MICROBIAL SOURCE TRACKING
IN TWO SOUTHERN MAINE WATERSHEDS
Merriland River, Branch Brook and Little River (MBLR) Watershed Report

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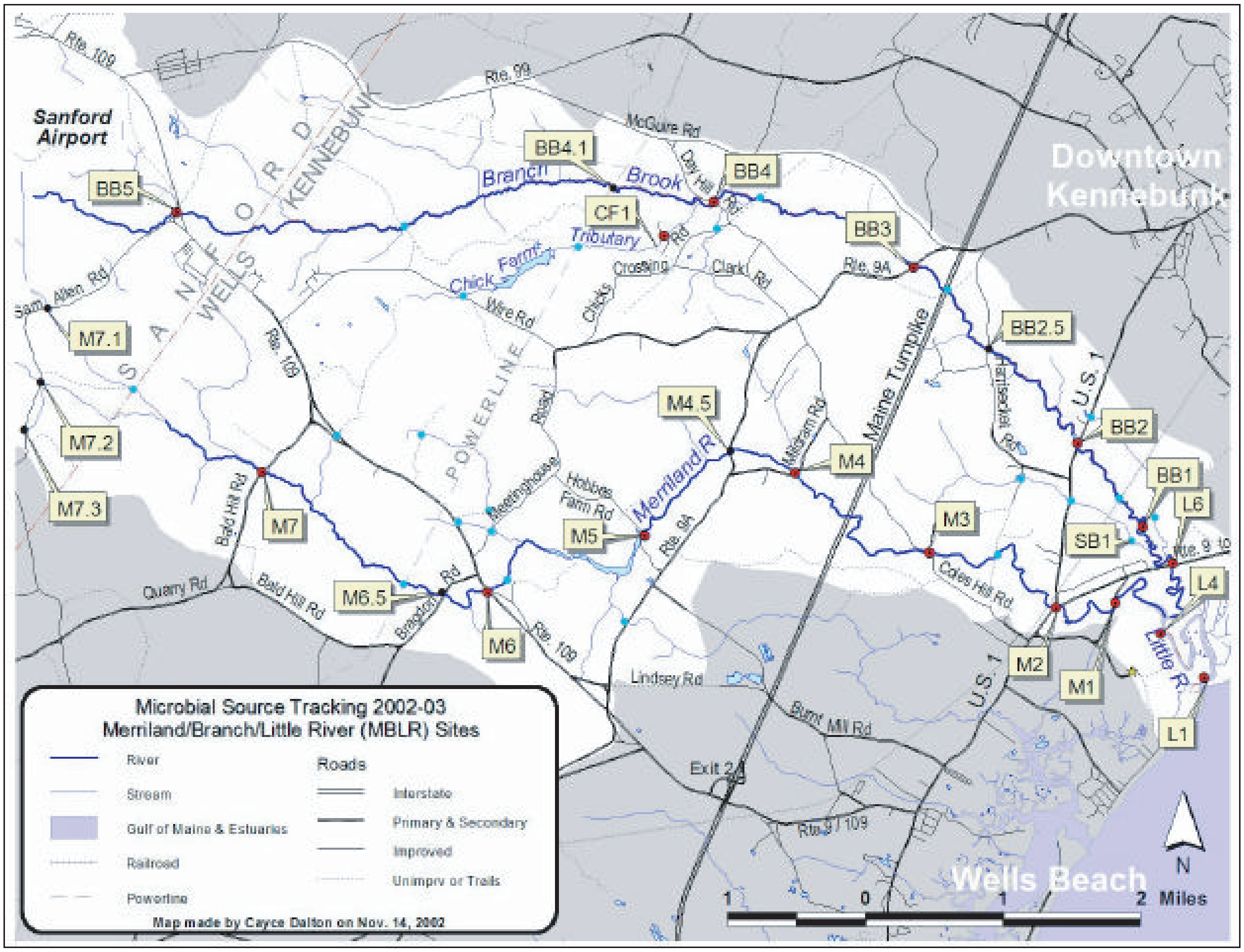

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ACKNOWLEDGEMENTS

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 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 providing DMR water quality monitoring data specific to this area and for her support throughout the duration of the project. Appendix 2 contains a full list of steering committee members. Dana and Cindy Johnson were also especially helpful in the collection and identification of animal scat samples.

Will Emmons and the staff at the Kennebunk Sanitary District provided a steady supply of influent for our bacterial analyses. Likewise, Bill Snyder and the staff at the Kennebunk, Kennebunkport and Wells Water District (KKWWD) for providing guidance on which areas of the watershed to investigate for potential fecal contamination sources. 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 ongoing support based on her extensive experience with microbial source tracking projects in seacoast New Hampshire. Finally, Michele Dionne, Research Director for the Wells National Estuarine Research Reserve (WELLS NERR), 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

Bacteria are Commonly Used as Indicators of Contamination

Microbial Source Tracking (MST) 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. Fresh water swimming beach standards, on the other hand, allow for up to 235 organisms per 100 mL of water sample for the indicator organism *E. coli* (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 not specific enough to make conclusions about the sources of the pollution.

In Maine and the U.S. there are Serious Impacts from Bacterial Contamination

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 2002 (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 (USFDA, NSSP Model Ordinance, 1999).[iv]

Standard Bacterial Testing has Weaknesses that MST Attempts to Address

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 such as microbial source tracking.

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 species specific sources of fecal contamination in surface waters. Ultimately, we hope the results from the *Microbial Source Tracking in Two Southern Maine Watersheds* project (hereafter referred to as the “MST Project”) will be 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 MBLR watershed with information regarding the microbial source(s) of fecal coliform bacterial contamination in this region.

- ***Goal 2:*** Educate community members living within the MBLR watershed regarding the results of this project as well as actions they can take to reduce contamination levels.

- ***Goal 3:*** Disseminate the project results to other watersheds in the Northeast region and the U.S.

EXPERIMENTAL DESIGN

This study focuses on the Merriland River, Branch Brook and Little River (MBLR) watershed in Wells, Kennebunk and Sanford Maine, where chronic and persistent bacterial contamination from unidentified sources has restricted shellfish harvesting.

STEP 1. Water and Scat Sampling

To meet the goals of the project, water sampling was conducted over a 6-month period beginning in December of 2002. Water sampling sites were selected on the basis of accessibility and proximity to suspected contamination sources. Scat was collected for 10 separate species (including humans) within the watershed.

STEP 2. Standard Bacterial Testing

Conventional bacterial testing for fecal coliform and *E. coli* (both indicator organisms of fecal contamination) was carried out for 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* contamination levels for the land areas draining into each sampling site.

STEP 3. Isolating Selected Samples

To further identify potential contamination sources, *E. coli* bacteria were isolated from some of the samples and delivered to the University of New Hampshire's Jackson Estuarine Laboratory (JEL) for genetic analysis.

STEP 4. Microbial Source Tracking

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.

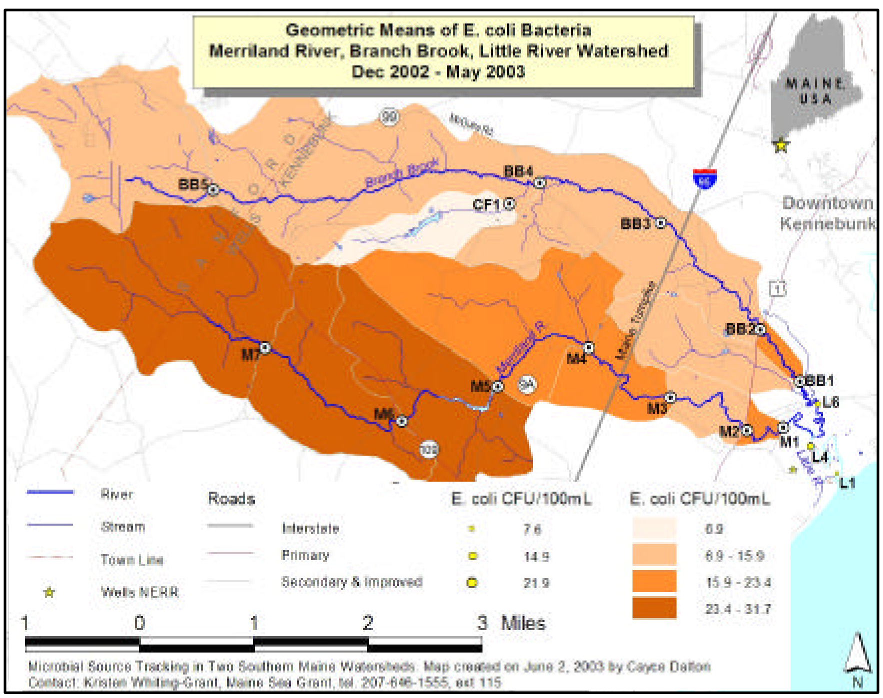
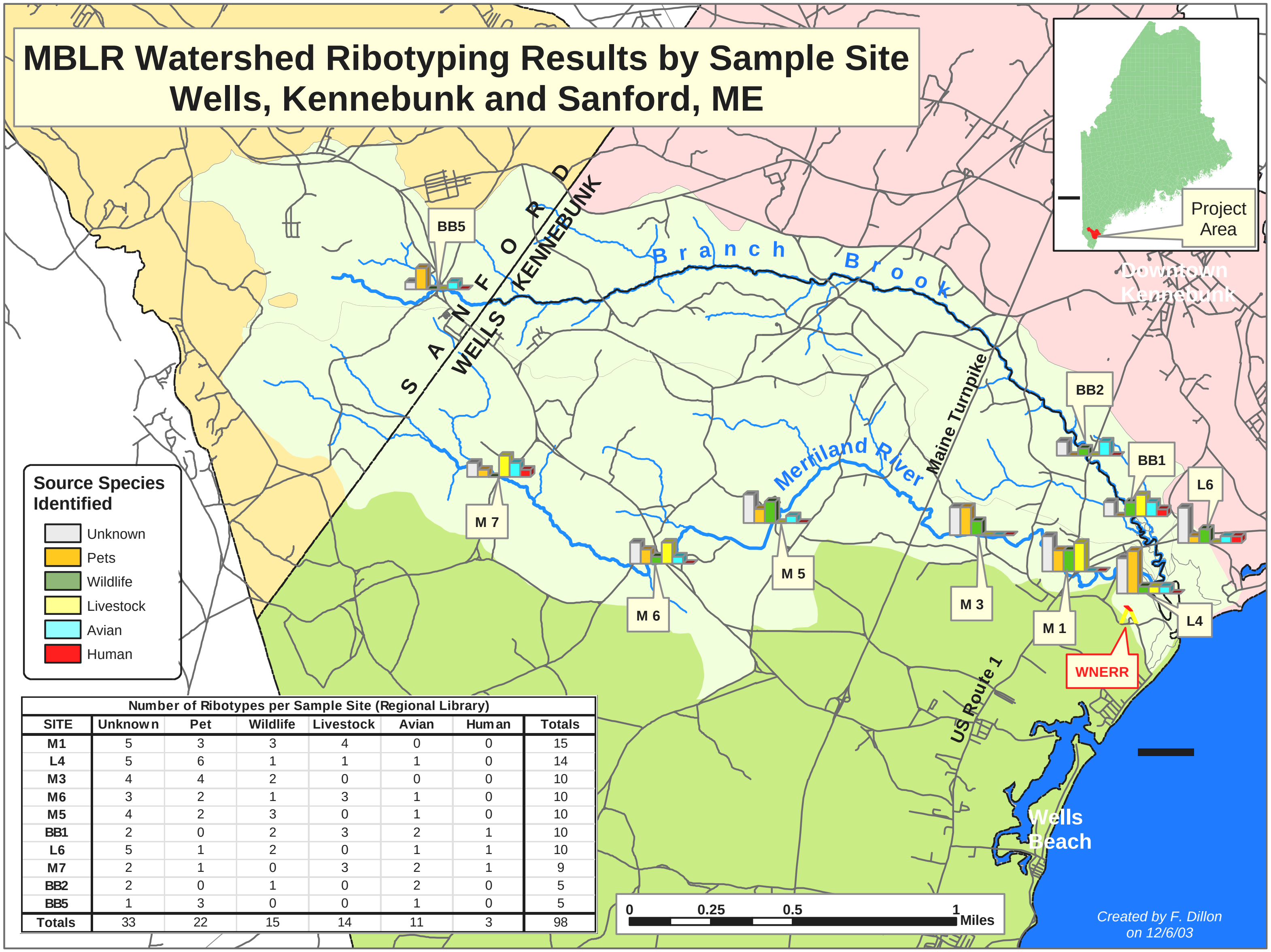


Figure A. *E. coli* bacteria levels for MBLR watershed sampling sites indicated by color for land drainage areas ("catchments"). Higher geometric mean values indicate higher contamination levels. Geometric mean is used (instead of average) to measure water quality statistics that show wide variability, because it minimizes the effects of low frequency, extreme values.



Number of Ribotypes per Sample Site (Regional Library)

SITE	Unknown	Pet	Wildlife	Livestock	Avian	Human	Totals
M1	5	3	3	4	0	0	15
L4	5	6	1	1	1	0	14
M3	4	4	2	0	0	0	10
M6	3	2	1	3	1	0	10
M5	4	2	3	0	1	0	10
BB1	2	0	2	3	2	1	10
L6	5	1	2	0	1	1	10
M7	2	1	0	3	2	1	9
BB2	2	0	1	0	2	0	5
BB5	1	3	0	0	1	0	5
Totals	33	22	15	14	11	3	98

Figure B. Geographic distribution and species composition of ribotypes in the MBLR watershed. Bar heights indicate number of ribotypes for each sampling site (actual numbers included in inset table). Source: Jones (2003).

STUDY RESULTS

Cats are most frequently identified source species; wildlife is next largest contributing category

Figure B (on page 6) provides a detailed summary of source species identification for each of the 10 water-sampling sites from which ribotypes were developed. It also helps determine which specific areas of the watershed should receive the greatest attention for remediation strategies. Each sample site is represented by a bar graph indicating the relative proportions of identified ribotypes along with the "unknowns," which are bacteria samples that could not be genetically identified by JEL. There is also an accompanying table that indicates the actual numbers of ribotypes for each sample site and each category type. As with Figure B above, the species categories are wildlife, pet, human, livestock, avian and unknowns.

The overall ribotyping results for the MBLR watershed are presented in Figure C. The most frequently identified single source of bacterial contamination was from cats (21%) while the next most significant combined species category of contributors was from wildlife (15%). Livestock and birds both played a lesser but still significant role at 14% and 11%, respectively. Also note that ribotypes for 35% of the bacteria samples analyzed by JEL could not be identified, which is to say that no matches could be established between known source species sample ribotypes and unknown water sample ribotypes.

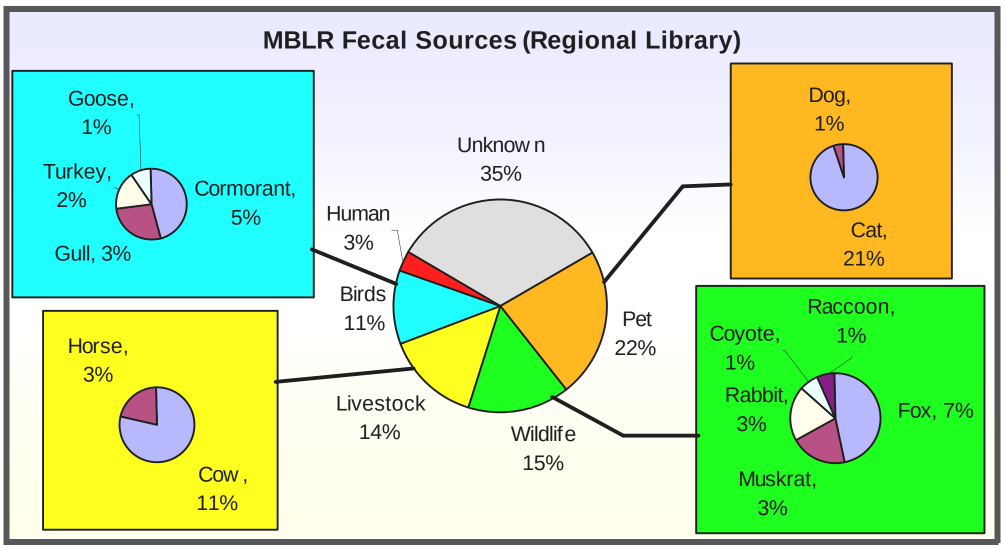


Figure C. Source species identification for MBLR watershed. Pets are the single largest type of contributor. (Jones, 2004).

MANAGEMENT RECOMMENDATIONS

The ribotyping results were used to develop a management plan for reducing fecal contamination in the MBLR watershed. Additional data sources used to corroborate the ribotyping results included: the work of previous researchers; field surveys for the MBLR watershed; maps of land cover/habitat types; and local knowledge of wildlife prevalence and distribution. The recommendations offered in this plan are summarized below for each of the identified sources.

<u>Wildlife Sources</u>

- Maintain or establish adequate riparian buffers to reduce volume of contaminated runoff.
- Work with municipalities to provide information to residents in watershed about ways to reduce attraction of problem species.
- Evaluate or consider developing local ordinances restricting the feeding of wildlife to reduce the congregation of animals and the potential concentration of their waste.

Pet Sources

- Increase efforts to promote proper handling and disposal of pet waste, and in particular cat waste.
- Evaluate or consider developing local ordinances targeting pet waste management.

Livestock Sources

- Cooperate with the municipalities, the Farm Management Bureau and University of Maine Cooperative Extension to identify all livestock owners in watershed and provide them with informational brochures about proper handling of livestock waste, such as not applying animal fertilizers on wet or frozen ground.
- Identify all sources of animal manure used as fertilizer (garden and nursery suppliers, local farms) and provide informational brochures for patrons purchasing manure at these locations on proper handling of animal fertilizers. Cooperate with University of Maine Cooperative Extension to share this information through Master Gardener programs.

Human Sources

- Provide information on proper septic system maintenance to all owners of septic systems in watershed.
- Recommend to the town or state the establishment of a septic system tracking program that establishes maintenance schedule for property owners. Also facilitate sharing of information between state agencies (Department of Marine Resource, Department of Human Services) for changes in septic system status discovered during site evaluations.
- Continue to work with Maine Department of Marine Resources to ensure that no overboard discharges exist along Little River estuary.

OTHER CONSIDERATIONS FOR THE USE OF MST IN THE FUTURE

Clearly, microbial source tracking methods represent a significant advancement over conventional bacterial test methods in attempting to more closely identify sources of fecal contamination in coastal and inland watersheds. However, due to the considerable expense of these methods, previous efforts to enlist upper-level state support in Maine for MST (at least throughout the 1990's) were not successful, particularly in light of budgetary constraints. A formal cost / benefit analysis may be needed before the state is likely to allocate significant resources to MST. Because MST – and ribotyping in particular – is an expensive process, a highly targeted approach for its use is recommended (Jones, 2004). With respect to the goal of opening clam harvesting areas we suggest the following:

- Identify and prioritize shellfish harvesting areas with very high resource value through close cooperation with community members and municipal officials.
- Establish baseline data (from both water quality monitoring and shoreline surveying) to determine where major contamination sources could be entering waterways.
- Determine the likelihood that *E. coli* from specific locations will enter the estuary in significant concentrations.
- Evaluate the most likely major sources of contamination and establish a targeted source library (especially for non-wildlife species).
- Conduct intensive, short-term water sampling in that region during the environmental conditions that historically produce the highest counts.

- Conduct MST on this targeted set of unknowns. As long as human, livestock or pet contamination is identified, then there is hope for correction and improvement in water quality and a reason to keep up the investigation.

Non-wildlife species are emphasized because management strategies are more likely to be successful in reducing fecal contamination from them rather than from wildlife species. We also suggest that the best places to target for MST work are those high priority areas where baseline data has been collected, resource value is high and the community capacity exists to help implement the resulting management plan.

NEXT STEPS

Ideally, fecal coliform and *E. coli* levels in the MBLR watershed will decrease following the implementation of these recommendations. An ongoing water quality monitoring program, using conventional bacterial test methods, will be needed to monitor any reductions in fecal contamination. Results from the DMR ongoing water sampling program in the Little River estuary will determine which areas are suitable for shellfish harvesting. However, it would also be helpful to establish an ongoing 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 MBLR 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 reduce bacterial contamination levels toward reopening shellfish harvesting areas in the Little River 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 technology 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 potential sources 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 could improve, potentially 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 MBLR watershed in the towns of Wells, Kennebunk and Sanford is plagued with unidentified sources of fecal contamination. The MBLR is a relatively small watershed (31.3 square miles) and its manageable size improves the likelihood that the origins of fecal contamination will be successfully identified. Clam flats have been closed for more than two decades in the Little River estuary due to chronically elevated fecal counts there (Dionne *et al*, 2002).

MST results will be used to guide local remediation plan development, potentially leading to reduced fecal counts and 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 MBLR watershed with information regarding the microbial source(s) of fecal coliform bacterial contamination in this region.
- ***Goal 2:*** Educate community members living within the MBLR watershed regarding the results of this project, as well as actions they can take to reduce contamination levels.
- ***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 (Fig. 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 much of the 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 (USEPA, 1996). The MST Project has adopted a nearly identical strategy.

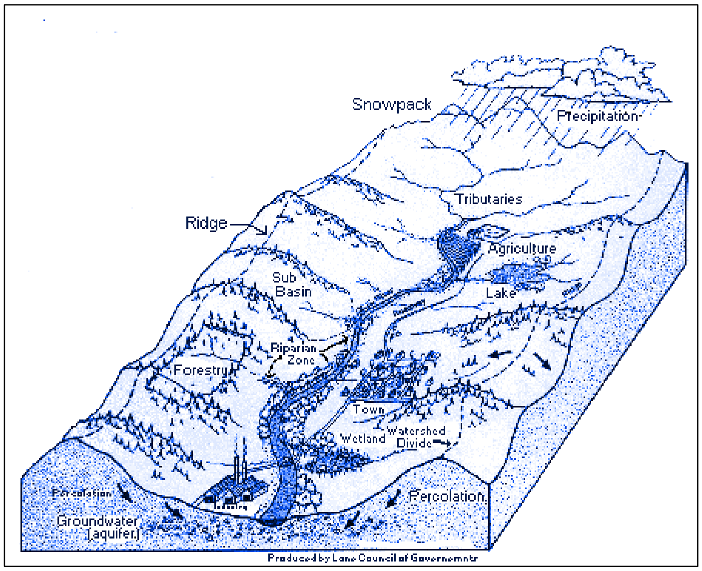


Figure 1. Cross-section of watershed

The MBLR 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, wildlife 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 fresh water swimming beach standards allow for up to 235 *E. coli* organisms per 100 mL of water (Maine DHS, 2002).[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 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 (Fig. 2). In 2002, nearly 2.5 million pounds of softshell clams were harvested with a commercial value of over $14.8 million.[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 (USFDA, 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.

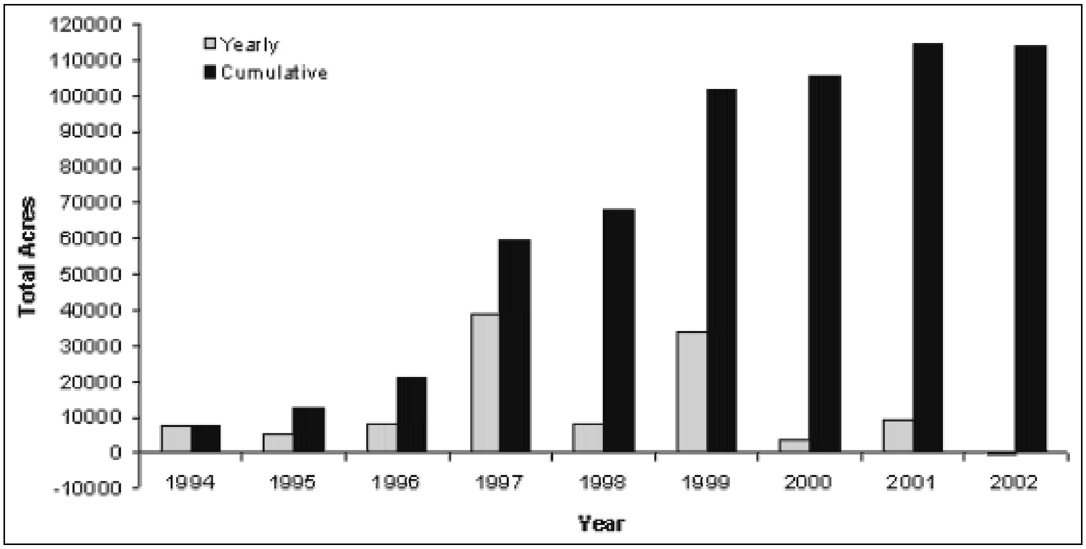


Figure 2. Acres of shellfish habitat reopened for harvest during 1994-2002 in Maine following pollution reduction and habitat restoration. (Source: Maine Department of Marine Resources, 2003).

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 (USFDA, 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 (USFDA, 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 MBLR watershed is designated as Class A (the second highest designation), which only allows bacteria concentrations to occur at natural levels (Maine Revised Statutes, Title 38, Chapter 3, § 465).

[2] Historical Maine Softshell Clam Landings (www.maine.gov/dmr/commercialfishing/softshellclam.htm)

The estuarine portion of the MBLR watershed is designated as Class SA (where “S” denotes saline water and “A” is the highest designation), which also only allows bacteria concentrations to occur at natural levels. For shellfish harvesting areas, Maine statutes ultimately defer to criteria specified by the National Shellfish Sanitation Program Model Ordinance (USFDA, 1999). The 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 MBLR RIVER WATERSHED

2.1 Hydrology, Habitat and Development

The MBLR watershed covers approximately 31.3 square miles and is made up of the Merriland River and the Branch Brook that converge within the salt marsh estuary to form the Little River before emptying into the Atlantic Ocean (Map 1). The two freshwater tributaries begin in the sandy outwash plains of Sanford near the municipal airport and flow southeast. The Merriland River has a drainage area of 15.7 square miles from Sanford through Wells, and the Branch Brook has a drainage area of 14.8 square miles starting in Sanford and traveling between Wells and Kennebunk, defining the town line. The Little River estuary occupies 0.8 square miles.

The meandering Merriland River and Branch Brook are influenced by the watershed’s topography and terrain created from glacial deposits. Both rivers originate in the Great Sanford Outwash Plains. The outwash plains of the Branch Brook consist of fine, medium, and coarse sand extending from Sanford to the Atlantic Ocean. Intermittently the plains were disrupted by local bedrock, till-covered knobs and dissected by deep, steep-sided streams that cut through the sand and into an underlying clay-silt strata of glaciomarine origin (Gerber, 1981). The Branch Brook and its small tributaries quickly wind through the ravine. East of Route 1, the land levels out where the Branch Brook drains into the Little River. The Merriland River bed is composed of glacial till, stratified sand and gravel, and the Presumpscot Formation clay with a series of end moraines (Huei Kuo, 1998). The Merriland River travels downstream through elevated land to Hobbs Pond, a pond created by a dam in the stream. Once beyond the Route 1 area, the land flattens as it flows into the Little River.

Land cover in the MBLR watershed has been variously described. For the present purposes (Fig. 3), land cover 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). Some land cover types may not be accurately portrayed at the local scale or most recent conditions since their primary use is for identifying habitat suitability.[5] However, the overall level of accuracy can be considered as satisfactory (Banner, 2002). 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. The majority of the MBLR watershed is undeveloped and consists mostly of forested land cover (upland forest at 58.7% and forested wetland at 13.5%) with only about 3% of developed land.

[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)

[5] Please refer to the Maine Office of GIS website at http://apollo.ogis.state.me.us/catalog/ for a full metadata report on the “gomlc7” digital data layer and the USFWS website at http://r5gomp.fws.gov/gom/habitatstudy/metadata/Landcover_Data_Methods.htm (Land Cover and Wetlands of the Gulf of Maine Watershed) for an explanation of data sources and relative accuracies.

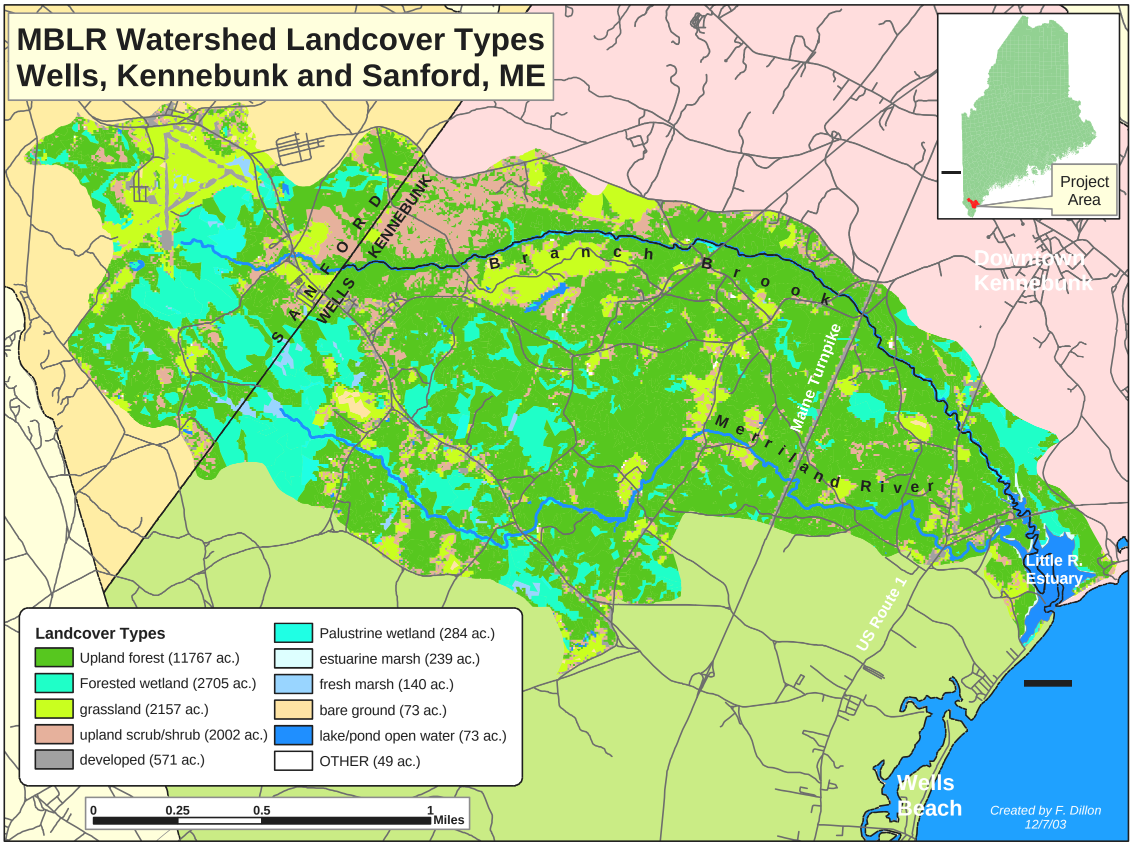


Figure 3. Land cover map of MBLR watershed (adapted from US Fish & Wildlife Service Gulf of Maine Habitat Mapping Project – 2001).

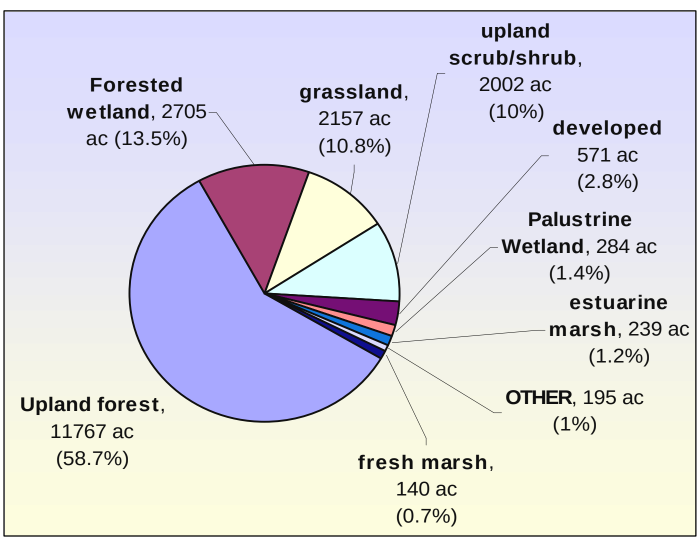


Figure 4. MBLR watershed land cover types.

LAND COVER TYPE	ACRES	LAND COVER TYPE	ACRES
Upland forest		**estuarine marsh**	**238.8**
upland mixed forest	6622.1		
upland deciduous forest	3874.4	**fresh marsh**	**139.9**
upland coniferous forest	1270.3		
	11766.9	**bare ground**	**72.9**
Forested wetland			
coniferous swamp	1484.2	**lake/pond open water**	**72.8**
deciduous swamp	1220.5		
	2704.7	**OTHER**	
		estuarine sand/mud shore	16.0
grassland	**2157.0**	cultivated	15.0
		estuarine open water	11.2
upland scrub/shrub	**2001.8**	marine sand/mud shore	3.8
		fresh submerged plants	1.1
developed	**571.0**	perennial stream	1.1
		marine open water	0.5
Palustrine wetland		estuarine shrub	0.4
deciduous shrub swamp	212.8		**49.1**
coniferous shrub swamp	71.4	**Total Acres:**	20059.0
	284.2	**Square Miles:**	31.34

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

2.2 Sewering of the Watershed

Southern Maine experienced a rapid period of growth and change in the 1990s. 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 Kennebunk and Wells where the year-round populations for the period from 1990 to 2000 increased by 31%, 21%, respectively. Sanford's population increase for the same period was negligible by comparison at only 2%. Housing units increased somewhat correspondingly for the same period in each of the towns at 24%, 49% and 6% for Kennebunk, Wells and Sanford, respectively (Southern Maine Regional Planning Commission, 2000). Most of this growth in the MBLR watershed occurred in areas without public sewer systems; therefore, most sanitary waste disposal is accommodated by septic systems.

2.3 Shellfish Growing Area Water Quality Monitoring Program

As noted above, 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 fecal contamination 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.

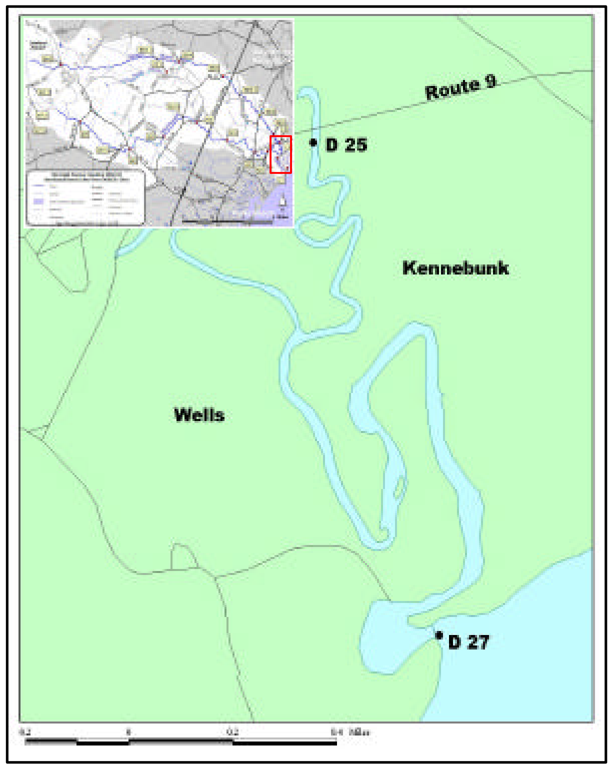


Figure 5. Maine Dept. of Marine Resources sampling stations for Little River estuary.

There are currently 2 DMR monitoring stations in the MBLR Estuary (Fig. 5). 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 MBLR Estuary are summarized in Table 2. As the table indicates, station D25 has routinely and persistently exceeded the NSSP's P90 standard. Station D27 has experienced declining P90 values since the year 2000; however, this is due in part to a recent change in the DMR's sampling regimen in the Little River estuary, which now occurs only during the winter months. Since year round sampling consistently resulted in excessive P90 values, DMR began sampling only during the clamming season. Consequently, while P90 values for station D27 meet NSSP standards for the period from October to April, year round water quality may not necessarily have improved.[6]

Little R. Estuary Yr. End P90's		
Station ID	**D25**	**D27**
1998	**413**	**55**
1999	**331**	**57**
2000	**324**	48
2001	**265**	39
2002	**285**	37
2003	**136**	23

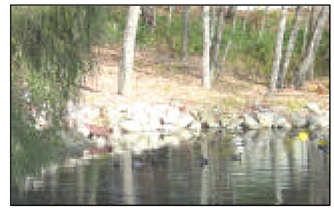
Table 2. Maine Department of Marine Resources Year-end 90th Percentile Results (MPN / 100 mL)

[6] 12/2/03 email communication from Laura Livingston, Maine Dept. of Marine Resources.

2.4 Watershed Surveys

In the summer and fall of 2001, the Wells National Estuarine Research Reserve (WELLS NERR) organized shoreland surveys in the MBLR watershed for a Maine DEP funded nonpoint source (NPS) pollution project. The surveys occurred within a 250 foot wide area on either side of the major tributaries in the MBLR since impacts to water quality were considered to be greatest from this zone. The surveys located and inventoried pollution sources and described other activities that were adversely affecting water quality. The most abundant source of NPS pollution identified was surface erosion followed by road erosion; bank erosion and trash; poorly designed culverts; inadequate vegetative buffers; construction activities; pipe discharge; and livestock and agricultural practices (Fig. 6). NPS pollution sources potentially contributing fecal contamination were identified as follows:

- Ducks and geese near site M6 (photo at right)
- Sloped horse paddock on Day Hill Rd near MST site BB4
- Pipe with unknown discharge near the intersection of Route 109 and Meetinghouse Road
- Pipe with unknown discharge near MST site BB5



Several of these sites were later determined as unlikely sources of bacteria. The pipe near Route 109 and Meetinghouse Road was thought to be intended as a wetland drainage pipe.[7] The pipe near MST site BB5 was determined to be associated with an aquaculture facility operated on an irregular basis.[8]

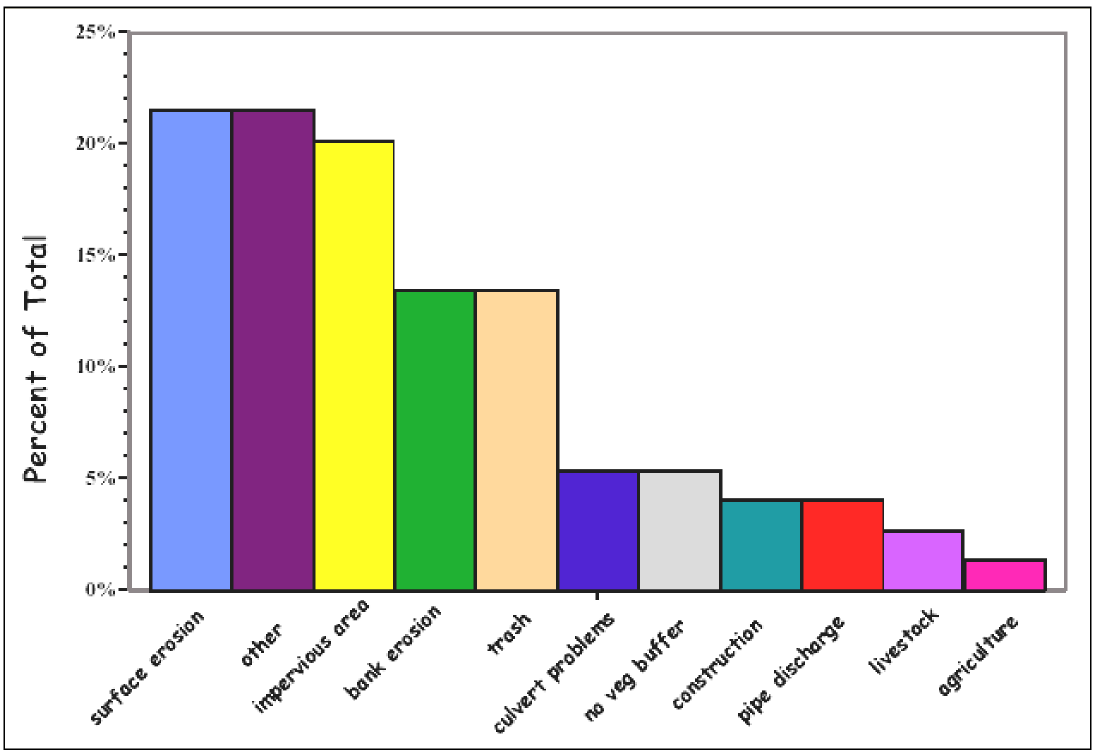


Figure 6: Type and frequency of NPS in MBLR watershed. (Source: Dionne *et al*, 2002).

Given the extensive watershed survey documentation already available and the relatively low incidence of NPS pollution from fecal sources, MST Project staff decided to focus on areas that were unexplored, had elevated fecal coliform counts or were noted as potential risks (Fig. 7). Several potential sources of fecal contamination not cited above were noted:

[7] Personal communication to MST Project staff member Cayce Dalton from Wells NERR Research Director Michele Dionne.
[8] Personal communication to MST Project staff member Cayce Dalton from KKWWD staff member Bill Snyder.

- Horses on Clark Road, near MST site BB4 ("BB" stands for Branch Brook – see Figure 13 o page 20 for complete map of MBLR samples sites)
- Horses on Sam Allen Road, near MST site M7.3 ("M" stands for Merriland River)
- Cows near Vintage Way upstream from MST site CF1 ("CF" stands for Chicks Farm tributary)
- Farm with livestock at intersection of Route 9A and Wire Road

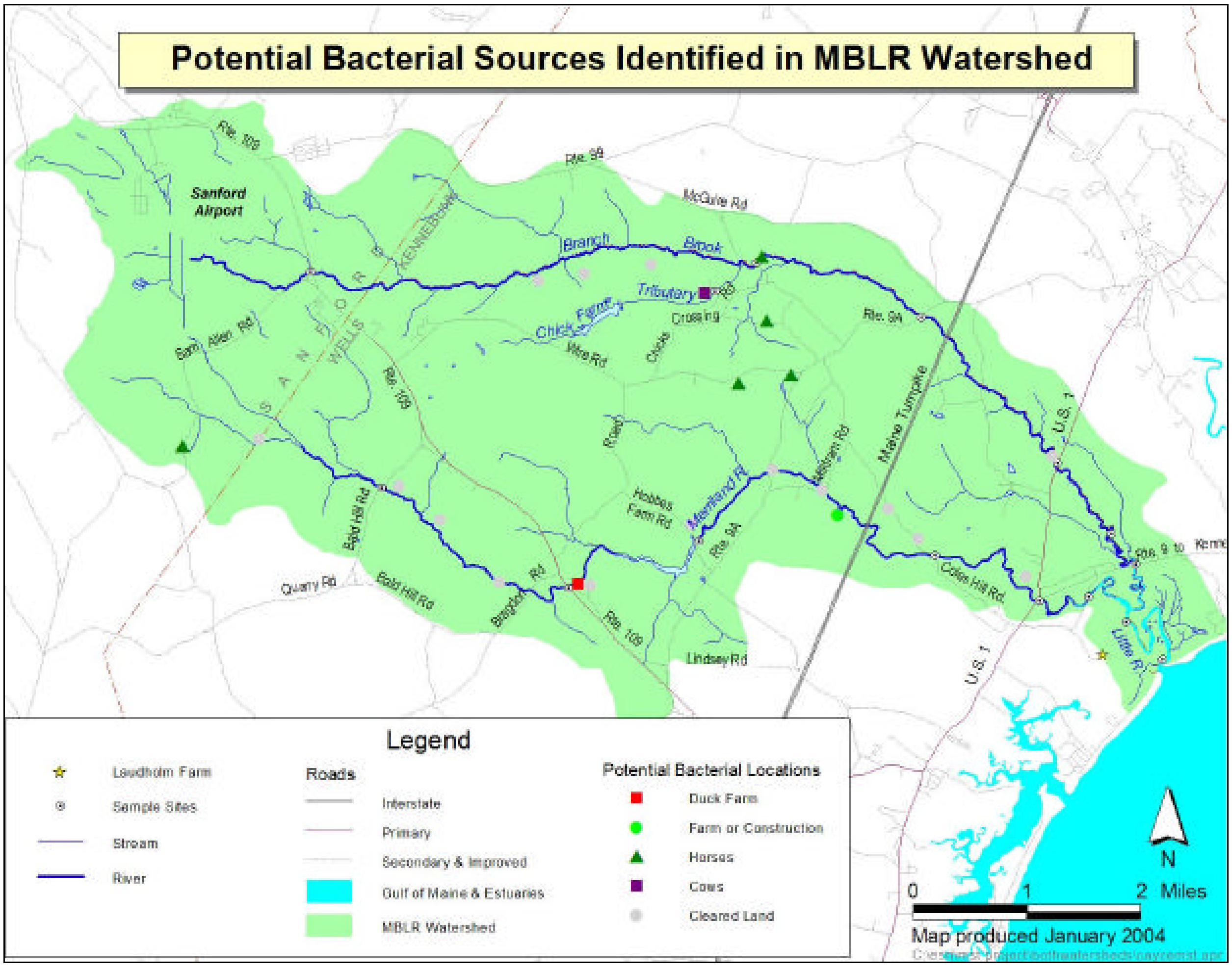


Figure 7: potential sources of bacterial contamination identified in early stages (ca. October 2002) of MBLR MST Project.

The Kennebunk, Kennebunkport and Wells Water District (KKWWD) was also consulted regarding their annual shoreline survey of Branch Brook. Their findings generally indicated that wildlife were the most likely contributors of fecal contamination within the Branch Brook watershed, though domestic dogs were also cited as possible sources. Apparently, dog packs are responsible for some of the deer kills that have occurred in the area. These findings are not surprising given that most of this land is undeveloped (see Figure 3 on pg. 14) and KKWWD has acquired a substantial portion of it for the purposes of watershed protection – as have other organizations (Fig. 8).

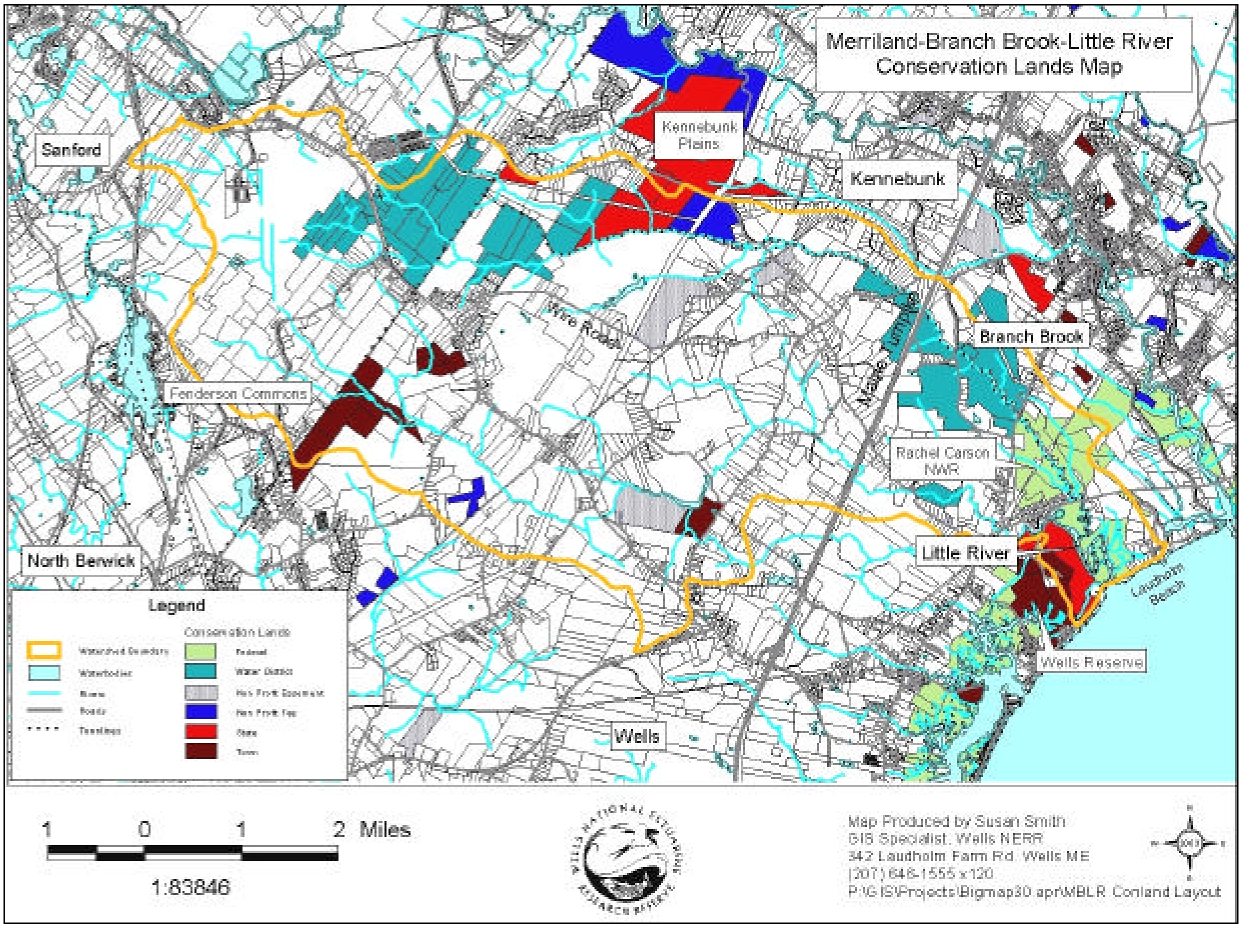


Figure 8: conservation lands in the MBLR watershed.

2.4.1 Watershed Survey Observations

April 11, 2003

In the area where Bragdon Road crosses the Merriland River (Fig. 9), otter and coyote scat samples were collected and later cultured for *E. coli* bacteria. These were eventually delivered to Jackson Estuarine Lab for genetic analysis. Scat for rabbit, muskrat, fox, and two deer was also collected and tested, but produced no bacterial growth, indicating that the samples were most likely too old. Fresh duck prints were observed in the snow, but very few other indications of animal presence were observed. We also unsuccessfully searched a beaver pond for beaver scat (Fig. 10).

Figure 9: volunteers Cindy and Dana Johnson help MST Project staff Fred Dillon (left) search for scat sample near Bragdon Road, in Merriland River watershed.

Figure 10: volunteer and professional trapper Dana Johnson goes to great lengths to find beaver scat sample in and around the middle reaches of the Merriland River.

May 19, 2003

MST Project staff Dalton walked portions of the Little River marsh (near WNERR's pontoon boat) and the trails along the marsh up the Merriland River behind Laudholm Farm Road. Potential wildlife sources that were observed included several chipmunks and squirrels. Completely dried coyote droppings were observed on the Little River marsh, and several deer tracks and trails were found. No fresh deer droppings were found, although behind Laudholm Farm Road an abundance of completely dried deer droppings were located on the bluff above the Merriland River (Fig. 11). One wild turkey was observed foraging in this area, and after a ten or fifteen minute observation, a fresh fecal sample was obtained. Several other older turkey droppings were found along the trails as well. Several adult and approximately ten young turkeys have been seen in the area around Laudholm Farm Road in 2003.

May 22, 2003

MST Project staff went to the upper part of the Branch Brook watershed. They observed a development with at least a 200' buffer and ATV trails with a buffer of at least 100' at the end of Wire Road (near Route 109 and Sanford town line); several marshy areas crossing the power line between Wire Road and Route 109; a new looking horse paddock (Fig. 12) on Chicks Crossing Road (east of Day Hill Road) and turkeys on Laudholm Farm Road.

Figure 11: deer scat behind the Alheim House (Laudholm Trust offices), on Laudholm Farm Road. This property abuts the lower Merriland River.

Figure 12: new livestock fencing seen from Chicks Crossing Road, near sites CF1 and BB4 (drainage to BB3 subcatchment).

August 14, 2003

Areas near sample sites that showed the highest geometric means were surveyed: M6 and M7. Most of the area is forested, so instead of following the entire length of the river through the brush, we focused on potential human or livestock sources. Using a 2001 Wells aerial photo, large open fields near the streams were noted as potential pastures.

We observed a gravel pit that appeared to be used by ATV traffic and was serviced by a power line access road at the corner of the power line and Bald Hill Road. No potential fecal source was apparent. One camper was found parked at the edge of the power line clearing on a private home access road. The distance from the river was approximately 310 meters over uneven terrain (as measured using GIS) and it was not clear whether the camper was inhabited or simply parked and vacant. Given the distance and the absence of a direct run-off path to the river, they determined that it was unlikely by itself to be a major source of fecal contamination. The next day, using GIS, it was determined that there is a trailer park near a tributary of the Merriland River, with some trailers being within 100 meters of the stream.

3.0 ASSESSMENT OF CURRENT FECAL CONTAMINATION

3.1 Sample Site Selection

MST Project staff selected sample sites on the basis of balancing the ease of accessibility and even geographic distribution throughout the watershed. GIS was used to determine intersections of roads and streams, and staff visited and photographed all of these potential sample sites. During preliminary site visits, staff documented accessibility issues, such as parking and walking to the stream banks, and potential sources of bacteria nearby, such as livestock or subdivisions. GIS software and contour data from Maine Office of GIS were used to delineate the subcatchment areas for potential sample sites.

Sixteen sample sites were chosen for consistent water quality monitoring. Among these, a higher density was selected close to the mouth of the rivers (Merriland River and Branch Brook) in order to capture bacterial data at head of tide and to account for the higher density of human development near the ocean and along US Route 1. Sites along these two rivers were named with M1 through M7 and BB1 through BB5. Three long-standing sample sites in the Little River estuary (L1, L4 and L6) monitored by the Watershed Evaluation Team (WET) program at the Wells NERR were chosen in order to provide overlapping data. Their WET names were retained for this project and two of them correspond with Maine DMR sampling sites where L1=D27 and L6=D25 (Figure 5 on page 15). Additional sites were also selected as possible locations to sample as conditions merited. These were labeled with decimals. For example, M6.5 was between M6 and M7. One large tributary near Chicks Crossing Road in Wells was also chosen (CF1) due to a nearby cow pasture. A second large tributary of the Branch Brook was sampled near the head of tide (SB1) due to its large subcatchment in the Route 1 area (Fig. 13).

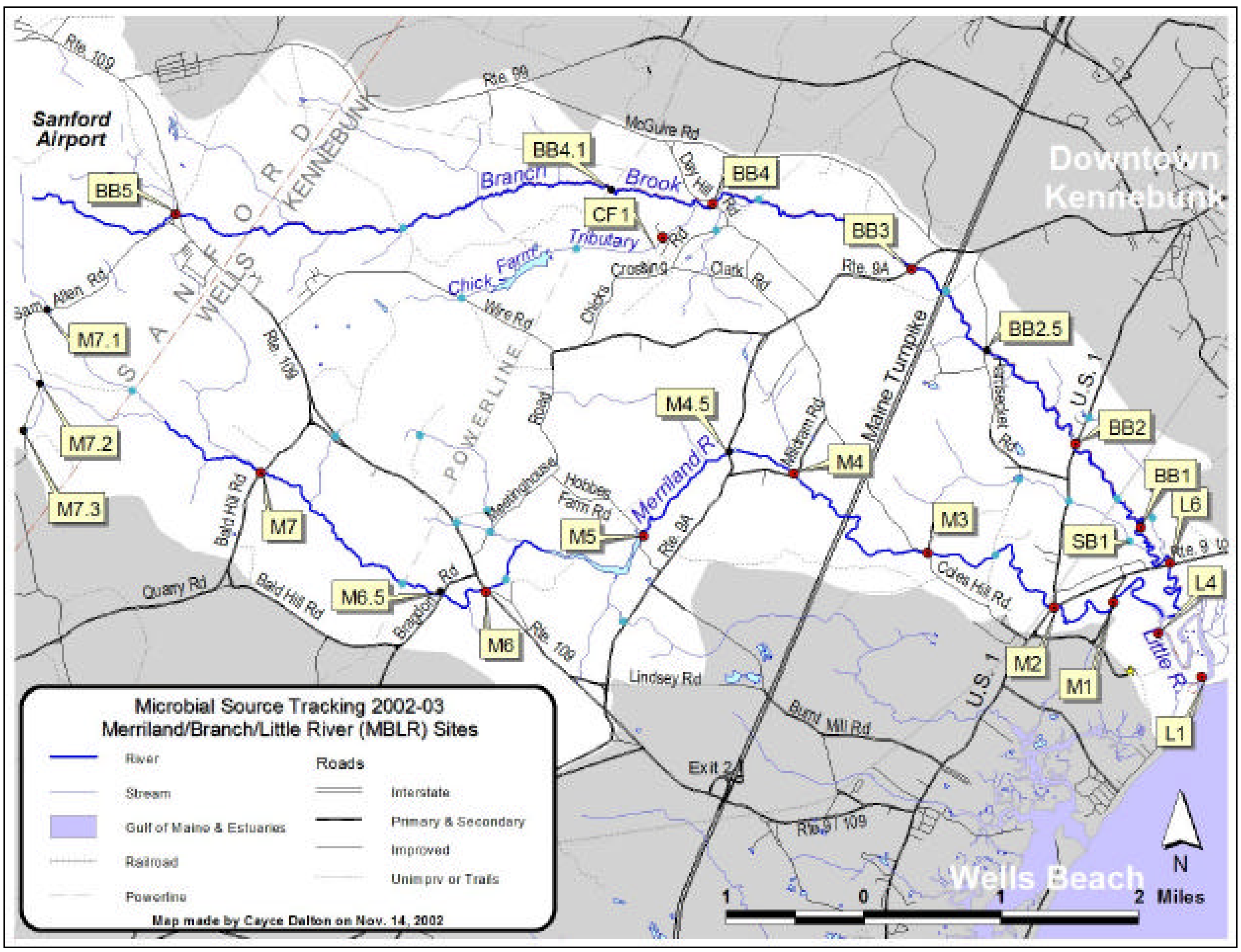


Figure 13: MBLR sample sites.

3.2 Sampling Design and Methods

Samples were collected between December and May – before, during and after the local clam harvesting season, which runs from January to March. One sampling date per month was pre-selected and the remaining dates were scheduled in relation to precipitation events during this period to allow for sample collection during and/or post-storm water samples. This winter/spring sampling period was intended to allow for an investigation of fecal counts related to snow melt during a January thaw and/or spring freshet.

This approach to sampling was based on the hypothesis that during winter months fecal material sits frozen on top of snow or frozen earth – often with little breakdown – until precipitation or thaw events release it directly into waterways. This in turn produces discrete pulses of fecal coliform (Mullan, C., Dionne, M., Whiting-Grant, K., 2001). Due to the small size of the MBLR watershed, these pulses were expected to begin soon after the start of a precipitation event or thaw. Therefore the targeted timeframe for water sampling was between two and 24 hours after the start of the event.

The initial sampling date included collection of a sample from every sampling site identified in the watershed. Selection of sampling sites on subsequent dates was guided by the data from previous sampling dates. Identified "hot spots" were followed-up with additional sampling in that general area. This flexible approach provided the opportunity to conduct intensive, focused sampling as a pattern of hot spots emerged. Water temperature was recorded at each site to investigate the hypothesis that fecal coliform will achieve higher concentrations in warmer environmental conditions. We reduced sampling during periods of extremely cold temperatures in January 2003 since fecal concentrations proved to be very low during this period (Fig. 14). Very low bacterial counts along the Branch Brook watershed were also reported by the KKWWD staff during this period.

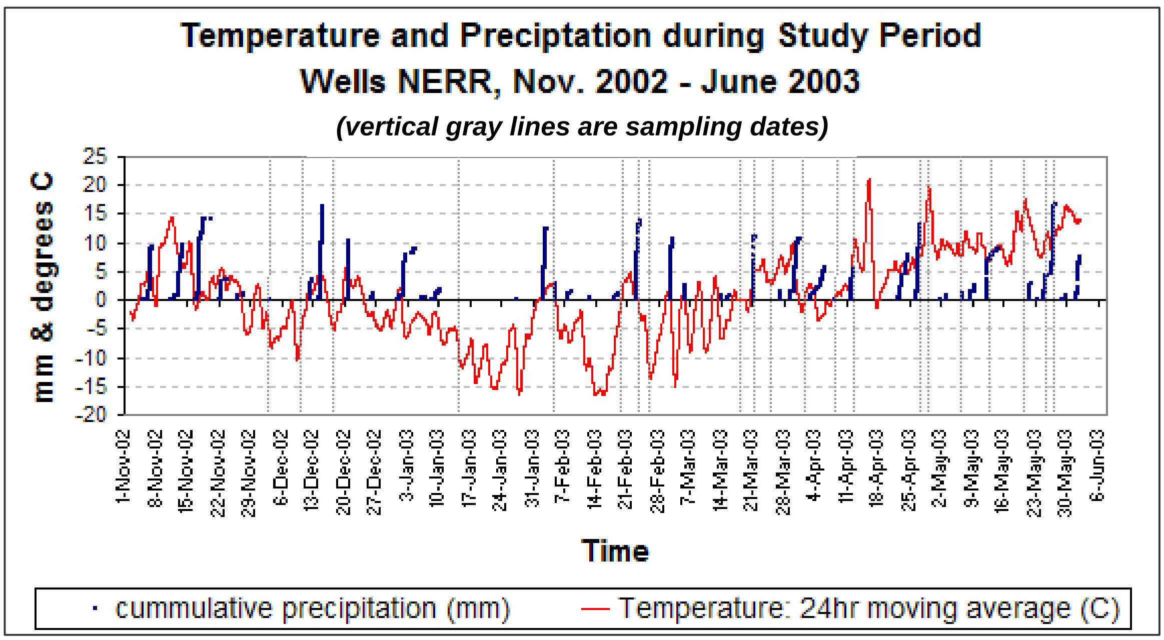


Figure 14: distribution of sample dates in relation to average temperatures and precipitation amounts.

Community volunteers and project staff collected water samples using the same procedures employed during the 2001-02 Webhannet 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" (Wells NERR, 2001). 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 (scat) for the MST reference library were collected by volunteers skilled in scat identification. Project staff collected the more easily identifiable samples including those from human sources. All fecal samples were collected from locations within the MBLR watershed representing the variety of potential sources. 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 WELLS NERR lab within six hours and refrigerated for later *E. coli* culturing and isolation. *E. coli* isolates were then transported to the University of New Hampshire's Jackson Estuarine Laboratory (JEL) along with sample collection forms (Appendix 4).

3.3 Defining Wet and Dry Weather Samples

Given the wide variety of potential precipitation conditions, high flow conditions were defined for each sampling event on a case-by-case basis. Sampling dates were classified by precipitation and wet weather conditions (Table 3). See Appendix 6 for graphs of precipitation, air temperature and fecal coliform counts. A "wet weather" designation was based on the approximate occurrence with one exception, 0.5 inches of precipitation in the previous 48 hours. On May 25, 2003, 0.43 inches of rain fell; however, all precipitation was within 24 hours of sampling.

Weather from the prior three days was considered in our interpretation of the results (Appendix 6), for each sampling event since a given amount of rain could conceivably have quite different effects on bacterial concentrations. For example, it is possible that a moderate rainfall which 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 flushed stored fecal matter into the waterways.

MBLR Sampling Dates	Inches of precipitation within 48 hours	Considered wet weather conditions
10-Dec-02	0.00	No
17-Dec-02	0.00	No
14-Jan-03	0.00	No
4-Feb-03	0.53	Yes
19-Feb-03	0.12	No
23-Feb-03	1.30	Yes
25-Feb-03	0.15	No
18-Mar-03	0.00	No
21-Mar-03	1.10	Yes
25-Mar-03	0.00	No
1-Apr-03	0.51	Yes
8-Apr-03	0.01	No
12-Apr-03	0.52	Yes
27-Apr-03	1.29	Yes
29-Apr-03	0.00	No
6-May-03	0.01	No
13-May-03	0.83	Yes
20-May-03	0.00	No
25-May-03	0.43*	Yes
27-May-03	1.20	Yes

Table 3: MBLR watershed sample collection calendar. * Normally, 0.5 inches of precipitation within 48 hours of sampling constituted wet weather conditions, although May 25, 2003, was defined as wet weather conditions because 0.43 inches occurred within 24 hours.

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[9] agar to detect the presence of *E. coli* and distinguish it from fecal coliform. A similar procedure for fecal coliform

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

testing (using mFC growth medium) has been employed by Wells National Estuarine Research Reserve (WELLS NERR) 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. (For a more detailed discussion of this procedure refer to "*E. coli* Isolation SOP" on file at the Wells National Estuarine Research Reserve).

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 and ArcView projects are archived at the Wells NERR on the MST Project computer and data CDs will be available from MST Project Manager Kristen Whiting-Grant.

3.6 Results – *E. coli*

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. For a few sample dates rainfall is associated with higher fecal counts, but in most cases the relationship between precipitation and fecal contamination appears to be quite weak (Table 4).

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; and town tax parcel boundaries from the towns of Wells, Kennebunk and Sanford. Project staff considered determining the general location of high bacterial counts an important first step in understanding the MBLR'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 (Fig. 15). This provides a way to quickly assess water quality in the MBLR watershed during the sampling period. Most of the Branch

Brook subcatchment had lower geometric mean values than the Merriland River subcatchment suggesting that the amount of protected or conserved land is related to water quality. (Recall that there is considerably more protected land in the Branch Brook subcatchment – see Figure 8 on page 18).

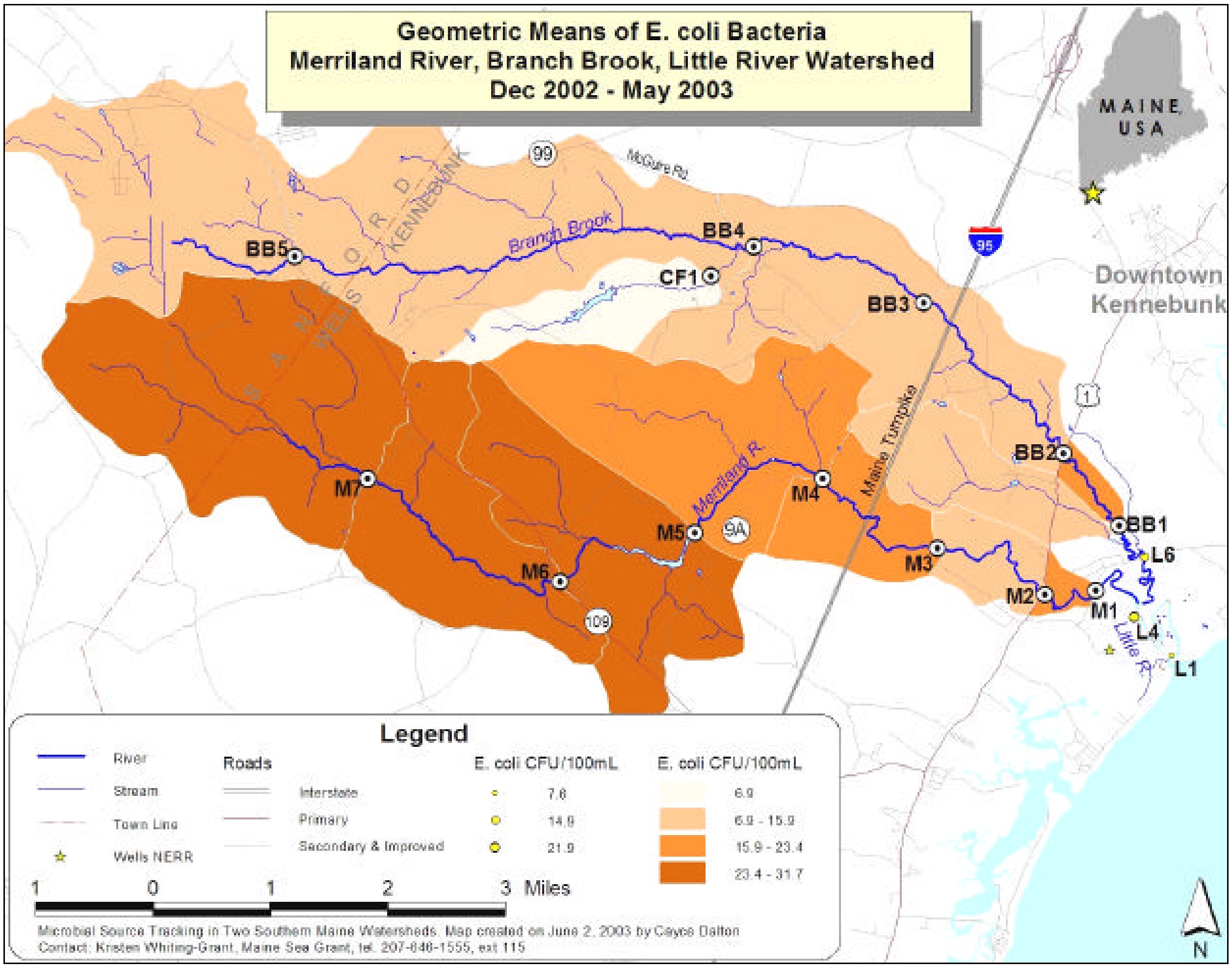


Figure 15: geometric means of *E. coli* concentrations in MBLR watershed by subcatchment area.

As mentioned in Section 1.3 above, all surface waters in the MBLR watershed are designated as Class A (or SA for the Little River estuary).[10] Consequently, *E. coli* concentrations cannot exceed naturally occurring levels. Overall bacterial concentrations in the MBLR watershed were quite low during the sampling period from December 2002 to May 2003. The highest geometric mean value was 31.7 *E. coli* colonies per 100 mL of sample (for site M7). However, there were several occasions when *E. coli* concentrations were quite high (Table 4).

[10] Maine Revised Statutes, Title 38, Chapter 3, § 468 and § 469.

	L1	L4	L6	M1	M2	M3	M4	M5	M6	M7	BB1	BB2	BB3	BB4	BB5	CF1	Precip.
3-Dec	0	5.8	12.5	7.3	9.1	5.8	9.2	7.5	3.3	49	13.3	10.8	15.8	39	10.8	63.5	0
10-Dec	3.3	-	3	6.4	2	6	3	3	4.2	5.8	0	5	6.7	8.2	14.2	0.8	0
17-Dec	1	140	18	217	184	186	159	207	139	159	14	5.5	12	35	10	10	0
14-Jan	3	12	-	9	6	1	2.5	6	1	2	-	0.5	1	-	0	-	0
4-Feb	-	-	-	5	4	4	5	51	6	3	5	8.8	3	3	-	-	0.53
19-Feb	-	-	1	1	2	-	-	0	0	-	-	-	4	-	1	-	0.12
23-Feb	-	-	-	9	-	10	37	5	5	9	-	2	1	3	4	-	1.3
25-Feb	6	6	5	17	-	-	14	22	13.5	4	4	4	0	6	0	0	0.15
18-Mar	1	-	1	4	3	4	4	25	160	76	2	1.5	23	19	1	2	0
21-Mar	-	36	12	135		42	52	44	375	455	37	64	43	25	0	6	1.1
25-Mar	10	13	27	31	23	18.3	15	39	105	91.7	8	9	4	5	5	22	0
1-Apr	9	3	13	8.3	7.5	11.7	15	15.8	22.5	17.5	16	11	15	4	7	9.5	0.51
8-Apr	10	3.3	8	5	2.5	5	8.3	8	17.5	15.8	9	4	6	1	19	4	0.01
12-Apr	-	-	84	-	-	-	-	-	-	60	-	-	-	-	-	-	0.52
27-Apr	-	126	90	94	-	110	-	185	-	96	-	62	-	25	21.7	-	1.29
29-Apr	46	57	12	20	17	12	16	28	14	18	12	12	12	0	10	0	0
6-May	6	4	12	26	42	28	6	12	14	8	36	14	40	22	92	6	0.01
13-May	10	48	75	93	46	48	78	208	186	82	136	70	20	18	64	6	0.83
20-May	26	24	4	78	38	20	16	20	12	50	10	16	24	26	89	9	0
25-May	34	108	156	194	228	336	98	36	390	114	258	488	372	90	90	44	0.43
27-May	> 400	130	700	730	280	400	1000	190	780	310	820	820	490	124	160	28	1.2
Max.	46	140	700	730	280	400	1000	208	780	455	820	820	490	124	160	63.5	
Min.	0	3	1	1	2	1	2.5	0	0	2	0	0.5	0	0	0	0	
Avg.	11.8	47.7	68.5	84.5	55.9	69.3	85.4	55.6	118.3	81.3	86.3	84.6	57.5	25.2	31.5	14.1	
Geom.	7.6	21.9	14.9	23.4	15.9	19.8	19.2	25.9	28.3	31.7	19.3	13.2	12.5	12.5	12	6.9	
Correl.	0.12	0.51	0.57	0.45	0.56	0.36	0.49	0.48	0.58	0.49	0.65	0.42	0.42	0.30	0.25	0.09	
R^2	0.01	0.26	0.32	0.20	0.31	0.13	0.24	0.23	0.34	0.24	0.42	0.17	0.18	0.09	0.06	0.01	

Table 4: *E. coli* results for MBLR watershed from December 2002 to May 2003. Averages (Avg.) and geometric means (Geom.) were calculated for each site. Correlation coefficients (Correl.) and linear regressions (R^2) were also calculated to determine the extent of relationship between *E. coli* concentrations and precipitation for the 48 hour period preceding each sampling event.

In general, the Merriland River sites tended to yield the highest *E. coli* concentrations while Branch Brook sites produced the lowest concentrations (Table 5). However, weather conditions were somewhat unusual during the sampling period with average temperatures being 2.8° F below normal and total precipitation being 4.6" below normal (National Weather Service Climate Data for Portland, ME). Consequently, the relationship between runoff events and *E. coli* concentrations may not have been as apparent for most of the sampling period. The final week of sampling (May 25th and May 27th) yielded much higher *E. coli* concentrations than any of the previous sampling events and many of these high results occurred at Branch Brook sampling sites (unlike any of the previous sampling events). Therefore, it is difficult to draw any definitive conclusions regarding the most problematic sampling sites in the MBLR watershed given the limited duration and unusual weather conditions of the sampling period.

Geometric Mean		Rank	Average		Rank
M7	31.7	1	M6	118.3	1
M6	28.3	2	BB1	86.3	2
M5	25.9	3	M4	85.4	3
M1	23.4	4	BB2	84.6	4
L4	21.9	5	M1	84.5	5
M3	19.8	6	M7	81.3	6
BB1	19.3	7	M3	69.3	7
M4	19.2	8	L6	64.9	8
M2	15.9	9	BB3	57.5	9
L6	14.9	10	M2	55.9	10
BB2	13.2	11	M5	55.6	11
BB3	12.5	12	L4	47.7	12
BB4	12.5	13	SB1	39.0	13
BB5	12.0	14	BB5	31.5	14
SB1	11.8	15	BB4	25.2	15
L1	7.6	16	CF1	14.1	16
CF1	6.9	17	L1	11.0	17

Table 5: ranked geometric means and arithmetic averages for *E. coli* concentrations in the MBLR watershed from December 2002 to May 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 MBLR watershed (Table 6). 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 abundance. Additional wildlife species were selected based on the various habitats present in the watershed. These included common mammals, such as muskrat, fox and coyote.

SPECIES	Ribotypes matched with Regional Library	% Total	Ribotypes matched with Local Library	% Total
Unknown	33	34%	43	44%
Pet				
Cat	21	21%	22	22%
Dog	1	1%	-	-
Wildlife				
Fox	7	7%	6	6%
Muskrat	3	3%	8	8%
Rabbit	3	3%	-	-
Coyote	1	1%	1	1%
Raccoon	1	1%	-	-
Livestock				
Cow	11	11%	11	11%
Horse	3	3%	-	-
Birds				
Cormorant	5	5%	-	-
Gull	3	3%	-	-
Turkey	2	2%	3	3%
Goose	1	1%	-	-
Human	3	3%	4	4%
TOTALS	98	100%	98	100%

Table 6. ribotyping results for MBLR watershed when matched against source species databases (regional and local reference libraries). (Jones, 2004).

Human samples were collected from three different sources: sewage (from the Kennebunk 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 MBLR watershed, the regional reference library developed by JEL (Table 6) 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. Samples for the regional library originated from Maine, New Hampshire, Vermont and Massachusetts – most from within 40 miles of the MBLR watershed.

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

Water 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 (even though all surface waters in the MBLR watershed are Class A). This rationale was used for Year 1 in the Webhannet watershed and it was continued in the MBLR watershed for consistency. 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 a limited number of *E. coli* isolates to be ribotyped. To select samples for ribotyping we focused on the sites that most consistently yielded the highest *E. coli* concentrations. Furthermore, ribotyping analysis was conducted on samples from different times of the season, and more or less equally between the Merriland River, Branch Brook and the Little River estuary portions of the watershed. 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. 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.

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

As described previously, water samples from the MBLR watershed were collected from December 2002 until May 2003. *E. coli* colonies were isolated from these samples and analyzed at the University of New Hampshire Jackson Estuarine Laboratory's ribotyping facility. Excerpts of the ribotyping report (Jones, 2004) are presented in the next two sections.

Site	Date	Weather Conditons	EC/100ml	Total # Isolates	Usable* Isolates	Ribotyping Success
L4	12/17/2002	dry	140	5	4	80%
	4/27/2003	wet	126	5	5	100%
	5/27/2003	wet	130	5	5	100%
L6	5/25/2003	wet	156	5	5	100%
	5/27/2003	wet	700	5	5	100%
M1	12/17/2002	dry	216.5	5	5	100%
	3/21/2003	wet	135	5	5	100%
	5/27/2003	wet	730	5	5	100%
M3	12/17/2002	dry	186	5	5	100%
	5/25/2003	wet	336	5	5	100%
M5	4/27/2003	wet	185	5	5	100%
	5/13/2003	wet	208	5	5	100%
M6	3/21/2003	wet	375	5	5	100%
	5/27/2003	wet	780	5	5	100%
M7	12/17/2002	dry	159	5	4	80%
	3/21/2003	wet	455	5	5	100%
BB1	5/13/2003	wet	136	5	5	100%
	5/27/2003	wet	820	5	5	100%
BB2	3/21/2003	wet	64	5	5	100%
BB5	5/6/2003	dry	92	5	5	100%
Totals				**100**	**98**	**98%**

Table 7: *E. coli* isolate selection for ribotyping analysis by sampling site, date and wet / dry weather conditions in the MBLR watershed. Pink cell values > 700 EC/100 mL; yellow cell values were between 336 and 455 EC/100 mL. (Jones, 2004).
* "Usable" isolates were those confirmed as *E. coli* by UNH / JEL.

Useable ribotypes resulted from samples collected on 7 different dates and 10 different sites (Table 7). 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 MBLR watershed and another larger regional database from Maine, New Hampshire, Vermont and Massachusetts (Table 6). 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 – 65% with the regional reference library as compared to 55% with the local reference library (Figs. 16, 17).

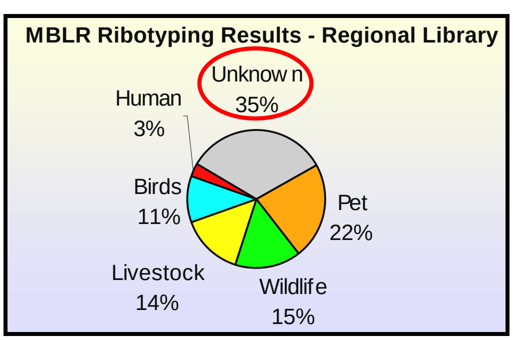


Figure 16. Source species identification using regional reference library. (Jones, 2004).

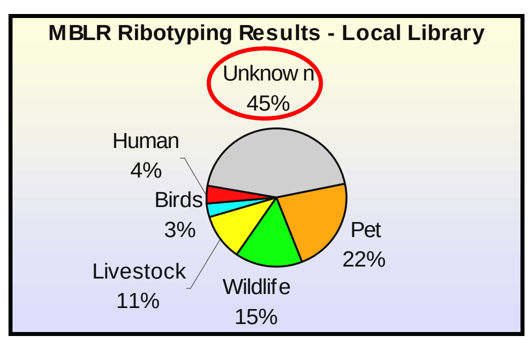


Figure 17. Source species identification using local reference library. (Jones, 2004).

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 (90%). 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 90% 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 90% threshold value; if the calculated value was below the threshold value, the water isolate sample was considered to be of unknown origin.

The overall ribotyping results (from the regional reference library) for the MBLR watershed are presented in Figure 18. Cats were the most frequently identified single source of bacterial contamination (21%); followed by cow (11%); fox (7%); cormorant (5%); human, rabbit, muskrat, horse and gull (all at 3%); turkey (2%); and goose, raccoon, coyote and dog (all at 1%). Also note that ribotypes for 35% of the bacteria samples analyzed by JEL could not be identified, which is to say that no clear matches could be established between ribotypes of known source species and ribotypes from unknown water samples. Many of these "unknowns" included some that had identical patterns for multiple species. This is considered to reflect 'garden-variety' strains of *E. coli* that can either exist temporarily in non-source species or are adapted to multiple species. These were included to allow for identification of patterns from "mixed" source species but were still ultimately considered as unidentifiable for the purposes of the current study.

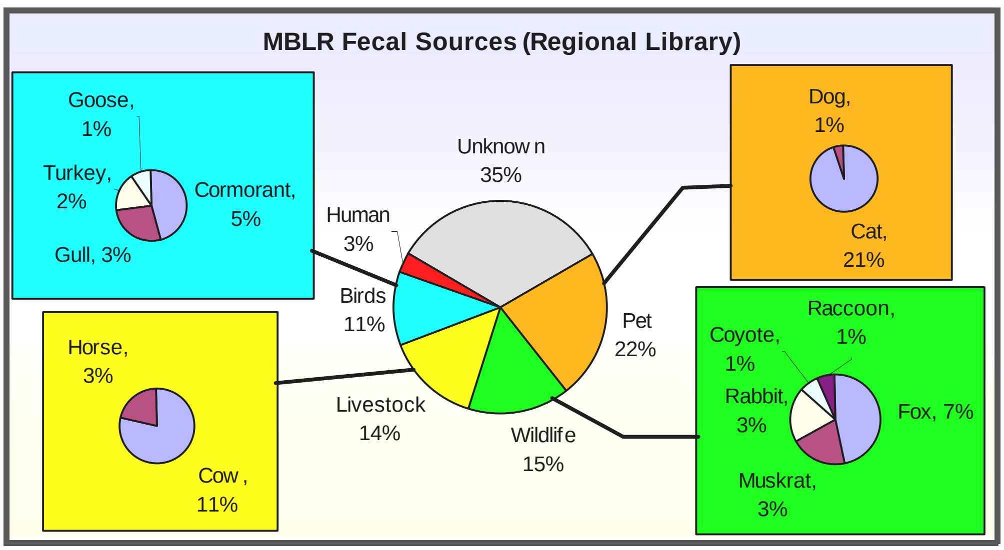
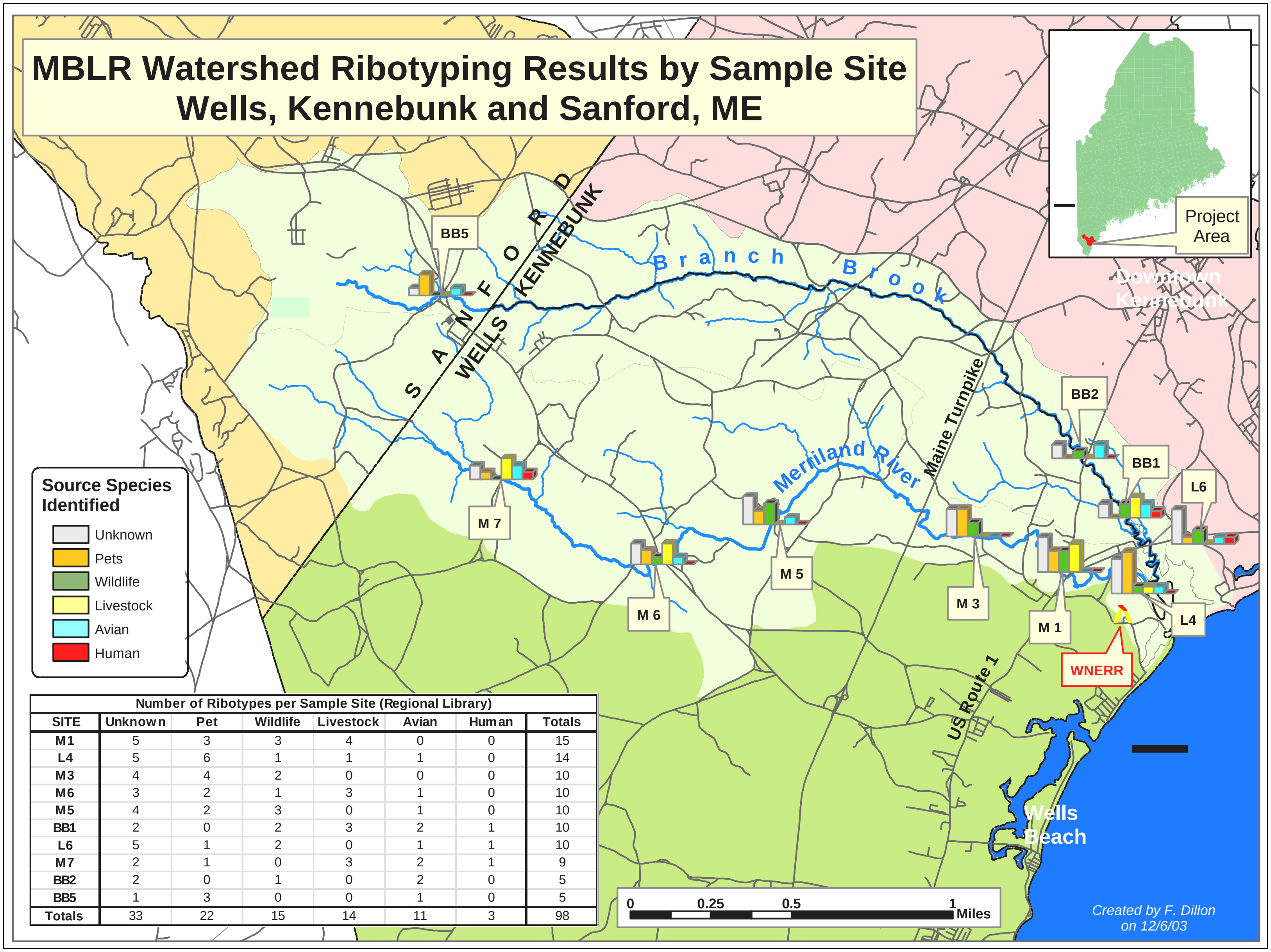


Figure 18. Source species identification for MBLR watershed using regional reference library. Pets – in particular cats – are the most frequently identified type of fecal contamination, followed by wildlife, livestock, birds and humans. (Jones, 2004).

3.10 Source Species Identification by Sampling Site and Subcatchment Area

Figure 19 provides a detailed summary of the identified fecal contamination source types for each of the 10 water sampling sites from which ribotypes were developed. Each site is represented by a bar graph, indicating the numbers of identified ribotypes along with those that could not be identified. Livestock was the most prevalent source identified at Merriland River sites M7, M6 and M1, while wildlife and pets were most prevalent at sites M5 and M3, respectively. Little River site L6 had a higher number of ribotypes for wildlife while pets were by far the most commonly identified fecal source at site L4. Pets were also most common at Branch Brook site BB5, birds were most common at BB2 and livestock was most common at BB1. From a subcatchment perspective, the Merriland River had the highest incidence of identified ribotypes from livestock and lowest incidence of ribotypes from human sources (Fig. 20, 21). For the Little River, the highest incidence of identified ribotypes was from pet and human-borne sources and the lowest incidence of ribotypes from livestock. In the Branch Brook the highest incidence of ribotypes was from birds and humans and the lowest incidence for pet-borne bacteria.



Number of Ribotypes per Sample Site (Regional Library)

SITE	Unknown	Pet	Wildlife	Livestock	Avian	Human	Totals
M1	5	3	3	4	0	0	15
L4	5	6	1	1	1	0	14
M3	4	4	2	0	0	0	10
M6	3	2	1	3	1	0	10
M5	4	2	3	0	1	0	10
BB1	2	0	2	3	2	1	10
L6	5	1	2	0	1	1	10
M7	2	1	0	3	2	1	9
BB2	2	0	1	0	2	0	5
BB5	1	3	0	0	1	0	5
Totals	33	22	15	14	11	3	98

Figure 19. geographic distribution and species composition of ribotypes in the MBLR watershed. Bar heights indicate number of ribotypes for each sampling site (actual numbers included in inset table). (Jones, 2004).

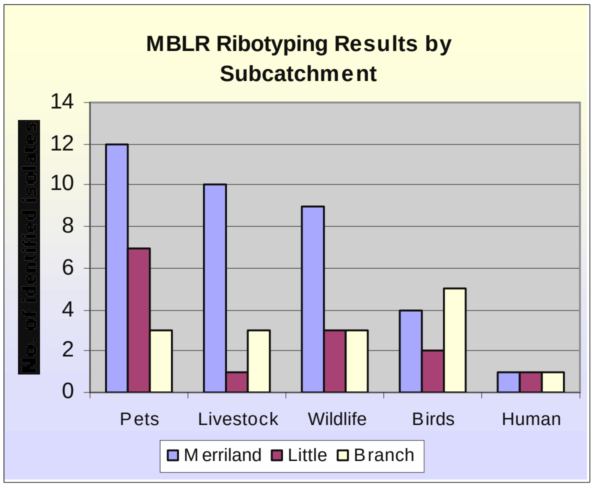


Figure 20. numbers of identified ribotypes by subcatchment. (Jones, 2004).

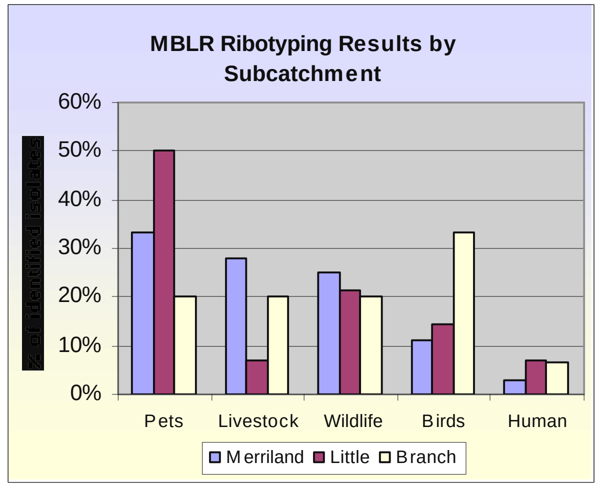


Figure 21. percent of totals for ribotypes identified within each subcatchment. (Jones, 2004).

3.11 Wet Versus Dry Weather Sources

Analysis of all isolates collected throughout the study period was separated into “wet” and “dry” weather samples (Table 7). Samples used for ribotyping were collected under wet conditions on 5 of the 7 sampling dates. There were 75 wet-weather isolates and 23 dry-weather isolates. Source species identified under wet weather included 49 of the 75 isolates collected (65%). The source species identified during dry weather included 16 of the 23 isolates collected (70%). The percentages of isolates identified by types of source species for wet and dry weather respectively were wild animals: 16 and 13%, humans: 4 and 0%, pets: 16 and 43%, livestock: 17 and 4% and birds: 12 and 9%. The percentage of isolates identified as wild animals and birds were similar for both wet and dry conditions, but there were large differences for humans, pets and livestock. Both human and livestock-borne isolates were much more prevalent during wet weather, while pets were much more prevalent under dry weather conditions.

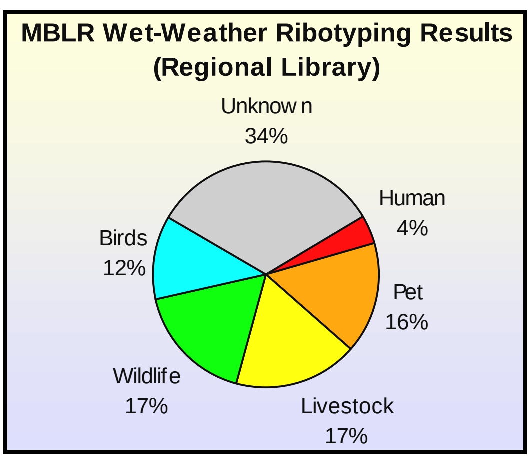


Figure 22. source species identification during wet weather. Source: Jones (2003)

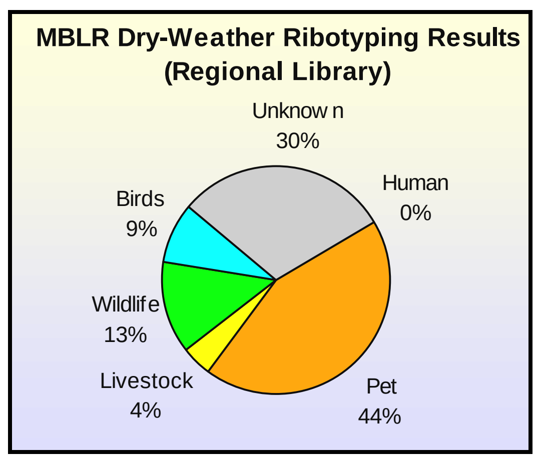


Figure 23. source species identification during dry weather. Source: Jones (2003)

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, stormwater runoff collection systems, and overboard discharges (OBDs).[11] As mentioned in Section 2.2 above, there is very little public sewer or stormwater infrastructure – and therefore very little potential for contamination from it – in the MBLR watershed. In 2001 the Wells National Estuarine Research Reserve (Wells NERR) conducted an extensive shoreland survey of the MBLR watershed's riparian zone (Dionne *et al*, 2002). Only two discharge pipes were identified, neither of which was determined as a source of fecal contamination, and DMR has not found any issues with OBDs. Apparently very little of the fecal contamination present in the MBLR watershed originates from point sources.

In addition to sewage, the potential effects of impervious surfaces (i.e., paved areas) on water quality can also be significant. 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)." While very little (if any) of the MBLR watershed can be considered "urban," water resource managers should be aware that urban runoff has been listed as an extremely difficult problem worthy of an ongoing implementation schedule by the TMDL[12] 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. Appendix 8 provides a list of documents (including model ordinances) developed by the Center for Watershed Protection and the Stormwater Manager's Resource Center to help municipalities protect local water resources.

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 (USEPA, 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,

[11] OBDs are individual or community domestic wastewater treatment systems that discharge directly to receiving waters.

[12] 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.

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 (USEPA, 2002). From the perspective of bacterial contaminants, NPS pollution can originate from a variety of sources including wildlife, improperly managed pet and livestock waste and failing septic systems. Each of these will be discussed in further detail below.

4.21 Wildlife Components

Given that only about 3% of the MBLR watershed is classified as developed (Figure 4, page 14), it is not surprising that the combined categories of wildlife and birds represent the most significant sources of identified fecal contamination. In the regional reference library, 40% of all ***identified*** ribotypes (excluding unidentifiable ribotypes) originated from wildlife and birds (Figs. 19, 24, 25). Foxes represent the most common species at 26% of all identified wildlife ribotypes, followed by cormorants at 18%; gulls, muskrats and rabbits all at 12%; turkeys at 8%; and coyotes, geese and raccoons all at 4%.

Controlling fecal contamination from wildlife sources has proven to be particularly difficult, both practically and politically. Management strategies have generally focused on removing animals from problem areas. MST 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). 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 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 late 2002 and 2003, the Town of Wells allowed for the taking of deer from Laudholm Farm and Drakes Island as part of an effort to reduce the burgeoning deer population (NOT to address issues with fecal contamination). The public meetings prior to these actions proved to be quite contentious with some residents voicing strong opposition. Therefore, given the potentially controversial nature of any initiative to further reduce animal populations beyond levels currently allowed through hunting and trapping[13], public input should be a crucial consideration before undertaking such initiatives. Also, current Maine Class A water quality standards – as in the MBLR watershed – allow for "naturally occurring" bacterial concentrations, which implies that fecal inputs from wildlife sources are not considered a serious detriment to water quality.

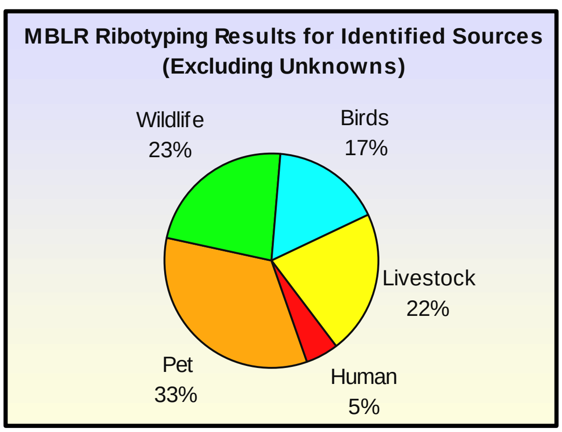


Figure 24. species composition for ***identified*** ribotypes (excluding unidentified ribotypes) in MBLR watershed. (Jones, 2004).

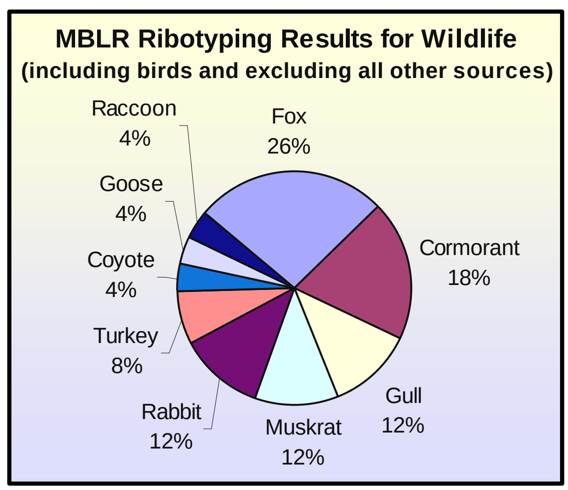


Figure 25: species composition for ***identified*** wildlife ribotypes in the MBLR watershed. (Jones, 2004).

4.22 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

[13] In the past, KKWWD has trapped beaver in the Branch Brook watershed in an attempt to reduce fecal contamination there.

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). Thus, if 3,000 people reside in the MBLR watershed there would be a canine fecal load of approximately 150 pounds per day – more than enough to significantly contribute to watershed fecal contamination. Microbial source tracking results for the MBLR watershed indicate that 21% of all ribotypes originated from cats while only 1% originated from domestic dogs (Table 6, page 26). For ***identified*** ribotypes (excluding unidentifiable isolates) ribotyping results for cats and dogs were 32% and 1.5%, respectively.

While residents typically seem to recognize that pet 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). The need to dramatically improve watershed education efforts to increase public recognition about the water quality and health consequences of pet waste are especially important given the unexpected finding that cats in particular were a major source of fecal contamination in the MBLR watershed. 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 – including cat litter. (Investigations by WNERR researchers in nearby watersheds have found cat litter dumped close to surface waters).

It is also possible that feral cat colonies are present in the MBLR watershed and may be contributing to fecal contamination there. If this is so, a trap/neuter/return (TNR) program might be worth considering. TNR involves humanely trapping feral cats and transporting them to a veterinary clinic for spaying or neutering. After surgery, the cat is returned back to the colony habitat. Since the cats are no longer reproducing, the colony gradually diminishes in size. One study in San Francisco found over a 50% reduction in feral cat population following the implementation of a TNR program.[14] New Hampshire passed legislation in 1994 that provided funding for cat spaying and neutering. Since then, animal shelters have experienced a significant decrease in intake and euthanasia rates. As of January 2004, Maine was in the process of developing a similar program that could help to address potential issues of fecal contamination from feral cat colonies.[15] Appendix 11 provides more information on the establishment of a pet waste management program.

4.23 Livestock Waste Management

Livestock represents 22% of identified fecal contamination sources in the MBLR watershed (Fig. 24), 79% from cows, and 21% from horses (Fig. 26). Agricultural management practices to reduce fecal contamination from livestock waste generally relate to proper manure storage and handling techniques along with keeping farm animals away from surface waters. The following strategies can be used to address runoff potential from livestock waste:

- Install drainage swales, buffers and filter strips on field edges that border surface waters;

- Protect fields from erosion to reduce the movement of manure fertilizers into surface waters;

[14] Friends of Feral Felines (http://home.maine.rr.com/feralfelines/factshet/TNR.htm)

[15] Personal communication on 1/29/04 between MST Project staff member Fred Dillon and Norma Worley, Director of Animal Programs for the Maine Department of Agriculture.

- Maintain adequate setbacks from surface waters and drainage ditches and avoid steep slopes when applying manure fertilizers;

- Apply manure fertilizers only during crop growing season (avoid spreading on frozen or saturated ground);

- Reduce manure application rate on poorly drained soils;

- Properly store manure to prevent runoff into nearby surface waters.

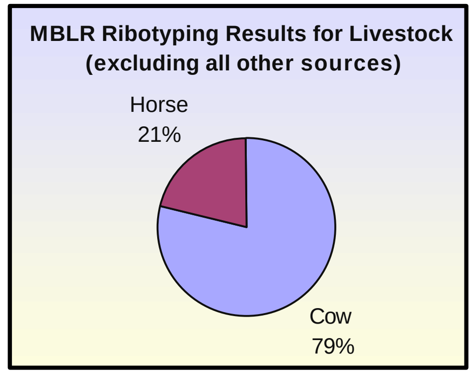


Figure 26: species composition for ***identified*** livestock ribotypes in the MBLR watershed. (Jones, 2004).

The Natural Resources Conservation Service in Alfred, ME can be contacted for more specific information regarding these management practices. Homeowners improperly using livestock manures for landscaping or gardening purposes also represent a potential threat to water quality. All of the recommendations above intended for larger scale agricultural operations also apply to smaller scale residential situations. Appendix 11 has more information for homeowners.

4.24 Septic System Controls and Inspection

Maine is a predominantly rural state and therefore relies heavily on privately owned subsurface disposal facilities (i.e., septic systems – Fig. 27). Most of the occupied buildings in the MBLR watershed use septic systems for domestic wastewater disposal. 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).

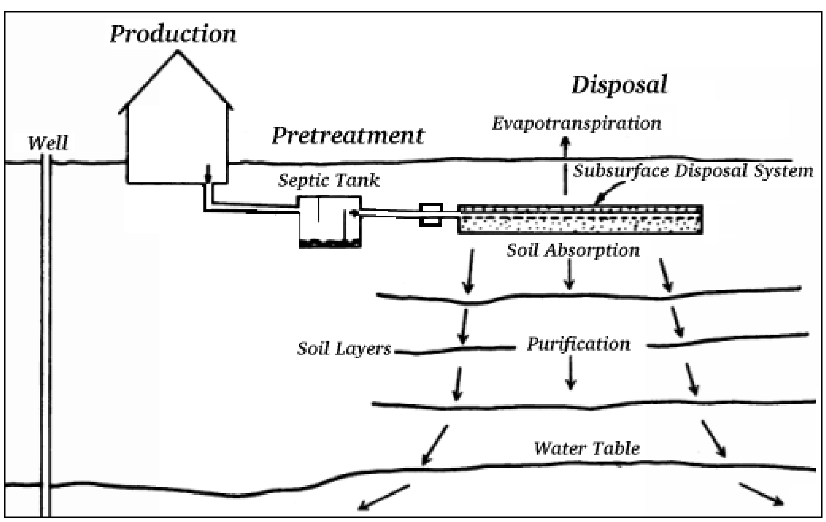


Figure 27. typical cross-section of septic system.

Given that most wastewater treatment in the MBLR watershed is provided by privately owned septic systems, it seems a reasonable hypothesis that most of the relatively small number of human ribotypes there (5% of all identified ribotypes –Fig. 24) originated from malfunctioning septic systems. As such, it would be useful to identify these properties by developing some type of septic system maintenance tracking system. One such strategy could simply involve cooperating with the Department of Human Services to query their databases for the issuance of all permits allowing the new construction or modification of septic systems before a certain date – perhaps 15 to 20 years ago. Here the assumption would be that older septic systems (installed shortly after the permit was issued when 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 scheduling system for septic tank pumping frequency. Communities around the country – including Brunswick, Maine – have established such programs to mitigate bacterial contamination from failing septic systems. In 2001, South Kingstown, Rhode Island, enacted a mandatory on-site wastewater management inspection program that established septic system maintenance schedules.[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 towns of Wells, Kennebunk and Sanford should consider one of these approaches.

<u>4.3 Summary of Management Recommendations</u>

The ribotyping results were used to develop a management plan for reducing fecal contamination in the MBLR watershed. Additional data sources used to corroborate the ribotyping results included: the work of previous researchers; field surveys for the MBLR watershed; maps of land cover/habitat types; and local knowledge of wildlife prevalence and distribution. The recommendations offered in this plan are summarized below for each of the identified sources.

<u>Wildlife Sources</u>

- Maintain or establish adequate riparian buffers to reduce volume of contaminated runoff.
- Work with municipalities to provide information to residents in watershed about ways to reduce attraction of problem species.
- Evaluate or consider developing local ordinances restricting the feeding of wildlife to reduce the congregation of animals and the potential concentration of their waste.

<u>Pet Sources</u>

- Increase efforts to promote proper handling and disposal of pet waste, and in particular cat waste.
- Evaluate or consider developing local ordinances targeting pet waste management.

<u>Livestock Sources</u>

- Cooperate with the municipalities, the Farm Management Bureau and University of Maine Cooperative Extension to 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 for patrons purchasing manure at these locations on proper handling of animal fertilizers. Cooperate with University of Maine Cooperative Extension to share this information through Master Gardener programs.

<u>Human Sources</u>

- Provide information on proper septic system maintenance to all owners of septic systems in watershed.

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[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)

- Recommend to the town or state the establishment of a septic system tracking program that establishes maintenance schedule for property owners. Also facilitate sharing of information between state agencies (Department of Marine Resource, Department of Human Services) for changes in septic system status discovered during site evaluations.

- Continue to work with DMR to ensure that no overboard discharges exist in the Little River estuary.

OTHER CONSIDERATIONS FOR THE USE OF MST IN THE FUTURE

Clearly, microbial source tracking methods represent a significant advancement over conventional bacterial test methods in attempting to more closely identify sources of fecal contamination in coastal and inland watersheds. However, due to the considerable expense of these methods, previous efforts to enlist upper-level state support in Maine for MST (at least throughout the 1990's) were not successful, particularly in light of budgetary constraints. A formal cost / benefit analysis may be needed before the state is likely to allocate significant resources to MST. Because MST – and ribotyping in particular – is an expensive process, a highly targeted approach for its use is recommended (Jones, 2004). With respect to the goal of opening clam harvesting areas we suggest the following:

- Identify and prioritize shellfish harvesting areas with very high resource value through close cooperation with community members and municipal officials.

- Establish baseline data (from both water quality monitoring and shoreline surveying) to determine where major contamination sources could be entering waterways.

- Determine the likelihood that *E. coli* from specific locations will enter the estuary in significant concentrations.

- Evaluate the most likely major sources of contamination and establish a targeted source library (especially for non-wildlife species).

- Conduct intensive, short-term water sampling in that region during the environmental conditions that historically produce the highest counts.

- Conduct MST on this targeted set of unknowns. As long as human, livestock or pet contamination is identified, then there is hope for correction and improvement in water quality and a reason to keep up the investigation.

Non-wildlife species are emphasized because management strategies are more likely to be successful in reducing fecal contamination from them rather than from wildlife species. We also suggest that the best places to target for MST work are those high priority areas where baseline data has been collected, resource value is high and the community capacity exists to help implement the resulting management plan.

For groups seriously contemplating the use of MST, the Massachusetts Office of Coastal Zone Management[18] has developed the following checklist:

Preliminary data and background information before beginning

- Document bacterial contamination (>1 year)
- Develop list of top contaminating candidates
- Know the time period of interest (e.g., bathing season)
- Establish community support / involvement in project

[18] Contact Todd Callaghan at 617-626-1233 or todd.callaghan@state.ma.us for more information.

Questions for contract lab or consultant

- When will the sampling program occur?
- Is there an established Quality Assurance plan for proper (water and scat) sample collection, handling and processing?
- Which MST method will be used?
- Will the MST method require a reference library?
- What is the cost per isolate?
- How many *E. coli* isolates will be selected for MST analysis (at least 10 should be collected from each sample)?
- For library dependent MST methods:
  - How many isolates and from which species will be in the scat reference library?
  - Will funds be available to build or augment the library?
  - Will a regional library be available to broaden the analysis?
  - What will be the threshold for similarity between knowns and unknowns?
- What will be the rate of correct classification?
- Has lab been validated with blind studies?
- What will be the timeframe for results?
- Are there references available from similar work performed elsewhere?

NEXT STEPS

Based on current understanding, fecal coliform and *E. coli* levels in the MBLR 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 Little River estuary will determine which areas are suitable for shellfish harvesting, though there is some question regarding how accurately year round water quality conditions are reflected with their current sampling regimen which runs from December to May (Section 2.3). In any event, it would also be helpful to establish an ongoing 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 MBLR 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 reduce bacterial contamination levels to allow reopening shellfish harvesting areas in the Little River 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 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 MBLR watershed. The process began by sending draft copies of this report to all members of the MBLR MST Steering Committee so their comments could be incorporated into the final version. This MBLR 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.

- Towns of Wells, Kennebunk and Sanford
- Southern Maine Regional Planning Commission
- Wells National Estuarine Research Reserve (WELLS NERR)
- 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 also will be (or have already been) 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, Kennebunk and Sanford Chambers of Commerce
- Wells Regional Weekly Publication (Making It At Home)
- WELLS NERR (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 local access cable TV stations of Wells, Kennebunk and Sanford; 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 (or have been) shared at public events occurring at WELLS NERR through the spring of 2004.

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.

Pet owners

Outreach efforts will be coordinated with the municipal offices of Wells, Kennebunk and Sanford. 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 dog. Similar materials will also be distributed to local veterinary practices, animal shelters and dog training establishments, making these informational materials available to pet owners during visits. The State of Maine is also expected to begin a spaying and neutering program in 2004. Informational materials for this program will be distributed to the locations cited above following its implementation.

Gardeners and farmers

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.

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. 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.

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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: MBLR Steering Committee List

Nancy Bayse	Great Works Regional Land Trust
Judy Bernstein	Town of Kennebunk
Jonathan Carter	Town of Wells
Ron Collins	Land owner, State Representative
Geoff Coombs	Natural Resource Conservation Service
Lois Dennet	Maine Council of Churches
Chris Feurt	Kennebunk Conservation Commission
Ward Feurt	Rachel Carson National Wildlife Refuge (RCNWR)
Keith Fletcher	The Nature Conservancy
Don Gobiel	Kennebunk, Kennebunkport, Wells Water District (KKWWD)
Diane Gould	US Environmental Protection Agency
Owen Grumbling	Wells Conservation Commission
James Gulnac	Town of Sanford
David Hardy	Wells Conservation Commission
Don Kale	Maine Department of Environmental Protection
Andrea Leonard	WELLS NERR
JT Lockman	Southern Maine Regional Planning Commission
RJ Mere	Kennebunk Conservation Commission (KCC)
Sue Schaller	Southern Maine Regional Planning Commission
Len Sevigney	Land owner
Tin Smith	WELLS NERR
Marilyn Smith Church	US Environmental Protection Agency
Marie Louise St. Onge	Kennebunk Land Trust
Esperanza Stancioff	Maine Sea Grant / Cooperative Extension Service
Betsy Stevens	Kennebunk Land Trust
John Storer	KKWWD
Graham Taylor	RCNWR
Heather True	WELLS NERR
John White	KCC

Appendix 3: Water Sample Collection Field Sheet

Water Sample Collection Field Sheet
Microbial Source Tracking in Two Southern Maine Watersheds, MBLR Watershed 2002-2003
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

<table>
<tr><td colspan="3">Microbial Source Tracking Project</td></tr>
<tr><td rowspan="2">Site Name:</td><td colspan="2">Type of Sample</td></tr>
<tr><td>Fecal:</td><td>Water:</td></tr>
<tr><td>Site Description:

Street:

Town:

Watershed:

Date Sample Collected:

Time:</td><td>Animal Species:

Location:</td><td>Water Temperature:
% DO Saturation:
DO:
pH:
Conductivity:

Location:
Instream
Seep
Swale
Storm Drain
Other___________

Air Temperature:</td></tr>
<tr><td colspan="2">Sampled By:</td><td>Weather:</td></tr>
<tr><td colspan="2">Parameters:

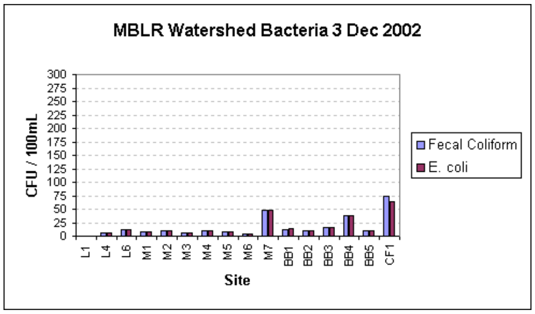
Air Temperature:</td><td>Laboratory Sample Notes:</td></tr>
<tr><td colspan="2">E. coli:</td><td>Results: FC______________cfu/100ml

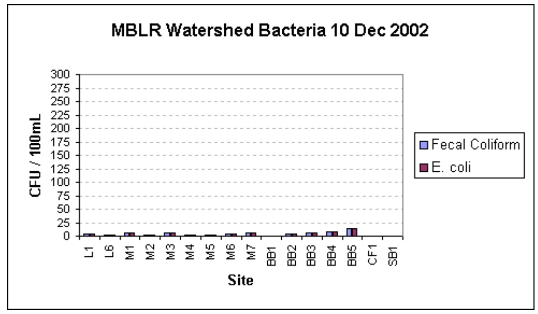
EC______________cfu/100ml</td></tr>
<tr><td colspan="2">Flow Rate: Yes:___________________ f/s No</td><td>Selected for ribotyping: Yes No
Date of ribotyping: ______________</td></tr>
<tr><td colspan="3">Comments and Site Sketch / Description:

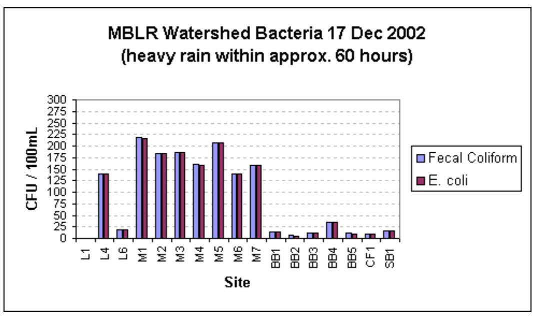
Site name (and T-soy plate labeling) indicates date of water sample collection (yyyymmdd), site designation (e.g. “B1”), and isolate designation “A-J” (with A-E on 1 T-soy plate and F-J on the other).</td></tr>
<tr><td colspan="3">Delivered to JEL Date:_____________________________ Time:______________________</td></tr>
</table>

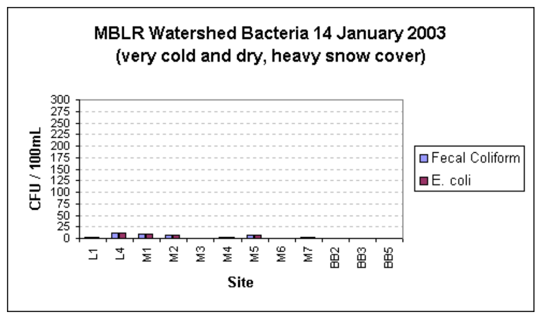
Appendix 5: Graphs of Merriland / Branch Brook / Little River watershed bacterial concentrations.

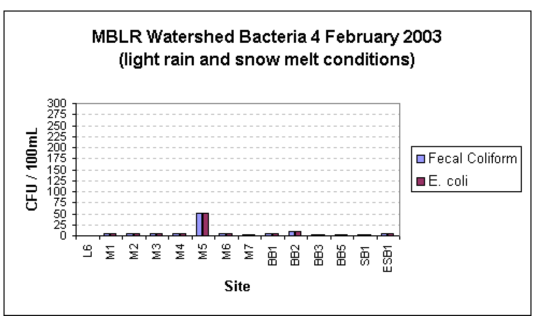
The graphs below describe the bacterial concentrations in the Merriland / Branch Brook / Little River watershed from December 2002 to May 2003. Both fecal coliform and *E. coli* concentrations were obtained using the mTEC + urea membrane filtration method and are measured in colony forming units (CFU) per 100mL of sample. Ribotyping was conducted on a subset of these bacteria. Note also that the vertical scale changes in the final two graphs to reflect higher bacterial levels in during warmer weather.

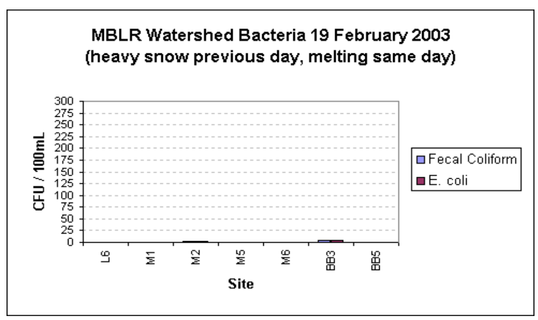


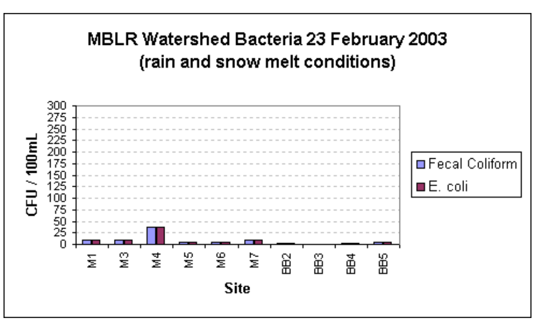


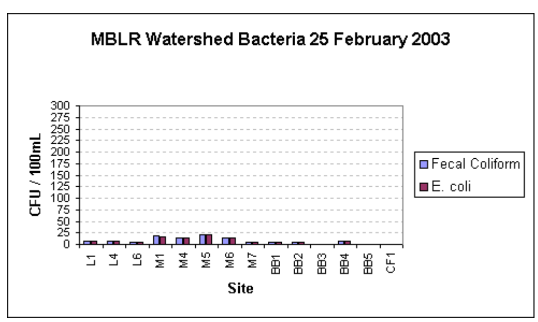


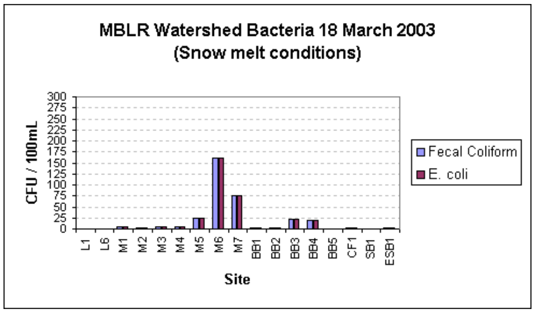


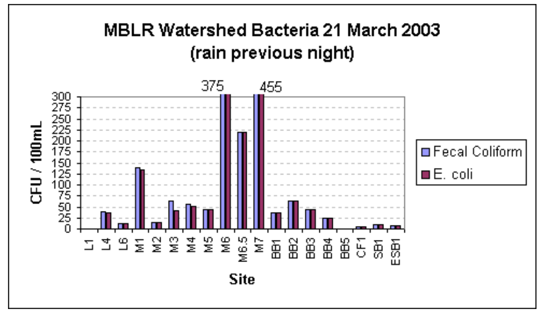


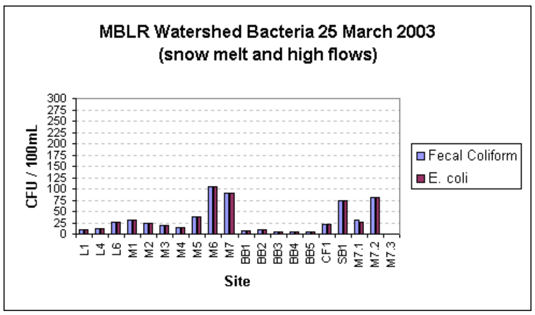


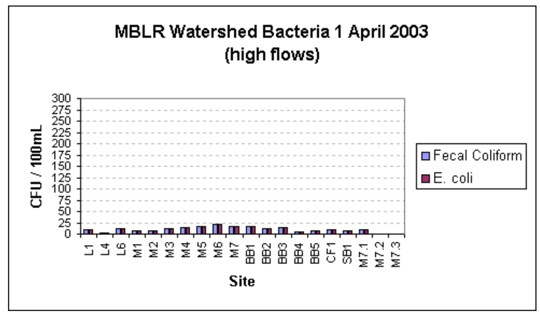


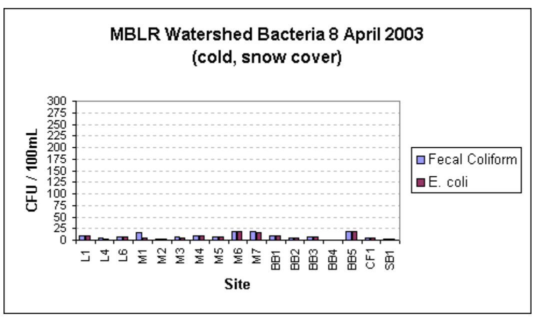


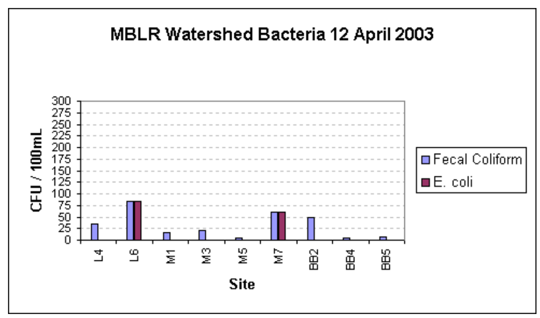

MBLR Watershed Bacteria 18 March 2003
(Snow melt conditions)
CFU / 100mL
Fecal Coliform
E. coli
L1 L6 M1 M2 M3 M4 M5 M6 M7 BB1 BB2 BB3 BB4 BB5 CF1 SB1 ESB1
Site

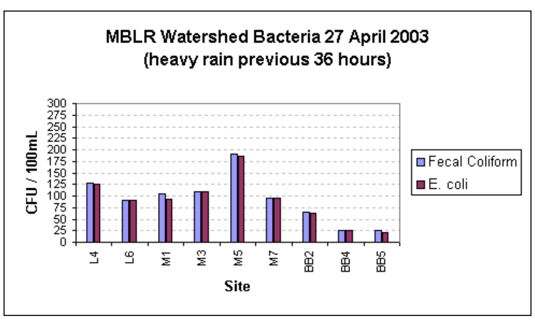

MBLR Watershed Bacteria 21 March 2003
(rain previous night)
375
455
CFU / 100mL
Fecal Coliform
E. coli
L1 L4 L6 M1 M2 M3 M4 M5 M6 M6.5 M7 BB1 BB2 BB3 BB4 BB5 CF1 SB1 ESB1
Site

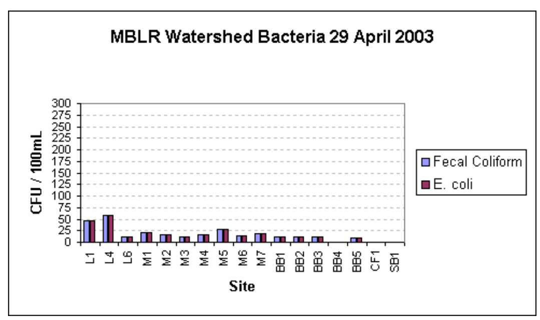

MBLR Watershed Bacteria 25 March 2003
(snow melt and high flows)
CFU / 100mL
Fecal Coliform
E. coli
L1 L4 L6 M1 M2 M3 M4 M5 M6 M7 BB1 BB2 BB3 BB4 BB5 CF1 SB1 M7.1 M7.2 M7.3
Site

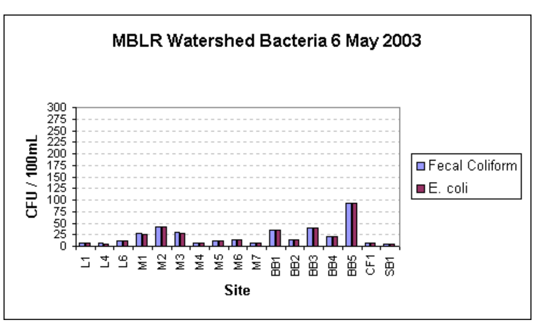

MBLR Watershed Bacteria 1 April 2003
(high flows)
CFU / 100mL
Fecal Coliform
E. coli
L1 L4 L6 M1 M2 M3 M4 M5 M6 M7 BB1 BB2 BB3 BB4 BB5 CF1 SB1 M7.1 M7.2 M7.3
Site

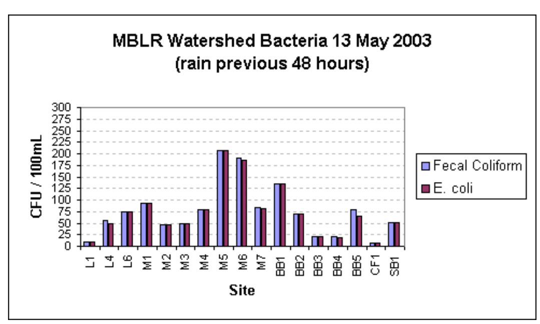

MBLR Watershed Bacteria 8 April 2003
(cold, snow cover)
CFU / 100mL
Fecal Coliform
E. coli
L1 L4 L6 M1 M2 M3 M4 M5 M6 M7 BB1 BB2 BB3 BB4 BB5 CF1 SB1
Site

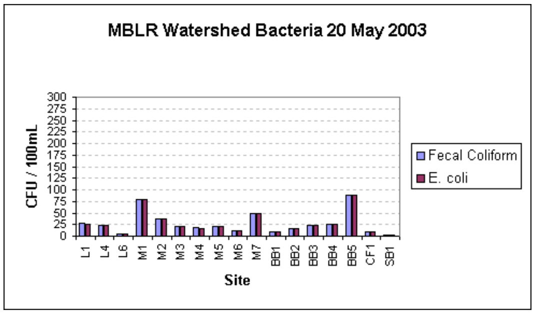

MBLR Watershed Bacteria 12 April 2003
CFU / 100mL
Fecal Coliform
E. coli
L4 L6 M1 M3 M5 M7 BB2 BB4 BB5
Site

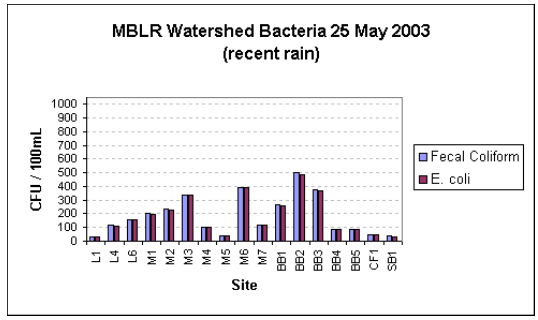

MBLR Watershed Bacteria 27 April 2003
(heavy rain previous 36 hours)
CFU / 100mL
Fecal Coliform
E. coli
L4 L6 M1 M3 M5 M7 BB2 BB4 BB5
Site

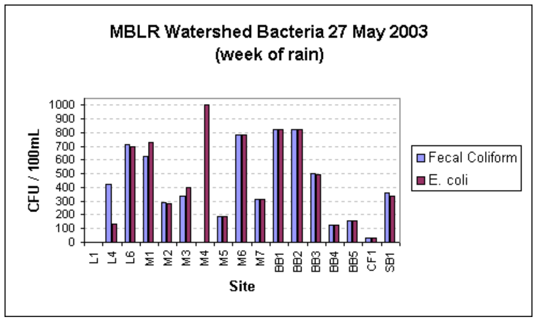

MBLR Watershed Bacteria 29 April 2003
CFU / 100mL
Fecal Coliform
E. coli
L1 L4 L6 M1 M2 M3 M4 M5 M6 M7 BB1 BB2 BB3 BB4 BB5 CF1 SB1
Site


MBLR Watershed Bacteria 6 May 2003
CFU / 100mL
Fecal Coliform
E. coli
L1 L4 L6 M1 M2 M3 M4 M5 M6 M7 BB1 BB2 BB3 BB4 BB5 CF1 SB1
Site


MBLR Watershed Bacteria 13 May 2003
(rain previous 48 hours)
CFU / 100mL
Fecal Coliform
E. coli
L1 L4 L6 M1 M2 M3 M4 M5 M6 M7 BB1 BB2 BB3 BB4 BB5 CF1 SB1
Site



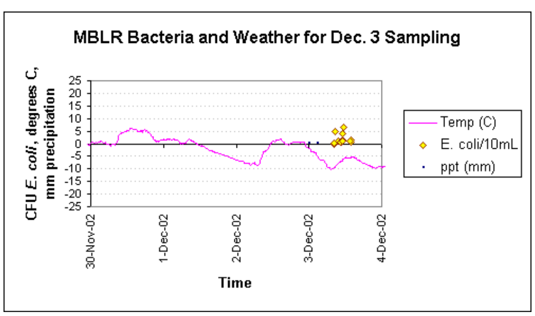


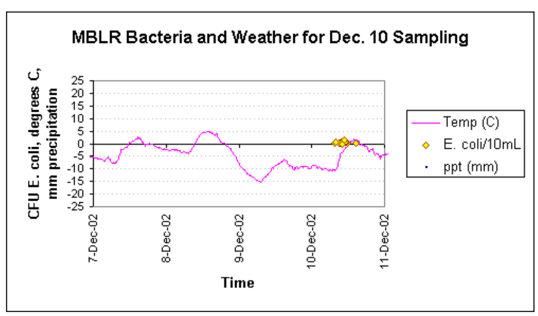


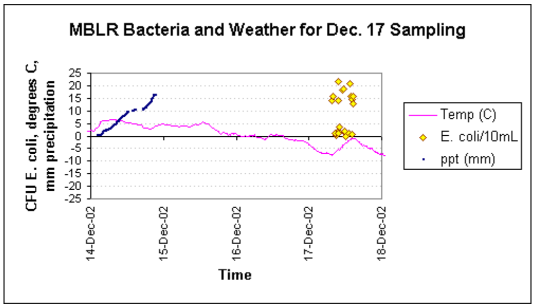
Appendix 6: Graphs of *E. coli* Concentrations and Weather Conditions

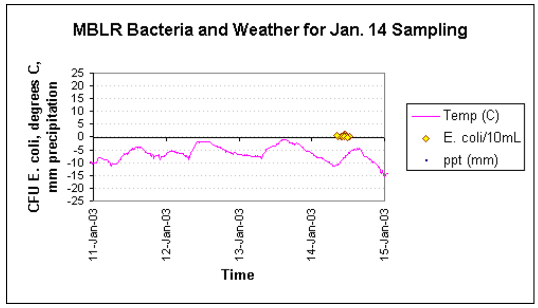
The following graphs show the concentrations of *E. coli* bacteria in the MBLR watershed in relation to air temperature and cumulative precipitation recorded by the weather station at the Wells National Estuarine Research Reserve. Cumulative precipitation amounts were reset to zero when more than 24 hours elapsed between precipitation recordings. For several sample dates, such as January 14 and February 19, temperatures were well below freezing in the three days leading up to the sampling event. Prolonged below-freezing temperatures are expected to correlate with much lower bacterial concentrations. On a some dates, significant precipitation events appear to correlate with higher bacterial concentrations as would be expected, such as on March 21, April 27, May 25 and May 27, although this does not appear to be the case on February 23 or 25.

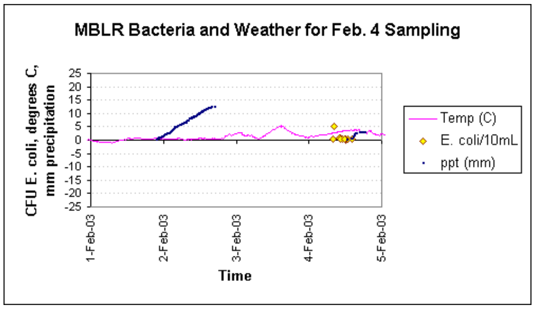
Note: Units were chosen in order to fit all data points on the same graph. Bacteria are in colony forming units (CFU) per 10 mL (instead of the standard 100 mL). To convert bacterial concentrations to per 100 mL, add a zero. Precipitation is shown in millimeters (instead of inches). Note also that the scale of the vertical axis changes for the last two graphs to accommodate higher bacterial concentrations.

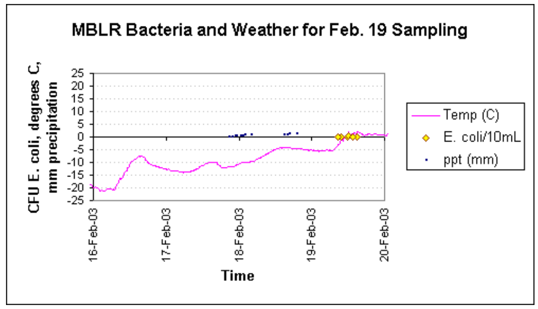


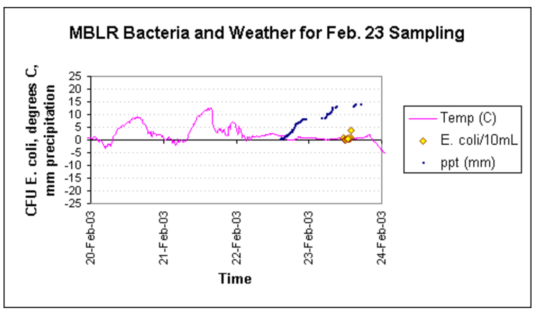


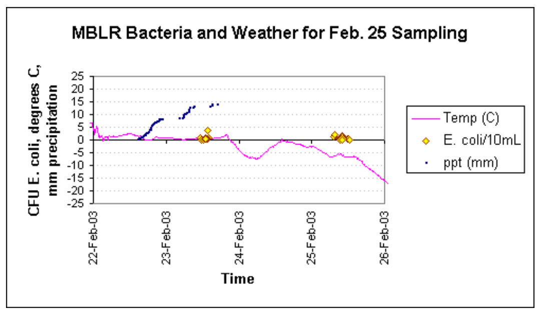

MBLR Bacteria and Weather for Dec. 17 Sampling
CFU E. coli, degrees C, mm precipitation
14-Dec-02
15-Dec-02
16-Dec-02
17-Dec-02
18-Dec-02
Time
Temp (C)
E. coli/10mL
ppt (mm)

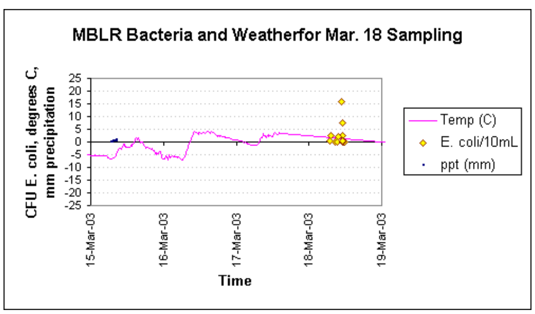

MBLR Bacteria and Weather for Jan. 14 Sampling
CFU E. coli, degrees C, mm precipitation
11-Jan-03
12-Jan-03
13-Jan-03
14-Jan-03
15-Jan-03
Time
Temp (C)
E. coli/10mL
ppt (mm)

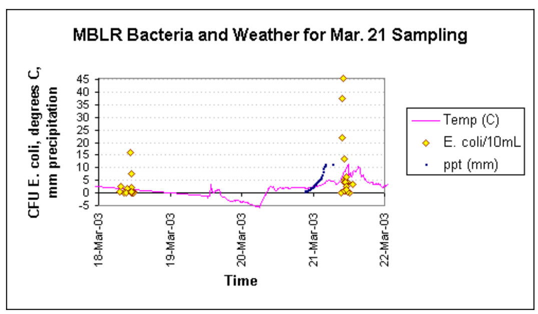

MBLR Bacteria and Weather for Feb. 4 Sampling
CFU E. coli, degrees C, mm precipitation
1-Feb-03
2-Feb-03
3-Feb-03
4-Feb-03
5-Feb-03
Time
Temp (C)
E. coli/10mL
ppt (mm)

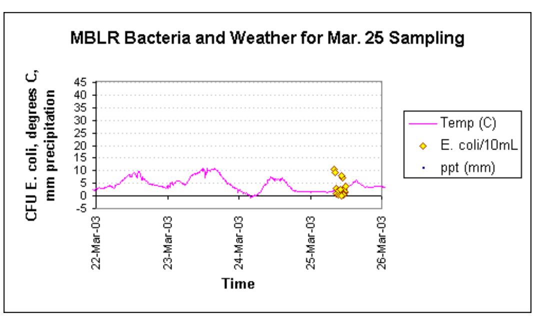

MBLR Bacteria and Weather for Feb. 19 Sampling
CFU E. coli, degrees C, mm precipitation
16-Feb-03
17-Feb-03
18-Feb-03
19-Feb-03
20-Feb-03
Time
Temp (C)
E. coli/10mL
ppt (mm)

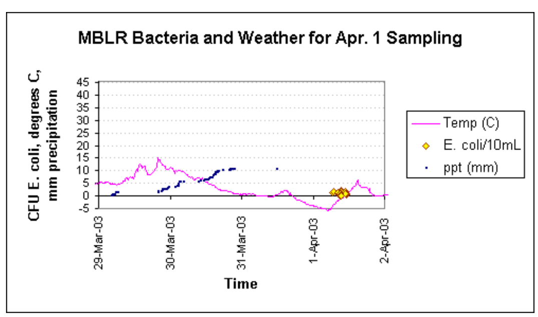

MBLR Bacteria and Weather for Feb. 23 Sampling
CFU E. coli, degrees C, mm precipitation
20-Feb-03
21-Feb-03
22-Feb-03
23-Feb-03
24-Feb-03
Time
Temp (C)
E. coli/10mL
ppt (mm)

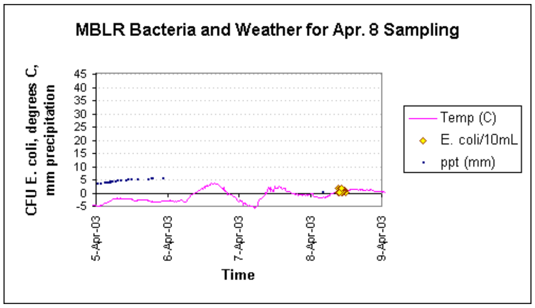

MBLR Bacteria and Weather for Feb. 25 Sampling
CFU E. coli, degrees C, mm precipitation
22-Feb-03
23-Feb-03
24-Feb-03
25-Feb-03
26-Feb-03
Time
Temp (C)
E. coli/10mL
ppt (mm)

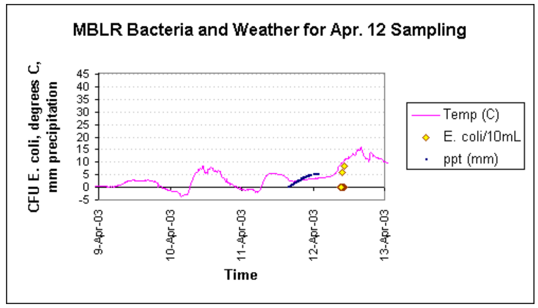

MBLR Bacteria and Weatherfor Mar. 18 Sampling
CFU E. coli, degrees C, mm precipitation
15-Mar-03
16-Mar-03
17-Mar-03
18-Mar-03
19-Mar-03
Time
Temp (C)
E. coli/10mL
ppt (mm)

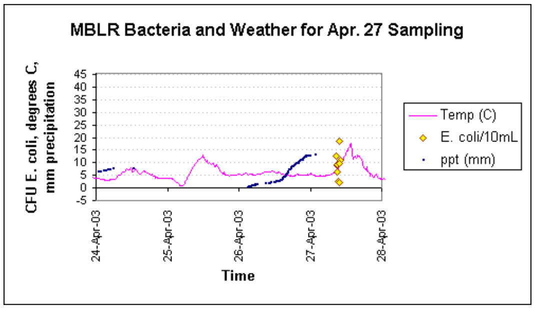

MBLR Bacteria and Weather for Mar. 21 Sampling
CFU E. coli, degrees C, mm precipitation
18-Mar-03
19-Mar-03
20-Mar-03
21-Mar-03
22-Mar-03
Time
Temp (C)
E. coli/10mL
ppt (mm)

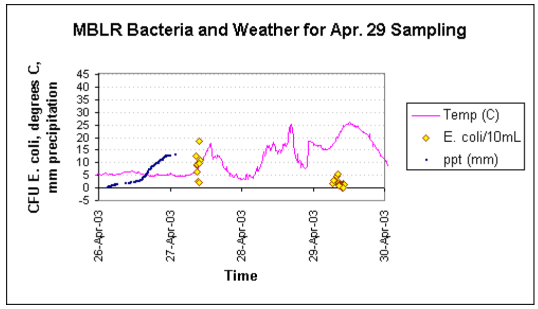

MBLR Bacteria and Weather for Mar. 25 Sampling
CFU E. coli, degrees C, mm precipitation
22-Mar-03
23-Mar-03
24-Mar-03
25-Mar-03
26-Mar-03
Time
Temp (C)
E. coli/10mL
ppt (mm)

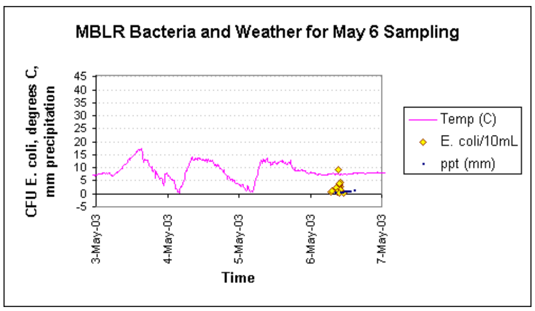

MBLR Bacteria and Weather for Apr. 1 Sampling
CFU E. coli, degrees C, mm precipitation
29-Mar-03
30-Mar-03
31-Mar-03
1-Apr-03
2-Apr-03
Time
Temp (C)
E. coli/10mL
ppt (mm)

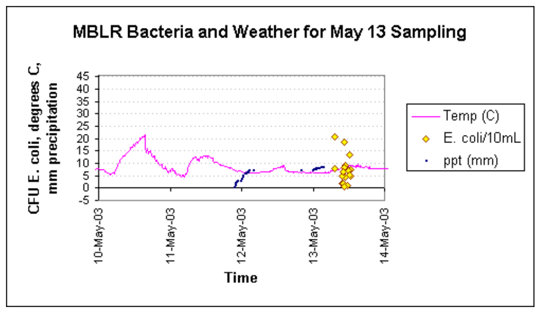

MBLR Bacteria and Weather for Apr. 8 Sampling
CFU E. coli, degrees C, mm precipitation
5-Apr-03
6-Apr-03
7-Apr-03
8-Apr-03
9-Apr-03
Time
Temp (C)
E. coli/10mL
ppt (mm)

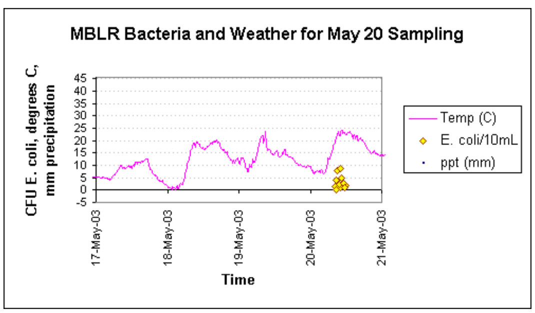

MBLR Bacteria and Weather for Apr. 12 Sampling
CFU E. coli, degrees C, mm precipitation
9-Apr-03
10-Apr-03
11-Apr-03
12-Apr-03
13-Apr-03
Time
Temp (C)
E. coli/10mL
ppt (mm)

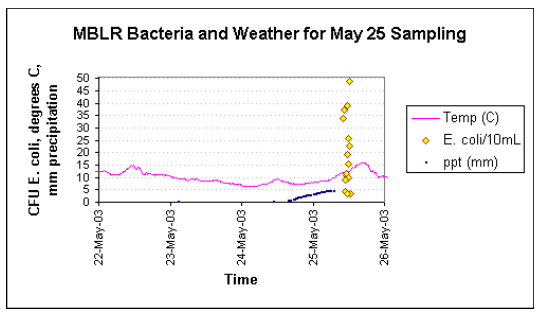

MBLR Bacteria and Weather for Apr. 27 Sampling
CFU E. coli, degrees C, mm precipitation
24-Apr-03
25-Apr-03
26-Apr-03
27-Apr-03
28-Apr-03
Time
Temp (C)
E. coli/10mL
ppt (mm)

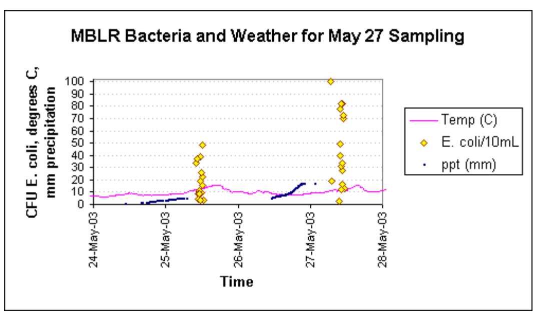

MBLR Bacteria and Weather for Apr. 29 Sampling
CFU E. coli, degrees C, mm precipitation
26-Apr-03
27-Apr-03
28-Apr-03
29-Apr-03
30-Apr-03
Time
Temp (C)
E. coli/10mL
ppt (mm)


MBLR Bacteria and Weather for May 6 Sampling
CFU E. coli, degrees C, mm precipitation
3-May-03
4-May-03
5-May-03
6-May-03
7-May-03
Time
Temp (C)
E. coli/10mL
ppt (mm)


MBLR Bacteria and Weather for May 13 Sampling
CFU E. coli, degrees C, mm precipitation
10-May-03
11-May-03
12-May-03
13-May-03
14-May-03
Time
Temp (C)
E. coli/10mL
ppt (mm)


MBLR Bacteria and Weather for May 20 Sampling
CFU E. coli, degrees C, mm precipitation
17-May-03
18-May-03
19-May-03
20-May-03
21-May-03
Time
Temp (C)
E. coli/10mL
ppt (mm)


MBLR Bacteria and Weather for May 25 Sampling
CFU E. coli, degrees C, mm precipitation
22-May-03
23-May-03
24-May-03
25-May-03
26-May-03
Time
Temp (C)
E. coli/10mL
ppt (mm)


MBLR Bacteria and Weather for May 27 Sampling
CFU E. coli, degrees C, mm precipitation
24-May-03
25-May-03
26-May-03
27-May-03
28-May-03
Time
Temp (C)
E. coli/10mL
ppt (mm)

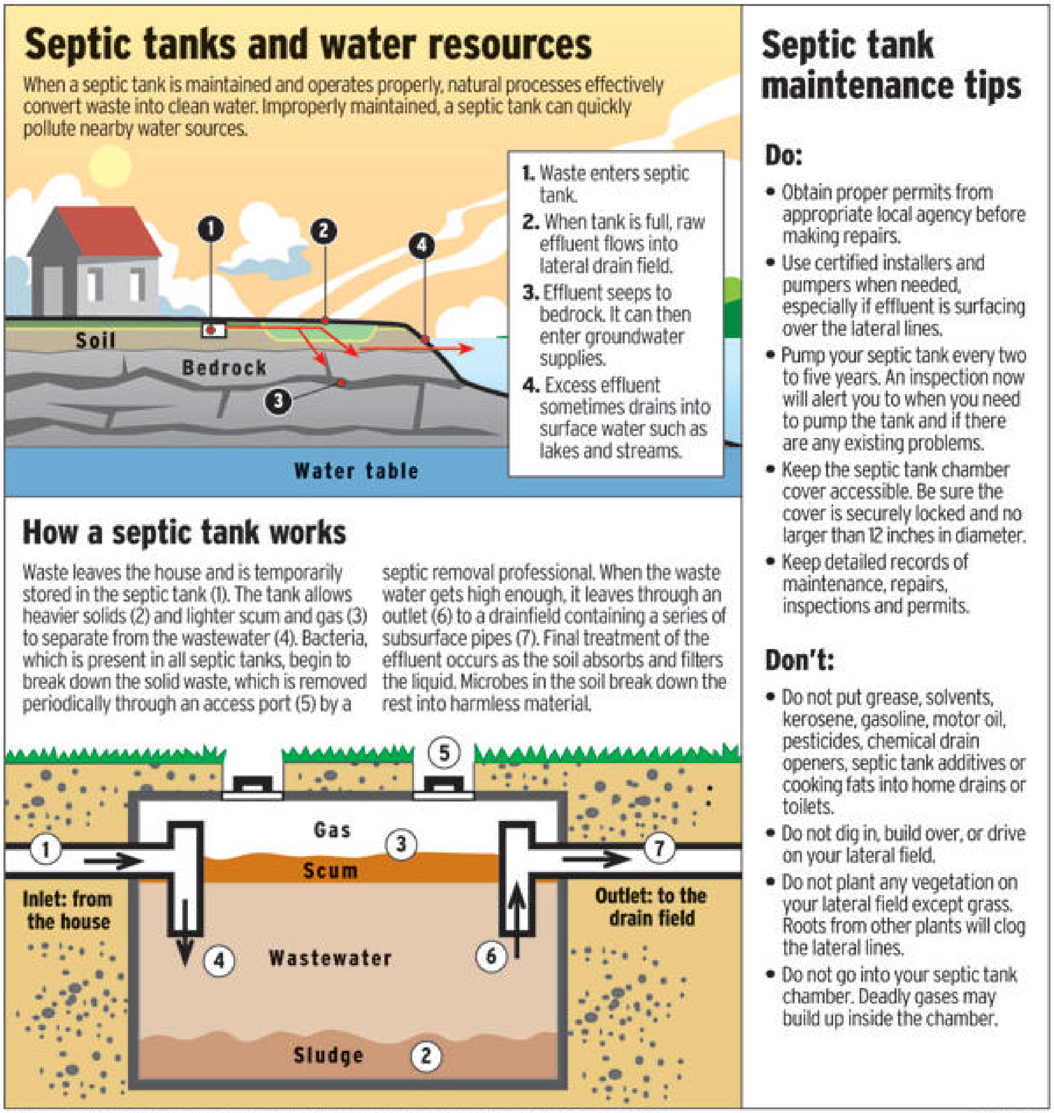
Appendix 7: Center for Watershed Protection: partial list of reference documents for protection of local water resources.

- 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 8: 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 9: 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 10: 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 11: Recommendations for residential fertilizer use[19]

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.

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