

# MICROBIAL SOURCE TRACKING IN TWO SOUTHERN MAINE WATERSHEDS

## *Executive Summary for Merriland River, Branch Brook and Little River (MBLR) Watershed*

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## 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 mammals (including humans) and birds, 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).<sup>i</sup> 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).<sup>ii</sup> 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).<sup>iii</sup> 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).<sup>iv</sup>



## 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

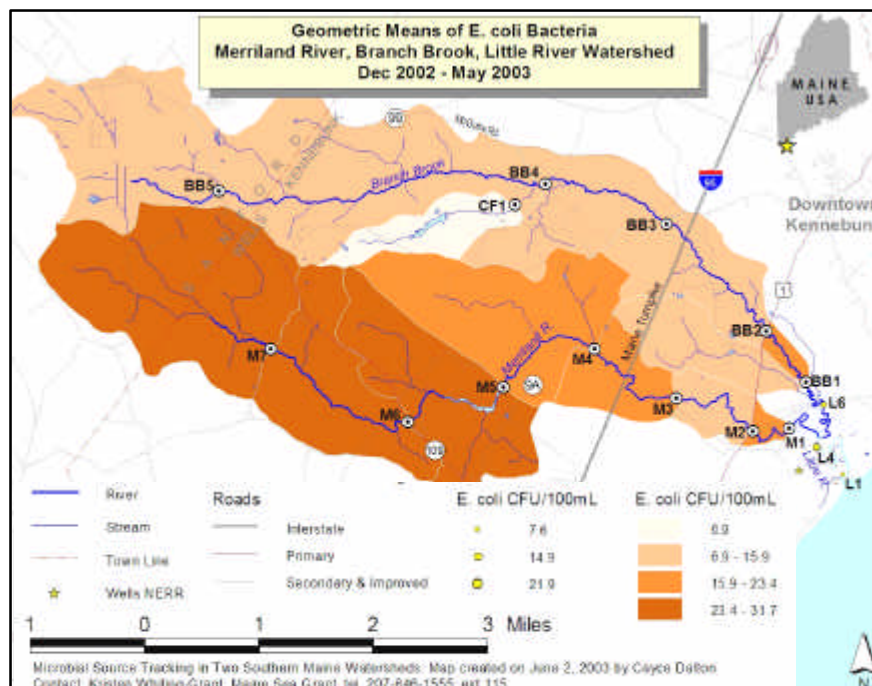
Conventional bacterial testing for fecal coliform and *E. coli* (both indicator organisms of fecal contamination) was carried out for all samples to determine contamination levels relative to state and federal water quality standards. The results from these analyses provided valuable information about 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.

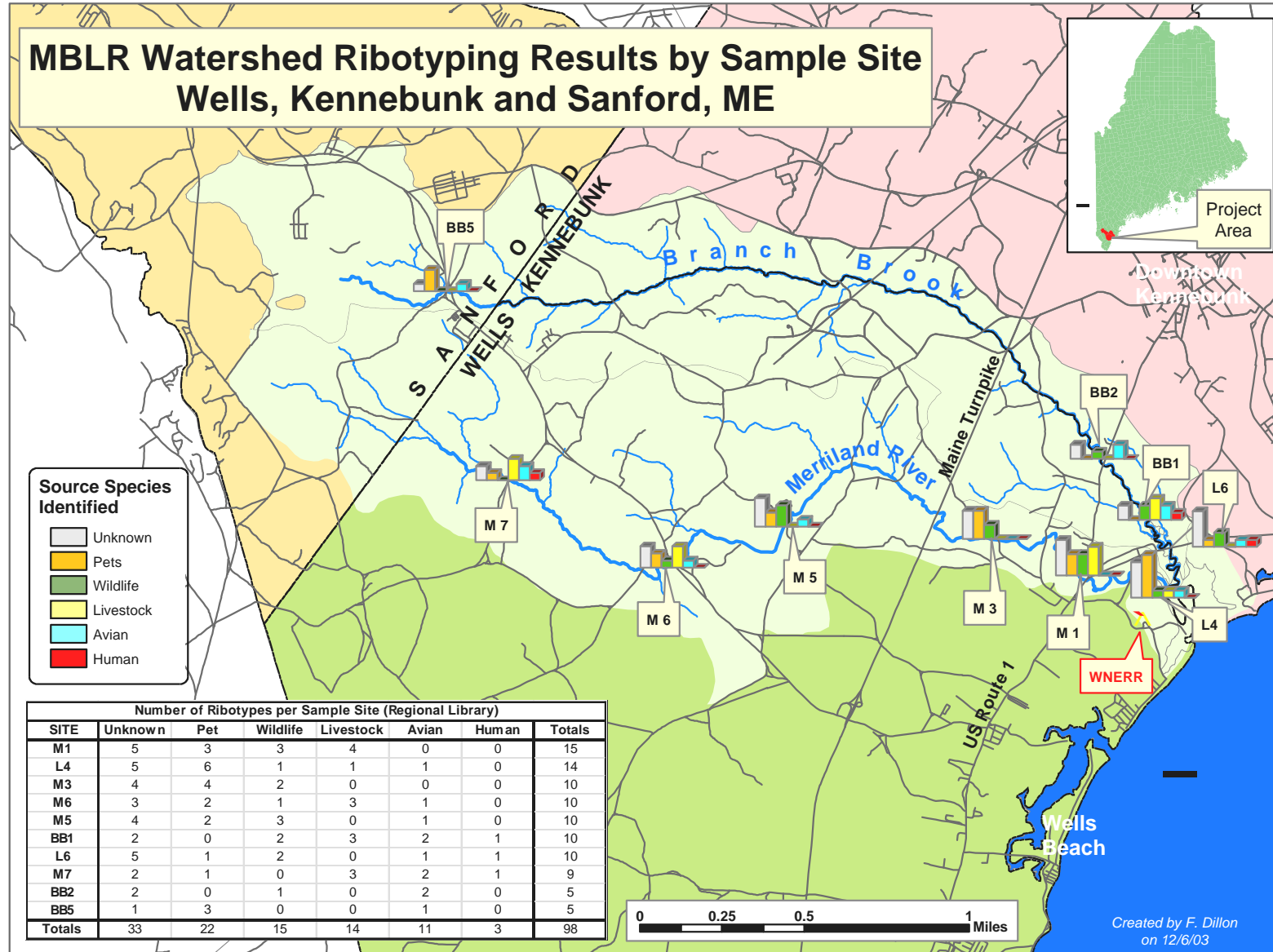
### STEP 4. Microbial Source Tracking

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.





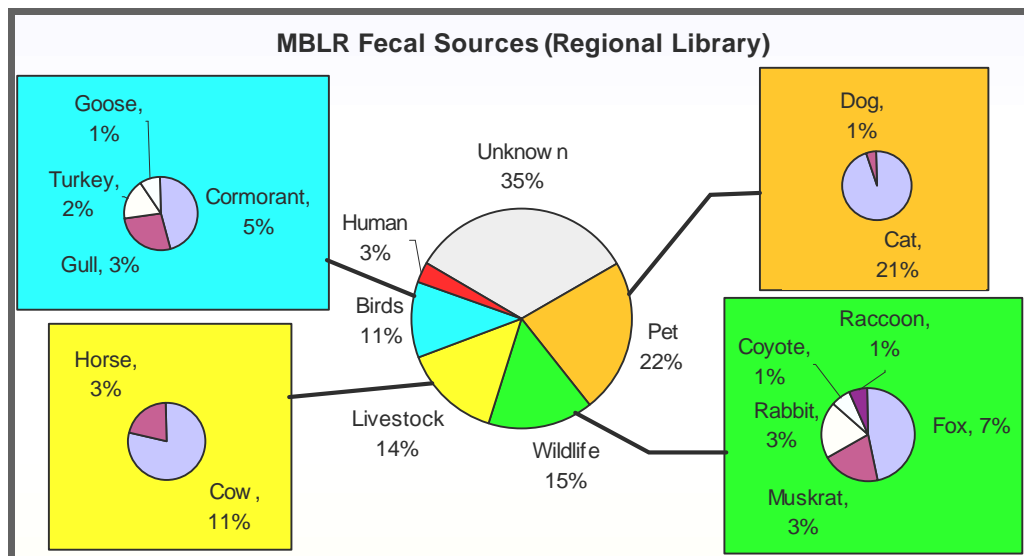
**Figure B.** Geographic distribution and species composition of ribotypes in the MBLR watershed. Bar heights indicate number of ribotypes for each sampling site (actual numbers included in inset table). Source: Jones (2003).

## 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.

### Wildlife Sources

- 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.

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

<sup>ii</sup> 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](http://spo.nos.noaa.gov/projects/95register/shellfish_one_pg.html))

<sup>iii</sup> 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](http://www.maine.gov/dmr/rm/2002annualreport/2002annualreport.htm))

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

