat docking facility. No contact. 5 slips/moorings available. There were no boats present at time of survey. There were no boating services provided, containers available for solid waste collection, sanitary facilities, boat holding tank pump-out facilities or portable toilet dump station facilities available at this location.

3. [redacted], [redacted]. Private boat docking facility. No contact. 2 slips/moorings available. Present at time of survey were 1 pleasure boat under 26' and 1 pleasure boat over 26'. The only boating service provided is an in-out ramp. There were no containers for solid waste collection, sanitary facilities, boat holding tank pump-out facilities or portable toilet dump station facilities available at this location.

SECTION E: CONTRIBUTES ANIMAL POLLUTION

20. *DIRECT* - [redacted]. Community association. No contact. Present at the time of survey were 26 domestic geese in a fenced area with a small pond. The pond drains directly into the Little Wicomico River. Waste disposal is unknown.

21. [redacted]. Dwelling- white frame 2 story with black trim. 2 persons. Present at time of survey were 10 cows in a fenced pasture approximately 35' from Bridge Creek. Manure is left on the ground.

APPENDIX B:

2) NOTICE AND DESCRIPTION OF SHELLFISH AREA CONDEMNATION NUMBER 105: LITTLE WICOMICO RIVER

REGISTRAR OF REGULATIONS

COMMONWEALTH Of VIRGINIA :

Department of Health

RANDOLPH L GORDON, M.D., M.P.H.
COMMISSIONER

P.O. BOX 2448
RICHMOND, VA 23218
TDD 1-800-828-1120

NOTICE AND DESCRIPTION OF SHELLFISH AREA CONDEMNATION NUMBER 105, LITTLE WICOMICO RIVER

EFFECTIVE 10 JUNE 1997

Pursuant to Title 28.2, Chapter 8, §§28.2-803 through 28.2-808, §32.1-20, and §9-6.14:4.1, B.16 of the Code of Virginia:

1. The "Notice and Description of Shellfish Area Condemnation Number 105, Little Wicomico River," effective 31 August 1993, is cancelled effective 10 June 1997.

2. Condemned Shellfish Area Number 105, Little Wicomico River, is established, effective 10 June 1997. It shall be unlawful for any person, firm, or corporation to take shellfish from area # 105 for any purpose, except by permit granted by the Marine Resources Commission, as provided in Section 28.2-810 of the *Code of* Virginia. The boundaries of the area are shown on map titled "Little Wicomico River, Condemned Shellfish Area Number 105, 10 June 1997" which is part of this notice.

3. The Department of Health will receive, consider and respond to petitions by any interested person at any time with respect to reconsideration or revision of this order.

BOUNDARIES OF CONDEMNED AREA NUMBER 105

A. The condemned area shall include all of that portion of Cod Creek and its tributaries lying upstream of a line drawn between Marine Resources Commission survey markers "Guckert" and "Cod."

B. The condemned area shall include all of that portion of the Little Wicomico River and its tributaries lying upstream of a line drawn from Marine Resources Commission survey marker "Long" northeasterly to the southernmost tip on the opposite shore.

Recommended by:

Director, Division of Shellfish S nation

Ordered by: Rand. Gordon MD by Don Stern MD Deputy 5-29-97

State He 1th Commissioner Date

I–DHVIRGINIA
DEPARTMENT
OF HEALTH
Protecting You and Your Environment

COMMONWEALTH of VIRGINIA -

Department of Health

RANDOLPH L GORDON, M.D., M.P.H.
COMMISSIONER

P O BOX 2448
RICHMOND, VA 23218
TOD 1-800-828-1120

NOTICE AND DESCRIPTION OF SHELLFISH AREA CONDEMNATION
NUMBER 180, LITTLE WICOMICO RIVER: BRIDGE CREEK
EFFECTIVE 10 JUNE 1997

Pursuant to Title 28.2, Chapter 8, §§28.2-803 through 28.2-808, §32.1-20, and §9-6.14:4.1, B.16 of the *Code of Virginia:*

1. The `Notice and Description of Shellfish Area Condemnation Number 180, Little Wicomico River: Bridge Creek," effective .6 February 1996, is cancelled effective 10 June 1997.

2. Condemned Shellfish Area Number 180, Little Wicomico River: Bridge Creek, is established, effective 10 June 1997. It shall be unlawful for any person, firm, or corporation to take shellfish from area #180 for any purpose, except by permit granted by the Marine Resources Commission, as provided in Section 28.2-810 of the *Code of Virginia.* The boundaries of the area are shown on map titled "Little Wicomico River: Bridge Creek, Condemned Area Number 180, 10 June 1997" which is part of this notice.

3. The Department of Health will receive, consider and respond to petitions by any interested person at any time with respect to reconsideration or revision of this order.

BOUNDARIES OF CONDEMNED AREA NUMBER 180

The condemned area shall include all of that portion of Bridge Creek and its tributaries lying upstream of a line drawn from Marine Resources Commission survey marker "Jamie" to the first prominent point upstream of Marine Resources Commission survey marker "Rail."

Recommended by:

Director, Division of Shellfis anitation

Ordered by: .~~................. ....„~: ~ -~'~,._ r~ ~~-.~~,-.~-.
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State Health Commissioner Date

VIRGINIA
DEPARTMENT
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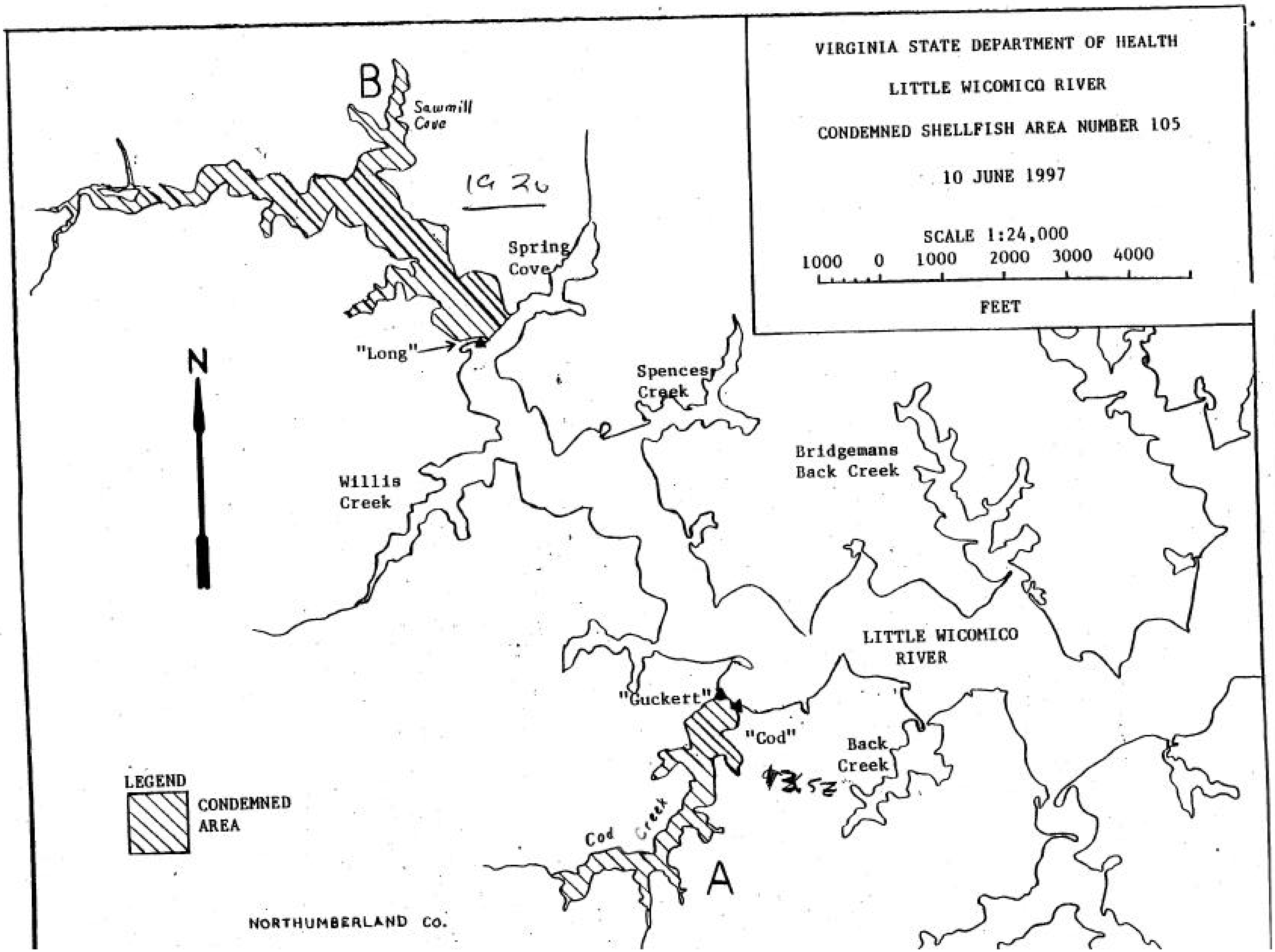
VIRGINIA STATE DEPARTMENT OF HEALTH
LITTLE WICOMICO RIVER
CONDEMNED SHELLFISH AREA NUMBER 105
10 JUNE 1997
SCALE 1:24,000
1000 0 1000 2000 3000 4000
FEET
B
Sawmill Cove
Spring Cove
"Long"
Spences Creek
Willis Creek
Bridgemans Back Creek
LITTLE WICOMICO RIVER
"Guckert"
"Cod"
Back Creek
Cod Creek
A
N
LEGEND
CONDEMNED AREA
NORTHUMBERLAND CO.

Appendix C

List of Attendees and Questions asked at the Public Meeting of July 22, 2003

List of Attendees for the Coan and Little Wicomico River TMDL Public Meeting Held on July 22, 2003 at the Northumberland County Courthouse Complex

Name	Organization
Mark Alling	Va. Department of Environmental Quality
Chester C. Bigelow, III	Va. Department of Environmental Quality
Charles Martin	Va. Department of Environmental Quality
Denise Moyer	Va. Department of Environmental Quality
Hoyt Wheeland	Va. Department of Conservation and recreation
Mike Harwood	NAPS
Lynton land	NAPS
Rosalie Coultrip	Va. Department of Health
Richard Cox	Three Rivers Health District
Rueben Varghese	Three Rivers Health District
Susan Lindsey	NAPS
Alfred C. Fischer	Northumberland County Planning Commission
Mary P. Cockrell	Landowner
Hugh Markham	Tidewater Resource Conservation District, USDA
Richard F. Haynie	Norhumberland County Supervisor
Stuart McKenzie	Northern Neck Planning District Commission
Thomas H. Tomlin	Norhumberland County Supervisor
E. luttrell Tadlock	Northumberland County government
W. H. Shirely	Northumberland County government

Questions asked at the Public hearing

Q1. Could you describe the BST water quality study? Was this something that EPA has bought into?

A: a) The BST study collects water quality samples for bacteria analysis. Using resistance to antibiotics as an indicator, the test helps us decide which animal the bacteria may be coming from. the theory is that because humans, their pets and livestock receive more antibiotics than natural populations we can distinquish their bacterial signature from these natural populations and from each other. It is an estimate of the relative contribution to the bacteria loading from humans, pets, livestock, wildlife and birds.
b) The Environmental protection agency is fully supportive of the ARA methodology to determine bacterial sources. Research does continue in to this and other tools.

Q.2 Is there a companion DNA based study to the BST anlysis? Are publications available?

A: Studies have been done doing DNA analysis. However it is impracticle to do them every time the ARA method is used. We have included the researchers BST studies with this report. A detailed reference list is included with each.

Q.3. Does the ARA account for geographic variations?

A: As part of each ARA based BST study, a library of scat bacteria samples is assembled to ensure that geographic variences are addressed.

Q.4. Is the library DNA analysis based or just ARA?

A: DNA analysis is incorporated into the library.

Q.5. Are you confident that the positioning of stations is not targeting human influence?

A: VDH has selected these station locations to accurately characterize the entire system. While human based sources of bacteria are a serious concern, it is all forms of bacteria at which the standard is focused. The is no intentional human bias though bacteria of human origin is a serious concern.

Q.6. Does the timeframe of sampling have an effect? What is the time frame, especially in light of spring rains?

A: Sampling is conducted once per month for a given year. Because we do not sample every day it takes several years of data to determine any seasonal effect.
Weather conditions which precede sampling events are recorded to help us with precipitation effects.

Q.7. VDH has worked on previous problems regarding failing septic systems. Why don’t we just stick with what they are currently doing?

Questions asked at the Public hearing (continued)

A: There is no intent to surplant the existing VDH program. Rather we hope to focus additional resource toward resolving these problems.

Q.8. Have you coordinated sampling events with biosolids applications?

A: We have not specifically done so in several years. However the Piedmont Regional office of DEQ will work with the County and concerned citizens to address this concern.

Q.9. What is the consequence if we still don't meet the standard after the TMDL and Implementation Plan are enacted?

A: The process has been staged to allow assessment of the effectiveness of implementation measures. Implementation is largely voluntary in non-point source dominated systems. Only areas which have permitted discharges would see immediate changes in the form of the TMDL load being made part of their permit.

Q.10. What about other pollutants in the future?

A: Should other pollutants result in violations of water quality standards sufficient to place the water body on the 303 (d) list then a TMDL would be required for that pollutant.

Q.11. Is the idea to use the least most costly BMP or identify the problem first?

A: The first step is to identify the problem and the target level to be met. BMP selection is done as the implementation plan is developed. Such a plan may be staged incorporating refined problem definition and applying less costly but focused BMP's and only using more costly solutions if the levels can be met no other way.

Q.12. Does the efect of water movement have an affect on the source ID?

A: The method selected for this water body treats all of the water as one block. It does not look at circulation or distribution and movement within the system. As we are using a watershed approach and attempting too address the entire surrounding areas problems as a solution to the in stream problem. This ensures that all areas receive equal attention and that the best solution to meeting the water quality standard is promoted.

Q.13. Who will do the implementation? Is the state providing funding? the localities? is this an unfunded mandate?

A: The implementation, like the TMDL development is considered to be a partnership between all of the stakeholders. These are the state, local government and citizens. As much of what needs to be accomplished is in the purview of the local health department etc… Some of the burden would fall to the local government though state agencies will do everything to provide assistance. At present there is very little additional funding for implementationn.

Q.14. What is the best estimate of the timeframe for this TMDL?

A: We anticipate that both the Coan and Little Wicomico River TMDL will be finalized by January of 2004.

Q.15. With the magnitude of the human source you found how many cranked tanks would this suggest?

A: That is difficult to say. VDH does surveys annually. A copy of the most recent one is included in the report,

Q.16. How many other shellfish TMDL's are you doing other than the Coan and Little Wicomico?

A: These two reports represent 9 TMDL's, there are an additional 19 or so in Northumberland County. There are more than 230 statewide.

Q.17. Do you see implementation within 5 years?

A: Implementation as soon as feasible is always desirable. It is however difficult to determine when implementation would actually begin. The plan can begin anytime after the TMDL is approved.

Q.18. When you see a large spike in the data does DEQ investigate?

A: The health Department takes the sample. By the time we know there is a spike several days or weeks have passed. Unless there is an accompanying fish kill or similar incident we would not know about it until much later. A persistent pattern of violations would be investigated.

Q.19. Why not look at the entire watersheds within the county? Can this report be expanded to include all of those in the county?

A: Part of what is driving this TMDL effort is a court order to address the impaired waters in Virginia and other states. Therefore those segments affected by the order must have priority. Also a report on the entire county wold be very difficult to complete in the timeframe we have available to us.

Q.20. If the standard was changed from shellfish to swimming how many areas would still be listed?

A: Many areas would no longer require TMDL’s, others would remain on the list. We do not have an exact number.

Q.21. Is there going to be an implementation fund?

A: There are currently no state funds specific to implementation.