MICROBIAL SOURCE TRACKING
IN TWO SOUTHERN MAINE WATERSHEDS

Webhannet River Watershed Report
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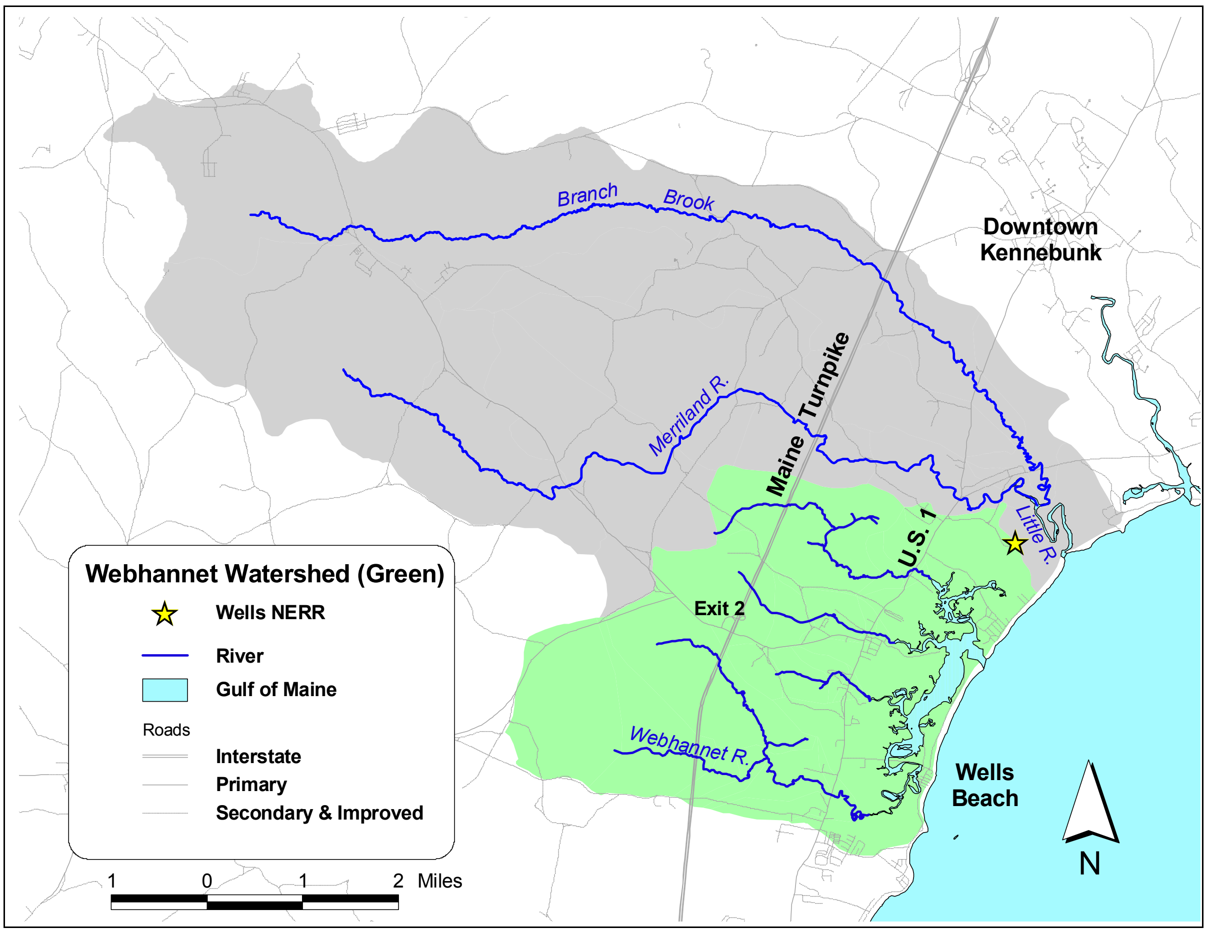

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Report Prepared by

Kristen Whiting-Grant
Maine Sea Grant
Wells National Estuarine
Research Reserve
342 Laudholm Farm Road
Wells, ME 04090

Cayce Dalton
Americorps / Maine
Conservation Corps
Wells National Estuarine
Research Reserve
342 Laudholm Farm Road
Wells, ME 04090

Fred Dillon
Maine Sea Grant
Wells National Estuarine
Research Reserve
342 Laudholm Farm Road
Wells, ME 04090

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ACKNOWLEDGEMENTS

The authors extend their appreciation to all of the citizen volunteers who provided assistance for this project. Without them, the research effort would not have been nearly as comprehensive. Due to their large number, a table is provided in Appendix 1 to identify each of them, along with their particular contributions. We would also like to thank the Webhannet Steering Committee who provided key feedback at critical junctures throughout the project. In particular, we would like to thank Laura Livingston from the Maine Department of Marine Resources (DMR) for conducting a shoreline survey of the Webhannet Estuary and providing DMR water quality monitoring data specific to this area. Appendix 2 contains a full list of steering committee members. Dana and Cindy Johnson were also especially helpful in the collecting and identifying animal scat samples, as was R.J. Mere.

Dennis Thayer and the staff at the Wells Sanitary District provided Geographic Information System (GIS) data for their wastewater collection system along with a steady supply of influent for our bacterial analyses. Likewise, Bill Snyder and the staff at the Kennebunk, Kennebunkport and Wells (KKW) Water District provided early technical assistance in setting up our lab facilities for bacterial testing. Throughout the duration of the project, Steve Jones, Tamara Bryant and Bethany O'Hara from the University of New Hampshire's Jackson Estuarine Laboratory (JEL) provided testing supplies and invaluable technical support for a variety of laboratory procedures. Natalie Landry with the New Hampshire Department of Environmental Services was also very helpful in providing suggestions based on her experience with microbial source tracking projects in seacoast New Hampshire. Finally, Michele Dionne, Research Director for the Wells National Estuarine Research Reserve (WNERR), provided technical review for the report while Susan White, Communications Director with Maine Sea Grant, graciously reviewed the report for editorial revisions.

Much of the outline for this report was developed from a synthesis of the following sources:

- Draft Fecal Coliform TMDL for the Narrow (Pettaquamscutt) River Watershed, Rhode Island. Rhode Island Department of Environmental Management. September 2001.

- Draft Bacteria TMDL for the Shawsheen River Basin. Massachusetts Department of Environmental Protection. February 2002.

- Total Maximum Daily Loads of Bacteria for Neponset River Basin. Massachusetts Department of Environmental Protection. May 2002.

- Tracking Bacterial Pollution Sources in Hampton Harbor. University of New Hampshire Jackson Estuarine Laboratory / New Hampshire Department of Environmental Services. April 2003.

EXECUTIVE SUMMARY

Microbial Source Tracking in Two Southern Maine Watersheds is a research project designed to identify more accurately the sources of fecal contamination in areas that have experienced persistent and elevated levels of bacteria. Various types of bacteria have long been used as indicators for assessing the quality and safety of water for its many uses. Bacteria provide convenient measures of water pollution because they are often associated with nonpoint and sewage pollution sources, and they are generally easy to count. Depending on the water body and its intended use, bacterial indicators have been selected and standards developed that are used to assess the risk of human illness as a result of ingestion or contact with the water body. For example, drinking water standards call for no detectable levels of coliform bacteria, which are indicators for the possible presence of disease-causing organisms. These bacteria originate from the intestinal tracts of warm-blooded mammals, including humans, and can also be found in soil. Swimming beach standards, on the other hand, allow for up to 104 organisms per 100 mL of water for the indicator organism enterococcus (MEDHS, 2002).[i] Similar standards have been developed for marine waters for both swimming and for shellfish growing area classification. While the use of these bacterial indicators provides a basis for evaluating water quality, conventional test methods are generally not specific enough to make conclusions about the sources of the pollution.

The National Shellfish Register indicates that there are 6.7 million acres of shellfish growing areas in the United States that are either restricted or closed to harvest (NOAA, National Shellfish Register, 1995).[ii] In Maine, unacceptable levels of fecal contamination forced the closure of 156,374 acres of productive shellfish harvesting areas by the end of last year (MEDMR, 2002).[iii] These closures represent both adverse environmental impacts and losses of economic opportunity and there are many efforts underway to increase the acreage opened to harvesting. Shellfish growing area closures are due either to elevated fecal coliform as determined through water quality monitoring, or increased risk of sewage pollution from known sources of human or animal waste (FDA, NSSP Model Ordinance, 1999).[iv] State regulating agencies responsible for investigating non-point pollution impacts on shellfish growing areas are often unable to identify the sources of fecal coliform found in closed areas. This represents an inherent weakness in the use of conventional test methods for bacterial indicators. Whereas fecal coliform is generally associated with fecal material from warm-blooded animals, the simple identification of this class of bacteria in a water sample lends no clues to the origin of the fecal material. Thus, it is virtually impossible to distinguish the sources of fecal contamination without more advanced testing methods.

MICROBIAL SOURCE TRACKING PROJECT GOALS

Microbial source tracking (MST) refers to a group of molecular, genetic and chemical methods used to identify specific strains of indicator bacteria or viruses in the environment. These methods attempt to overcome the limitations of conventional bacterial testing by providing information about the actual sources of fecal contamination in surface waters. Results from the *Microbial Source Tracking in Two Southern Maine Watersheds* project are being used to guide local remediation plan development in an effort to reduce fecal coliform to levels low enough for the reopening of shellfish harvesting areas. This could also provide significant cost savings to municipalities, as well as the state, by increasing the likelihood that remediation effectively targets the true sources of contamination. Additionally, this project can be used as a model for similar watersheds throughout the state and the nation. The main goals of the project are:

- ***Goal 1:*** Provide resource managers in the Webhannet watershed with information regarding the microbial source(s) of fecal coliform bacterial contamination in this region.
- ***Goal 2:*** Educate community members living within the Webhannet watershed regarding the results of this project.
- ***Goal 3:*** Disseminate the project results to other watersheds in the Northeast region and the U.S.

EXPERIMENTAL DESIGN AND STUDY RESULTS

This study focuses on the Webhannet watershed in Wells, Maine, where chronic and persistent bacterial contamination from unidentified sources has restricted shellfish harvesting. To meet the goals of the project, water sampling was conducted over a 10-month period beginning in December of 2001. The upper freshwater portions of the watershed were sampled from December to May to correspond with the local shellfish harvesting season (January to April) and the estuary was sampled from June to September to focus on contamination sources during peak tourist season. Conventional bacterial testing for fecal coliform and *E. coli* (both indicator organisms indicative of fecal contamination) was done on all samples to determine contamination levels relative to state and federal water quality standards. The results from these analyses provided valuable information about which areas of the watershed were most contaminated. Figure A indicates *E. coli* concentrations at particular water sampling sites by dot size and color. It also indicates contamination levels for the land areas draining into each sampling site. To further identify potential contamination sources, *E. coli* bacteria were removed from some of the samples and delivered to the University of New Hampshire's Jackson Estuarine Laboratory (JEL) for genetic analysis. JEL uses a microbial source tracking method known as ribotyping, which produces a DNA banding pattern (or ribotype) of the *E. coli*. Ribotypes from water samples are compared to those from confirmed animal scat samples to determine the most likely source of *E. coli* contamination.

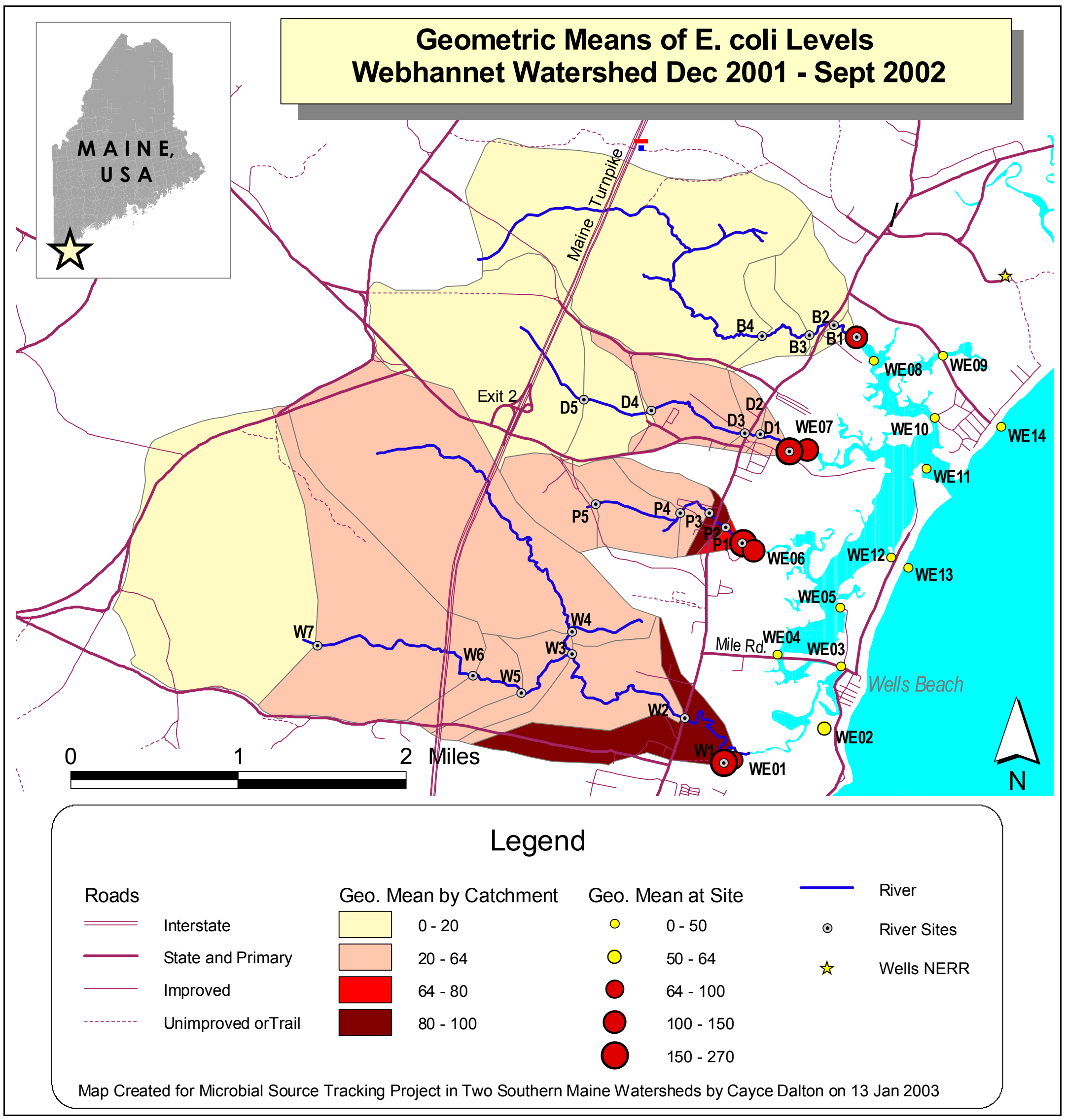


Figure A. *E. coli* bacteria levels for Webhannet watershed sampling sites. *E. coli* concentrations for sampling sites are indicated by dot size and color and for land drainage areas ("catchments") by color. Higher geometric mean (a type of average) values indicate higher levels of contamination.

The ribotyping results for the Webhannet watershed are presented in Figure B. The single largest source species of bacterial contamination came from humans (18%) while the most significant overall category of contributors was from wildlife (29%). Livestock and pets both played a more minor role at 11% and 9%, respectively. Also note that ribotypes for 30% of the bacteria samples delivered to JEL could not be identified. This occurred due to the inherent limitations of the ribotyping method and can generally be improved upon by increasing the number of animal scat samples (known as the source species reference library) that serve as the basis for comparison with water samples. JEL is continuously expanding their reference library with new scat samples to improve their ability to accurately identify bacterial contamination sources.

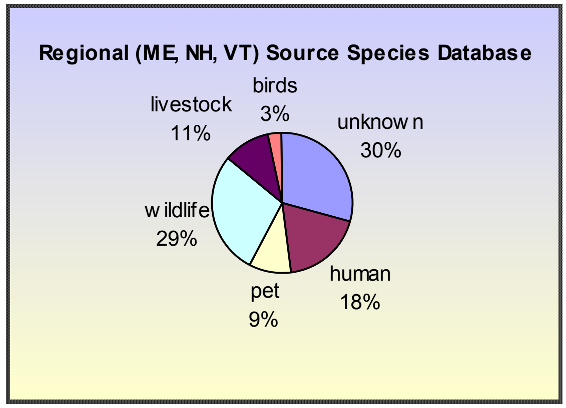
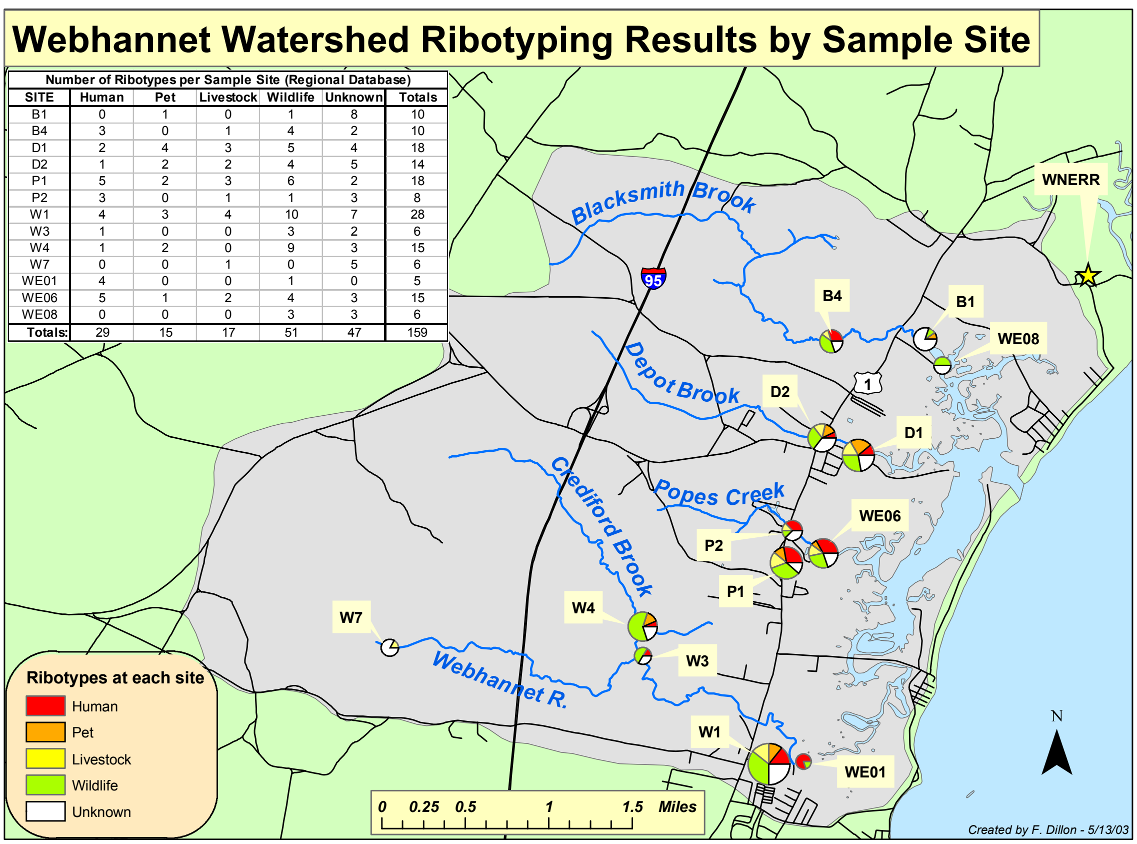


Figure B. Source species identification for Webhannet watershed. Humans are the largest single contributor Source: Jones (2003)

Figure C provides a detailed summary of source species identification for each of the 13 water-sampling sites from which ribotypes were developed. It also helps to determine which specific areas of the watershed should receive the greatest attention for remediation strategies. Each sample site is represented by a pie chart indicating the relative proportions of identified ribotypes along with those that could not be identified ("unknowns"). There is also an accompanying table that indicates the actual numbers of ribotypes for each sample site and each species type. The species categories are wildlife (including birds), humans, pets, livestock and unknowns. Surprisingly, significant levels of human contamination occurred in the publicly sewered portions of the watershed, particularly near the outlets of Popes Creek and the Webhannet River. As expected, wildlife contributions were highest in the undeveloped upper portions of watershed, particularly along Blacksmith Brook and the Webhannet River. Wildlife was also significant along the edges of the marsh area of the Webhannet estuary. Ribotypes for livestock and pet waste generally occurred in conjunction with human ribotypes.



Number of Ribotypes per Sample Site (Regional Database)

SITE	Human	Pet	Livestock	Wildlife	Unknown	Totals
B1	0	1	0	1	8	10
B4	3	0	1	4	2	10
D1	2	4	3	5	4	18
D2	1	2	2	4	5	14
P1	5	2	3	6	2	18
P2	3	0	1	1	3	8
W1	4	3	4	10	7	28
W3	1	0	0	3	2	6
W4	1	2	0	9	3	15
W7	0	0	1	0	5	6
WE01	4	0	0	1	0	5
WE06	5	1	2	4	3	15
WE08	0	0	0	3	3	6
Totals:	29	15	17	51	47	159

Figure C. Geographic distribution and species composition of ribotypes in the Webhannet watershed. Pie sizes indicate number of ribotypes for each sampling site (actual numbers included in inset table). Source: Jones (2003).

MANAGEMENT RECOMMENDATIONS

The ribotyping results were used to develop a management plan for reducing fecal contamination in the Webhannet watershed. Additional data sources used to corroborate the ribotyping results included: the work of previous researchers; field surveys for the Webhannet Estuary and upper freshwater portions of the watershed; customized maps of land cover/habitat types and public sewer line locations; a correlation analysis of precipitation and sewage flow data for the Wells Wastewater Treatment Facility; and local knowledge of wildlife prevalence and distribution. The recommendations offered in this plan are summarized below for each of the identified sources.

Human Sources

- Identify any remaining septic systems in sewered portion of watershed and inspect for proper functioning. Malfunctioning systems should either be repaired or replaced with public sewer.
- Identify oldest septic systems in unsewered portion of watershed and provide informational brochures (see Appendix) to property owners of these systems. Also consider an inspection program to identify malfunctioning systems.
- Provide informational brochures to all owners of septic systems in watershed.
- Consider establishing septic system tracking program that establishes maintenance schedule for property owners. Refer to models established by municipalities elsewhere (see Appendix).
- If none of the above measures noticeably reduce fecal contamination levels in areas where human sources were identified then re-evaluate public sewer system for existence of infiltration and inflow (I&I) in these areas. Repair leaking pipe sections as appropriate.
- Increase efforts to promote use of boat pumpout facilities at Harbor Marina through dissemination of informational brochures to boat owners.
- Continue to work with Maine Department of Marine Resources to ensure that no overboard discharges exist along Webhannet estuary.

Wildlife Sources

- Solicit comprehensive public input before considering reduction plan (relocation or hunting) for problem species (coyote, raccoon, fox and deer).
- Provide informational brochures at local civic buildings and commercial establishments informing all residents in watershed about ways to reduce attraction of problem species.

Livestock Sources

- Identify all livestock owners in watershed and provide them with informational brochures about proper handling of livestock waste.
- Identify all sources of animal manure used as fertilizer (garden and nursery suppliers, local farms) and provide informational brochures at these locations on proper handling of animal fertilizers.

Pet Sources

- Increase efforts to promote proper handling and disposal of pet waste.

CONCLUSIONS

Ideally, fecal coliform and *E. coli* levels in the Webhannet watershed will decrease following the implementation of these recommendations. An ongoing water quality monitoring program, using conventional bacterial test methods, will be needed to measure any reductions in fecal contamination. Results from the Maine Department of Marine Resource's (MEDMR) ongoing water sampling program in the Webhannet estuary will determine which areas are suitable for shellfish harvesting. However, it would also be helpful to establish a monitoring program in the upper watershed to identify specific areas that might persist in contributing to elevated bacterial contamination levels. Findings from this study could be used in conjunction with an upper watershed monitoring program to suggest potential sources of fecal contamination. The Watershed Evaluation Team at the Wells National Estuarine Research Reserve might be able to expand their sampling activities to include sites in the upper Webhannet watershed. MST project staff will also be conducting a variety of outreach activities (press releases, articles, public access TV) to inform the public about the findings from this report. The ultimate aim of these combined efforts is to reopen shellfish harvesting areas in the Webhannet Estuary, while also serving as a model for similar efforts elsewhere.

[i] Maine Department of Human Services Beach Water Safety Testing Guidelines. June, 2002. (www.state.me.us/dep/blwq/docbeach/testguide.pdf)

[ii] National Oceanic and Atmospheric Administration. *The 1995 National Shellfish Register of Classified Growing Waters* (http://spo.nos.noaa.gov/projects/95register/shellfish_one_pg.html)

[iii] Maine Department of Marine Resources Bureau of Resource Management. *Annual Report for 2002 and 2003 Research Plan* (www.maine.gov/dmr/rm/2002annualreport/2002annualreport.htm)

[iv] US Food and Drug Administration. *National Shellfish Sanitation Program Model Ordinance*. (http://vm.cfsan.fda.gov/~ear/nsspotoc.html)

1.0 INTRODUCTION

1.1 Project Goals and Anticipated Outcomes

The intent of *Microbial Source Tracking in Two Southern Maine Watersheds* (hereafter referred to as the MST Project) is to further explore the use of Microbial Source Tracking (MST) to identify more accurately the bacteria found in water samples, while also attempting to validate a tool that has been developed for determining the sources of fecal pollution in coastal ecosystems. Specifically, isolates of *E. coli* were selected from fecal coliform positive samples and analyzed using MST techniques. Initial activities focused in York County where over 11,000 acres of shellfish growing areas are currently prohibited to harvesting (DMR, 2003) and the sources of fecal pollution are poorly documented. This technique can provide regulatory agencies and municipal officials with more specific clues about the source of nonpoint pollution and allow for more efficient investigation and remediation of those sources. Significant savings of resources used for water quality sampling, laboratory analysis, pollution source investigation, and pollution source remediation can result from the information obtained from the MST analysis. Through these investigations, coastal water quality will improve, resulting in better ecological and human health through safe shellfish harvesting areas and swimming beaches.

While the MST Project focuses on estuaries in southern Maine where water quality problems persist, the findings and resulting tools are applicable to other estuaries and coastal areas. Numerous coastal watersheds in southern Maine fit this profile and need research that will identify the microbial sources of contamination in the region. In particular, the Webhannet watershed in the town of Wells is plagued with unidentified sources of fecal contamination. The Webhannet is a relatively small watershed (13.5 square miles) and its manageable size improves the likelihood that the origins of fecal contamination will be successfully identified. Clam flats that have been open in recent years are currently being targeted for closure in the Webhannet estuary, due to a new pattern of elevated fecal counts.

MST results will be used to guide local remediation plan development, leading to reduced fecal counts and, ultimately, to the reopening of clam harvesting areas. This could also provide significant cost savings to municipalities, as well as the state, by increasing the likelihood that remediation effectively targets the true sources of contamination. Moreover, successful source identification and remediation that leads to the reopening of harvesting areas has the added benefit of returning management of the shellfish harvesting area back to the local level. Once a harvesting area is closed, management of that area is assumed by the state and local stewardship efforts are minimized. Additionally, this project can be used as a model for similar watersheds throughout the state and the nation. The main goals of the project are the following:

- ***Goal 1:*** Provide resource managers in the Webhannet watershed with information regarding the microbial source(s) of fecal coliform bacterial contamination in this region.
- ***Goal 2:*** Educate community members living within the Webhannet watershed regarding the results of this project.
- ***Goal 3:*** Disseminate the project results to other watersheds in the Northeast region and the U.S.

1.2 Watershed Dynamics and Microbial Contamination

The MST Project adopts a watershed approach in seeking to identify microbial sources of contamination. A watershed is a geographic area in which all sources of water – including lakes, rivers, estuaries, wetlands, and streams, as well as groundwater – drain to a common surface water body (Figure 1). Because all watersheds are defined by natural hydrology and ultimately drain to coastal waters, they are good focal points for managing coastal resources. The U.S. Environmental Protection Agency (USEPA) established a watershed approach in the mid-1990s as a strategy for effectively protecting and restoring aquatic ecosystems and protecting human health. This strategy derives from the premise that many water quality and ecosystem problems are best solved at the watershed level rather than at the levels of individual water bodies or dischargers. Major features of the EPA's watershed approach are: targeting priority problems, promoting a high level of stakeholder involvement, integrating solutions that make use of the expertise and authority of multiple agencies, and measuring success through monitoring and other data gathering (EPA, 1996). The MST Project has adopted a nearly identical strategy.

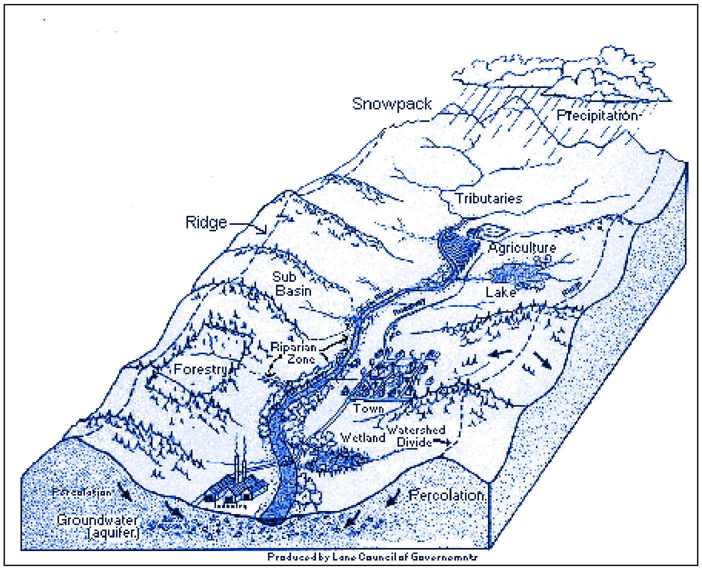


Figure 1. Cross-section of watershed

The Webhannet is a coastal watershed consisting of several parts, from the upland headwaters to the estuary. Headwaters often include wetlands, and wetlands often are adjacent to the flowing waters of rivers or streams. As the streams and rivers flow to the estuary, they are influenced by many land and water uses. They pass through upland areas used for a variety of purposes, such as farming, housing, businesses, recreation, and conservation. The rivers and streams empty into the estuary, which provides a unique habitat for a diverse group of organisms. Among other habitat functions, rivers and estuaries provide breeding and feeding grounds for a variety of aquatic and terrestrial animals. Nearshore waters, the areas directly offshore from the beach, are part of the coastal watershed because they are influenced by the activities going on along the shoreline and by pollutants coming from the land. Farther offshore are other habitats that are part of the coastal watershed and are also influenced by its drainage.

Since a watershed is made up of several components, it is important to remember that what happens on the land can affect the water. For example, if a river or stream flows through an agricultural area, it can pick up fertilizer, manure, and pesticides from farming operations that run off the land after a rainstorm. As it passes urbanized and suburbanized areas, it might gather fertilizers that wash off lawns, untreated sewage from failing septic tanks, sediment from construction sites, and runoff from impervious surfaces like parking lots. These diffuse, hard-to-measure inputs are referred to collectively as nonpoint source (NPS) pollution. Upon reaching the coast, the stream or river can be affected by commercial and recreational boating, discharges from industrial and municipal facilities, and recreational activities on beaches. All of these areas – agricultural, suburban, urban, and coastal – can have an impact on marine resources. Pathogens are a particular type of pollution that originates from microbial organisms like bacteria and viruses. They come from untreated or poorly treated sewage, pet and farm animal waste, and improperly handled medical waste. Pathogens in coastal waters in unsafe amounts can result in beach closures, shellfish bed closures, fish kills, and human health problems.

Microbial indicators have long been used for assessing the quality and safety of water for its many uses. Bacteria provide convenient measures of water pollution because they are often associated with nonpoint and sewage pollution sources, and they are generally easy to enumerate. Depending on the water body and its intended uses, bacterial indicators have been selected and standards developed that are used to assess the risk of human illness as a result of ingestion or contact with the water body. For example, drinking water standards call for no detectable coliform bacteria, while swimming beach standards call for less than 100 colony-forming units (CFU) of enterococcus per 100 mL of water (EPA, 1999).[1] Similar standards have been developed for marine waters for both swimming and for shellfish growing area classification. While the use of these bacterial indicators provides a metric on which to evaluate water quality, they are often not specific enough to make conclusions about pollution sources.

The National Shellfish Register indicates that there are 6.7 million acres of shellfish growing areas in the United States that are either restricted or closed to harvesting (NOAA, National Shellfish

[1] Bacterial standards vary by state, intended use and which regulatory agency has jurisdictional authority. In Maine, recreational standards for freshwater are for *E. coli* per Department of Environmental Protection (DEP) regulations while recreational standards for marine water are for enterococcus per Department of Marine Resources (DMR) regulations. Shellfish harvesting standards are for fecal coliform per DMR regulations.

Register, 1995). In Maine, the Department of Marine Resources (DMR) restricted or closed approximately 8.5% (or 156,000 acres) of the state's shellfish areas to harvesting in 2002. These closures represent both an adverse environmental impact and a loss of economic opportunity, and there are many efforts under way to increase the overall harvestable acreage (Figure 2). Shellfish growing area closures are due either to elevated fecal coliform as determined through water quality monitoring, or increased risk of sewage pollution from known sources of human or animal waste (FDA, NSSP Model Ordinance, 1999). State regulatory agencies responsible for investigating nonpoint pollution impacts on shellfish growing areas are often unable to identify the source of fecal coliform found in closed areas. This represents an inherent weakness in the use of bacterial indicators. Whereas fecal coliform is generally associated with fecal material from warm-blooded animals, the simple identification of this class of bacteria in a water sample lends no clues to the origin of the fecal material.

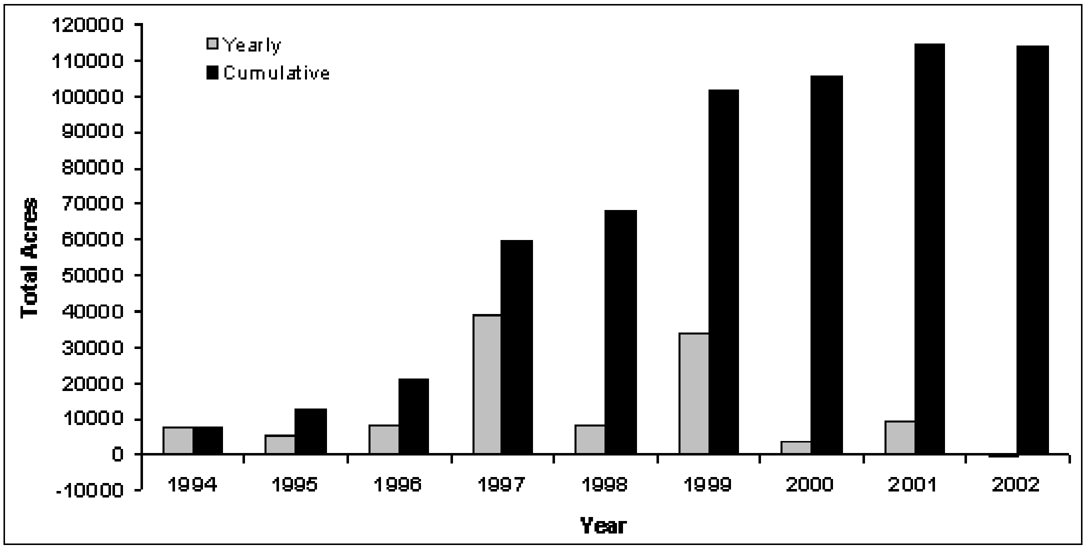


Figure 2. Acres of shellfish habitat reopened for harvest during 1994-2002 in Maine following pollution reduction and habitat restoration. (Source: Maine Department of Marine Resources, 2003).

Fecal coliform can originate from humans, wildlife, and domestic animals but, until recently, the national standards for classifying shellfish growing areas required closures for the presence of fecal coliform regardless of its sources (FDA, 1999). National standards now allow investigators to perform risk assessments of human pathogens related to elevated fecal coliform levels in determining the safety of a shellfish growing area. In their pollution source investigations, regulatory agencies and municipal officials attempt to identify and eliminate these sources. In many instances, the source of the problem cannot be determined and expensive corrective measures are either not possible or fruitless. If remediation is not possible, and a determination is made that human sewage is not involved, a risk assessment of the pollution source and possible associated human and animal pathogens may allow areas to be opened to harvest (FDA, 1999).

1.3 Applicable Water Quality Standards

Maine has a water classification system that establishes water quality goals for directing the State's management efforts in protecting surface waters for designated uses (Maine DEP, 1999). The classification system specifies the criteria needed to protect these designated uses, which derive from the federal Clean Water Act's minimum fishable-swimmable standards. This system is intended to function more as a hierarchy of risk than as a hierarchy of use or quality. Risks are understood as natural or anthropogenic events that result in ecosystem degradation. Maine has four classes for freshwater rivers and three classes for marine and estuarine waters. The entire freshwater portion of the Webhannet watershed is designated as Class B, which has the corresponding instantaneous and seasonal average[2]

[2] Standards apply from May 15th – September 30th and the average is determined by a geometric mean calculation. (MRSA, Title 38, Chapter 3, § 465)

E. coli limits of 427 bacterial colonies per 100 mL of sample and 64 bacterial colonies per 100 mL of sample, respectively.

The estuarine portion of the Webhannet watershed is designated as Class SB (where "S" denotes an estuary), which has the corresponding instantaneous and seasonal enterococcus bacteria limits of 54 per 100 mL and 8 per 100 mL, respectively. For shellfish harvesting areas, Maine statutes ultimately defer to criteria specified by the National Shellfish Sanitation Program Model Ordinance (FDA, 1999). The Maine Department of Marine Resources (DMR) analyzes the 30 most recent samples from a given location on an annual basis. Each location has six to 12 samples collected per year so that 30 samples are collected over 2.5 to 5 years. The fecal coliform median, or geometric mean, cannot exceed 14 MPN[3] per 100 mL and the estimated 90th percentile (P90) cannot exceed 49 MPN per 100 mL. If either limit is exceeded, then the area must be reclassified as prohibited or, if the P90 is less than 88, reclassified as restricted for depuration harvesting.[4]

2.0 DESCRIPTION OF WEBHANNET RIVER WATERSHED

2.1 Hydrology, Habitat, and Development

The Webhannet watershed covers approximately 13.5 square miles and consists of six tributaries from north to south: Blacksmith Brook, Depot Brook, Popes Creek, the Webhannet River, Crediford Brook and an unnamed stream. The first four of these empty directly into the Webhannet estuary with freshwater contributions of approximately 55% from the Webhannet River, 25% from Blacksmith Brook, 13% from Depot Brook and the remaining 7% from Popes Creek (Ward, 1994). All tributaries flow across sand and gravel deposits near their headwaters and the impermeable sandy muds of the Presumpscot Formation in their lower reaches (Smith, 1977, Caswell and Caldwell 1979, Thompson and Borns 1985). Consequently, the Webhannet River and Blacksmith Brook likely receive some flow from groundwater recharge (Ward, 1994). Water circulation patterns in the estuary have been described as well mixed during all parts of the tidal cycle (Mariano, 1989). The dominant influences on these mixing patterns are tide and wind characterized by a longitudinal salinity gradient with no appreciable vertical or lateral gradients (Holden, 1997). Tidally driven water circulation patterns can be significant when considering the transport effects of fecal contamination in estuarine environments. As such, most estuarine samples were collected on an outgoing tide.

Land uses in the Webhannet watershed have been variously described. For the present purposes (Figure 3), land use types are based on a habitat analysis conducted by the U.S. Fish and Wildlife Service (USFWS) for endangered species protection in the Gulf of Maine watershed (Banner and Schaller, 2001). Figure 4 presents the land uses and a summary of the relative acreages and proportions of each type within the watershed. Table 1 provides the complete list of USFWS land cover types and the groupings used for the present analysis. Given the large undeveloped tracts of land to the west of the Maine Turnpike, upland forest represents the largest land cover type in the Webhannet watershed. This mostly contiguous area consists of a variety of habitats, supporting several species of potential interest for the MST Project. Forested wetlands represent the next largest land cover type that also provide habitats for several species of potential interest, as do estuarine marsh and grassland areas. Developed land essentially consists of areas in the built environment with impervious surfaces (residences, commercial establishments, parking lots and roads). Most development in the Town of Wells is concentrated along the Route 1 corridor and beaches to the east. These land uses are potentially significant sources of contaminated urban runoff. The remaining categories together make up less than 10% of land area in the watershed, though they also provide habitats for species of potential interest.

[3] MPN means "most probable number" and is based on certain probability formulas that estimate the mean density of coliforms in a given sample. Coliform density provides an assessment of the sanitary quality of untreated water.
[4] Email communication on 2/25/03 from L. Livingston (DMR) to F. Dillon (MST Project)

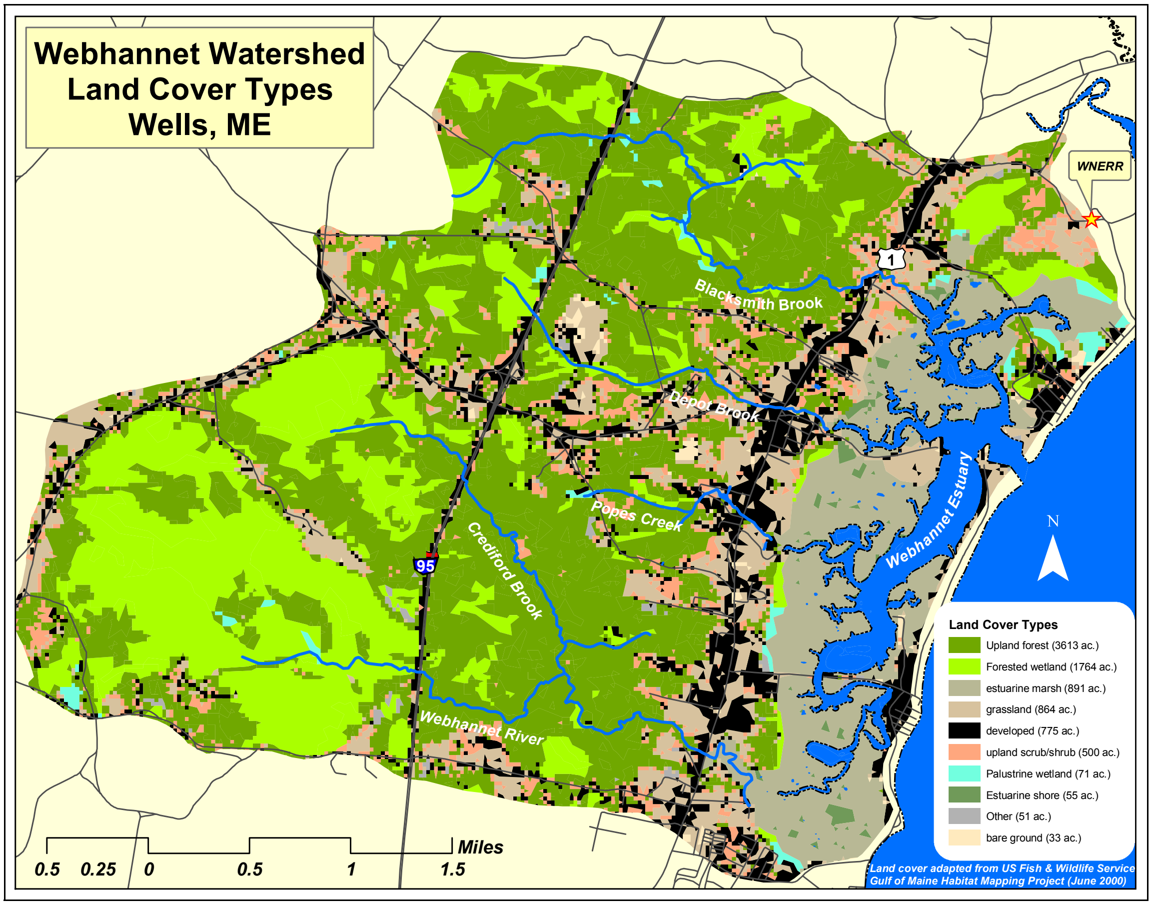


Figure 3. Land cover map of Webhannet watershed (adapted from US Fish & Wildlife Service Gulf of Maine Habitat Mapping Project – 2001).

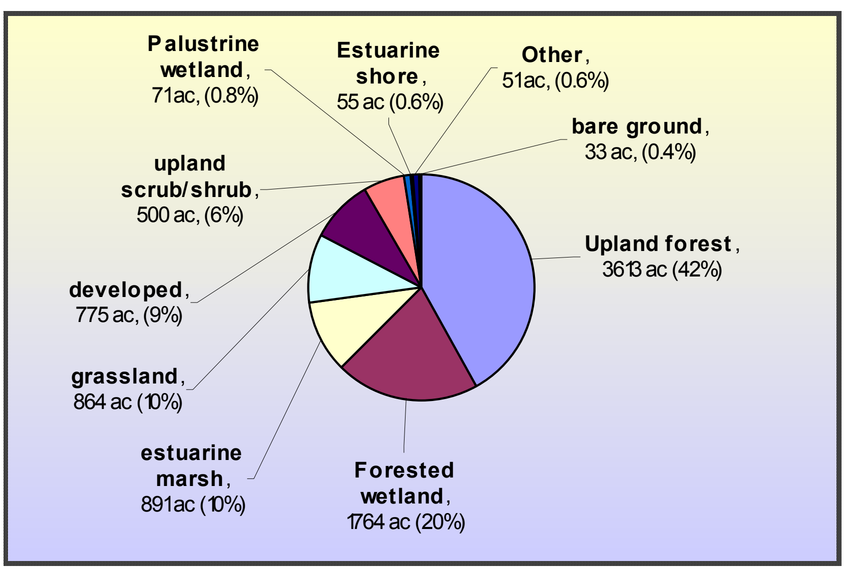


Figure 4. Webhannet watershed land cover types.

LAND COVER TYPE	ACRES	LAND COVER TYPE	ACRES
Upland forest		**Estuarine shore**	
upland coniferous forest	509.6	estuarine rocky shore	0.1
upland deciduous forest	864.1	estuarine sand/mud shore	55.0
upland mixed forest	2239.6		**55.1**
	3613.3	**OTHER**	
Forested wetland		**Open water**	
coniferous swamp	1098.6	estuarine open water	7.7
deciduous swamp	665.6	lake/pond open water	34.3
	1764.1	marine open water	0.1
		cultivated	4.3
estuarine marsh	**890.8**	**fresh marsh**	4.1
		Marine shore	
grassland	**864.2**	marine rocky shore	0.2
		marine sand/mud shore	0.8
developed	**775.1**		**51.4**
upland scrub/shrub	**500.4**	**bare ground**	**32.7**
Palustrine wetland			
coniferous shrub swamp	1.6		
deciduous shrub swamp	69.2	**Total Acres:**	**8617.9**
	70.8	**Total Square Miles:**	**13.5**

Table 1. Adaptation of US Fish & Wildlife Service land cover types for Webhannet watershed study

2.2 Sewering of the Watershed

The final decade of the 20th century marked a period of rapid change and growth for southern Maine. Population increased by 13.5% in York County from 1990 to 2000, as compared to an increase of 3.8% for the State as a whole during the same period (U.S Census Bureau, 2003). Growth was even more pronounced in Wells where the year-round population rose from 7,778 in 1990 to 9,400 in 2000, an increase of nearly 21% (Southern Maine Regional Planning Commission, 2000). Population exceeds 30,000 at the peak of the summer tourist season. To accommodate this growth, the town's wastewater infrastructure has undergone a variety of improvements. The Wells Sanitary District completed a major plant upgrade in January of 2002 and developed a geographic information system (GIS) database for its entire wastewater collection system (Figure 5). This GIS database provides a comprehensive catalog for all sewer system components – such as manholes, gravity sewer lines and force mains – and delineates pumping station catchment areas, which can be very useful in identifying potential problems with sewer system leakage (also known as infiltration and inflow, or I&I). Most of the public sewer system lies east of the Maine Turnpike with the greatest concentration along Route 1 from Route 9 in the north to the Ogunquit town line in the south. Sanitary waste treatment for the remaining developed areas of the town is provided by privately owned septic systems.

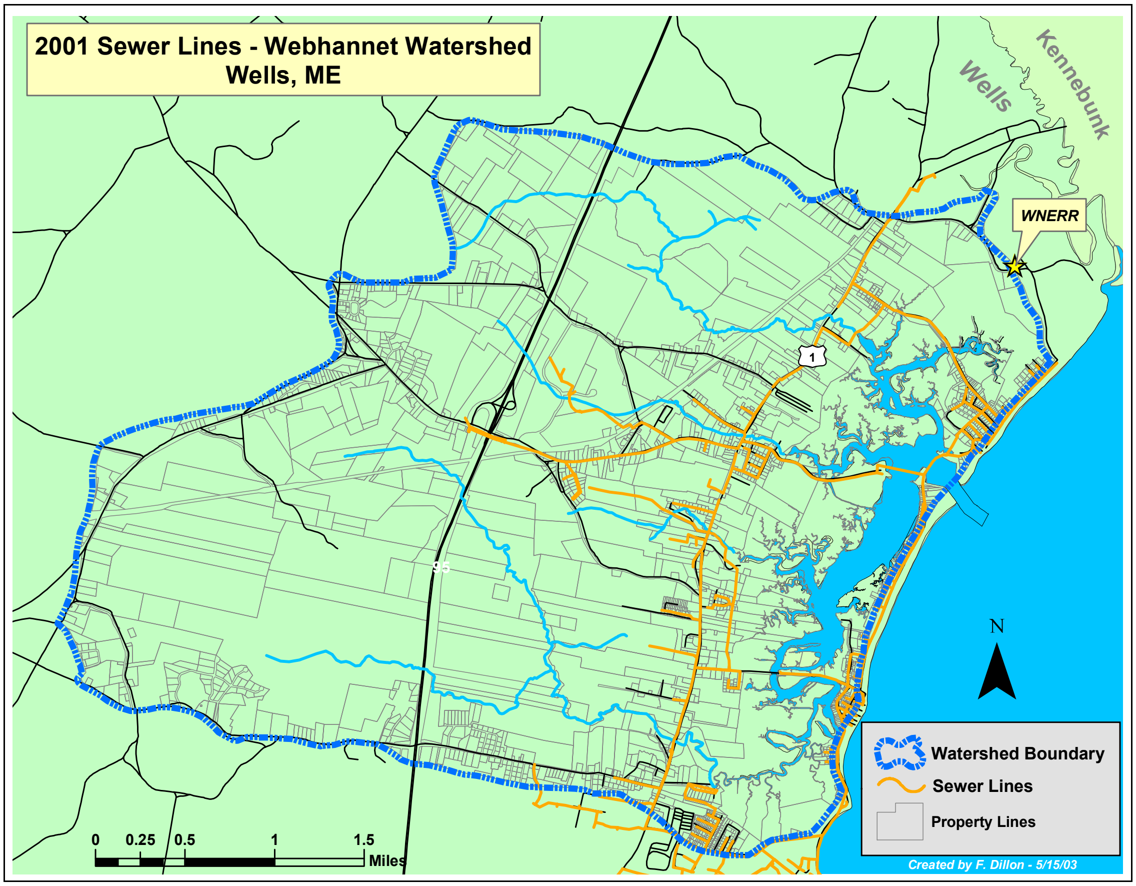


Figure 5. Sewered areas within Webhannet watershed (Data source: Wells Sanitary District / Wright-Pierce)

2.3 Shellfish Growing Area Water Quality Monitoring Program

As noted above, the Maine Department of Marine Resources (DMR) monitors coastal waters for fecal contamination to determine the cleanliness of shellfish harvesting areas. Their Shellfish Growing Area Classification Program uses the standards outlined in the National Shellfish Sanitation Program (NSSP) to establish marine water quality limits and to conduct shoreline surveys. Water samples are collected by volunteers and analyzed for fecal coliform at the Boothbay Harbor microbiological laboratory. Shoreline surveys involve a visual inspection of the coast to determine the location and magnitude of potential sewage pollution problems. The information from these two projects is compiled into a Sanitary Survey. This document is then used to classify areas where shellfish grow along the Maine coast as being suitable for shellfish harvesting all of the time, part of the time under certain conditions, or not at all.

There are currently 10 DMR monitoring stations in the Webhannet Estuary (Figure 6). As specified in the NSSP's Model Ordinance, year end 90th percentile (P90) fecal coliform results cannot exceed 49 MPN per 100 mL. DMR water quality monitoring results for the Webhannet Estuary are summarized in Table 1. As the table indicates, stations D13 and D16 have routinely and persistently exceeded the NSSP's P90 standard. Year end P90 values for stations D14, D17 and D18 also increased beyond the 49 MPN limit in 2002; stations D20 and D21 are both on the verge of exceeding this standard. Consequently, most of the estuary is subject to conditional approval, with the area south of Mile Road restricted to shellfish harvesting.

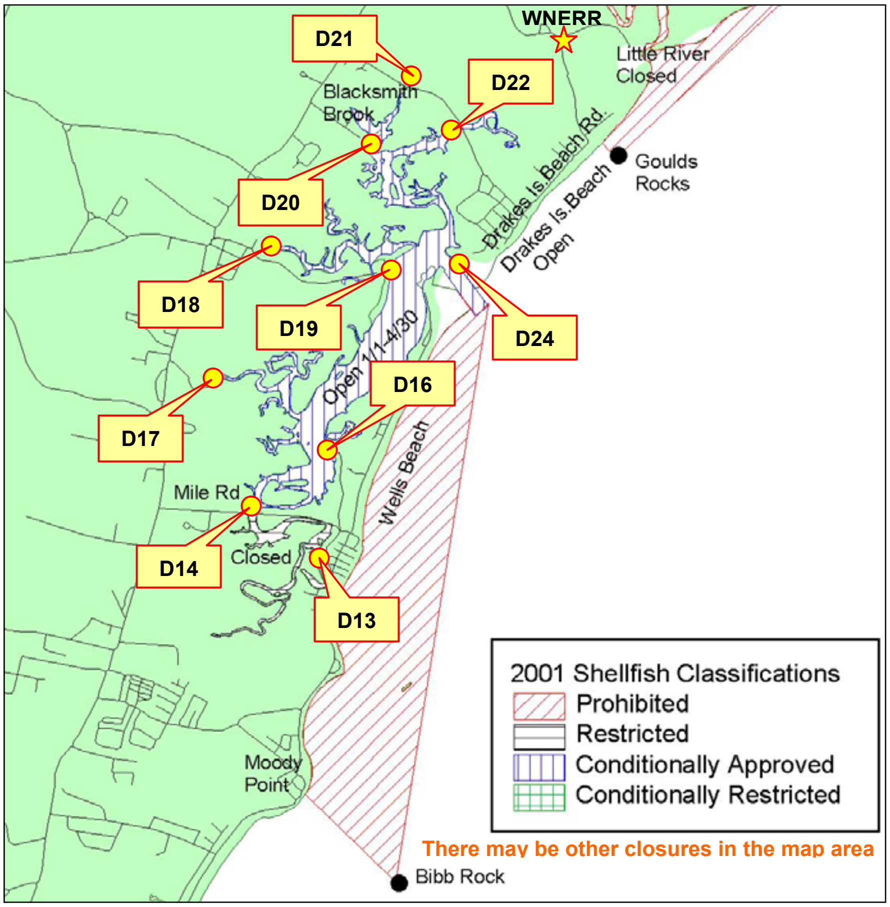


Figure 6. Maine Department of Marine Resources Webhannet Estuary sampling stations and shellfish harvesting area classifications

Webhannet Estuary Year End P90's for Fecal Coliform					
Station ID	**1998**	**1999**	**2000**	**2001**	**2002**
D13	***139.3***	***150***	***159***	***159***	***98.7***
D14	48	***93***	48	48	***107.7***
D16	***93***	***93***	***76.8***	***76.8***	***76.8***
D17	***240***	***93***	43	48	***107.7***
D18	***62.7***	23	48	48	***93***
D19	43	25	23	23	25
D20	48	43	43	43	48
D21	48	***93***	***93***	48	48
D22	43	43	43	23	***107.7***
D24	25	23	25	10.49	25

Table 2. Maine Department of Marine Resources Year-end 90th Percentile Results (MPN / 100 mL)

2.4 Watershed and Shoreline Surveys

In 1995, a sanitary survey was conducted for the Webhannet watershed that consisted of the following three components (Bright, 1996):

- **Sanitary survey:** a visual inspection of all waterfront properties NOT connected to the town sewer. These included dwellings located on the beach, on the Webhannet River, on salt- and freshwater marshes, and on streams and storm ditches draining into the estuary. Properties were inspected for faulty septic systems.

- **Shoreline survey:** a visual inspection of the shoreline in the area proposed to be opened for shellfish harvesting. The shoreline was inspected for open pipes and any other elements which may have been causing fecal contamination of the water.

- **Water sampling:** collection of samples from the beach, the Webhannet River, salt- and freshwater marshes, streams and storm ditches draining into the estuary. Samples were collected at various stages of tide and runoff conditions and were analyzed at DMR for fecal coliform and salinity.

The findings from the sanitary survey portion of this study did not identify any obvious problems with properties using septic systems, though the work was conducted during a drought period, which made it difficult to determine fecal coliform sources in the watershed. However, there were cases where some septic systems had never been cleaned. These were cited as posing potential water quality and public health threats in the future if not adequately serviced. The shoreline survey examined only the northern portion of the Webhannet estuary because it was the most likely area to meet approved water quality standards. The southern portion of the estuary was not surveyed. Water sampling results were remarkably similar to those listed above in Table 2.

Despite the difficulties encountered in locating sources of fecal contamination, due to the unusually dry weather during the study period, several recommendations were offered. The main points are:

- Continue water quality monitoring in the estuary on a monthly basis and conduct dye testing at several suspect properties if nearby results are high.

- Periodically sample clam meat for fecal contamination.

- Locate and sample drainage ditches into the marsh during high runoff periods.

- Conduct shoreline survey in the southern portion of the Webhannet estuary.

- Investigate possible fecal contributions from recreational vehicles (RVs)

- Post signs informing dog owners to clean up after their pets

- Encourage homeowners to pump out septic systems on a regular periodic basis

On May 1, 2002, Laura Livingston from Maine DMR, and MST staff members Fred Dillon and Cayce Dalton conducted a selective shoreline survey of the Webhannet Estuary. All properties abutting the estuary were believed to be on public sewer, based on the map provided by the Wells Sanitary District. As such, a thorough search for malfunctioning septic systems was not completed. Dennis Thayer, superintendent of the Wells Sanitary District, later stated that at least a few homes near Depot Brook had septic systems in the summer of 2002, but that an extension of the sewer lines was soon planned for that area (see below). The lobster pound and restaurant on Harbor Road (adjacent to Depot Brook) were also investigated. There were intake and/or discharge lines of estuarine water apparently being used for the lobster tanks. These were not considered to be a potential source of fecal contamination. The Ocean View campground on Harbor Road was also carefully inspected. All observed campers had pipes going from the camper into the public sewer system (Figure 7). A few water samples were collected from pipe outfalls and a sample was collected below a culvert on Depot Brook (Figure 8). Virtually all of these pipes carry stormwater which, according to DMR test results, rarely indicate high bacterial concentrations.

Figure 7. Typical connection from camper to public sewer system

Figure 8. Laura Livingston from Maine DMR collecting sample near Harbor View campground

On May 13, 2002 MST Project staff Fred Dillon and Cayce Dalton conducted a survey of the riparian zone along the north branch of the Webhannet River (Crediford Brook). Most of this area resides within the Morse Tree Farm and consists of woods and dirt logging roads. No significant potential human sources of fecal bacteria were found, but several indications of possible wildlife sources were discovered and photographed. The two most visible potential sources were moose and beaver.[5] Many moose droppings were found upstream from the W4 site, as was a moose skeleton. One pile of scat was found in a small tributary, indicating a direct fecal load to the water (Figure 9). Later laboratory testing of moose scat from this same area (collected on October 15, 2002) indicated a high coliform density, entirely covering a petri dish even after diluting by six orders of magnitude. Presence of beaver was also quite clear. A large beaver dam was found at the W4 site (Figure 10), and a second dam was found approximately one kilometer upstream. In addition, fresh signs of tree felling were seen and photographed. A beaver lodge was located next to these signs (Figure 11).

Figure 9. Moose scat in tributary to Webhannet's north branch (Crediford Brook).

Figure 10. Large beaver dam adjacent to W4 site.

Figure 11. Beaver lodge and felled trees upstream from W4.

On May 14, 2002, MST project staff Fred Dillon and Cayce Dalton conducted a survey of the riparian zone along the south branch of the Webhannet River starting at W7 and walking downstream past the Maine Turnpike. Weather conditions were cloudy with occasional light rain. No abutting development beside the Turnpike was observed, and no direct potential sources of fecal bacteria to the river were noted except for wildlife. The only direct evidence observed of wildlife contribution to fecal bacteria was one example of scat from a small animal. Given the large tracts of undeveloped woods and fields along this stretch of river, significant wildlife presence is likely. However, except for material deposited very near a watercourse, fecal contaminated runoff is somewhat unlikely, due to the relatively flat terrain in this area. The W7 site is where a gravel road crosses the Webhannet's south branch (Figure 12). Six plastic tube culverts of approximately 3 feet in diameter and approximately 20 feet long provide passage for the river under the road. Nonetheless, behind the road there was pooled and slow-moving water. Lands near the river at W7 had been cleared a few years prior (for a golf course and condominium resort that has yet to be completed), and showed signs of early succession from field to forest. Several drainage ditches were seen holding water near the turnpike. The algae in one of these ditches suggested nutrient loading, but no direct link to fecal sources was observed.

[5] Neither moose nor beaver samples are included in the local or regional scat reference libraries (see section 3.7 below for further explanation).

Figure 12. Sample site W7, with standing water upstream and abandoned field in background.

Figure 13. A second culvert downstream from W7, also with standing water.

Figure 14. The Webhannet River's south branch crossing at the Maine Turnpike.

Project staff Fred Dillon and Cayce Dalton conducted a survey of riparian zones adjacent to the W1 site, Depot Brook and Popes Creek in the Webhannet Estuary on May 28, 2002. As mentioned above, this entire area was thought to be on public sewer until Dennis Thayer of the Wells Sanitary District later stated that there were a few septic systems present in the Depot Brook area (Brook Lane), one of which was known to be leaking. Thayer said that a public sewer line would be installed within a year, and the owner of the leaking system intended to connect to it. In addition to septic systems, several other potential sources of fecal contamination were found. The first concern was the campground near W1. Although it seemed that all of the camp sites were connected to public sewer, its proximity to the sample site and the somewhat rundown nature of the facilities suggested a potential risk. The second concern was the wildlife at P1. A groundhog was observed at the site (Figure 15), and volunteer samplers have repeatedly reported waterfowl and a fox at this site on other occasions. The surrounding marsh area can also be considered a likely source, due to waterfowl and other wildlife. Another potential fecal contamination source was the pond on Pope's Creek, which might be an attractive spot for waterfowl or other wildlife (Figure 16). One last possibility for fecal contamination is leakage from underground sewer pipes, although project staff observed no indication of this on this day or any other.[6] What appeared to be a privately owned sewage pump station was discovered on Depot Brook between D4 and D5. The station appeared to be in good working order and therefore was not considered as a source of fecal contamination.

Figure 15. Groundhog at sample site P1. Waterfowl and fox have also been observed here.

Figure 16. Pond and lawn between sites P3 and P4; noted as possible waterfowl site.

Figure 17. Webhannet River at US Route 1. Sewer line crossing bridge.

On May 31, 2002, volunteer Jessica Szafranski and MST Project staff member Cayce Dalton walked from W3 to W6 looking for possible sources of fecal contamination to the Webhannet River. The only potential sources documented were moose scat in the woods downstream of W5 on the Webhannet's south branch and unidentified animal tracks near W6. Sites W3, W5, and W6 were photographed (W4 is on the north branch of the Webhannet – Crediford Brook – and was walked on 5/13/02).

[6] A sewer line blockage occurred near site D1 in September 2002 – see section 4.11 below for further explanation.

Figure 18. Sample site W3.

Figure 19. Sample site W5.

Figure 20. Sample site W6.

3.0 ASSESSMENT OF CURRENT FECAL CONTAMINATION

3.1 Sample Site Selection

Preliminary sample site selection guidelines for the MST Project were provided by a nonpoint source pollution (NPS) study completed for the Webhannet watershed in 2000. This earlier work delineated subcatchments for each of the four major tributaries discharging directly into the Webhannet estuary (Blacksmith Brook, Depot Brook, Popes Creek, and the Webhannet River). It focused on watershed inputs based on the hypothesis that high fecal coliform levels in the estuary were originating from wildlife and domestic animal sources in the upper freshwater sections of the watershed (Mullan, Dionne, and Whiting-Grant, 2001). Human sources around the estuary (by far the most densely populated region of the watershed) were not expected to be significant since all of this area was serviced by public sewer. Four sample sites that corresponded with existing DMR sampling stations were established at the heads of tide for each of the main tributaries discharging into the estuary. This was intended to allow for comparisons between sampling programs even though different bacterial test methods were used.[7] An additional 17 sites were established throughout the freshwater portions of the watershed. These sites were selected primarily on the basis of adjacent land uses and wildlife habitats suspected of being potential fecal contamination sources.

The MST Project essentially retained all of the 21 sites (Figure 21) from the 2000 NPS study since it clearly demonstrated that most of the fecal contamination in the estuary was originating from the freshwater portions of the watershed. Twelve additional estuarine and two beach sites were established to identify more accurately potential sources of fecal contamination in these areas. The additional estuarine sites were selected, based on known or suspected areas of fecal contamination. The two beach sites – one north and one south of the mouth of the estuary – were selected to identify any relationships between estuarine and beach bacterial concentrations. The original 21 sites from the mostly freshwater portions of the watershed were sampled from December 2001 until May 2002 to coincide with the clamming season (which ran from January 2002 to April 2002). This upstream sampling approach was based on the hypothesis that, during winter months, fecal material sitting atop frozen snow or earth would be released by precipitation events and thaws – often with little breakdown of the material – directly into waterways. Consequently, discreet and measurable pulses in fecal coliform levels would be produced (Mullan, Dionne, and Whiting-Grant, 2001). The summer sampling period ran from June 2002 to September 2002 to coincide with fecal contamination related to the peak tourist season. All 12 estuarine sites – including the four from each of the main tributaries located at the heads of tide – and the two beach sites were sampled during this period. The head of tide sites were retained to screen for a pattern between freshwater and estuarine bacterial concentrations.

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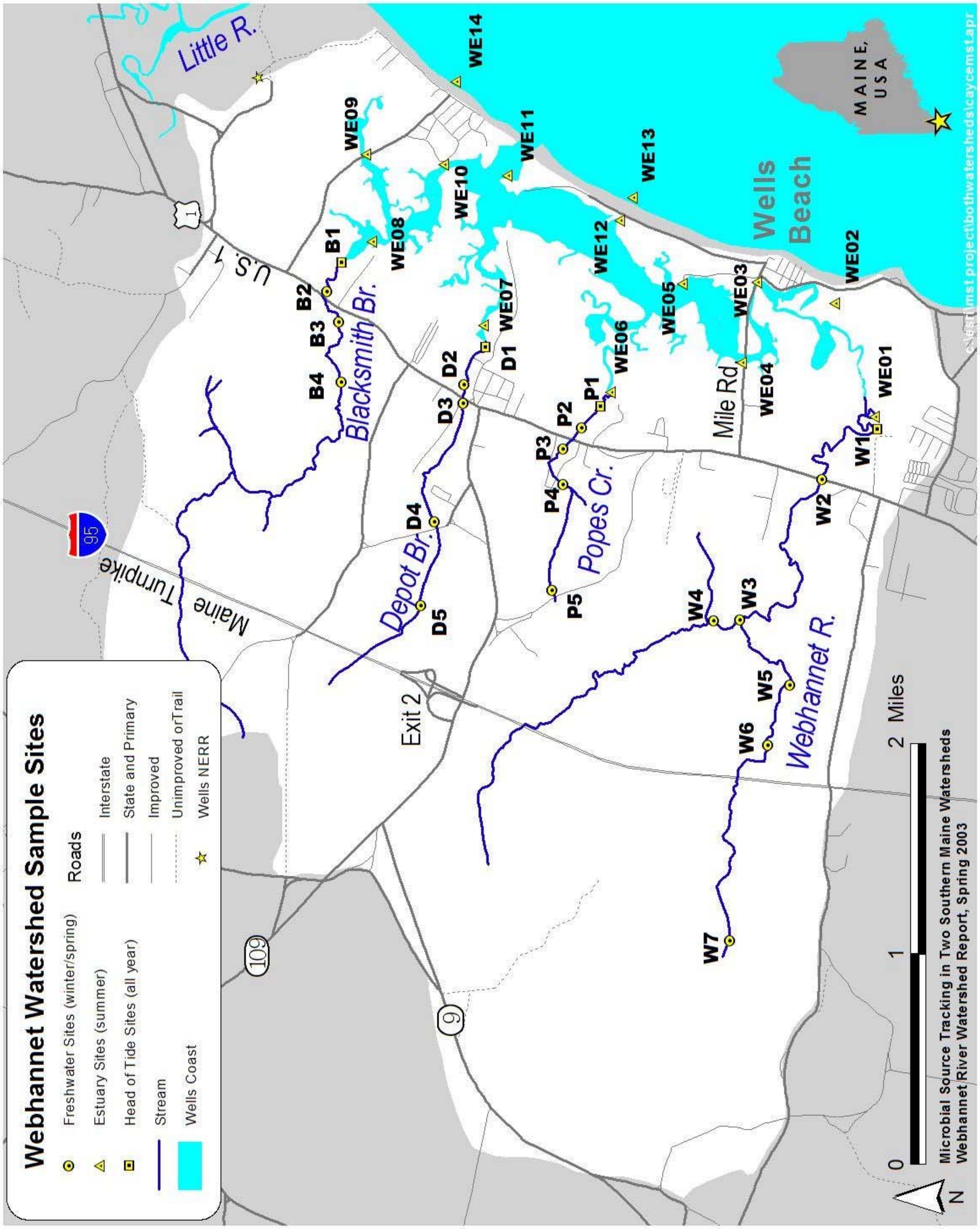
[7] DMR uses a multiple tube fermentation method (A1) to analyze fecal coliform whereas the NPS study used membrane filtration (with mFC growth medium) to test for fecal coliform. The MST Project used membrane filtration with mTEC growth medium to test for fecal coliform and *E. coli*. Technically, results from the multiple tube fermentation and membrane filtration methods are not directly comparable, though they do provide some relative basis for comparison.



Webhannet Watershed Sample Sites
Freshwater Sites (winter/spring)
Estuary Sites (summer)
Head of Tide Sites (all year)
Stream
Wells Coast
Roads
Interstate
State and Primary
Improved
Unimproved or Trail
Wells NERR
Little R.
U.S. 1
Maine Turnpike
95
109
9
Exit 2
Blacksmith Br.
Depot Br.
Popes Cr.
Webhannet R.
Mile Rd.
Wells Beach
MAINE, USA
B1
B2
B3
B4
D1
D2
D3
D4
D5
P1
P2
P3
P4
P5
W1
W2
W3
W4
W5
W6
W7
WE01
WE02
WE03
WE04
WE05
WE06
WE07
WE08
WE09
WE10
WE11
WE12
WE13
WE14
0
1
2 Miles
N
Microbial Source Tracking in Two Southern Maine Watersheds
Webhannet River Watershed Report, Spring 2003

3.2 Sample Collection Procedures and Design

Community volunteers and project staff collected water samples using essentially the same procedures employed during the 2000 NPS study. Samples were collected using sterile Whirl-Pak bags and tongs, temperature was measured, and basic observations about the site were recorded on a water sample field sheet (see Appendix 3). All volunteers were trained by project staff, and follow-up training was conducted as needed. Methods were designed to minimize the possibility of the sampler contaminating the sample. Sample collection procedures are outlined in detail in the "MST *E. coli* SOP" (on file at the Wells National Estuarine Research Reserve in Wells, ME). As discussed above, there were two distinct sampling periods used to distinguish between potential sources of fecal contamination (i.e., upland freshwater vs. estuarine). Each period consisted of approximately 10 sampling events (for a total of 20 sampling events from December 2001 to September 2002), and roughly half of these occurred during dry weather conditions while the other half occurred during wet weather conditions (i.e., runoff from thaws and / or precipitation). Most samples from estuarine sites were collected during an outgoing tide to reflect more closely the conditions in the immediate proximity of these sample locations and to minimize confounding factors associated with the estuarine mixing regime.

Most animal fecal samples for the MST reference library were collected by volunteers with specialized skills in accurately identifying the sources of particular samples. Project staff collected the more easily identifiable samples along with those originating from human sources. All fecal samples were collected from locations within the Webhannet watershed to reflect the types of potential contamination sources found there. Sampling protocol consisted of collecting only "fresh" unfrozen fecal material to increase the likelihood of *E. coli* viability. Samples were placed in a sterile Whirl-Pak bag labeled with the animal source species, date, time and general location from which the sample was collected. Samples were then transported to the WNERR lab within six hours and refrigerated for later processing at the University of New Hampshire's Jackson Estuarine Laboratory (JEL). In the first year of the MST Project, raw fecal samples were delivered directly to JEL for *E. coli* culturing and subsequent ribotyping. However, by September of 2002, project staff members were culturing and isolating *E. coli* from fecal samples before delivery to JEL. (Some fecal samples were collected after water sample collection was completed in September 2002.) In all cases, raw fecal samples or isolates were accompanied with sample collection forms that specified the date, time, and location of sample collection; species identification; the sample delivery date; and relevant comments (Appendix 4).

3.3 Defining Wet and Dry Weather Samples

Originally, the MST Project proposal defined wet weather conditions to exist after 1 inch of rainfall in 24 hours. However, given the wide variety of possible precipitation conditions, project staff took a more flexible approach in defining high flow conditions by considering each sampling event on a case-by-case basis. Precipitation conditions, along with the decision to designate a sampling event as occurring during a wet weather period, are recorded in Table 3. See Appendix 5 for graphs of precipitation, air temperature and fecal coliform counts. The rationale for determining if conditions merited a "wet weather" designation were based roughly on the idea of 0.25 inches of rainfall in the previous 24 hours. If significantly less rain fell, but the timing was much closer to sampling, than it was considered a wet weather event. If rainfall was spread out over a longer period of time, then more precipitation was generally needed for staff to assign the wet weather designation. Drought conditions in 2002 made wet weather sampling difficult to conduct. Weather from the prior three days was considered for each sampling event since a given amount of rain could conceivably have quite different effects on bacterial concentrations. For example, a moderate rainfall that was preceded by a long dry period could create significant fecal loading to streams if there was a large store of pet and wildlife waste on the ground. Alternately, a heavy rain might have little effect if it had rained several times in the prior week, because earlier rains could have already washed the ground surface clean. In any event, unusually low precipitation amounts generally resulted in fairly weak relationships with fecal coliform and *E. coli* concentrations.

Sample Date	Precipitation (inches)	Duration of Precipitation Event	Weather Station	Post-Precipitation?
December 4, 2001	0.00		Portland, ME	no
January 8, 2002	0.20	36 hours	WNERR	no
January 24, 2002	0.48	48 hours	Portland, ME	yes
January 31, 2002	0.36	48 hours	Portland, ME	yes
February 21, 2002	0.84	8 hours	WNERR	yes
March 4, 2002	2.00	36 hours	WNERR	yes
March 11, 2002	0.52	36 hours	WNERR	yes
April 15, 2002	1.50	60 hours, with 1.23" in previous 8 hours	WNERR	yes
May 6, 2002	0.00		WNERR	no
May 15, 2002	4.70	60 hours, no rain in previous 14 hours	WNERR	yes
May 30, 2002	negligible		WNERR	no
June 15, 2002	0.55	8 hours	WNERR	yes
June 20, 2002	0.40	8 hours	WNERR	yes
July 18, 2002	0.00		WNERR	no
August 16, 2002	0.00		WNERR	no
August 27, 2002	0.00		WNERR	no
September 3, 2002	0.10	9 hours	WNERR	yes
September 12, 2002	0.00		WNERR	no
September 18, 2002	1.38	2 days prior, none within 48 hrs	WNERR	no
September 23, 2002	1.14	24 hours	WNERR	yes

Table 3: Webhannet watershed sample collection calendar

3.4 Laboratory Methods & Analytical Procedures

Bacterial analysis for water samples was done in accordance with EPA Method 1103.1 (*Escherichia coli* in Water by the Membrane Filter Procedure, 1985). This procedure uses mTEC[8] agar to detect the presence of *E. coli* and distinguish it from fecal coliform. A similar procedure for fecal coliform testing (using mFC growth medium) has been employed by Wells National Estuarine Research Reserve (WNERR) staff for over 10 years and has proven highly reliable in identifying bacterial contamination. The mTEC medium encourages the growth of *E. coli* bacteria – a subset of fecal coliform – and inhibits the growth of other bacteria by using a very precise and relatively high temperature range. *E. coli* are provided with essential nutrients contained in the mTEC medium while the growth of other bacteria types is inhibited. Dyes cause the *E. coli* to produce a characteristic yellow or yellow-brown color. The incubation at 44.5°C favors the high temperature-tolerant *E. coli* over other bacteria types. At this temperature, colonies of *E. coli* and other bacteria form. *E. coli* produce a yellow color while other bacteria remain purple in color. Placing *E. coli* on a urea substrate at a pH of 4.5 distinguishes it from fecal coliform and other types of bacteria. The *E. coli* colonies remain yellow while other bacteria types become red or purple.

Project staff also performed procedures to isolate *E. coli* from water and fecal samples for eventual genetic analysis (ribotyping). Before *E. coli* from fecal samples could be isolated it first had to be cultured. This was accomplished by creating a slurry of fecal material and analyzing a series of diluted samples for *E. coli*. Subsequent isolation procedures involved transferring ten distinct and separate *E. coli* colonies from each water or fecal sample to the general purpose growth medium, tryptic soy agar (TSA). These *E. coli* isolates were then transported to the University of New Hampshire's Jackson Estuarine Laboratory (JEL) and preliminarily screened to determine their suitability for genetic analysis. JEL estimates that approximately 85% of all isolates submitted for screening are actually confirmed as *E. coli* (due to false positives on the remaining 15%) and therefore appropriate for subsequent ribotyping. (For a more detailed discussion of this procedure refer to "*E. coli* Isolation SOP" on file at the Wells National Estuarine Research Reserve).

[8] mTEC stands for membrane filtration method, Thermotolerant, *E. coli*

3.5 Data Management

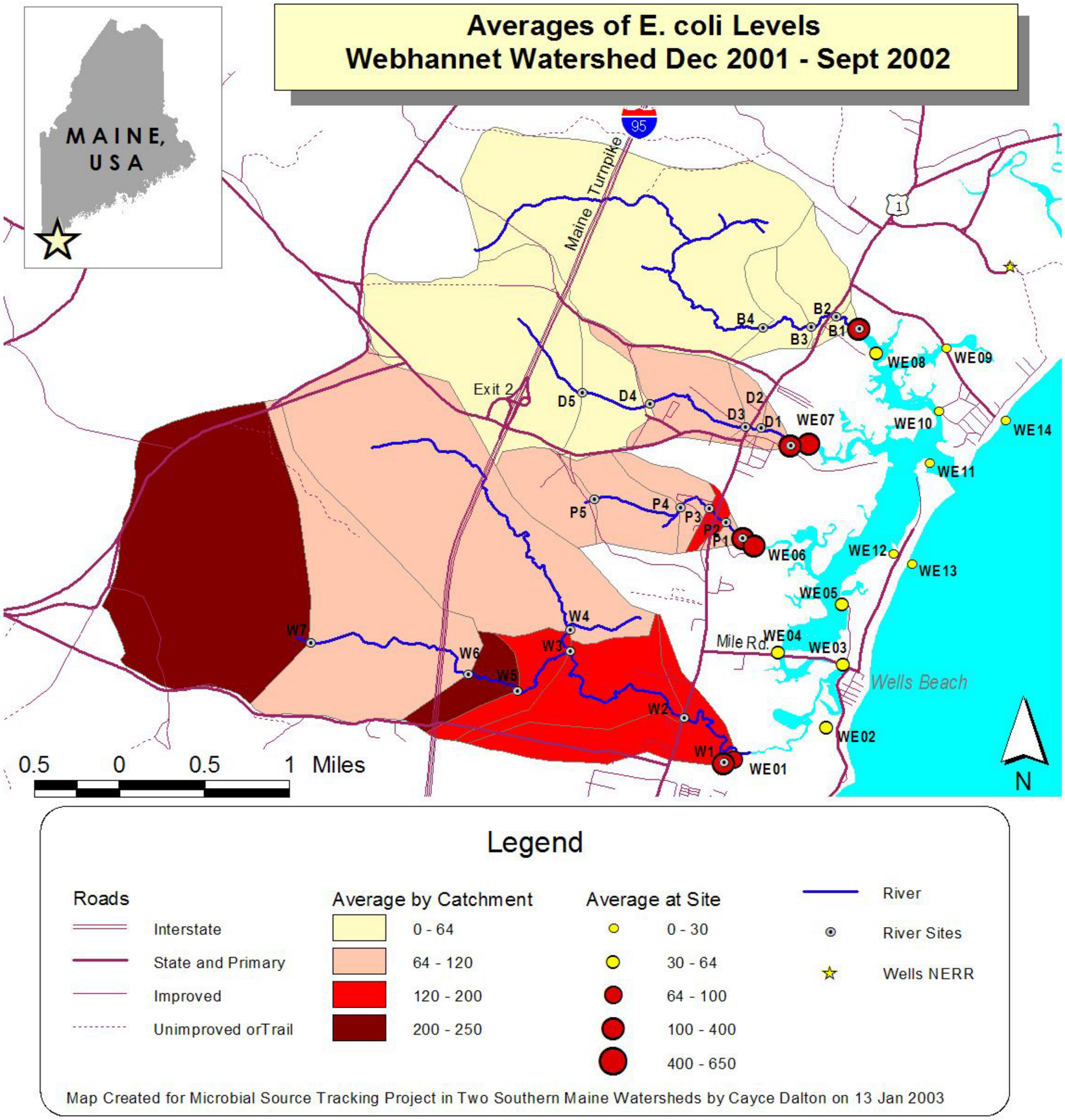
For each water sample collected, volunteers and project staff recorded time and date, water temperature, water flow, approximate water depth, ice coverage, whether sample was taken from edge or middle of stream, and any other pertinent comments regarding the site. As volunteers delivered samples, project staff noted arrival date and time to record the chain of custody. Project staff also recorded all laboratory work done, including membrane filtration and counting dates, person(s) conducting filtration, time in and out of incubator, dilution volumes, fecal coliform and *E. coli* colony counts, person(s) conducting bacterial colony counts and any other pertinent comments. Data from all forms were entered into an Excel spreadsheet by project staff for tabulation and graphing. Weather data obtained from the Wells NERR weather station were also converted into Excel format and graphed against bacterial concentration data. Each Excel file explains in detail the methods used for organizing and managing the data. Original field sheets, laboratory forms and scat/isolate delivery forms are archived at Wells NERR. The Excel files (C:\Dell MST\Data Files\2001-2002 Webhannet Data.xls and C:\Dell MST\Data Files\Statistical Analyses\Weather Analysis.xls) and ArcView projects are archived at the Wells NERR.

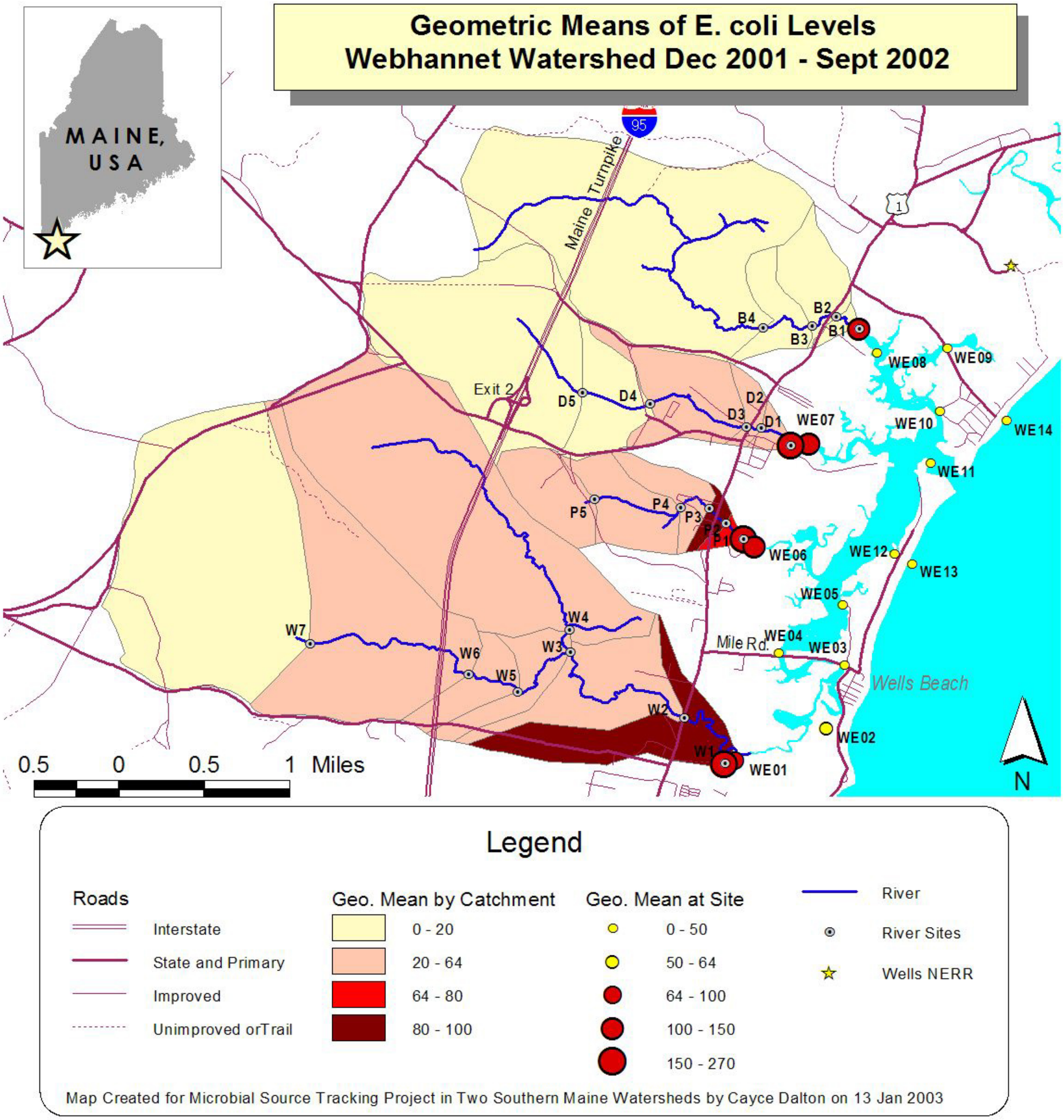
3.6 Analysis of *E. coli* Data for Water Samples

Initial fecal coliform and *E. coli* data analysis was conducted by project staff using Excel spreadsheet software. The averages and geometric means for each site were calculated, as were graphs of each sample date showing fecal coliform and *E. coli* concentrations. Generally, fecal coliform and *E. coli* concentrations were observed to be closely related. In addition, graphs of precipitation, rainfall and fecal coliform counts were created. Relationships between weather and fecal contamination were much more varied, except for the general pattern of much higher counts in the summer season. For a few sample dates, rainfall is associated with higher fecal counts.

Project staff relied on ArcView GIS software to geographically analyze the bacterial concentrations. Several sources of geographical data were used, such as watershed boundaries, contour lines, rivers, streams, town boundaries, streets and digitally corrected orthoquad aerial photos from the Maine Office of GIS; data of sample site locations from the Wells NERR's Trimble handheld GPS collected in the field by project staff; town tax parcel boundaries from the town of Wells; and Wells Sanitary Sewer system data provided by Wright-Pierce Engineers. Project staff considered determining the general location of high bacterial counts an important first step in understanding the Webhannet's fecal contamination. Using contour lines provided by Maine Office of GIS, subcatchments were drawn for each sample site. Bacterial concentrations for each site were imported from Excel into ArcView and joined to this geographic data, allowing subcatchments to be color-coded based on the bacterial concentration found at each site. Since estuarine sites are not associated with subcatchments, their bacterial data were mapped with symbol size relating to bacterial concentration. Maps were made for each sample date, as well as for the average and geometric mean of each site (see Figures 22 and 23).

The data indicate that *E. coli* concentrations are highly variable in the watershed. Sites that show high average or geometric means of *E. coli* occasionally show very low concentrations. In a few cases, sites that were consistently clean showed a one-time concentration that was very high. General patterns did emerge, however. Blacksmith Brook tended to show very low bacterial concentrations. The Webhannet River, Pope's Creek and Depot Brook tended to show high, and sometimes very high, bacterial levels. The geometric means and the averages both indicate that bacterial concentrations are at their highest levels at sites south of Route 1. The high average of site W7 (upstream site on the Webhannet River) is due to one very high count. The geometric mean de-emphasizes single extreme counts, indicating that all the other counts for W7 were generally quite low. The estuary sites, particularly those not immediately adjacent to freshwater inputs tended to show moderate to low counts, with the geometric means of all estuary sites falling below 64 CFU/100mL.


Averages of E. coli Levels
Webhannet Watershed Dec 2001 - Sept 2002
MAINE, USA
95
Maine Turnpike
1
Exit 2
Mile Rd.
Wells Beach
B1
B2
B3
B4
D1
D2
D3
D4
D5
P1
P2
P3
P4
P5
W1
W2
W3
W4
W5
W6
W7
WE01
WE02
WE03
WE04
WE05
WE06
WE07
WE08
WE09
WE10
WE11
WE12
WE13
WE14
0.5 0 0.5 1 Miles
N
Legend
Roads
Interstate
State and Primary
Improved
Unimproved orTrail
Average by Catchment
0 - 64
64 - 120
120 - 200
200 - 250
Average at Site
0 - 30
30 - 64
64 - 100
100 - 400
400 - 650
River
River Sites
Wells NERR
Map Created for Microbial Source Tracking Project in Two Southern Maine Watersheds by Cayce Dalton on 13 Jan 2003


Geometric Means of E. coli Levels
Webhannet Watershed Dec 2001 - Sept 2002
MAINE, USA
95
Maine Turnpike
1
Exit 2
B1
B2
B3
B4
D1
D2
D3
D4
D5
P1
P2
P3
P4
P5
W1
W2
W3
W4
W5
W6
W7
WE01
WE02
WE03
WE04
WE05
WE06
WE07
WE08
WE09
WE10
WE11
WE12
WE13
WE14
Mile Rd.
Wells Beach
N
0.5 0 0.5 1 Miles
Legend
Roads
Interstate
State and Primary
Improved
Unimproved orTrail
Geo. Mean by Catchment
0 - 20
20 - 64
64 - 80
80 - 100
Geo. Mean at Site
0 - 50
50 - 64
64 - 100
100 - 150
150 - 270
River
River Sites
Wells NERR
Map Created for Microbial Source Tracking Project in Two Southern Maine Watersheds by Cayce Dalton on 13 Jan 2003

3.7 Selection of Source Species for *E. coli* Reference Libraries

The experimental design for the MST Project established a minimum number of 10 species for the development of a local source reference library. Fecal samples for this library originated from a combination of domestic animals, wildlife and human sources that resided within the Webhannet watershed. Individual species were selected on the basis of local knowledge about their relative prevalence. Anecdotal information and observation suggested that domestic dog, waterfowl and deer were all present in great abundance. Additional wildlife species were selected, based on the various habitats present in the watershed. These included common mammals, such as moose, coyote, red and grey fox, raccoon, squirrel, beaver, and otter. The species for which fecal samples were actually obtained are listed in Table 4. Human samples were collected from three different sources: sewage (from the Wells Wastewater Treatment Plant influent), septage (from a local septic hauler) and raw fecal material (from an individual living within the watershed). The rationale for obtaining samples from these different human sources was based on the fact that each of them often produces different genetic fingerprints (or ribotypes). Given the limited number of total species used to establish the local reference library for the Webhannet watershed, the regional reference library developed by JEL (Table 4) was included in the ribotyping analysis to provide a more comprehensive basis for comparing ribotypes from unknown water samples with those from known fecal samples.

Species	Local Reference Library	Regional Reference Library
Pets		
Cat	-	2
Dog	6	15
Humans		
Stool sample	10	14
Septage	17	17
Wastewater	13	55
Wildlife		
Coyote	10	15
Deer	3	41
Grey Fox	3	3
Muskrat	-	3
Racoon	4	28
Red Fox	3	26
Squirrel	4	4
Livestock		
Cow	-	30
Horse	-	14
Chicken	-	2
Birds		
Cormorant	-	13
Duck	-	4
Goose	-	19
Grouse	2	2
Pigeon	-	2
Robin	-	3
Seagull	-	5
Total Isolates	75	317
Total Species	11	22

Table 4. Source species database for ribotyping analysis of Webhannet watershed
Source: Jones, 2003

3.8 Selection of *E. coli* Isolates for Ribotyping Analysis

Samples were initially selected for *E. coli* isolation based on the Maine Department of Environmental Protection (DEP) seasonal (May 15th to Sept. 30th) water quality standard of 64 colonies / 100 mL for Class B surface waters. Given that the Webhannet River and all of the other tributaries in the watershed are designated as Class B by the State, this seemed a reasonable (if somewhat arbitrary) value to determine a cutoff point for creating isolates. All water samples with *E. coli* concentrations greater than 64 colonies / 100 mL were selected for isolation. As described above, this process involved transferring 10 distinct and separate colonies from each water sample exceeding the cutoff value to the general purpose growth medium, tryptic soy agar (TSA). *E. coli* concentrations from water samples routinely exceeded the threshold value throughout the sampling period. Consequently, many more isolates were created than could be processed for ribotyping.

The MST Project budget allowed for the ribotyping of approximately 200 isolates and JEL had reported that only about 85% of all isolates submitted for ribotyping are ultimately confirmed as *E. coli*. Therefore, to provide enough genetic material for ribotyping, approximately 235 isolates were selected from both the upper freshwater and lower estuarine sections of the watershed. The number of isolates selected from each section was determined by the relative proportion of each sample collection period. The sampling period for the upper watershed spanned six months (Dec.-May) and the estuarine sampling period spanned four months (June-Sept). Thus 60% of the isolates (about 140) were chosen from the upper watershed while 40% (about 95) were chosen from the estuary.

Deciding which isolates to select from within each watershed section was done primarily by focusing on the sites that most consistently yielded the highest *E. coli* concentrations. A significant part of the MST Project's intent is to help resource managers develop targeted mitigation strategies to reduce fecal loadings in the estuary and thereby reopen closed shellfish harvesting areas. As such, it seemed reasonable to hypothesize that sites with consistently high counts represented the greatest potential fecal loadings to the estuary (assuming they also originated from an area with a significant flow contribution). Isolates were also selected from one "clean" site (with consistently low *E. coli* concentrations) to reflect conditions from a relatively undisturbed area of the watershed. See Appendix 6 for a more detailed discussion of the selection methodology.

3.9 Source Species Identification for *E. coli* Isolates from Unknown Water Samples

As described previously, water samples from the Webhannet watershed were collected from December 2001 until September 2002. *E. coli* strains were isolated from these samples and analyzed at the University of New Hampshire Jackson Estuarine Laboratory's ribotyping facility. Dr. Steve Jones has developed a report with a detailed discussion of the results from the source species identification for the Webhannet water samples. What follows in this and the next two sections are excerpts taken directly from his report.

Useable ribotypes resulted from samples collected on 12 different dates and 13 different sites (Table 5). The DNA of all culturable strains were processed for ribotype profile analysis to identify source species for isolates from water samples, using isolates from known sources of fecal samples as references. The fecal source *E. coli* ribotypes used for references included a small database from the Webhannet watershed and another larger regional database from New Hampshire, Maine and Vermont. The larger regional database provides a more comprehensive basis for comparison between water sample ribotypes and fecal sample ribotypes. Consequently, more source species were successfully identified – 70% with the regional reference library as compared to 53% with the local reference library (Figures 24 and 25).

Site Date	Weather conditions	*E. coli* cfu/100 ml	Total isolates	Usable ribotypes	Ribotyping success
W1					
4/15/2002	wet	490	10	8	80%
5/30/2002	dry	54	10	6	60%
6/15/2002	wet	600	10	9	90%
8/27/2002	dry	1380	10	5	50%
W3					
4/15/2002	wet	735	10	6	60%
W4					
12/4/2001	dry	123	10	6	60%
1/31/2002	wet	555	10	9	90%
W7					
4/15/2002	wet	2335	10	6	60%
WE01					
9/23/2002		900	10	5	50%
WE06					
6/15/2002	wet	1700	10	9	90%
8/27/2002	dry	22	10	6	60%
WE08					
6/15/2002	wet	4800	10	6	60%
B1					
5/15/2002	wet	54	10	10	100%
B4					
4/15/2002	wet	388	10	10	100%
D1					
2/21/2002	wet	490	10	6	60%
6/15/2002	wet	730	10	3	30%
8/16/2002	dry	1040	10	1	10%
9/23/2002	wet	2320	10	8	80%
D2					
12/4/2001	dry	355	10	7	70%
4/15/2002	wet	235	10	7	70%
P1					
3/11/2002	wet	195	10	8	80%
5/15/2002	wet	189	10	5	50%
8/16/2002	dry	460	10	1	10%
9/12/2002	dry	320	10	4	40%
P2					
12/4/2001	dry	70	10	4	40%
3/11/2002	wet	795	10	1	10%
5/30/2002	dry	426	10	3	30%
Totals			270	159	59%

Table 5. Yield of successful and identifiable ribotype patterns from Webhannet watershed *E. coli* isolates. Source: Jones (2003)

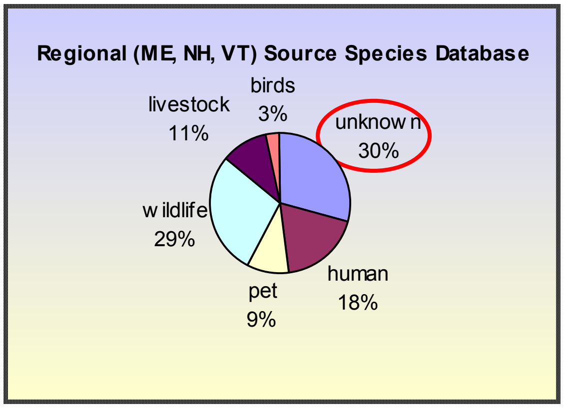


Figure 24. Source species identification using regional reference library. Source: Jones (2003)

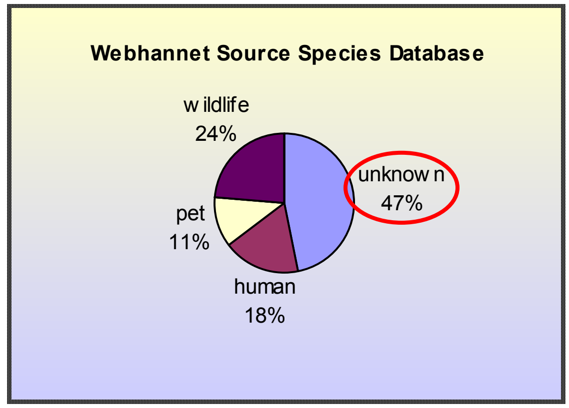


Figure 25. Source species identification using local reference library. Source: Jones (2003)

Using these databases, the results of data analysis provide information on the identification of source species for the water samples, with a defined degree of certainty (80%). The degree of certainty refers to a calculated similarity index between each water sample ribotype and the most closely matching fecal sample ribotype. For this study, the predetermined threshold similarity index that was considered to be a minimum value for identifying source species was 80% for comparisons with both source species databases (local and regional). Thus, the identification of a source species was considered successful for a given water isolate sample if its match was equal to, or greater than, the 80% threshold value; if the calculated value was below the threshold value, the water isolate sample was considered to be of unknown origin.

As indicated in Figures 24 and 25, the variety of source species can be grouped according to five types: humans, pets, livestock, birds, and wildlife. Grouping identified source species by these types helps in considering management approaches needed to eliminate pollution sources based on these results. Human-related sources (feces, septage, wastewater treatment plant influent) were the most common identified single source/species and accounted for 18% of sources, but the wildlife species (raccoon, coyote, deer, fox, squirrel) were the most commonly (29%) identified types of sources. Overall, it appears that there is a relatively important level of human sources that contribute to the fecal contamination of the watershed, but a larger fraction of the contamination appears to be of non-human (wildlife, livestock, birds and pets) origin. The significance of livestock as a source should be investigated because this type of source was initially perceived as not being significant and was excluded from the local database.

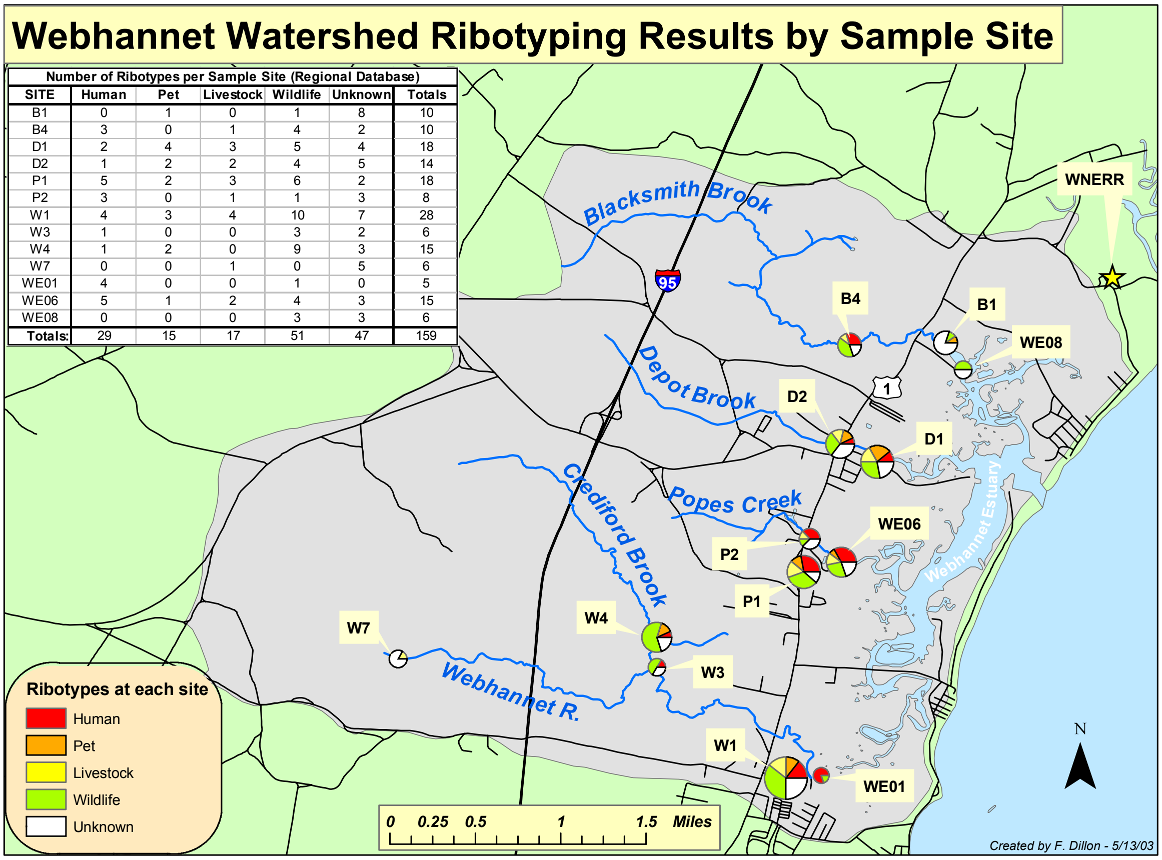
3.10 Source Species Identification

The identified source species are summarized by each of the four tributaries and matching estuarine sites in Table 6. In the Webhannet River, wildlife species constituted a majority of the isolates identified, based on analyses using both the local and regional databases. This finding corresponds well with the earlier work conducted in the Webhannet watershed by Mullan, Dionne, and Whiting-Grant (2001) where they hypothesized that most of the fecal contamination was originating from wildlife sources in the upper freshwater reaches of the watershed. Indeed, given the fact that much of the Webhannet River subcatchment is comprised of undeveloped land cover types – mostly forested wetland and upland forest (see Figure 3) – this result is not surprising. Wildlife species also constituted the largest fraction of identified source species for samples from Blacksmith and Depot brooks, although the analysis using the Webhannet database suggests humans are also important in Blacksmith Brook and pets are important in Depot Brook. Here again, given the large undeveloped tracts of land in the upper reaches of these subcatchment areas, wildlife might be expected to be the most prominent source. Humans were identified for the largest fraction of isolates from Popes Creek. Using the regional database for one of the tributaries (Popes Creek) with matching estuarine sites, the type of source species most commonly identified in the tributary was also the most common type of source species found at the estuarine site (human). However, the dominant type of source species found at WE01, at the mouth of the Webhannet River, were humans, despite being a relatively small fraction of identified isolates in the tributary compared to wildlife species. The identification of humans in all cases may lend more credence to concerns regarding malfunctioning septic systems, particularly in those areas where public sewer is available.

Webhannet Database								
	Webhannet River	WE01	Popes Creek	WE06	Blacksmith Brook	WE08	Depot Brook	TOTALS
Ribotyped isolates	55	5	26	15	20	6	32	159
Isolates >80%	29	4	17	9	7	2	17	85
Raccoon	4	-	2	0	0	-	1	7
Coyote	9	-	3	2	1	2	4	21
Deer	2	-	2	0	1	-	0	5
Dog	5	-	3	2	1	-	7	18
Fox	2	-	0	0	0	-	2	4
Human	7	4	7	5	3	-	3	29
Squirrel	0	-	0	0	1	-	0	1
Regional Database								
Isolates >80%	38	5	21	12	10	3	23	112
Raccoon	6	-	2	0	2	-	0	10
Cow	4	-	4	2	1	-	4	15
Coyote	9	-	2	2	0	2	4	19
Deer	1	-	1	1	1	-	1	5
Dog	5	-	2	1	1	-	6	15
Fox	4	1	0	1	1	1	3	11
Goose	1	-	0	0	0	-	0	1
Seagull	1	-	2	0	0	-	1	4
Horse	1	-	0	0	0	-	1	2
Human	6	4	8	5	3	-	3	29
Squirrel	0	-	0	0	1	-	0	1

Table 6. Source species identification for E. coli isolates by tributary. Source: Jones (2003)

Figure 26 provides a detailed summary of source species identification for each of the 13 water sampling sites from which ribotypes were developed. It also helps to determine which specific areas of the watershed should receive the greatest attention for remediation strategies. Each sample site is represented by a pie chart, indicating the relative proportions of identified ribotypes along with those that could not be identified ("unknowns"). There is also an accompanying table that indicates the actual numbers of ribotypes for each sample site and each species type. The species categories are wildlife (including birds), humans, pets, livestock and unknowns. Surprisingly, significant levels of human contamination occurred in the publicly sewered portions of the watershed, particularly near the outlets of Popes Creek and the Webhannet River. As expected, wildlife contributions were highest in the undeveloped upper portions of the watershed, particularly along Blacksmith Brook and the Webhannet River. Wildlife was also significant along the edges of the marsh area of the Webhannet estuary. Ribotypes for livestock and pet waste generally occurred in conjunction with human ribotypes.



Number of Ribotypes per Sample Site (Regional Database)

SITE	Human	Pet	Livestock	Wildlife	Unknown	Totals
B1	0	1	0	1	8	10
B4	3	0	1	4	2	10
D1	2	4	3	5	4	18
D2	1	2	2	4	5	14
P1	5	2	3	6	2	18
P2	3	0	1	1	3	8
W1	4	3	4	10	7	28
W3	1	0	0	3	2	6
W4	1	2	0	9	3	15
W7	0	0	1	0	5	6
WE01	4	0	0	1	0	5
WE06	5	1	2	4	3	15
WE08	0	0	0	3	3	6
Totals:	29	15	17	51	47	159

Figure 26. Geographic distribution and species composition of ribotypes in the Webhannet watershed. Pie sizes indicate number of ribotypes for each sampling site (actual numbers included in inset table). Source: Jones (2003).

3.11 Wet Versus Dry Weather Sources

Analysis of all isolates collected throughout the study period was separated into "wet" and "dry" weather samples. Wet and dry weather designations for each sample date are summarized in Table 5. Samples used for ribotyping were collected under wet conditions on seven of the 12 dates, including all of the winter samples, three of the four spring samples, and one of the autumn samples. Samples were collected under dry conditions on five dates, including all of the summer samples, one spring sample and one autumn sample. Using the Regional database, source species identified under wet weather conditions included 81 of the 116 isolates collected (70%). The source species identified under dry weather conditions included 31 of the 43 isolates collected (72%). The percentage of isolates identified by types of source species for wet and dry weather were: wildlife, 28% and 30%; humans, 18% and 19%; pets, 10% and 7%; livestock, 9% and 14%; and birds, 3% and 5%. The largest difference was for livestock, reflecting a greater percentage of the identified sources under dry compared to wet conditions. The results for weather conditions using the regional database are illustrated in Figures 27 and 28.

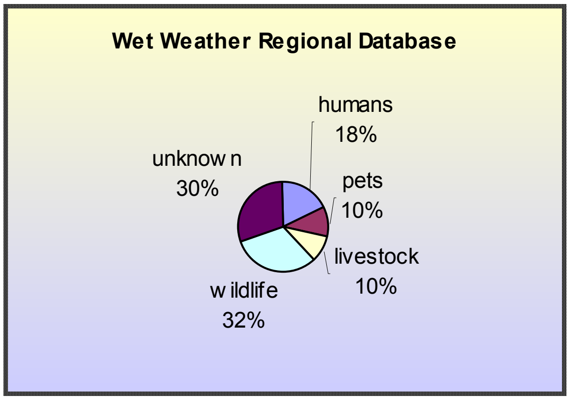


Figure 27. Source species identification during wet weather. Source: Jones (2003)

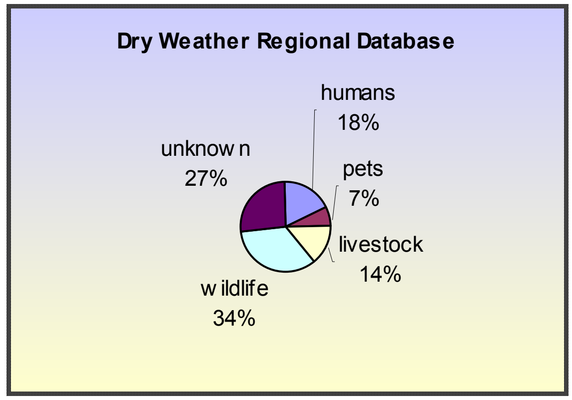


Figure 28. Source species identification during dry weather. Source: Jones (2003)

4.0 RECOMMENDED MANAGEMENT ACTIONS

4.1 Control of Point Sources

The greatest potential source of human microbial contamination from point sources is raw sewage (Massachusetts Department of Environmental Protection, 2002). The 1972 amendments to the Federal Water Pollution Control Act (known as the Clean Water Act or CWA) provided the statutory basis for regulating the discharge of pollutants from point sources to waters of the United States. The CWA required the EPA to develop and implement the National Pollutant Discharge Elimination System (NPDES), a national program that required all facilities discharging pollutants to obtain a discharge permit for these activities. It also established discharge limits for the removal of a variety of pollutants – one of which is fecal coliform bacteria. (Federal authority for the administration of the NPDES Program was delegated to the State of Maine in 2001. Authorized wastewater discharges are now referred to as "MEPDES" permits). Potential point sources of sewage include those from wastewater treatment facilities, urban stormwater runoff collection systems, and overboard discharges (OBDs).[9] Each of these is discussed in detail below. The only permitted discharge under the NPDES program currently existing in Wells is for the town's wastewater treatment facility.

4.11 Investigation of Wastewater Treatment Infrastructure

Raw sewage, although not usually discharged intentionally, can reach water bodies through leaks in sanitary sewer systems, overflows from surcharged sanitary sewers, illicit connections of sanitary sewers to storm sewer systems, or unidentified broken sanitary sewer lines. According to the Center for Watershed Protection, as much as 10% of all storm sewer outfall pipes in some communities may discharge dry weather flow. If only a few of these discharge sewage, the result can be very high bacteria concentrations, due to low stream flow volumes (CWP, 1999). Illicit sewer connections can have as large an impact as broken or leaking sewer pipes and represent a direct threat to public health since they also result in discharges of partially treated or untreated human wastes. Quantifying these contamination sources is extremely speculative without direct monitoring of the source because the magnitude is directly proportional to the volume of the sources and their proximity to surface waters. Typical values of fecal coliform in untreated domestic wastewater range from 10^6 to 10^7 MPN/100 mL[10] (Metcalf and Eddy, 1991).

The sanitary sewer system in Wells was constructed in the late 1980s. Therefore, it is relatively new from a design-life perspective, which is typically 50 years for more modern construction practices and materials. Such systems are expected to experience very little leakage during their design life assuming proper design and installation. Daily flows from the Wells Wastewater Treatment Plant (WWTP) were compared to daily precipitation values to identify the extent of sewer system leakage. Sewer systems

[9] OBDs are individual or community domestic wastewater treatment systems that discharge directly to receiving waters.
[10] MPN means "most probable number" and is based on certain probability formulas that estimate the mean density of coliforms in a given sample. Coliform density provides an assessment of the sanitary quality of untreated water.

prone to leakage would be expected to show strong correlations with significant precipitation events. Conversely, a lack of correspondence between these two variables would generally indicate a relatively "tight" (or leak-free) system. As Figure 29 indicates, the Wells sanitary sewer system appears to fall into the latter category. Significant precipitation events do not result in corresponding increases in plant flows (which increased independently of precipitation during the summer months in response to the large influx of tourists). Indeed, the relationship between plant flow and precipitation is virtually nonexistent in terms of the correlation coefficient value, which was calculated at -0.01.

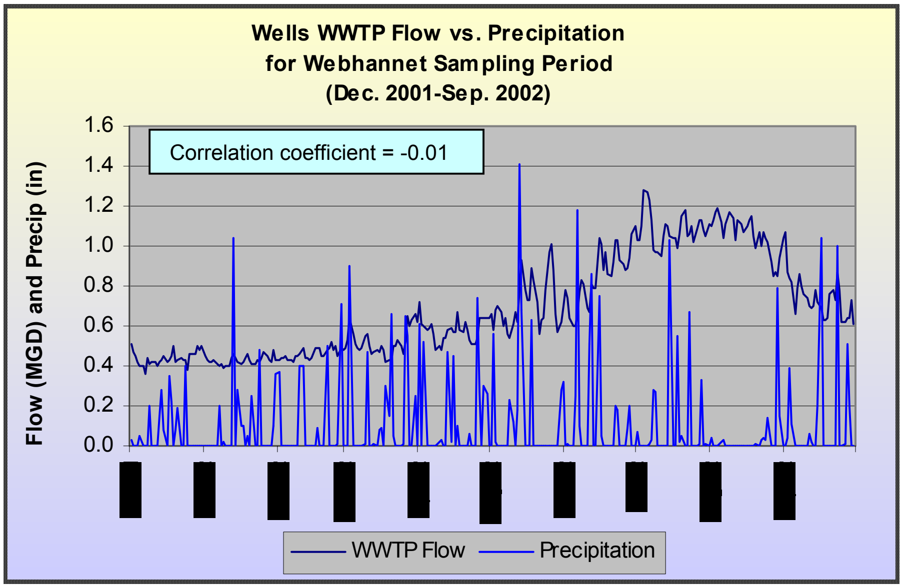


Figure 29: Wells Wastewater Treatment Plant flow vs. precipitation during Webhannet sampling period

The potential for sanitary sewer system overflows[11] is also unlikely given that all nine of the town's pumping stations have standby power generators. Additionally, the sewer system still has considerable hydraulic capacity for most areas of the town and plans are currently being developed to address those areas nearing design capacity. Storm sewer infrastructure in Wells is virtually nonexistent (less than 1000 lineal feet) and none of it is connected to the sanitary sewer.[12] Therefore, combined sewer overflows (CSO's) – the combination of sanitary sewers and storm sewers – do not appear to pose a water quality problem for the town since they simply do not exist. However, in September 2002 water quality monitoring for the MST Project identified exceptionally high bacterial levels resulting from a sewer line blockage in the area of Depot Brook (at site D1). The Wells Sanitary District repaired the problem by September 19th but residual fecal contamination persisted through the last water quality sampling event on September 23rd.

Aside from this single overflow incident and based on the cursory analysis of the relationship (or lack thereof) between plant flow and precipitation, the town's wastewater infrastructure appears to pose minimal risks to shellfish harvesting areas in the Webhannet estuary. Circumstantial support for this hypothesis is provided by the DEP, which requires all wastewater treatment facilities in the state to submit a Discharge Incident Report whenever untreated or partially treated sewage is released to Maine surface waters. The only such incident on file since 2000 is the one described above.[13] The DMR also has established an Emergency Response Plan for the Wells Sanitary District that requires similar notification (Appendix 7).

However, there were significant numbers of human ribotypes identified to the east of Route 1 in the publicly sewered portion of the watershed, particularly near the outlets of Popes Creek and the Webhannet River (Figure 26). An investigation should be conducted to determine whether any properties

[11] Sanitary sewer overflows are the discharge of untreated sewage to surface water bodies.
[12] Conversation with Wells Sanitary District Superintendent, Dennis Thayer (4/4/03).
[13] Email correspondence with Matt Hight, Maine DEP (4/6/03)

in these areas are not connected to the public sewer and are instead using septic systems for wastewater disposal. If there are malfunctioning septic systems present, they should be repaired or replaced with public sewer. If no malfunctioning septic systems are discovered, then the human sources originated either from the public sewer system or further up in the unsewered portions of the watershed. Consequently, the public sewer in these areas should be carefully evaluated for possible extraneous leakage. Suspect sewer lines should be selected for isolation flow gauging during periods of wet and dry weather to determine net infiltration (and potential exfiltration) rates. Any line segments experiencing significant I&I should be repaired. Section 4.22 will provide a discussion on developing a process to identify malfunctioning septic systems in the unsewered portions of the watershed.

4.12 Urban Runoff

Urban runoff, while generally considered a nonpoint source of pollution, is also considered a point source from a regulatory perspective since many municipalities must now have permits requiring them to mitigate water quality impacts from stormwater outfalls. In December 1999, the EPA released its Storm Water Phase II Final Rule which requires operators of regulated small municipal separate storm sewer systems (MS4's) to obtain a National Pollutant Discharge Elimination System (NPDES) permit and develop a stormwater management program designed to prevent harmful pollutants from being washed by storm water runoff into the MS4 and then discharged from the MS4 into local water bodies (EPA, 2002). Currently, Wells is not designated as a regulated MS4 and therefore does not have to comply with this rule. (Note: regulated MS4 designation results from being located within the boundaries of an "urbanized area" as defined by the U.S. Census Bureau; being identified by the NPDES permitting authority as having a stormwater collection system that causes or has the potential to cause adverse impacts to water quality; or substantially contributing to the pollutant load of a regulated MS4 through physical interconnection).

Despite the relative lack of a stormwater collection system in Wells, the Center for Watershed Protection has stated that "bacteria levels in urban stormwater are so high that watershed practices will need to be exceptionally efficient to meet current fecal coliform standards during wet weather conditions (1999)." Watershed managers should be aware that urban runoff has been listed as an extremely difficult problem worthy of a long implementation schedule by the TMDL[14] Federal Advisory Committee (MA DEP, 2002). Furthermore, it should be noted that it may be very difficult to reduce urban stormwater fecal coliform concentrations so that water quality standards are met. The Center for Watershed Protection has concluded that "current stormwater practices, stream buffers and source controls have a modest potential to reduce fecal coliform levels, but cannot reduce them far enough to meet water quality standards in most urban settings (CWP, 1999)." Consequently, more intensive "good housekeeping" practices, such as proper pet waste removal, street sweeping, and reductions in the amount of impervious surfaces, are likely to be necessary to decrease stormwater bacteria loadings (MA DEP, 2002). All of these practices should be considered as essential components in the development of an urban stormwater management plan. The Center for Watershed Protection and the Stormwater Manager's Resource Center have developed a variety of reference documents (including model ordinances) to help municipalities protect local water resources (see Appendix 8 for a list of these).

4.13 Overboard Discharges

As discussed previously, most of the coastal properties in Wells – particularly the area around the estuarine portion of the Webhannet watershed – are serviced by public sewer. Additionally, the targeted shoreline survey conducted with the assistance of Laura Livingston from the DMR in May of 2002 did not identity the presence of overboard discharges adjacent to the estuary. Based on the earlier work of Bright (1996) and recent communications with Wells Sanitary District Superintendent Dennis Thayer, it appears that most attention regarding privately owned wastewater disposal systems should be focused on potentially malfunctioning septic systems. This is discussed in detail below.

[14] The TMDL (or total maximum daily load) defines how much of a pollutant would be the maximum amount that could be discharged daily into a water resource from all sources in a surrounding area, while still allowing the water to be used for drinking water, fishing, swimming and other purposes.

4.2 Control of Nonpoint Sources

According to the EPA, the single largest cause of water quality degradation today results from nonpoint source (NPS) pollution. NPS is the primary reason that nearly 40% of the rivers, lakes and estuaries surveyed throughout the country fail to meet basic water quality use classifications for swimming or fishing (EPA, 2002). As discussed in section 1.2, NPS occurs when rainfall, snowmelt, or irrigation runs over land or through the ground, picks up pollutants, and deposits them into rivers, lakes, and coastal waters or introduces them into groundwater. It also produces adverse changes to the vegetation, shape, and flow of streams and other aquatic systems. For example, improper development or excessive pollutant loads can damage wetlands plants that provide protection against sudden increases in stream flow and harmful alterations of watercourses. NPS pollution is widespread because it can occur any time activities disturb the land or water. Agriculture, forestry, grazing, urban runoff, construction, physical changes to stream channels, and habitat degradation are all potential sources of NPS pollution. Careless or uninformed household waste disposal also contributes to NPS pollution problems (EPA, 2002). From the perspective of bacterial contaminants, NPS pollution can originate from a variety of sources, including improperly managed livestock and pet waste, failing septic systems, improperly treated boat waste, leaking sewer lines and wildlife. Each of these will be discussed in further detail below.

4.21 Wildlife Components

As might be expected, wildlife (including waterfowl) represents potentially significant sources of fecal contamination in many coastal watersheds, as is certainly the case in the Webhannet watershed. The ribotyping results clearly implicate the combined category of wildlife as the largest overall contributor of fecal contamination. In both the local and regional reference library databases, 45% of all ***identified*** ribotypes originated from wildlife. These values drop to 24% and 32%, respectively, when including unidentified ribotypes (Figures 24 and 25). Figure 26 indicates the relative geographic distribution of wildlife ribotypes throughout the watershed and the animals representing the most significant proportions of identified ribotypes are indicated in Figures 30 and 31. In both the local and regional databases, coyotes are by far the largest contributors, followed by raccoon, fox and deer. These findings correspond well with anecdotal information provided by local animal trappers.[15]

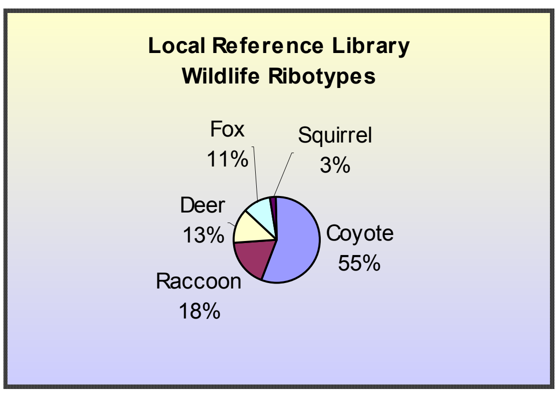


Figure 30. Species composition of ribotypes from local reference library.

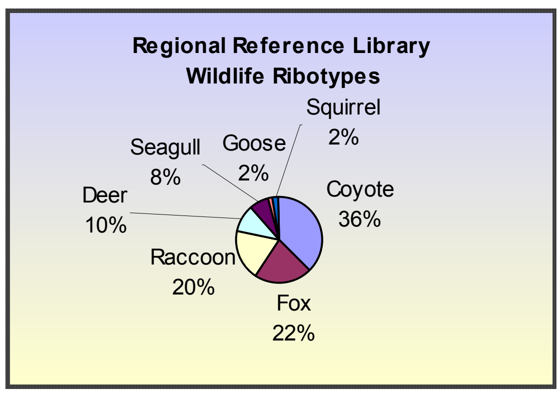


Figure 31. Species composition of ribotypes from regional reference library.

Possible control strategies for reducing fecal loadings from wildlife include relocating or killing a number of animals from problem species. Researchers in other parts of the country have had some measure of success with these strategies. In Maryland, a local shellfish harvesting area was reopened after removing approximately 100 raccoons over a six-month period (Smith, 1996). In late 2002, the Town of Wells allowed for the taking of approximately 28 deer from Laudholm Farm and Drakes Island as part of an effort to reduce the burgeoning population there. However, as noted by microbial source tracking Virginia Tech researcher George Simmons, "What do we do when several of the citizens in the watershed say they don't want the animals trapped or shot and prefer to sacrifice the water quality

[15] Dana and Cindy Johnson (also known as "The Creature Catchers") have extensive first hand knowledge of and experience with wildlife populations in the Town of Wells. They provided information on species distributions and abundance to MST Project staff.

because no one is using it for economic gain anyway? In other words, why kill the animals just for the sake of reducing the fecal coliform numbers (Blankenship, 1996)?" In April 2003 the Maine Legislature enacted a bill to prohibit coyote snaring after a series of highly contentious public hearings. Many of the public comments were offered by animal welfare advocates. Therefore, given the potentially controversial nature of any initiative to further reduce animal populations beyond levels currently allowed through hunting and trapping, public input should also be a crucial consideration before undertaking such initiatives.

4.22 Septic System Controls and Inspection

Maine is a predominantly rural state and ~~is~~ therefore relies heavily on privately owned subsurface disposal facilities (i.e., septic systems – Figure 32). Most of the area to the west of Route 1 in the Webhannet watershed uses septic systems for domestic wastewater disposal (Figure 5). These systems, when properly designed and installed, can effectively treat wastewater without threatening surface water quality. However, septic system effectiveness is also strongly dependent on regular and timely inspection and pumpout. Malfunctioning systems can potentially discharge bacteria to surface waters in concentrations ranging from 10^6 to 10^7 MPN/100 mL (Metcalf and Eddy, 1991). National and local studies have indicated that septic systems experience significant failure rates that typically range between 1% and 5% per year – and sometimes much higher depending on the region (De Walle, 1981). As discussed in section 4.11, it appears that the collection system for the Wells Wastewater Treatment Facility is relatively leak free since there was virtually no correlation between precipitation and plant flows. Therefore, it may be reasonable to hypothesize that most of the human ribotypes identified in the Webhannet watershed originated from failing septic systems. Earlier work by Bright (1996) and recent comments from the Wells Sanitary District Superintendent also appear to support this hypothesis. As discussed in section 3.10, the most significant sources of human ribotypes are clustered to the east of Route 1 in an area almost entirely serviced by public sewer near the outlets of Popes Creek and the Webhannet River (sample sites WE06 and WE01, respectively, in Figure 26).

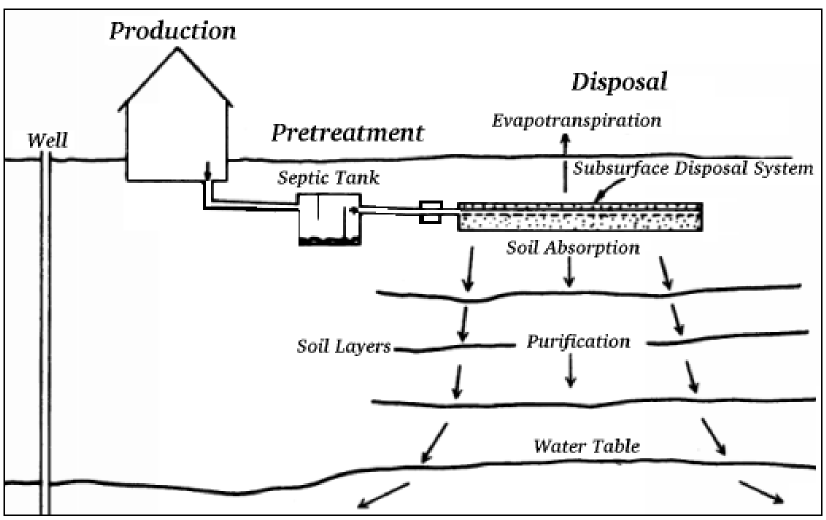


Figure 32. Typical cross-section of septic system.

Identifying all of the properties in the sewered areas of town that are still using septic systems should be the first step in attempting to mitigate bacterial contamination from human sources. Septic systems for each of these properties should then be inspected to determine whether they are functioning properly. Systems determined to be malfunctioning should either be repaired or replaced with pubic sewer. Since some of the human ribotypes could also have originated from further up in the unsewered portions of the watershed, it would be useful to identify all of the properties there that rely on septic systems. This could be accomplished by comparing the town's parcel database with the Sanitary District's sewer user database. All built parcels with bathroom facilities not included in the Sanitary District's sewer user database would necessarily have to be relying on septic systems for wastewater disposal. There are approximately 6,500 records in the parcel database and approximately 2,800 records in the sewer user database (most records in both databases derive from the Webhannet watershed).

While not all parcels are occupied by buildings and many sewer user records apply to the same parcel (for multiplexes and commercial properties), the number of parcels on septic systems is clearly quite considerable. Therefore, database management strategies should be developed for narrowing the list of potentially suspect septic systems.

One such strategy might involve joining building permit records with septic system records and then querying the combined database for all building permits issued before a certain date – perhaps 15 to 20 years ago. Here the assumption would be that older septic systems (installed when the permit was issued and the house was built) are more likely to fail as they approach the end of their design life. Once these systems have been identified, informational brochures could be sent to the property owners summarizing the public health risks associated with failing systems (see Appendix 9 for an example). A more elaborate and ambitious strategy would involve developing a tracking and mandatory scheduling system for septic tank pumping frequency. Communities around the country – including Brunswick, Maine – are beginning to implement such systems to mitigate bacterial contamination from human sources. In 2001, South Kingstown, Rhode Island, enacted a mandatory on-site wastewater management inspection program that establishes septic system maintenance schedules by using specialized computer software.[16] The Massachusetts Department of Environmental Protection has developed free software[17] to be used by municipalities for this purpose. Given the relatively high likelihood that a significant portion of bacterial contamination from human sources is originating from malfunctioning septic systems, the Town of Wells or Wells Sanitary District may want to seriously consider one of these approaches.

4.23 Boat Waste

Marinas and recreational boating are increasingly popular uses of coastal areas. The growth of recreational boating, along with the growth of coastal development in general, has led to a growing awareness of the need to protect waterways. When these facilities are poorly planned or managed they may pose a threat to the health of aquatic systems (USEPA, 1993). Fecal coliform levels in marinas and mooring fields become elevated near boats during periods of high boat occupancy and usage. National Oceanic and Atmospheric Administration (NOAA) has identified boating activities (the presence of marinas, shipping lanes, or intracoastal waterways) as a contributing source in the closure to harvesting of millions of acres of shellfish-growing waters on the east coast of the United States (Leonard et al., 1989). Two of the most important factors in successfully preventing sewage discharge are providing "adequate and reasonably available" pumpout facilities and conducting a comprehensive boater education program (USEPA, 1991).

Wells operates a harbor area that contains a private marina, restaurant and the Wells Harbor Community Park. Wells Harbor was dredged in 2001 and new mooring facilities were established along with the dredging project. The harbor provides 88 slips and 62 moorings for vessels up to 44 feet in length. According to the town's assistant harbormaster, only about 30% of these vessels are equipped with toilet facilities.[18] To provide an environmentally responsible disposal option for sanitary waste, the town installed a boat pumpout station last year that discharges directly into the public sewer. Use of these facilities was initially quite limited, which appears to support the notion that few vessels have toilets. It could also be due to a lack of awareness on the part of boat owners regarding the availability of pumpout facilities. DMR water quality monitoring data from a sampling location adjacent to the marina indicate that fecal coliform levels have consistently met state standards for shellfish harvesting. Indeed, fecal coliform results from this location (DMR site D19) are among the lowest in the estuary (Table 1). Thus, it appears that boat waste poses a minimal risk to the shellfish harvesting areas in the estuary. However, a boater education program may still be warranted to continue this trend. Appendix 10 contains information on the essential elements of such a program.

[16] Town of Kingstown, RI Department of Public Services (www.southkingstownri.com/code/pw_onsitewaste.cfm)
[17] SepTrack Septic System Software Tracking Page (www.buzzardsbay.org/septrfct.htm)
[18] Conversation with Doug Knox on 4/21/03.

4.24 Pets and Pet Waste

Pet waste represents a potentially significant source of fecal contamination in surface waters, particularly in urbanized areas. Microbial source tracking research in Seattle found that nearly 20% of identifiable bacterial isolates originated from dogs (Trial, 1993). These bacteria can pose health risks to humans and other animals from the potential spread of disease. Feces from household pets, such as cats and dogs also contain greater concentrations of fecal coliform than human feces (Scott, 2003). It has been estimated that for watersheds of up to 20 square miles draining to small coastal bays, two or three days of improperly managed droppings from a population of about 100 dogs would contribute enough bacteria and nutrients to temporarily close a bay to swimming and shellfishing (USEPA, 1993). A rule of thumb for determining the number of dogs in a particular locale is approximately 1 dog per 10 people producing an estimated 0.5 pound of feces per dog per day (MADEP, 2002). According to the most recent U.S. Census figures, there were 9,400 year-round residents living in Wells in the year 2000. This year, the Wells town clerk issued 1,307 dog licenses.[19] Thus, there are approximately 1.4 dogs for every 10 year-round residents in Wells with a combined fecal load of approximately 650 pounds per day – more than enough to potentially contaminate the 13.5 square mile Webhannet watershed. Microbial source tracking results for the Webhannet watershed indicate that over 13% of identifiable isolates originated from domestic dogs (none were identified for cats).

While residents typically seem to recognize that dog waste can be a water quality problem (Hardwick, 1997; Swann, 1999), they generally rank it as the least important local water quality problem (Syferd, 1995 and MSRC, 1997). These findings strongly suggest the need to dramatically improve watershed education efforts to increase public recognition about the water quality and health consequences of dog waste. Pet waste collection as a means of reducing fecal contamination involves using a combination of educational outreach and enforcement activities to encourage residents to clean up after their pets. Pet waste collection programs use pet awareness and education, signs, and pet waste control ordinances to alert residents to the proper disposal techniques for pet droppings. In some parts of the country, the concept of parks or portions of parks established specifically for urban dog owners has gained in popularity. With provisions for proper disposal of dog feces and siting and design to address stormwater runoff, these parks may represent another option for protecting local water quality. The town of Wells currently provides dog waste collection stations at several beach locations and has an ordinance (Chapter 80, Article 1, § 80-5) specifically prohibiting improper dog waste disposal. Additional measures that could be used to further promote proper pet waste disposal include providing informational brochures to residents when they register their dogs and publishing appropriate pet waste disposal methods in the local Chamber of Commerce's monthly newsletter for visiting tourists. Appendix 11 provides more information on the establishment of a pet waste management program.

4.25 Summary of Management Recommendations

The ribotyping results were used to develop a management plan for reducing fecal contamination in the Webhannet watershed. Additional data sources used to corroborate the ribotyping results included the work of previous researchers; field surveys for the Webhannet Estuary and upper freshwater portions of the watershed; customized maps of land cover / habitat types and public sewer line locations; a correlation analysis of precipitation and sewage flow data for the Wells Wastewater Treatment Facility; and local knowledge of wildlife prevalence and distribution. The recommendations offered in this plan are summarized below for each of the identified sources.

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[19] Personal communication with Wells town clerk, 7/23/03.

Human Sources

- Identify any remaining septic systems in sewered portion of watershed and inspect for proper functioning. Malfunctioning systems should either be repaired or replaced with public sewer.
- Identify oldest septic systems in unsewered portion of watershed and provide informational brochures (Appendix 9) to property owners of these systems. Also consider an inspection program to identify malfunctioning systems.
- Provide informational brochures to all owners of septic systems in watershed.
- Consider establishing septic system tracking program that establishes maintenance schedule for property owners. Refer to models established by municipalities elsewhere (see Appendix 9).
- If none of the above measures noticeably reduce fecal contamination levels in areas where human sources were identified, then re-evaluate public sewer system for existence of extraneous leakage (known as infiltration and inflow, or I&I) in these areas. Repair leaking pipe sections as appropriate.
- Increase efforts to promote use of boat pumpout facilities at Harbor Marina through dissemination of informational brochures to boat owners.
- Continue to work with Maine Department of Marine Resources to ensure that no overboard discharges exist along Webhannet estuary.
- Consider use of portable toilet facilities in areas where people may be defecating directly on the ground (i.e., vacant lot next to old Dexter Shoe store and Webhannet River Park).

Wildlife Sources

- Solicit comprehensive public input before considering reduction plan (relocation or hunting) for problem species (coyote, raccoon, fox and deer).
- Provide informational brochures at local civic buildings and commercial establishments informing all residents in watershed about ways to reduce attraction of problem species (see Appendix 12).

Livestock Sources

- Identify all livestock owners in watershed and provide informational brochures about proper handling of livestock waste.
- Identify all sources of animal manure used as fertilizer (garden and nursery suppliers, local farms) and provide informational brochures at these locations on proper handling of animal fertilizers.

Pet Sources

- Increase efforts to promote proper handling and disposal of pet waste.

4.3 Additional (Future) Monitoring

Ideally, fecal coliform and *E. coli* levels in the Webhannet watershed will decrease following the implementation of these recommendations. An ongoing water quality monitoring program using conventional bacterial test methods, will be needed to measure any reductions in fecal contamination. Results from the DMR's ongoing water sampling program in the Webhannet estuary will determine which areas are suitable for shellfish harvesting. However, it would also be helpful to establish a monitoring program in the upper watershed to identify specific areas that might persist in contributing to elevated bacterial contamination levels. Findings from this study could be used in conjunction with an upper watershed monitoring program to suggest potential sources of fecal contamination. The Watershed Evaluation Team at the Wells National Estuarine Research Reserve might be able to expand their sampling activities to include sites in the upper Webhannet watershed. MST project staff will also be conducting a variety of outreach activities (press releases, articles, public access TV) to inform the public about the findings from this report. The ultimate aim of these combined efforts is to reopen shellfish harvesting areas in the Webhannet estuary while also serving as a model for similar efforts elsewhere.

5.0 PUBLIC PARTICIPATION / PUBLIC OUTREACH

5.1 Volunteer Participation

Volunteers were recruited as water sample collectors for the project by posting fliers at local universities, libraries and churches; placing an announcement on the local public access TV channel; asking the Wells NERR volunteer coordinator for references; and contacting local high schools and inviting volunteers from the 1999-2000 project to join. Throughout the project year, new volunteers became involved and were appropriately trained. Their dedication greatly helped the project. Over 30 volunteers (Appendix 1) collectively logged a total of 261 hours of water sampling, lab assistance and watershed surveying through the winter, spring and summer. In addition to contributing their time and energy, they developed a much greater appreciation for the relationships between human activities and impacts to the local environment, particularly with respect to water quality degraded by bacteria in their watershed. Feedback questionnaires were provided to all volunteers at the end of their service (Appendix 13). Without exception, the responses were overwhelmingly positive in terms of how much knowledge each of them gained over the course of the project.

5.2 Web Site Development

In mid-March, 2002, MST Project staff member Cayce Dalton developed and uploaded a Web site dedicated to this project (www.umseagrant-mst.org). The Web site is intended as an outreach tool for researchers, volunteers, state and municipal officials, steering committee members and the general public. The site, created and maintained using Dreamweaver Web design software, contains tables and graphs of all sampling results, a maps section, downloadable field sheets, news, and slideshows. It has proven to be a valuable resource for volunteers interested in tracking the project's progress, as well as for other interested parties from around the country, including water quality monitoring groups, state and federal agency personnel and MST researchers.

5.3 Conference / Workshop Presentations

MST Project staff gave numerous presentations to local, state, regional and national audiences. These included hands-on demonstrations that allowed participants to conduct mock membrane filtration analyses, overviews of analytical activities in the Wells National Estuarine Research Reserve lab, poster displays and conference presentations.

- November 28, 2001: Wells Clam Commission. Description and status of project.
- March 16, 2002: Going Green, Wells, ME. MST demonstration booth.
- April 21, 2002: Earth Day, Wells, ME. MST demonstration booth (including hands-on membrane filtration station).
- April 25, 2002: MST overview for Watershed Evaluation Team, Wells, ME.
- May 23, 2002: New England Interstate Water Pollution Control Commission's 13th Annual Nonpoint Source Conference, Boothbay, ME.
- June 19, 2002: Casco Bay Estuary Project's 2nd State-of-the Bay Conference, Freeport, ME.
- July 15, 2002: Maine Sea Grant Extension State of Maine Beaches Conference, Saco, ME.
- September 20, 2002: Maine Wastewater Control Associations Annual Conference, Phippsburg, ME.
- October 5, 2002: National Estuaries Day, Wells, ME.
- October 24, 2002: Northeast Beaches Conference, Woods Hole, MA.
- November 7, 2002: MBLR Rivers at Risk Workshop, Wells, ME.
- January 22, 2003: University of New England Service Learning Group, Biddeford, ME.
- January 28, 2003: EPA Technology Transfer Conference, Cocoa Beach, FL.
- February 6, 2003: Maine Conservation Corps Monthly Meeting

- March 15, 2003: Going Green for St. Patrick's Day Fair organized by Wells NERR, held at York Public Library, ME
- March 27, 2003: Maine Department of Environmental Protection Stream Team Summit, Bowdoin College, ME.
- April 16, 2003: Maine Water Conference, Augusta, ME.
- May 8, 2003: Northeast Shellfish Sanitation Association, Danvers, MA.
- May 9, 2003: New England Estuarine Research Society / Southern New England Chapter of American Fisheries Society Joint Meeting
- June 7, 2003: Student Research Symposium, National Consortium of Specialized Secondary Schools for Mathematics, Science and Technology,. Wells National Estuarine Research Reserve, Wells, ME.

5.4 Media Relations

Several media outlets were used to disseminate information in the form of a PowerPoint presentation and articles regarding the Webhannet MST project. Among these were:

- Wells local access cable TV station, Channel 3
- Maine Sunday Telegram – July 21, 2002
- EPA Coastlines – December 2002, Issue 12.6
- National Small Flows Clearinghouse / Small Flows Quarterly – Spring 2003, Volume 4, Number 2

5.5 Community Outreach for Plan Implementation

As stated previously, one of the main project goals is to conduct outreach activities to assist in the implementation of management recommendations that will reduce fecal contamination in the Webhannet watershed. The process began by sending draft copies of this report to all members of the Webhannet MST Steering Committee so their comments could be incorporated into the final version. This Webhannet watershed report will be forwarded to the following local, regional and state agencies and presentations of findings will also be given to allow for discussion and action planning based on study results.

- Town of Wells
- Southern Maine Regional Planning Commission
- Wells National Estuarine Research Reserve (WNERR)
- Maine State Planning Office / Maine Coastal Program (MCP)
- Maine Department of Marine Resources (DMR)
- Maine Department of Environmental Protection (DEP)
- US Environmental Protection Agency (EPA), Region 1

The findings will also be presented at meetings and conferences of related regional / national professional organizations, including at least one of the following regional or national professional organizations: the Northeast Shellfish Sanitation Association (NESSA) and the Interstate Shellfish Sanitation Conference (ISSC). Outreach activities to inform the general public about the study findings will also be conducted in several ways. A press release summarizing the findings will be forwarded to local and regional news media including:

- York County Coast Star
- Portland Press Herald / Maine Sunday Telegram
- Maine Public Radio

Articles summarizing the findings will be included in the newsletters of cooperating organizations including:

- Wells Chamber of Commerce (Wells Guide, Chamber Newsletter)

- Wells Regional Weekly Publication (Making It At Home)
- WNERR (Watermark)
- MCP (Maine Coastline)
- MCP / Maine Sea Grant / DEP / DMR (Maine Shore Steward)
- York County Cooperative Extension (Extension Horizons)

A brief PowerPoint presentation summarizing the findings will be produced and broadcast on the Wells local access cable TV station; it will also be posted on the MST Project web site (and other cooperating governmental agencies if possible) along with the entire final report, executive summary and press release. Finally, the study findings will be shared at public events occurring at WNERR during the summer of 2003.

As mentioned previously, outreach materials will be provided to specific audiences regarding actions that can be taken to reduce fecal contamination in the Webhannet watershed.

Homeowners with septic systems

Efforts will be made to reach homeowners with septic systems in the sensitive shoreland zone abutting the watershed's rivers and tributaries. Shoreland property owners who are not connected to the town's sewer system will be identified. A direct mailing will be done to these property owners that briefly shares the study findings, discusses the economic impact of closed clam flats and recommends simple steps for proper septic system maintenance (Appendix 9). Similar outreach materials will be distributed through town public service centers where homeowners routinely visit such as the transfer station, library, post office and town offices. Outreach materials will also be disseminated to local real estate agents to pass along to new residents who purchase shoreland property with septic systems. The same would be done with septic pump out service providers to distribute to their customers.

Registered boat owners in Wells

In cooperation with the Town of Wells, outreach materials (Appendix 10) will be mailed to registered boat owners summarizing the results of this study and encouraging the use of the Wells Harbor boat pump out facility.

Pet owners in Wells

Outreach efforts will be coordinated with the municipal offices that administer dog licensing and beach pass programs in Wells. Public information materials on dog waste management (Appendix 11) and a summary of the results from this study will be distributed to dog owners when registering their dogs or acquiring their beach passes. Similar materials will also be distributed to local veterinary practices and dog training establishments, making these informational materials available to dog owners during visits.

Gardeners and farmers in Wells

Given the potential for fecal contamination from animal manures used by homeowners as fertilizers, outreach materials will be provided at local commercial greenhouses and gardening outlets. Appendix 14 provides a summary of recommendations for proper use of both chemical fertilizers and animal manures.

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APPENDICES

Appendix 1: Citizen Volunteer List

Andrea Leonard	Denise Jarrett *	Liz Hogan
Alex Radcliffe	Derek Thibault	Mandy Sumner
Andrew Stafford	Don Emery **	Mary Anne Hawkins
Barbara Perry	Ed Baker*	Michael Nadeau
Bruce McGarry	Elizabeth Brockaway*	Michelle Dennis*
Cara Ellis	Erica Lindgren	Michelle Somers*
Carol Davis*	Erick Carlson	Naomi Shike
Carol Thompson	Glorya Laughton	Olive Morest *
Cathy Walker	Jamie Koehler	Richard Lane **
Charles Lord	Jan Wirth*	RJ Mere
Cindy Johnson	Jean Hamlin	Robin Stanley **
Dan Doolittle **	Jen Bridges	Roy Bishoff **
Dana Johnson	Jessica Szafranski	Sarah McKay
Dana Knudson	Kate Durost	Ted Cunningham
Daria Micheletti	Kate Ostergren*	Wayne Cronin **
Dawn Morse	Lily Pearmain*	Will Heiser

* Sampled for 5-9 dates
** Sampled for more than 10 dates

Appendix 2: Webhannet Steering Committee List

Kristen Whiting-Grant	Principal Investigator / Project Manager – Maine Sea Grant
Stephen Jones	Co-principal Investigator – UNH Jackson Estuarine Laboratory
Michele Dionne	Co-principal Investigator – Wells National Estuarine Research Reserve
Cayce Dalton	Volunteer Leader – Americorps / Maine Conservation Corps
Fred Dillon	Graduate Assistant – Maine Sea Grant / Muskie School of Public Service
Laura Livingston	Maine Department of Marine Resources
Jonathan Carter	Town of Wells
JT Lockman	Southern Maine Regional Planning Agency
Caitlin Mullan	Brown University Ph.D. Candidate (and former Webhannet watershed researcher)
Don Kale	Maine DEP
Geoff Coombs	York County Natural Resources Conservation Service

Appendix 3: Water Sample Collection Field Sheet

Water Sample Collection Field Sheet
Microbial Source Tracking in Two Southern Maine Watersheds, Webhannet Watershed 2001-2002
Wells National Estuarine Research Reserve, Fred Dillon and Cayce Dalton, 207-646-8645 x 103

Samplers: Hours Volunteered Today:	Date: Current Weather:____________________ Air Temp:_____ Start of Last Rain:

Sample Site	Time of Sample	Water Temp (C°)	Water Flow / Level *	Ice Cover **	Sample Taken From	Depth Where Sample Taken	Comments
					Edge / middle		
					Edge / middle		
					Edge / middle		
					Edge / middle		
					Edge / middle		
					Edge / middle		
					Edge / middle		
					Edge / middle		
					Edge / middle		

*Water Flow / Level: Very Low, Low, Medium, High, Very High
** Ice Cover: Partial, Full

Given by:_______________________________________ Date: ______________ Time: ______________

Received by: ____________________________________ Date: ______________ Time: ______________

Appendix 4: JEL Sample Delivery Form

Microbial Source Tracking in Two Southern Maine Watersheds
Researchers: Fred Dillon & Cayce Dalton, Wells National Estuarine Research Reserve, 207-646-1555, ext 103
fdillon@wellsnerrcec.lib.me.us, cayce@wellsnerrcec.lib.me.us

Microbial Source Tracking Project		
Site Name:	Type of Sample	
	Fecal:	Water:
Site Description: Street: Town: **Wells, Maine** Watershed: **Webhannet Watershed** Date Sample Collected: Time:	Animal Species: Location:	Water Temperature: % DO Saturation: DO: pH: Conductivity: Location: Instream Seep Swale Storm Drain Other__________ Air Temperature:
Sampled By:		Weather:
Parameters: Air Temperature:		Laboratory Sample Notes:
E. coli:		Results: FC_____________cfu/100ml EC_____________cfu/100ml
Flow Rate: Yes:__________________ f/s No		Selected for ribotyping: Yes No Date of ribotyping: ______________

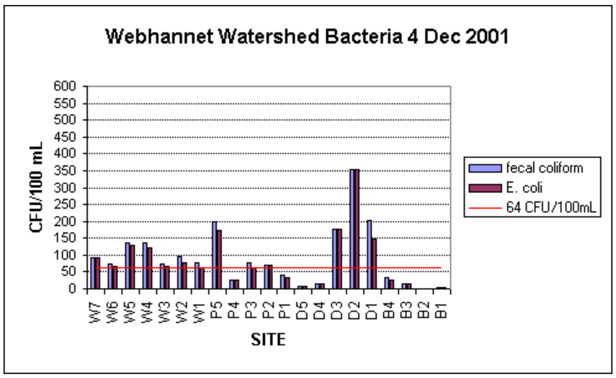
Comments and Site Sketch / Description:

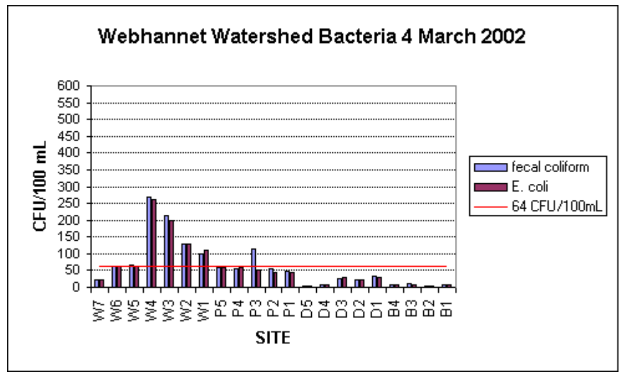
```
Site name (and T-soy plate labeling) indicates date of water sample collection
(yyyymmdd), site designation (e.g. "B1"), and isolate designation "A-J" (with A-E on 1 T-
soy plate and F-J on the other).

Sample sites with "1" designations are at the mouths of rivers / streams and higher
numbers denote sites higher in the watershed.

   WE = Webhannet Estuary
   W = Webhannet River (predominantly freshwater)
   P = Popes Creek (predominantly freshwater)
   D = Depot Brook (predominantly freshwater)
   B = Blacksmith Brook (predominantly freshwater)
```

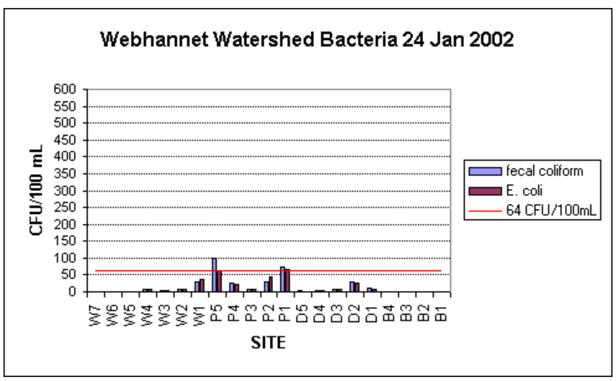
Delivered to JEL Date:________________________________ Time:______________________

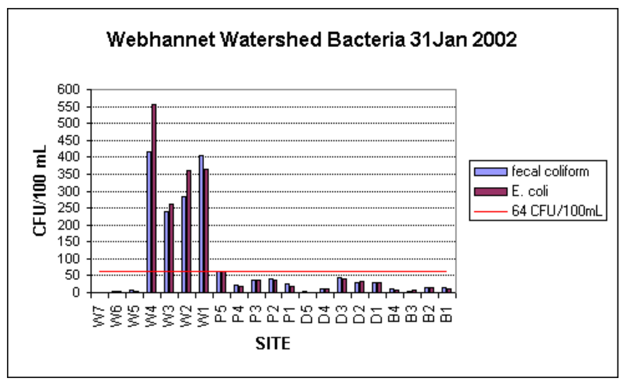
Appendix 5: Graphs of Webhannet watershed bacterial concentrations

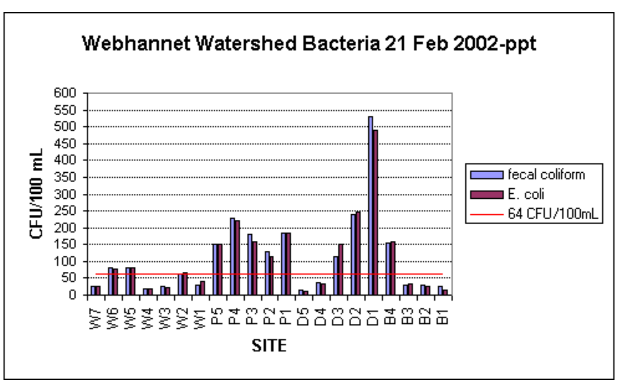
The graphs below describe the bacterial concentrations in the Webhannet watershed from December 2001 to September 2002. From December to May, the freshwater tributaries to the estuary were tested (Webhannet River, Popes Creek, Depot Brook, and Blacksmith Brook). From June to September, the estuary was tested, along with the approximate head of tide sites for each stream. Both fecal coliform and *E. coli* concentrations were obtained using the mTEC + urea membrane filtration method and are measured in colony forming units (CFU) per 100mL of sample. Ribotyping was conducted on a subset of these bacteria. Note also that the vertical scale changes between these two sampling periods to reflect higher bacterial levels in the summer. The line indicating 64 CFU/100mL is shown to represent the seasonal standards for class B recreational waters in Maine, although this standard did not necessarily apply to all waters tested.

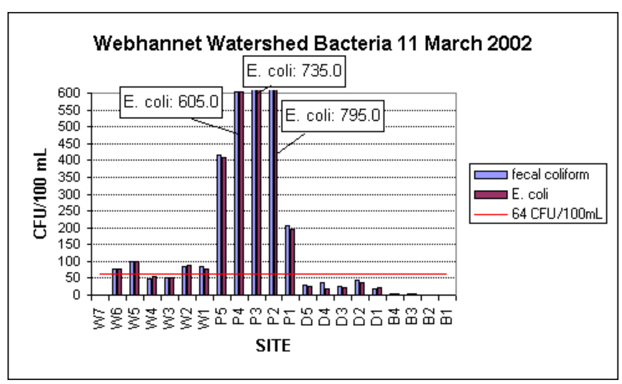


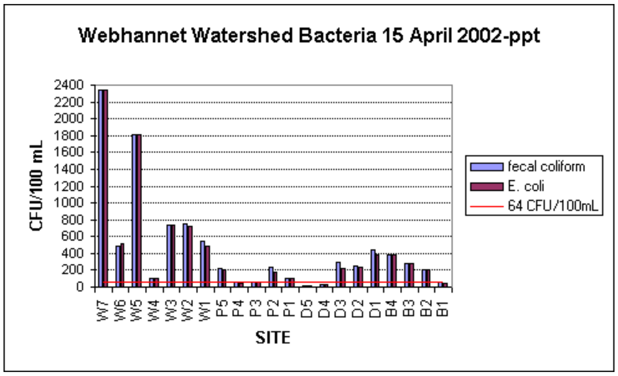


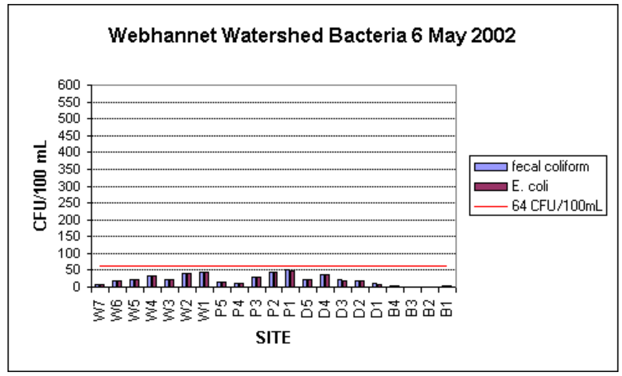


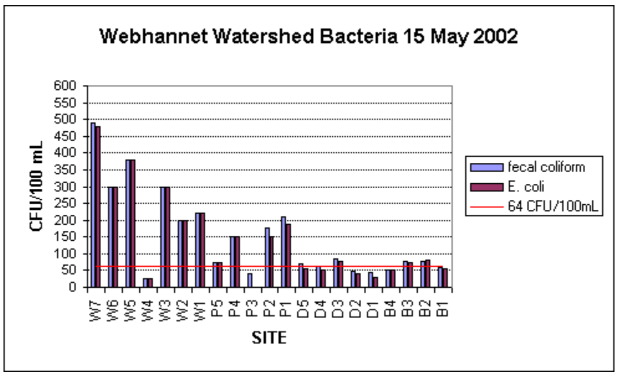


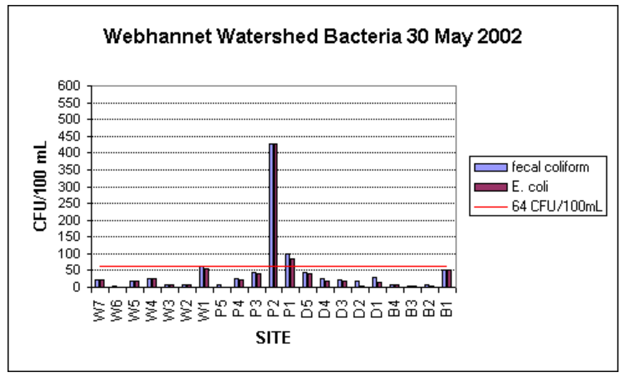


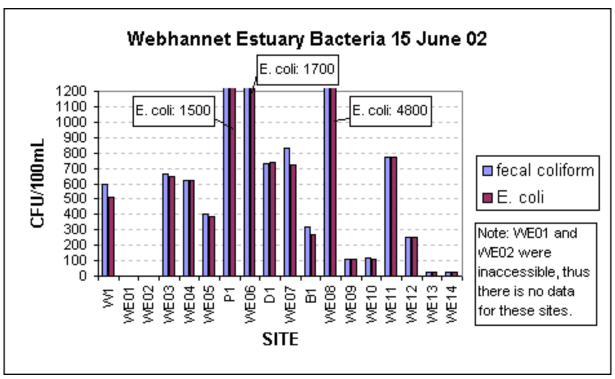


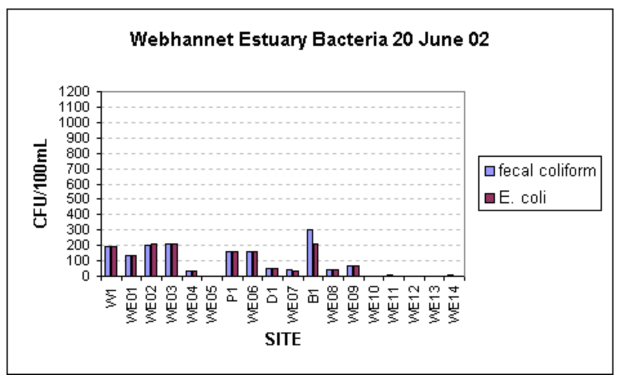

Webhannet Watershed Bacteria 15 April 2002-ppt
CFU/100 mL
SITE
W7 W6 W5 W4 W3 W2 W1 P5 P4 P3 P2 P1 D5 D4 D3 D2 D1 B4 B3 B2 B1
fecal coliform
E. coli
64 CFU/100mL

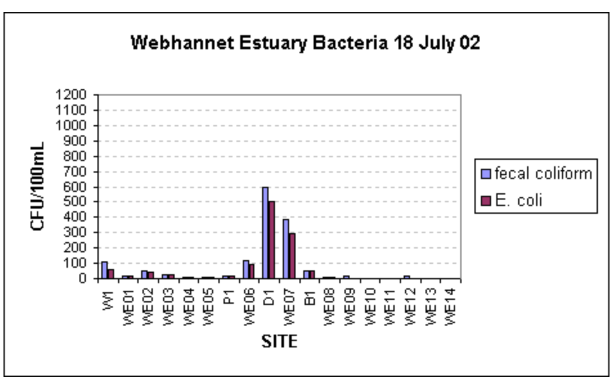

Webhannet Watershed Bacteria 6 May 2002
CFU/100 mL
SITE
W7 W6 W5 W4 W3 W2 W1 P5 P4 P3 P2 P1 D5 D4 D3 D2 D1 B4 B3 B2 B1
fecal coliform
E. coli
64 CFU/100mL

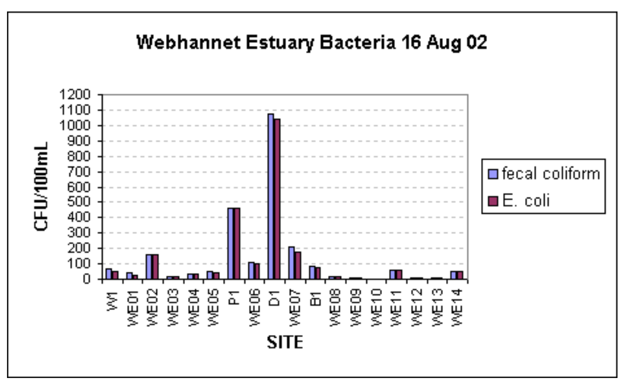

Webhannet Watershed Bacteria 15 May 2002
CFU/100 mL
SITE
W7 W6 W5 W4 W3 W2 W1 P5 P4 P3 P2 P1 D5 D4 D3 D2 D1 B4 B3 B2 B1
fecal coliform
E. coli
64 CFU/100mL

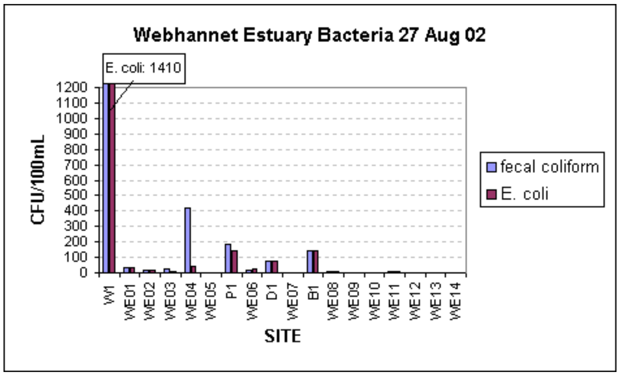

Webhannet Watershed Bacteria 30 May 2002
CFU/100 mL
SITE
W7 W6 W5 W4 W3 W2 W1 P5 P4 P3 P2 P1 D5 D4 D3 D2 D1 B4 B3 B2 B1
fecal coliform
E. coli
64 CFU/100mL

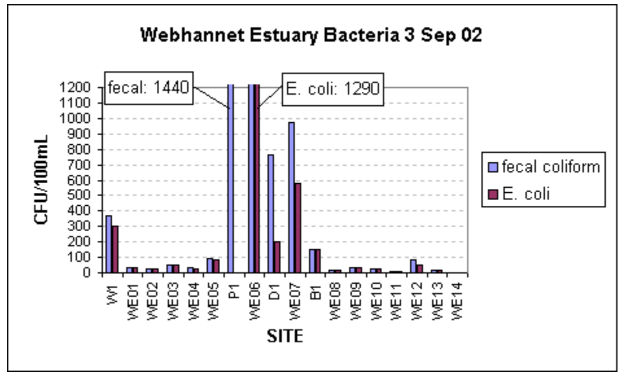

Webhannet Estuary Bacteria 15 June 02
E. coli: 1700
E. coli: 1500
E. coli: 4800
CFU/100mL
SITE
W1 WE01 WE02 WE03 WE04 WE05 P1 WE06 D1 WE07 B1 WE08 WE09 WE10 WE11 WE12 WE13 WE14
fecal coliform
E. coli
Note: WE01 and WE02 were inaccessible, thus there is no data for these sites.

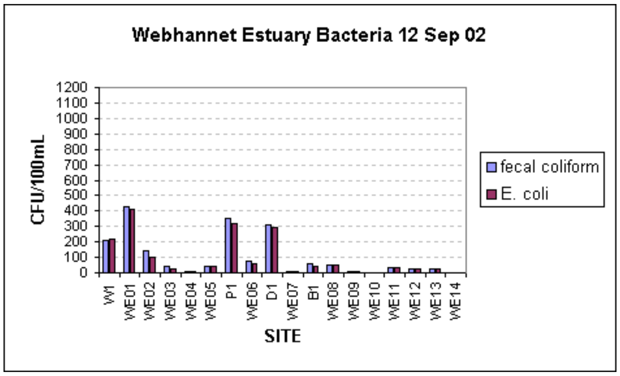

Webhannet Estuary Bacteria 20 June 02
CFU/100mL
SITE
W1 WE01 WE02 WE03 WE04 WE05 P1 WE06 D1 WE07 B1 WE08 WE09 WE10 WE11 WE12 WE13 WE14
fecal coliform
E. coli

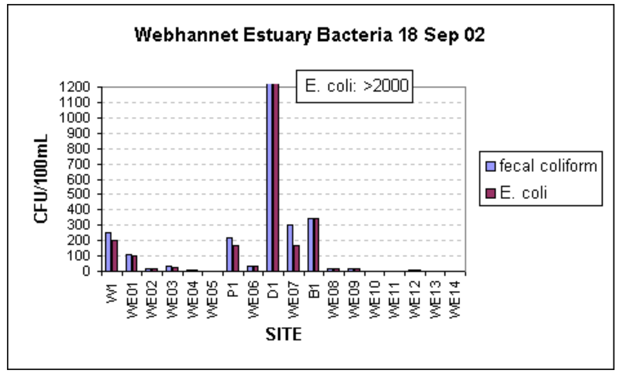

Webhannet Estuary Bacteria 18 July 02
CFU/100mL
SITE
W1 WE01 WE02 WE03 WE04 WE05 P1 WE06 D1 WE07 B1 WE08 WE09 WE10 WE11 WE12 WE13 WE14
fecal coliform
E. coli

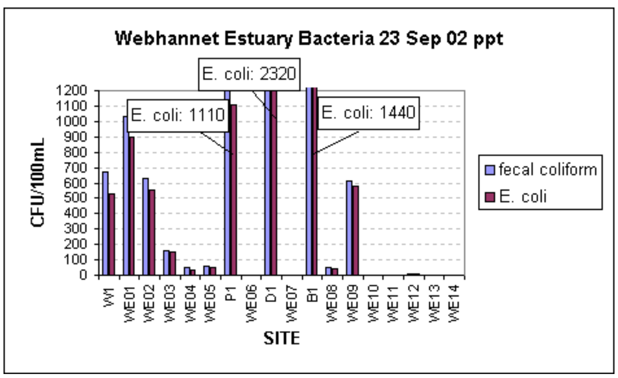

Webhannet Estuary Bacteria 16 Aug 02
CFU/100mL
SITE
W1 WE01 WE02 WE03 WE04 WE05 P1 WE06 D1 WE07 B1 WE08 WE09 WE10 WE11 WE12 WE13 WE14
fecal coliform
E. coli


Webhannet Estuary Bacteria 27 Aug 02
E. coli: 1410
CFU/100mL
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
W1
WE01
WE02
WE03
WE04
WE05
P1
WE06
D1
WE07
B1
WE08
WE09
WE10
WE11
WE12
WE13
WE14
SITE
fecal coliform
E. coli


Webhannet Estuary Bacteria 3 Sep 02
fecal: 1440
E. coli: 1290
CFU/100mL
SITE
fecal coliform
E. coli


Webhannet Estuary Bacteria 12 Sep 02
CFU/100mL
SITE
fecal coliform
E. coli


Webhannet Estuary Bacteria 18 Sep 02
E. coli: >2000
CFU/100mL
SITE
fecal coliform
E. coli


Webhannet Estuary Bacteria 23 Sep 02 ppt
E. coli: 2320
E. coli: 1110
E. coli: 1440
CFU/100mL
SITE
fecal coliform
E. coli

Appendix 6: *E. coli* isolate selection criteria for ribotyping (upper watershed)

Sample Date	W7	W6	W5	W4	W3	W2	W1	P5	P4	P3	P2	P1	D5	D4	D3	D2	D1	B4	B3	B2	B1
4-Dec-01	91.0	67.0	128.0	123.0	68.0	78.0	62.5	173.3	27.0	64.0	70.0	32.0	8.0	14.0	176.0	354.5	148.0	25.0	14.0	1.0	4.0
8-Jan-02	3.3	12.5	11.7	34.0	15.8	11.7	39.0	86.0	19.0	10.0	37.0	28.0	1.7	2.5	24.0	185.0	155.0	1.0	1.0	1.0	5.0
24-Jan-02	1.0	1.0	1.7	7.5	4.2	6.7	36.0	62.0	23.3	6.8	44.1	65.0	0.8	2.5	7.5	27.0	6.7	1.0	1.0	1.0	1.0
31-Jan-02	1.7	3.3	4.2	555.0	260.0	360.0	365.0	64.0	19.2	36.0	36.0	19.0	0.8	9.5	40.0	33.0	29.0	9.0	6.0	15.0	12.0
21-Feb-02	27.0	78.0	80.0	17.5	22.0	65.0	41.0	150.0	220.0	160.0	115.0	185.0	10.8	32.0	150.0	245.0	490.0	160.0	33.0	25.0	14.0
4-Mar-02	21.0	62.0	61.0	260.0	200.0	130.0	110.0	60.0	58.0	53.0	46.0	44.0	2.0	7.5	31.0	22.0	30.0	8.0	9.0	4.0	6.0
11-Mar-02	1.7	77.0	100.0	57.0	52.5	88.3	79.0	410.0	605.0	735.0	795.0	195.0	24.0	16.7	23.0	37.0	21.0	2.0	2.0	1.0	1.0
15-Apr-02	2335.0	515.0	1815.0	98.3	735.0	715.0	490.0	205.0	49.5	60.0	175.0	110.0	7.5	24.0	215.0	235.0	390.0	387.5	279.0	206.0	43.5
6-May-02	8.2	17.3	23.0	32.0	22.0	39.0	43.0	16.4	10.0	30.0	44.0	48.0	21.0	37.5	20.0	17.3	9.1	2.4	1.8	1.0	3.6
15-May-02	480.0	300.0	380.0	27.0	300.0	200.0	~~220.0~~	74.0	~~152.4~~	42.0	150.0	189.1	54.0	51.0	76.0	40.0	28.0	52.0	~~75.0~~	80.0	54.0
30-May-02	21.0	1.0	20.0	25.7	5.7	5.7	54.3	1.0	22.9	40.0	425.7	85.7	40.0	19.0	20.0	2.9	14.3	7.0	2.0	4.5	50.0
Isolate Sets	3	5	6	4	5	7	6	8	2	5	7	8	0	0	4	4	4	2	1	2	3
Max	2335.0	515.0	1815.0	555.0	735.0	715.0	490.0	410.0	605.0	735.0	795.0	195.0	54.0	51.0	215.0	354.5	490.0	387.5	279.0	206.0	54.0
Min	1.0	1.0	1.7	7.5	4.2	5.7	36.0	1.0	10.0	6.8	36.0	19.0	0.8	2.5	7.5	2.9	6.7	1.0	1.0	1.0	1.0
Average	271.9	103.1	238.6	112.5	153.2	154.5	140.0	118.3	109.7	112.4	176.2	91.0	15.5	19.7	71.1	109.0	120.1	59.5	38.5	30.9	17.6
Geo. Mean	18.6	25.3	45.2	53.3	52.0	62.0	89.3	62.2	47.9	47.8	98.5	68.3	6.9	13.6	42.8	50.5	46.0	10.6	7.4	5.4	8.0
Col/100 mL																					
< 64	8.0	6.0	6.0	7.0	6.0	4.0	6.0	4.0	8.0	8.0	5.0	5.0	11.0	11.0	7.0	7.0	7.0	9.0	9.0	9.0	11.0
64-427	1.0	4.0	4.0	3.0	4.0	6.0	4.0	7.0	2.0	2.0	5.0	6.0	0.0	0.0	4.0	4.0	3.0	2.0	2.0	2.0	0.0
>427	2.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0
Geomean > 64	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0

Samples were selected for *E. coli* isolation based on the Maine DEP's seasonal (May 15th-Sept. 30th) water quality standard of 64 colonies / 100 mL for Class B surface waters. Thus, all orange or yellow shaded cells indicate isolated samples (except perhaps for crossed out cells, for which we have no isolate records). Additionally, cells enclosed with red borders were isolated per our records. Totals for the number of isolate sets for each sample site are indicated directly below each site column. Isolate sets consisted of two T-soy agar plates with 5 isolates per plate for each selected site (for a total of 10 isolates per selected sample).

According to the language in the original project proposal, approximately 200 isolates can be ribotyped for the Webhannet River watershed. Since only about 85% of the isolates are presumptively confirmed as *E. coli*, 235 isolates will be selected to provide enough material for ribotyping. The *E. coli* results listed in the table above are for the upper and predominantly freshwater portion of the watershed and represent 60% of the total sampling period (6 of 10 months). Estuarine sampling began in June and will end September. Selecting isolates for ribotyping based on equal weighting for each portion of the watershed results in approximately 140 isolates (or about 14 isolate sets) for the upper freshwater section and 95 isolates (or 9½ isolate sets) for the lower estuarine section.

Deciding which isolates from the upper watershed to ribotype was done primarily by selecting the sites that consistently yielded the highest *E. coli* counts. A significant part of the project's intent is to help resource managers develop targeted mitigation strategies to reduce fecal loadings in the estuary and hopefully reopen closed shellfish harvesting areas. Therefore, it seems reasonable to hypothesize that those sites with consistently high counts potentially represent the greatest fecal loading to the estuary (assuming that they also originate from an area with a significant flow contribution).

The bold red sample site designations in the first row represent those with the highest overall fecal concentrations in terms of geometric and (in most cases) arithmetic means; ten isolate sets were selected for ribotyping from these sites. The remaining isolate sets were selected from sites that either had exceptionally high counts for a particular sampling date or, in the case of B1, to provide background data from a “clean” site. Isolates were also selected throughout the entire sampling period and were more heavily weighted from the Webhannet (5 isolate sets) since it provides the greatest flow contribution to the estuary. Pope's Creek has the next highest number of isolates (4 isolate sets) since it had the highest overall *E. coli* concentrations, followed by Depot Brook with 3 isolate sets and Blacksmith Brook with 2 isolate sets.

Appendix 7: Wells Sanitary District Emergency Response Plan for Sewage Discharge

As of 5/23/01:

WELLS SANITARY DISTRICT
EMERGENCY RESPONSE PLAN

Most of the homes in Wells are connected to the Wells sewage collection system. The Wells Beach is classified prohibited, but the Webhannet River is classified conditionally approved and open from January 1 through April 30. A release of undisinfected effluent from the treatment plant outfall or a break in a sewer line or a malfunction at a pump station may result in sewage impacting water quality in this area.

In the event that there is a release of undisinfected effluent from the treatment plant outfall or an overflow of sewage, from a break in a sewer line or from a pump station, which may jeopardize water quality in the Wells tidal waters, the Wells Sewage Treatment Plant personnel will initiate the following emergency response plan:

On the day of the overflow, or on the first working day after the overflow, plant personnel will give a report on the overflow to the DMR Water Quality personnel (1-207-633-9500 - ask to speak to someone in Water Quality).

The following participant agrees to accept responsibility for carrying out the emergency response plan as indicated:

____________________________________ ______________

Wells Sewage Treatment Plant Date

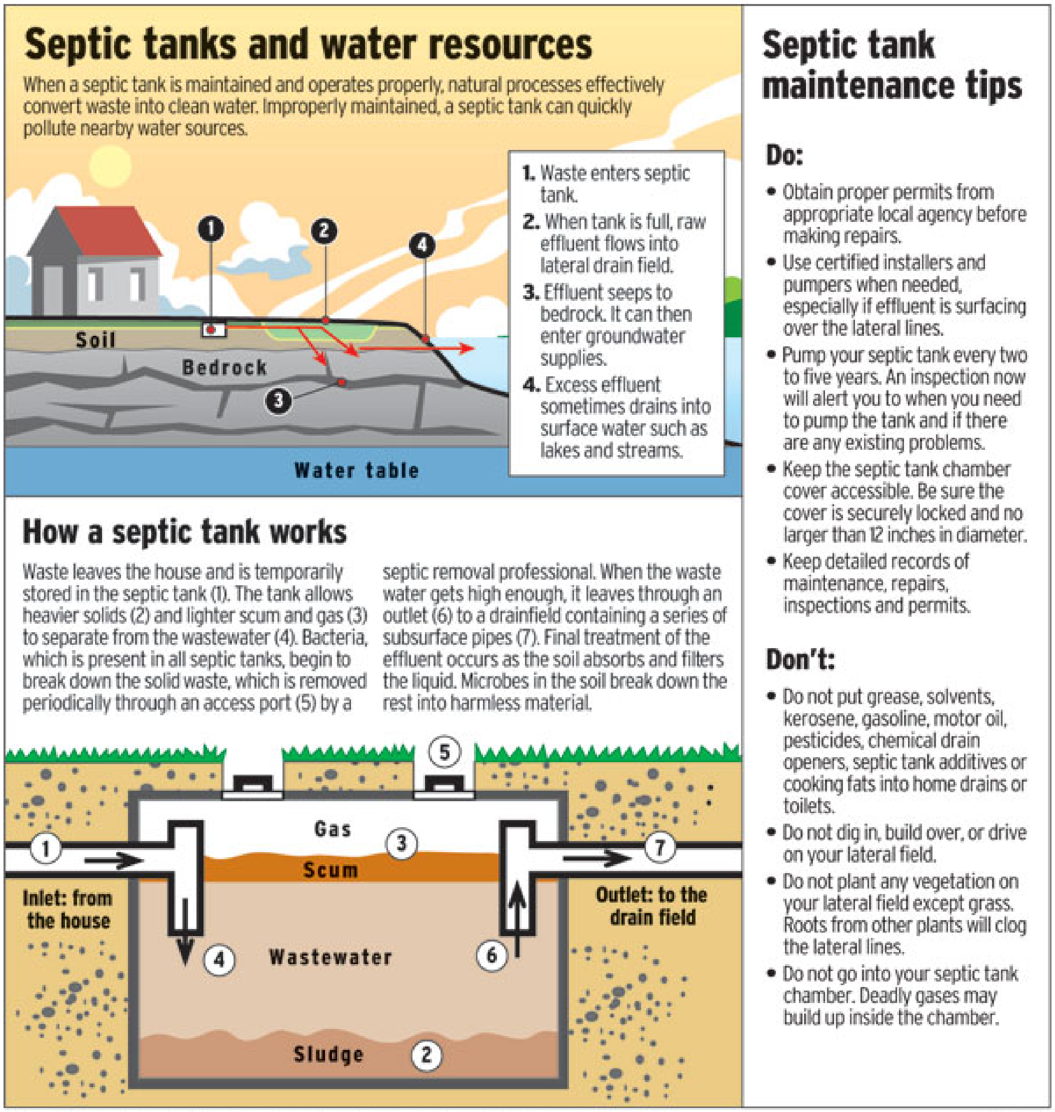
Appendix 8: Center for Watershed Protection: partial list of reference documents for protection of local water resources.

- A Better Guide to Site Planning: www.cwp.org/SPSP/INTRO.PDF (very good resource)
  - Chapter 1 – A Stream Protection Strategy (www.cwp.org/SPSP/chapter_one.pdf)
  - Chapter 2 – The Importance of Imperviousness (www.cwp.org/SPSP/chapter_two.pdf)
  - Chapter 3 – Watershed-Based Zoning (www.cwp.org/SPSP/chapter_three.pdf)
  - Chapter 4 – Stream Protection Clusters (www.cwp.org/SPSP/chapter_four.pdf)
  - Chapter 5 – The Architecture of Stream Buffers (www.cwp.org/SPSP/chapter_five.pdf)
  - Chapter 6 – Headwater Streets (www.cwp.org/SPSP/chapter_six.pdf)
- Site Planning Model Development Principles: www.cwp.org/22_principles.htm
- Codes and Ordinances Worksheet: www.cwp.org/COW_worksheet.htm
- Rapid Watershed Planning Handbook: http://centerforwatershedprotection.goemerchant7.com/index.cgi
- Eight Lessons Learned from the Local Site Planning Roundtable Process: www.cwp.org/lessons.htm
- Model Ordinances for Aquatic Resource Protection: www.stormwatercenter.net/

Contact information

Center for Watershed Protection
8391 Main Street
Ellicott City, MD 21043
Phone: 410-461-8323
Fax: 410-461-8324
Web: www.cwp.org/index.html
Email: center@cwp.org

Appendix 9: Septic System Maintenance Resources



SOURCE: VIRGINIA COOPERATIVE EXTENSION, MISSOURI DEPARTMENT OF NATURAL RESOURCES, NEWS-LEADER RESEARCH NEWS-LEADER

Recordkeeping Folder and Information Package on Septic Systems

- The National Small Flows Clearinghouse (NSFC) offers a septic system information folder, which was developed by the NSFC and reviewed in collaboration with the National Onsite Wastewater Recycling Association (NOWRA) and the Pennsylvania Septic Management Association (PSMA). **The Homeowner Onsite System Recordkeeping Folder (Item #WWBLPE37)** provides a place to record and store information about your system and its maintenance. On the cover of the folder are sections for permit and local health department information and for a description of the system. This description consists of a checklist that covers septic tank and pump size, soil treatment system dimensions, accessories, and household information. Inside are tips for locating your system, space to sketch the location of the system, a safety checklist, and a section for recording the names, addresses, and certification numbers of your system's designer, installer, operation and maintenance provider, and pumper. The cost of this folder is 40 cents plus shipping.

- In addition, the NSFC offers a **Homeowner Septic Tank Information Package (Item #WWPKPE28)**, which provides you with this folder packed with materials that give an overview on septic systems for homeowners. Included are the three brochures mentioned above on how to maintain a septic system and how to recognize potential problems. Also included are the three issues of *Pipeline* also described above that focus on septic system operation and maintenance, management, and what happens when you have your system inspected. A fact sheet on various alternative household cleaning solutions is included that offers safe alternatives over chemical cleansers. The package costs two dollars plus shipping.

  To order the information packages contact the National Small Flows Clearinghouse at:

  National Environmental Services Center
  West Virginia University
  PO Box 6064
  Morgantown, WV 26506-6064
  Phone: (800) 624-8301 / (304) 293-4191
  Fax: (304) 293-3161

Also see Stormwater Center Septic System Fact Sheets at:

- Pollution Prevention Fact Sheet: Septic System Controls
  www.stormwatercenter.net/Pollution_Prevention_Factsheets/SepticSystemControls.htm
- Non-Stormwater Fact Sheet: Septic Systems
  www.stormwatercenter.net/Assorted%20Fact%20Sheets/Tool7-Non_Stormwater/SepticSystems.htm

Appendix 10: Example of Boater Education Program: Rhode Island Sea Grant[20]

Vessel Discharge of Sewage Education Summary

Provide educational information about the pumpout service to customers.

Many boaters are unaware of current state and federal regulations that require the use of properly operating marine sanitation devices (MSDs), or how their on-board system works. Marinas can act as the most efficient source of accurate information to boaters. Even if you do not currently have a pumpout station, let boaters know where the nearest facility is located. Methods for sharing information about pumpouts and MSD regulations are numerous and can include:

1. Pamphlets and Flyers-There is a great deal of information being produced by the government and many nonprofit organizations that can be handed out at your facility, perhaps in the ship's store or at the fuel/pumpout dock. Most of the information is free and carries no copyright. Some sources for pumpout information include:

Coastal Resource Management Council: Stedman Government Center, Tower Hill Road, Wakefield, RI 02879 (401-277-2476)

State Department of Environmental Management, Division of Water Resources, or Narragansett Bay Project: 291 Promenade Street, Providence, RI 02908. (Water Resources, 401-277-3961; Narragansett Bay Project, 401- 277-3165)

RI Sea Grant, University of Rhode Island Bay Campus, Narragansett, RI 02882-1197 (401-792-6842). Fact sheets available on-line. (http://seagrant.gso.uri.edu/BMP/sewage_fs.html)

US Coast Guard, Marine Safety Office, 20 Risho Avenue, East Providence, RI 02914 (401-435-2300)

US EPA Region I, Waters Program: J. F. K Federal Building, Boston, MA 02203-2211 (617-565-3420)

Save the Bay: 434 Smith Street, Providence, RI 02908 (401-272-3540)

International Marina Institute: 35 Steamboat Avenue, Wickford, RI 02852 (401-294-9558)
RI Marine Trade Association: (401-885-5044)

2. Newsletter-If you provide a newsletter to your customers, perhaps you could consider a section highlighting different steps you are taking to improve the environment. This is also a great way to advertise the pumpout service and could be distributed to boaters who are not customers.

3. Inserts-Billing statements provide an opportunity to let your customers know about your pumpout service.

4. Meetings-Once a pumpout station is installed, consider hosting a meeting for your tenants and other boaters to explain the services and rules relating to the MSDs and pumpout stations. A demonstration of how cleanly and efficiently a pumpout operates may make people more likely to use it. Your local harbormaster or Coast Guard Auxiliary/Power Squadron unit should be able to assist you in conducting meetings.

5. Inspections-Consider offering an additional service to your customers by inspecting their existing MSDs and correcting any problems that may lead to improper operations. This could become another step in the winterization or spring commissioning process. Providing holding tank installation services will also help boaters easily comply with new no-discharge laws.

[20] http://seagrant.gso.uri.edu/BMP/sewage-edu.html

The Coast Guard Auxiliary is also available to conduct free boating safety inspections, which include a check of the MSD and overboard discharge valve.

6. Slip leasing agreement-You can use your tenant contracts to inform boaters about the use of the pumpout station. Although having no legal authority to enforce state laws, marinas can declare themselves no-discharge marinas and require tenants to use pumpout stations and ensure that Y valves are sealed to prevent incidental overboard discharge. In most facilities with these requirements, the penalty for discharging within the facility is expulsion.

There are other ways to help the boater understand the value in using a pumpout station and having a properly operating MSD. It is also important to have any member of the staff who will be operating the pumpout understand state and federal laws pertaining to MSDs and pumpout stations. This will enable them to answer questions that boaters may have.

APPENDIX 11: Information on Pet Waste Management Programs

From USEPA (very good references)

- Pollution Prevention/Good Housekeeping for Municipal Operations
  http://cfpub.epa.gov/npdes/stormwater/menuofbmps/poll_3.cfm

- Public Education and Outreach on Storm Water Impacts
  http://cfpub.epa.gov/npdes/stormwater/menuofbmps/edu_8.cfm

APPENDIX 12: Information on Wildlife Damage Control

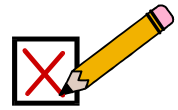
- Wisconsin Department of Natural Resources Nuisance Wildlife (Urban and Suburban) – good informational clearinghouse. www.dnr.state.wi.us/org/land/wildlife/damage/urbsub.htm

- University of Wisconsin Extension Controlling Nuisance Birds & Wildlife
  http://cf.uwex.edu/ics/infosource/birds.cfm

- New York State Department of Environmental Conservation – Wildlife Damage Control
  www.dec.state.ny.us/website/dfwmr/wildlife/damage.htm

Appendix 13: MST Volunteer Feedback Questionnaire

Volunteer Feedback Questionnaire
Microbial Source Tracking Project



Thank you for volunteering on the MST project! Please take a moment to let us know how it's going. We'll use your responses to improve our volunteer program. Responses will be kept strictly confidential. Do not put your name on the survey unless you wish to.

Please comment further on any question if you'd like to. Your feedback is valuable.

1. Approximately how long have you volunteered for the MST project? (please circle)
   More than 2 months   1 – 2 months   Less than 1 month

2. What did you volunteer for? (circle all that apply)
   Water Sampling   Lab   Other___________________

3. Is the volunteer position what you expected it would be from descriptions by the staff?
   Absolutely   *Mostly*   *Somewhat*   *Not at all*

4. Do you feel you have you been adequately trained to perform your volunteer assignment?
   Absolutely   *Mostly*   *Somewhat*   *Not at all*

5. Do you feel your efforts as a volunteer are important and provide a valuable service to the community?
   Absolutely   *Mostly*   *Somewhat*   *Not at all*

6. Is volunteering on the MST project interesting, enjoyable or rewarding?
   Absolutely   *Mostly*   *Somewhat*   *Not at all*

7. How would you rate your knowledge of bacterial water quality in southern Maine?
   Before volunteering: *none*   *modest some*   *well-informed*
   After volunteering: *none*   *modest some*   *well-informed*

8. Have you visited the MST project website? (http://www.umseagrant-mst.org)
   Yes   *No*

9. Do you have any suggestions for how to improve the volunteer experience, or can you think of any new ways volunteers can serve the project? (use back if necessary)

10. Overall, how would you rate your volunteer experience with the MST project?
    (1 = Horrible, 10 = Great)
    1   2   3   4   5   6   7   8   9   10

<u>Results</u>

Surveys sent: 20   Surveys received: 14   Response rate: 70%

Question 1:	More than 2 months: 12 (86%)		1-2 months: 2 (14%)	
Question 2:	Water sampling: 14 (100%)		Lab: 5 (36%)	
Question 3:	Absolutely: 14 (100%)			
Question 4:	Absolutely: 13 (93%)		Mostly: 1 (7%)	
Question 5:	Absolutely: 8 (57%)		Mostly: 4 (29%)	
Question 6:	Absolutely: 11 (79%)		Mostly: 3 (21%)	
Question 7:	12 (86%) improved knowledge, with 11 (79%) saying "some" or "well-informed"			
Question 8:	Yes: 6 (43%)		No: 8 (57%)	
Question 10:	5: 2 (14%)	8: 1 (7%)	8½: 1 (7%)	10: 10 (71%)

APPENDIX 14: Recommendations for residential fertilizer use[21]

Step five: Fertilization

Like any other living organism, grass needs basic nutrients for survival. But how much and what kind?

Phosphorus in fertilizer is rarely essential for established Maine lawns. Since a soil test will likely reveal ample phosphorus, use phosphorus-free fertilizer on existing lawns. Small amounts of phosphorus may be desirable, however, for improved germination when seeding a new lawn. Mix starter phosphorus into root zone and never apply on soil surface. Follow soil test recommendations.

How much green is too green? The iridescent, emerald-green lawn acquired by overfeeding with fertilizers, especially nitrogen, is actually unhealthy turf that's under stress. In this condition, the lawn is vulnerable to plant diseases, weeds and drought.

A soil test analyzes existing fertility of the soil and its pH (degree of acidity or alkalinity). This information is essential for developing a nutrient program.

Soil pH must read between 6.0 - 7.0. Most Maine soils are acidic with a pH of 4.8 to 5.2. Lime increases pH and can be applied anytime during the growing season. Pelletized dolomitic limestone works best.

Measure your lawn area to determine square footage. Then calibrate your spreader to apply the correct amount of fertilizer. Excessive use harms the environment, is costly, increases need for mowing and can burn grass plants.

Nitrogen in fertilizer is the element needed in the greatest quantities by the grass plant, but it should never be over applied. Treat your lawn **only** when a soil test indicates the need. Best time to apply is late August or September. Use slow release formulations of nitrogen (water insoluble nitrogen, some manures, activated sludge, sulfur-coated urea) that "spoon feeds" small amounts of the nutrient over many weeks. Do not apply before heavy rainfall! Excess nitrogen washed into Casco Bay promotes algae growth and chokes marine life.

Animal manure fertilizers contain bacteria that can be carried into surface waters when inappropriately used and stored. To prevent bacterial contamination, maintain adequate setback distances (100') from water courses, private wells and steep slopes and do not apply before heavy rainfall! Excessive bacterial contamination results in closed shellfish harvesting areas and swimming beaches.

[21] Maine Board of Pesticides Control BayScaper Program (modified to include specific reference for animal manures). (www.state.me.us/agriculture/pesticides/bayscaper/homepage.htm)

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