BACTERIAL SOURCE TRACKING IN THE VERNON RIVER WATERSHED

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Abstract. The Vernon River basin drains 16,000 acres of densely populated, urbanized area that includes portions of the City of Savannah and Chatham County and all of the City of Vernonburg. The upper reaches of the basin are comprised of several urban storm water drainage canals, including Casey Canal, which discharge into Hayners Creek, one of many small feeder creeks draining to the Vernon River. Hayners Creek and Casey Canal are listed on Georgia's 303(d) list as not supporting their designated Fishing classification due to fecal coliform, dissolved oxygen, and fish consumption guideline water quality criteria. Conventional methods to determine the source of fecal coliform bacteria have been unsuccessful to date**.**

Stakeholders within the Vernon River Watershed, including the Skidaway Institute of Oceanography, the cities of Savannah and Vernonburg, the Chatham County Heath Department, and Integrated Science & Engineering, Inc, understand that is critically important to identify the sources of fecal contamination in order to develop a workable management plan to eliminate the identified sources. Through Georgia Environmental Protection Division 319 grant funding assistance, the Vernon River Stakeholders' Project Team will utilize Assessing Multiple Antibiotic Resistance (MAR) and Ribosomal RNA (rRNA) Fingerprinting to track and identify the source of bacterial contamination in the Vernon River basin. This project will also evaluate viral tagging of fecal bacteria in septic systems as an effective indicator of septic functionality.

Once the source(s) of fecal contamination are identified through the bacterial source tracking program, the Project Team will develop best management practices to address and eliminate those identified sources. Once established, these BMPs will likely be incorporated into the future TMDL Implementation Plan for the Vernon River basin. Because this basin is typical of coastal communities in the region, the BMPs developed through this project will likely be applicable on a regional scale.

PROJECT BACKGROUND

The Vernon River is a meandering tidal creek that drains a narrow stretch of salt marsh forming part of the southeast boundary between the City of Savannah and the southeast portion of unincorporated Chatham County. The town of Vernonburg is on a peninsula of high ground at an oxbow bend on the west bank of the river, and is bordered on all other sides by the city of Savannah. The Vernon River is one of many tidal creeks that make up the Ogeechee River Estuary. The topography of the area is low and flat with most of the land surface at a height of 10 to 20 feet above mean sea level.

Land use in the basin is a combination of commercial areas and single and multi-family residential areas. Most of the City of Savannah to the north and west, and some neighborhoods to the east of the Vernon River are served by sanitary sewers. The sewage treatment plants serving these areas discharge their effluent to the Savannah River several miles north of this basin. There are approximately 2500 septic systems within this basin, approximately 80% of which are in unincorporated Chatham County. The Town of Vernonburg and a few neighborhoods within Savannah also rely on septic systems.

The Casey Canal and Hayners Creek are both listed as impaired waterways due to fecal coliform water contamination, yet the source or sources remain allusive. Conventional dye testing, smoke testing and even televising of sanitary sewer lines have all failed to identify leaks and or cross connections between sanitary sewers and stormwater systems. .

Septic systems in the Vernon River Basin are a suspected source of fecal bacteria contamination. Drainage ditches in areas served by individual septic systems have tested high for fecal coliform bacteria. Soil types found within this basin are not optimal for septic system function, and the tidal nature of the basins could also affect riparian septic systems' effectiveness. Traditionally the Health Department has utilized dye tracers to test the integrity of septic systems, however, there is some question as to the efficacy of this method. To date, dye testing of

individual septic systems has failed to identify malfunctioning systems.

PROJECT APPROACH

Purpose

The primary goal of this project is to develop workable management plans to identify and eliminate sources of fecal bacteria.

Methodology

In order the accomplish this goal, the Project Team will utilize Multiple Antibiotic Resistance and rRNA fingerprinting to track the source of the bacterial contamination in the Vernon River basin. MAR is a technique whereby samples of *E. coli* are tested for their resistance to several different antibiotics. Each samples "resistance record" is then compared to a library of known samples. Matches are considered positive source identification. rRNA fingerprinting will be utilized to confirm the results of the MAR or to attempt to classify sources that could not be identified through MAR. rRNA fingerprinting assesses the genes found in *E. coli* samples and compares them to the library of known samples. Again, a match is considered positive identification.

It is also essential that an effective method for testing the functionality of septic systems be developed. Therefore, this project includes the evaluation of viral tagging as a method for assessing septic system function. *E.coli*-specific bacteriophage will be released into septic systems to infect *E. coli* bacteria and propagate. Unlike dyes that are rapidly diluted when released into a sewer or septic system, E.coli-specific bacteriophage will replicate themselves if a source of fecal contamination is present facilitating their detection even if the original source of contamination is diluted. The presence of this phage would thus indicate a failing system. Should viral tagging prove to be a good indicator of septic functionality, procedures will be developed to incorporate it into the future TMDL Implementation Plan.

Project Deliverable

The final task of this project is the development of practical BMPs to eliminate fecal bacteria contamination in the Casey Canal and Hayners Creek in the Vernon River basin. Once the sources of fecal contamination are identified, source specific BMPs will be developed. Implementation of the BMPs will be inter-jurisdictional in nature, and performed in conjunction with local NPDES Stormwater Management Plans and Watershed Protection Plans. Watershed-wide and site-specific bacterial source tracking techniques will be identified and a revised long-term water quality monitoring plan will be developed for the Vernon River basin.

WORKPLAN

The following Workplan outlines the water quality monitoring procedures to track bacteria sources and determine the effectiveness of local septic systems.

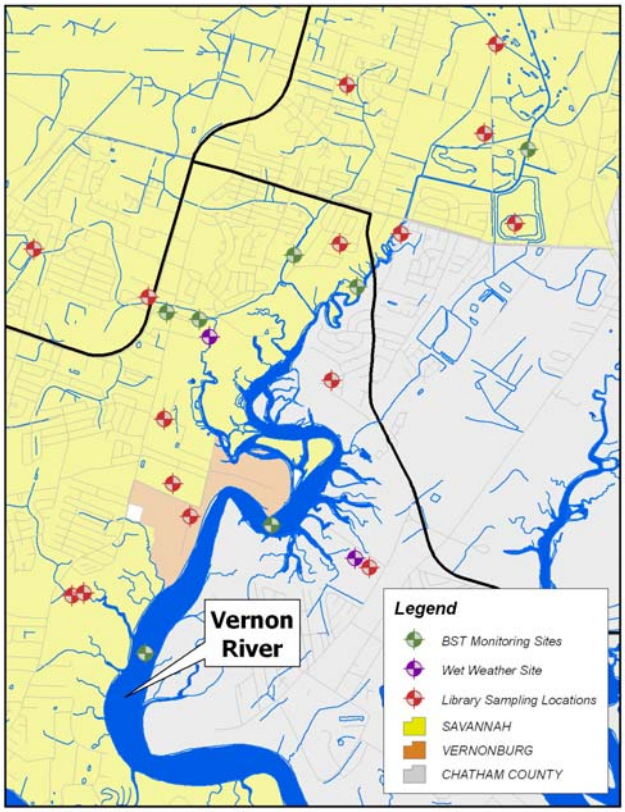
MAR and Ribosomal RNA Analysis

MAR profiling and Ribosomal RNA fingerprinting requires a library of samples to be built from isolated strains of *E.coli* sampled from known fecal coliform sources within the Vernon River Basin. This library will be used for source discrimination of fecal bacteria samples taken at hotspots throughout the basin. A minimum of 500 *E.coli* isolates will be characterized to ensure sufficient library size for source discrimination. (Wiggins et al., 2003.)

The *E. coli* isolates will include four (4) samples of fecal matter that will be collected from various sources (such as septic systems, sanitary sewage, dog wastes, wildlife, etc.). Sampling teams will attempt to collect samples within previously identified hotspot areas. Each hotspot area consists of a location where consistently high levels of fecal coliform have been identified through previous sampling, as well as an upstream and downstream reach. Wherever possible, each of the four samples will be collected in separate hotspot areas representing different environmental and socio-economic areas within the Vernon Basin. This will account for differences in antibiotic resistance in humans and domestic animals related to socio-economic status and will ensure that the library has a comprehensive representation of the antibiotic resistance of *E.coli* associated with each of the above-mentioned sources.

Bacterial sampling will be conducted at various points throughout the watershed (See the map in Figure 1.) Samples will be collected during consecutive tidal cycles (incoming and outgoing tides), and sampling events will be planned during dry and wet weather conditions. This sampling program will be repeated for two annual cycles. The sampling schedule is designed to help isolate the potential sources of fecal contamination. If high numbers of fecal bacteria are identified during dry weather, then the source is likely to be an illicit discharge. If fecal contamination is more pronounced during wet weather, then the source is likely non-point, i.e. pet waste and/or septic systems. By sampling both the incoming and outgoing tides, the Project Team will also be able to more accurately pinpoint the location of the fecal contamination.

Figure 1: Sampling Locations



Septic System Viral Tracing

The Project Team will identify septic systems for testing that have a relatively high probability, i.e. the right conditions, for failure. Targeted systems will have drain fields that lie in sandy soil within 50 feet of the Vernon River or a tributary of the Vernon River. Household conditions must also be a consideration such that the residents must be present throughout the duration of the experiment to ensure that there is adequate input into the system.

Identified septic systems will be seeded with *E.coli* bacteriophages. Bacteriophages will attach themselves to *E. coli* bacteria and will, if detected, positively indicate the presence of that specific E. coli source. This particular bacteriophage was chosen because it can be sensitively detected, and is able to persist for days to weeks in receiving waters. Water samples will be collected in receiving waters directly adjacent to and downstream of the drain field's closest proximity to the river. Samples will be collected hourly for the first 48 hours and then twice a day for the next five days. (Paul et al., 1995.)

Dye tracing will be conducted in conjunction with the viral tracing described above to test the efficacy of one method against the other. Septic systems will be tested one at a time, beginning at the most downstream location, to ensure that any bacteriophages detected can be traced to a specific source septic system. Additionally, tests will be carried out in dry and wet weather to account for differences in hydrology likely to affect sanitary sewage discharge.

Best Management Practices (BMPs)

Upon completion of the water quality sampling, MAR and rRNA fingerprinting, the Project Team will assess the results and develop BMPs that address the identified sources of fecal bacteria contamination. BMPs may include (depending on identified sources) pet/domestic animal waste education and ordinances, a sanitary sewer inspection program, and a septic system inspection and operation program. The comprehensive septic system program will be designed based on the results of the viral tracing and will include the following tasks:

- Utilize viral tracing techniques to determine an effective setback for drain fields.
- Develop and ordinance to stipulate appropriate setbacks, as well as an inspection and maintenance schedule.
- Design a comprehensive monitoring and inspection program will be designed and implemented to identify failing septic systems.
- Develop a capital improvement plan for conversion of septic systems to sanitary sewer, in appropriate areas.

Upon completion of this project, these BMPs will be included in the future TMDL Implementation Plan for the Vernon River Basin.

LITERATURE CITED

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