Synopsis

Proposal Title: A Molecular Approach to Determine the Origin of Fecal Bacteria in Catoma Creek of the Alabama River Basin

Principal Investigators: Yucheng Feng, C. Wesley Wood, and Joey N. Shaw

Funding Period: March 2003 – February 2004 with a no-cost extension to May 2004

Statement of the Problem and Research Objectives

Fecal pollution impairs the quality of streams and rivers for recreational use and adversely affects fish and aquatic life. Fecal contamination can originate from both human and non-human sources including surface runoff from land application of animal wastes or farm animal feedlots, inadequate septic or sewer systems, improper waste disposal, and wildlife impact. Determining the sources of fecal contamination is necessary to develop effective pollution control strategies. Presence of fecal indicator bacteria is the second most common cause of surface water impairment in Alabama. Thirty percent of the 181 impaired water bodies in the state are attributed to fecal indicator bacteria according to Alabama’s 2002 Section 303 (d) List. Catoma Creek in Montgomery County is a tributary of the Alabama River. Both agricultural and urban land uses exist in the watershed. A 23-mile segment of Catoma Creek has been included on the State 303(d) list due to impairment of fecal pollution.

The objectives of this research were to construct a library of rep-PCR DNA fingerprints from *Escherichia coli* strains isolated from a wide range of collection of human and animal feces, identify the sources of fecal contamination in the Catoma Creek watershed using the constructed library.

Research Methodology

To achieve the project objectives, first of all, experiments were conducted to isolate *E. coli* from the feces of eight types of warm-blooded animals (including humans) in the watershed, obtain a DNA fingerprint of each isolate using the rep-PCR DNA fingerprinting technique, and construct a host origin library of *E. coli* DNA fingerprints. Secondly, water samples were collected monthly at eight locations in the Catoma Creek watershed (Figure 1) from May of 2003 to April of 2004. Both chemical and microbiological analyses of the water samples were performed. Membrane filtration procedure was used to determine *E. coli* concentrations in the water samples using modified membrane-thermotolerant *Escherichia coli* agar (mTEC). The rep-PCR DNA fingerprints of *E. coli* isolates from the water samples were also obtained. Identification of the sources of fecal contamination will be achieved by matching the DNA fingerprints from the water isolates against fingerprints contained in the source library.

Principal Findings and Significance

Fecal samples were collected from (the number of fecal samples indicated in parentheses) cattle (20), chicken (20), horse (21), waterfowl (21), deer (28), wild turkey (15), dog (18), and human (15). A total of 484 *E. coli* isolates was obtained and characterized. Differences in the rep-PCR DNA fingerprints among different animals were observed. Construction of the rep-PCR DNA fingerprint library of known sources will soon be completed.
Monthly water sample collection was initiated in May of 2003 and continued through April of 2004 at 8 locations in the watershed, with 3 locations on the main stem of Catoma Creek and 5 on the tributaries. During the one-year sampling period, *E. coli* densities ranged from 18 to 12,650 CFU/100 ml, with 70% of the water samples exceeding the EPA criterion of 126 CFU/100 ml for full body contact recreation, and 41.6% above the criterion of 298 CFU/100 ml for moderate full body contact recreation. There was a high correlation between *E. coli* density and stream flow. Chemical analyses of the water samples showed that the concentration ranges for NH$_4$-N, NO$_3$-N, phosphorus, and potassium were 0.19 to 0.78 mg/l, 0.12 to 0.38 mg/l, 0.13 to 0.65 mg/l, and 0.16 to 4.74 mg/l, respectively. During the same period, 480 *E. coli* isolates were obtained from the water samples. Figure 2 shows an example of the rep-PCR DNA fingerprint patterns of *E. coli* isolates from the watershed.

The presence of high *E. coli* densities in the Catoma Creek watershed limits the recreation use of the streams by the local communities. Relatively high concentrations of phosphorus suggest that eutrophication may occur in the watershed. Once the sources of fecal contamination in the Catoma Creek watershed are determined, corrective actions can be developed to target the contamination source(s). It will also assist water resource managers in designing TMDL implementation plans and lead to improved water quality.

**Publications/Presentations**


**Students Supported on the Project**

R. Udenika Wijesinghe, MS student, Department of Agronomy and Soils  
Amiel Ollie, BS student, Environmental Science major
Figure 1. Water sampling sites in the Catoma Creek watershed.

Figure 2. rep-PCR DNA fingerprint patterns of E. coli isolates obtained from water samples collected in May of 2003.