

Tracking Geographic and Taxonomic Sources of Fecal Microbes in Plaster Creek Tributaries Harry Ervin and Luke Tilma DeJong Lab Summer 2016 Research Summary

The problem of bacterial pollution in the Plaster Creek watershed is frequently recognized by high levels of *E. coli* which exceed the state guidelines for safe contact. In the summer of 2015, the DeJong lab used *E. coli* counts and qPCR methods to determine the five most problematic tributaries to Plaster Creek in regards to their fecal bacterial levels as well as the taxonomic sources of the fecal pollution. Though they found all tributaries to be contributing bacterial pollution, they determined Whiskey Creek, Little Plaster, an unnamed tributary referred to as Indian Trails, to have likely human contamination. They also determined to have 76th street tributaries to have bovine contamination and some evidence of human contamination.

This work has been continued in the tributaries identified. We have conducted sub-sampling along the length of each tributary in the hopes of narrowing the sources of the contamination. Over 90 samples were collected and brought back to the lab for two different evaluations. All samples were subjected to the first test of *E. coli* levels. It is run by adding two milliliters of the water sample to a Coliscan media to grow bacterial colonies in an incubator for approximately 24 hours. The media causes different forms of bacterial colonies to grow various colors. The cell cultures were then counted for *E. coli* present in the sample by identifying purple/blue colonies. Samples were collected on both “wet” and “dry” days for comparison. Consistent with the previous year, “wet” samples typically had higher *E. coli* levels. Every tributary had multiple sampling sites which yielded *E. coli* estimates that exceeded state standards.

One sample from each location was filtered through 0.45 micron paper, trapping bacteria onto the filter paper, and providing the material for DNA extraction. We performed DNA extraction on all of these samples, and they are ready for qPCR testing. We also tested and optimized new genetic markers from the literature. We have now begun the process of subjecting this year's samples to previously used and to new genetic markers. Initial results confirm several of the tributaries as having human contamination detectable at multiple sites.

Samples have also been sent to scent-trained canines (Environmental Canine Services, ECS) for analysis, and revealed human sources in Indian Trails, Whiskey Creek, Little Plaster, and the 76th St. tributaries. We have observed ECS in action, and are making plans for when and how to perform an assessment on the ground. We believe that together, the results from these different strategies will help us locate some of the point sources of fecal bacteria pollution.

This summer has changed the way we perceive the natural world. We no longer simply see things. We wonder the condition of things, how they got that way, and what can be done to solve any problems. It has also been beneficial in helping Harry narrow down the possible paths for his future. It has been amazing to see the process of science in action and learn how to handle the difficulties that may come our way. This summer has prepared us to find our next problem and face it.