Tracking Fecal Pollution Sources in the Upper Reaches of the Horse Creek Watershed in Aiken County, SC

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Introduction

The Horse Creek watershed in Aiken County, SC, is known for its history of high coliform pollution. Previous studies have identified one particular tributary, Sand River, as being a major contributor to the upper portions of the watershed, but the source(s) remain unknown. Sand River drains Hitchcock Woods, an urban forest that is heavily used by equestrians; is transected by both old and new sewer lines; and is surrounded by older homes, some of which depend upon aging septic systems. In addition, Sand River in Hitchcock Woods receives an enormous volume of stormflow from the downtown area during rain events. This study focused on fecal pollution in two of Sand River's smaller tributaries, Calico Creek and Cuthbert Branch.

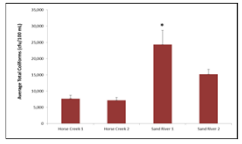


Figure 1. Total coliform studies in 2011 identified Sand River as the major contributor of fecal pollution to downstream locations, including Langley Pond. (Harmon et al. *Environmental Monitoring and Assessment,* in press)

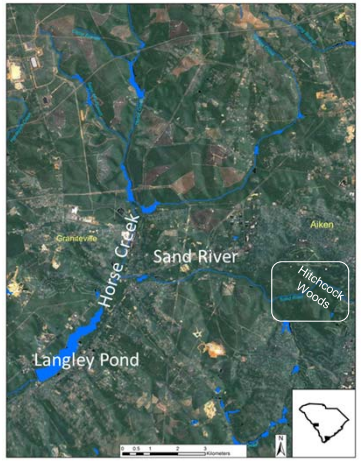
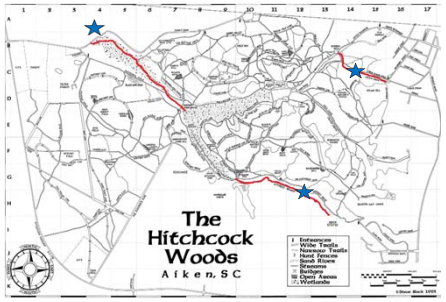


Figure 2. Sand River drains Hitchcock Woods, an urban forest that is heavily used by equestrians. This area of the city also includes a high number of horses and their associated stables and barns.



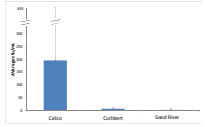


Figure 3. The highest concentrations of fecal coliforms were found in Calico Creek, with an average of 195 cfu/mL. Error bars represent one standard deviation.

Objectives

1. Evaluate fecal coliform counts in Sand River and two tributaries, Calico Creek and Cuthbert Branch.
2. Use antibiotic resistance analysis as a microbial source tracking technique to identify likely sources of fecal coliforms in these two small streams.

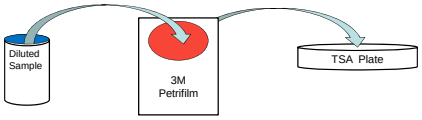
Methods

Feces from known species were collected within the Horse Creek watershed. These included samples from horses, waterfowl, dogs, and one sample of mixed sewage inflow from the Horse Creek Wastewater Treatment Plant. Fecal samples were homogenized under sterile conditions in the laboratory, and approximately 1 gram of homogenate was suspended in 10 mL of sterile Nanopure water. Samples were then diluted, and fecal coliform bacteria were isolated as shown below.

Fecal coliforms from unknown sources were isolated from Sand River, Calico Creek, and Cuthbert Branch in Hitchcock Woods (Figures 2-4).

Figure 4. Collection pole and sterile tube used to collect samples.

Fecal Coliform Isolation and Antibiotic Resistance



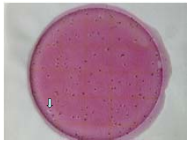


Figure 5. 1mL of each sample was pipetted onto a 3M Petrifilm and incubated overnight at 44°C. Gas-producing colonies (indicated by an arrow) were transferred to Tryptic Soy Agar (TSA) Petri plates and incubated at 37°C for 24 hours.

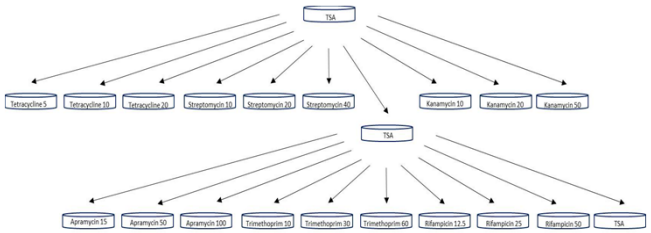


Figure 6. Fecal coliform isolates were transferred to TSA plates containing antibiotics to test for resistance. An isolate was considered to be resistant to a particular antibiotic concentration if growth occurred after 24 hours of incubation at 37°C. All antibiotic concentrations are expressed as µg/mL. TSA plates without antibiotics served as controls.

Results

A total of 643 isolates were obtained from known sources, and their patterns of antibiotic resistance were determined and analyzed for the ARA library. This library then served as a point of reference for the identification of unknown fecal pollutant sources shown in Table 2. Discriminant function analysis indicated that 75.3% of isolates from known sources were correctly classified.

ARA Comparison

A total of 384 isolates were obtained in 2014 from Calico Creek, Cuthbert Branch, and Sand River. These antibiotic resistance profiles were compared to the ARA library using discriminant function analysis (SPSS statistical software). Grouping of unknown isolates are shown in Table 2 below.

Table 2. Comparison of fecal coliform isolates from Calico Creek, Cuthbert Branch, and Sand River to the ARA library of known sources.

		Predicted Group Membership				
Sample	**n**	**Horse (%)**	**Waterfowl (%)**	**Dog (%)**	**Sewage (%)**	**Total (%)**
Horse	179	81.0	4.5	2.2	12.3	100
Waterfowl	177	15.8	65.0	13.0	6.2	100
Dog	143	2.8	11.2	80.4	5.6	100
Sewage	144	13.2	4.2	6.9	75.7	100
Calico Creek	177	43.5	5.1	4.5	46.9	100
Cuthbert Branch	82	30.5	20.7	14.6	34.1	100
Sand River	125	12.0	6.4	11.2	70.4	100

Conclusions

Calico Creek contributed the greatest number of fecal coliforms to the watershed with an average of 195 cfu/mL vs. 7 cfu/mL for Cuthbert Branch and 3 cfu/mL for Sand River.

Based on these results, we can conclude that the majority of the fecal coliform pollution load in this stream originate from horse and sewage.

- Calico Creek: ~ 90% grouped with horse (43.5%) and sewage (46.9%).
- Cuthbert Branch:~ 65% grouped with horse (30.5%) and sewage (34.1%).
- Sand River: ~ 82% grouped with horse (12.0%) and sewage (70.4%).

Fecal coliform isolates from Calico Creek and Cuthbert Branch grouped differently than those from Sand River. Sand River isolates grouped predominantly with sewage.

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