MICROBIAL SOURCE TRACKING
IN TWO SOUTHERN MAINE WATERSHEDS

Executive Summary for Webhannet River Watershed

Report Number: MSG-TR-03-02

August 2003

A Maine Sea Grant Project
funded by the
Cooperative Institute for Coastal and Estuarine Environmental Technology

Prepared by

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

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

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

INTRODUCTION

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

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

MICROBIAL SOURCE TRACKING PROJECT GOALS

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

- ***Goal 1:*** Provide resource managers in the Webhannet watershed with information regarding the microbial source(s) of fecal coliform bacterial contamination in this region.

- ***Goal 2:*** Educate community members living within the Webhannet watershed regarding the results of this project.

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

EXPERIMENTAL DESIGN AND STUDY RESULTS

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

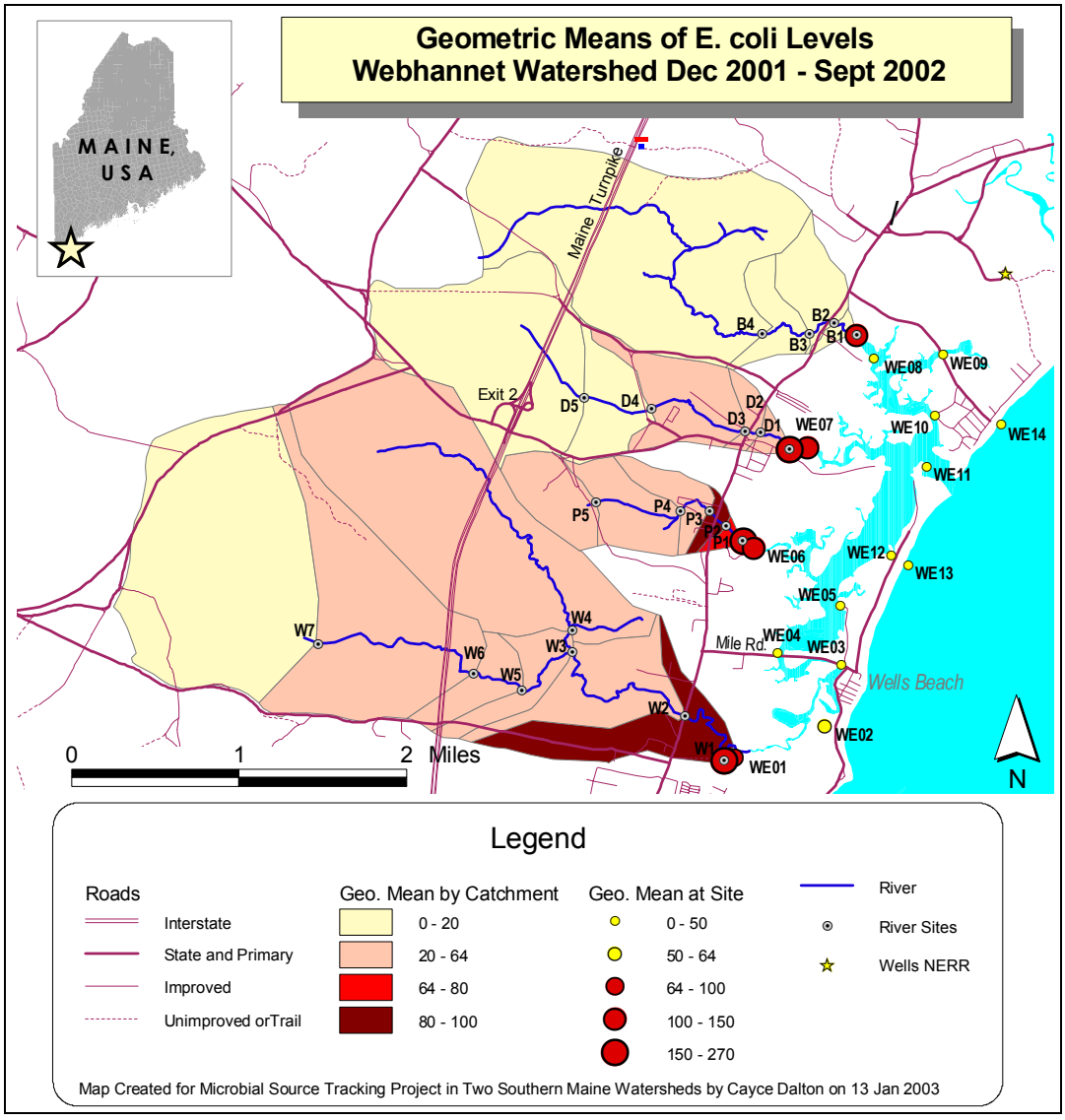

Figure A. *E. coli* bacteria levels for Webhannet watershed sampling sites. *E. coli* concentrations for sampling sites are indicated by dot size and color and for land drainage areas ("catchments") by color. Higher geometric mean (a type of average) values indicate higher levels of contamination.

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

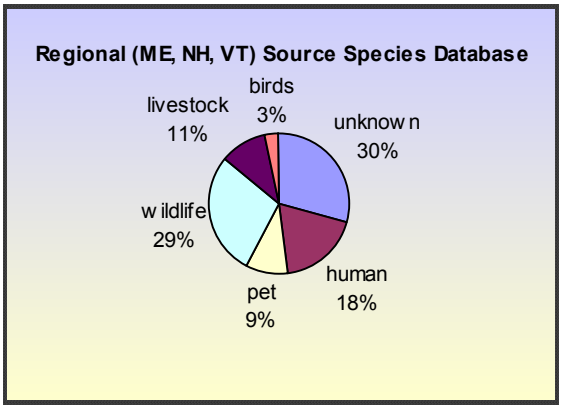

Figure B. Source species identification for Webhannet watershed. Humans are the largest single contributor Source: Jones (2003)

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

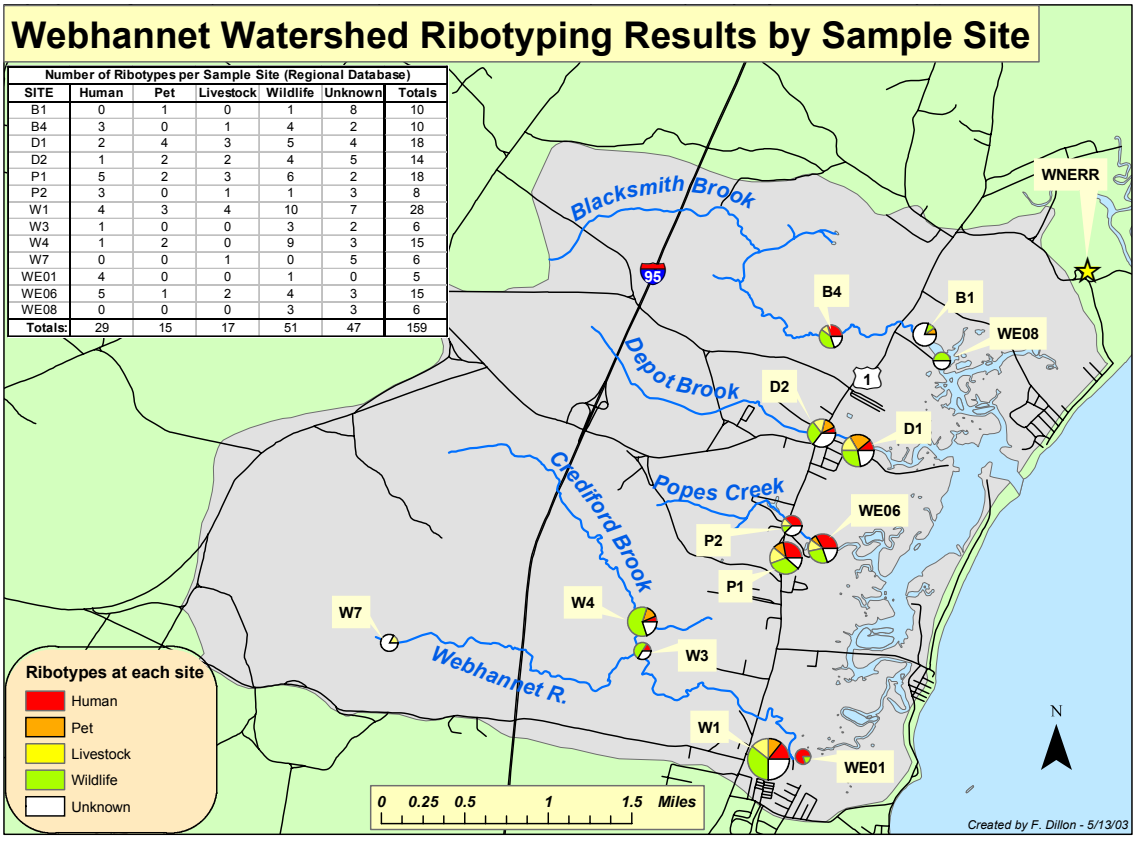

Figure C. Geographic distribution and species composition of ribotypes in the Webhannet watershed.

MANAGEMENT RECOMMENDATIONS

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

Human Sources

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

Wildlife Sources

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

Livestock Sources

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

Pet Sources

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

CONCLUSIONS

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

---

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

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

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

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