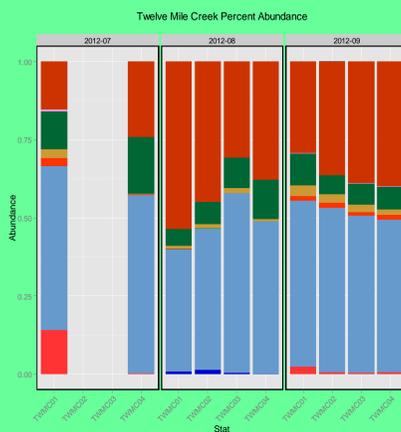
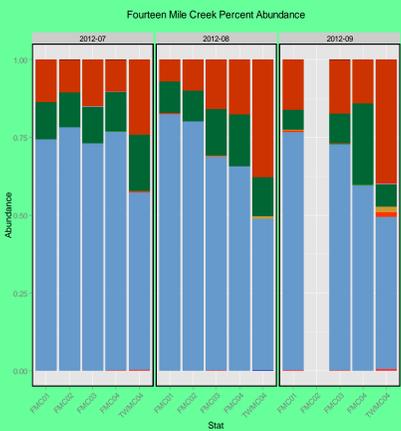
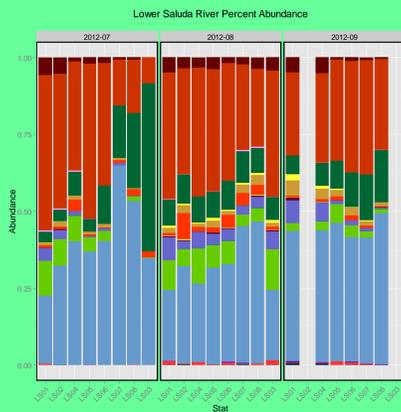
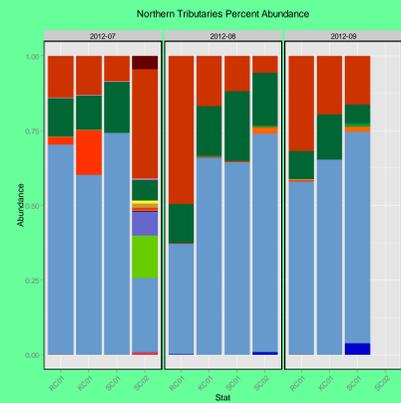


Microbial Source Tracking to Assess Human Contributions of Fecal Bacteria to a Freshwater Receiving Stream

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Bacterial Community Structure



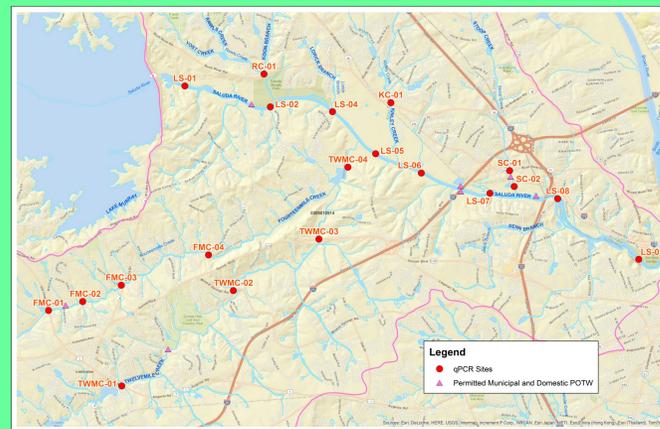
Abstract

The State of South Carolina, Department of Health and Environmental Control (SCDHEC) is charged with the responsibility to ensure that waters of the State support classified uses and are free from pollutants in excess of State standards. Crucial to any plan to achieve compliance with *E. coli* standards is an understanding of the probable sources of fecal bacteria (i.e., human vs. animal).

Metagenomics (sequencing and analysis of DNA from environmental samples) and quantitative polymerase chain reaction (qPCR) are genetic methodologies that can be used for microbial source tracking: the identification of microbial organisms specific to potential sources of fecal bacteria pollution. Metagenomic methods were used first to sequence DNA present in receiving stream water samples. The 16S rRNA amplicon was analyzed using BLAST, Greengenes, etc., to derive and contrast the microbial community composition. Then, qPCR methodology was used to quantify the presence of known genetic markers to substantiate and confirm the presence of potential human sources of fecal bacteria pollution. This assessment strategy was employed on an urban freshwater stream system, the Lower Saluda River, from its "origination" from a major impoundment, to its confluence with the Congaree River.

A comprehensive study conducted July – September 2012 included eight Lower Saluda River sites, 10 sites on tributary streams with municipal wastewater treatment plant discharges and two sites on tributaries without discharges. A generally increasing trend in human markers downstream in the Lower Saluda River was noted. The most specific human marker, *M. smithii*, was seen immediately downstream of two municipal wastewater treatment plants and also from an impoundment outfall. Community structure between tributaries appeared to be substantially different. The extent of these differences and their influence on the Lower Saluda River are being evaluated.

Lower Saluda River watershed - Study Sites



Sample Collection

Tributary samples were collected from bridge crossings and Lower Saluda River samples were collected by boat. Each water sample was collected in 2 L Sterile Nalgene sample bottles at surface (0.3 m) depth. Samples were iced and a temperature blank used to ensure that the samples were kept at the proper temperature. Samples were then delivered to the NOAA CCEHBR laboratory personnel on-site.

Methodology

Water Filtration

Water samples (1 L each) were filtered through 0.45 µm nitrocellulose filters to capture microorganisms, and filters were placed in 2 mL bead beating vials for DNA extraction.

DNA Extraction

Bead beating vials were processed on a FastPrep (MP Biomedicals) instrument to disrupt the filter and attached microbial cells. DNA was extracted using the DNEasy Plant Mini Kit (Qiagen) following the manufacturer's protocol.

Methodology (cont.)

PCR Amplification and Sample Pooling

- The forward primer contained the 454 Life Sciences primer B, the broadly conserved bacterial primer 27F, and a 2-base linker sequence ("TC").
- The reverse primer contained the 454 Life Sciences primer A, the bacterial primer 338R, a "CA" inserted as a linker between the barcode and the rRNA primer, and a unique 12-bp error-correcting Golay barcode used to tag each PCR product. PCRs consisted of 0.25 µL (30 µM) of each forward and reverse primer, 3 µL of template DNA, and 22.5 µL of Platinum PCR SuperMix (Invitrogen).
- A composite sample for pyrosequencing was prepared by pooling approximately equal amounts of PCR amplicons from each sample.
- For each sample, we amplified the 16S rRNA gene using a primer set similar to that described in Hamady *et al.* (2007) that was found to be well-suited for the phylogenetic analysis of pyrosequencing reads.
- Once quantified, the DNA was precipitated, centrifuged, and resuspended. The sample was sent to the Selah Genomics Facility at the University of South Carolina (Columbia) for pyrosequencing on a 454 Life Sciences Genome Sequencer FLX (Roche) machine.

Phylogenetic Analyses

- Sequences were processed and analyzed following the procedure described in Hamady *et al.* (2007).
- Only those sequences >200 bp in length with an average quality score >25 and no ambiguous characters were included in the analyses.
- Sequences were assigned to samples by examining the 12-bp barcode. Phylotypes were identified by using megablast to identify connected components (nearest neighbor) sets of similar sequences.
- A representative sequence was chosen from each phylotype by selecting the most highly connected sequence, i.e., the sequence that had the most hits more significant than the BLAST threshold to other sequences in the dataset.
- The set of all representative sequences was aligned by using NAST (parameters: minimum alignment length, 190; sequence identity, 70%) with a PH lanemask (<http://greengenes.lbl.gov/>) to screen out hypervariable regions of the sequence. A relaxed neighbor-joining tree was built by using Clearcut, employing the Kimura correction.
- Unweighted UniFrac was run by using the resulting tree and the sequences annotated by environment type. Taxonomic identity of the phylotypes was assigned with BLAST against the Greengenes database by using an *E* value cutoff of 1e-10 and the Hugenholtz taxonomy.
- The statistical significance of differences in microbial community composition between sample categories was determined by using the G test on relative phylotype abundances.

Summary

Bacterial Community Structure

- In August and September, in the absence of significant rainfall, bacterial community structure appears to remain relatively stable between months for each stream system.
- In general, there appears to be a decrease in percent of Proteobacteria and an increase in percent of Actinobacteria in Fourteen mile and Twelve Mile Creeks, from upstream to downstream, which does not appear to be the case in the Lower Saluda River.
- Diversity is less in tributary streams than in the Lower Saluda River. Twelve Mile Creek is the largest tributary watershed and appears to be the most similar to the Lower Saluda River.
- Nitrospirae and Planctomycetes are significant throughout the Lower Saluda River and do not appear in tributaries with the exception of SC02 in July.

Bacterial Indicators of human sources

- Human Bacteriodes and Lactospiraceae are present throughout the Lower Saluda and most tributary streams.
- There is a general increase of universal Bacteriodes, human Bacteriodes and Lactospiraceae, from upstream to downstream, in the Lower Saluda River. This trend is not evident in tributary streams.
- M. smithii* is absent from Fourteen Mile Creek; Human Bacteriodes appears only at FMC04 in July and FMC02 in September.
- M. smithii* appears only downstream of waste water treatment plants on Stoops Creek and Twelve Mile Creek, and below Corley Mill pond (TMC04) in August.

Impact of Rainfall on Community Structure

- There was 3.29 inches of rainfall in the 24 hours prior to sample collection in July. There was zero inches of rainfall recorded prior to sampling in August and September. However, light scattered showers were visually noted in August.
- The dramatic changes in bacterial community structure in Stoops Creek (SC02) and stations downstream of LS04 in the Saluda River are attributed to heavy rainfall in July. Most notably, the presence of Nitrospirae and Planctomycetes in Stoops Creek and the increase of Bacteriodes in the Lower Saluda River.

Bacterial indicators of human sources

