Geographic and Taxonomic Sources of Fecal Microbes in the Plaster Creek Watershed

Harry Ervin, Luke Tilma, Dr. Randall DeJong

Calvin COLLEGE 1876

Introduction

Plaster Creek is a highly polluted stream draining a 58 square mile area in the Lower Grand River watershed, spanning much of south-east Grand Rapids, suburban Kentwood and Caledonia, as well as rural agricultural lands to the south. Among its host of problems is fecal pollution, as indicated by the presence of *E. coli*. Levels of *E. coli* in Plaster Creek and its tributaries consistently exceed state guidelines for partial and full body contact with the water. One of the keys to restoring the cleanliness of Plaster Creek's waters is to identify the geographic and taxonomic sources of fecal contamination.

Previously, the DeJong lab focused on identifying the 5 worst tributaries in regards to levels of *E. coli* and potential taxonomic inputs. This summer's research investigated these five tributaries in an attempt increase source specificity. We continued evaluation of *E. coli* levels in these tributaries, with a greater focus on the analysis of DNA present in water samples using quantitative PCR (qPCR), a method utilizing genetic markers to identify which species the DNA originates from.

Objectives

- Continue evaluation of E. coli levels
- Collect water samples for qPCR analysis
- Achieve greater specificity as to the geographic and taxonomic sources of pollution

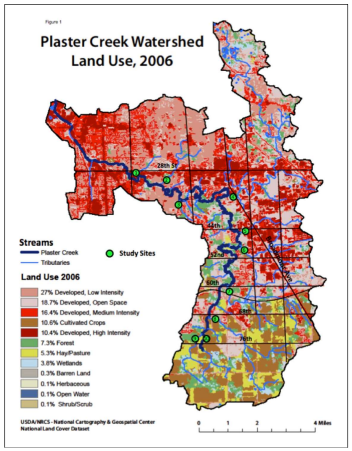

Figure 1. Plaster Creek watershed land usage map showing 10 sampling sites previously investigated.

Methods

Sampling

1-3 samples were taken at each subsampling location depending on the width of the stream. One sample consisted of 250-300 mL of water collected via a sterile bottle attached to a sampling pole. Tributaries subsampled include Indian Trails, Cross Creek, Whiskey Creek-Little Plaster, and 76th Street (on Figure 1 these are sites 2, 3, 4, and 9/10, respectively).

Figure 2. Harry (left) and Luke (right) retrieving a water sample.

I. Coliscan EasyGel

1. 2 mL of every sample were combined with Coliscan Easygel and plated.
2. Incubated at 37°C.
3. *E. coli* colonies were counted after 24 hrs.

II. DNA Filtration and Extraction

One sample from each location was processed this way.

1. Water was filtered on 0.45 micron filters.
2. These samples were then extracted using the MO BIO PowerWater DNA Isolation Kit.

Quantitative PCR

Quantitative PCR was performed on a *Fluidigm Biomark HD* in order to test for presence of certain species' DNA in the water. This analysis was performed on all extracted samples (with the exception of Cross Creek) using a variety of different genetic markers, based on mitochondrial DNA (mtDNA), animal-associated *Bacteroides*, as well as some animal-associated species of Archaea. Before and during use with water samples, markers were tested with positive controls to determine efficacy and accuracy.

Results

E. coli Counts

Though this was not the main concern of this year's research, we did observe a continuation of previous years' trends—*E. coli* counts consistently over the safe limits set forth by the State (1000 CFU's/100 mL for partial body contact, and 300 CFU's/100 mL for full body contact).

Quantitative PCR

Overall, we observed the most positives for the presence of human fecal contamination rather than other species within the watershed, although there were some positives for dog, goose, and ruminant species (likely deer or cow).

Human

- Human-associated *Bacteroides* DNA was detected in Little Plaster (4), Indian Trails (2), and 76th St. (9/10) tributaries (qPCR $C_t < 20$)
- Indian Trails (2) indicated the highest human contamination (qPCR $C_t < 10$ on multiple occasions)

Other

- Ruminant markers indicate ruminant contamination in the 76th St. area (9/10)
- The Whiskey Creek tributary (4) seems to carry dog and goose contamination

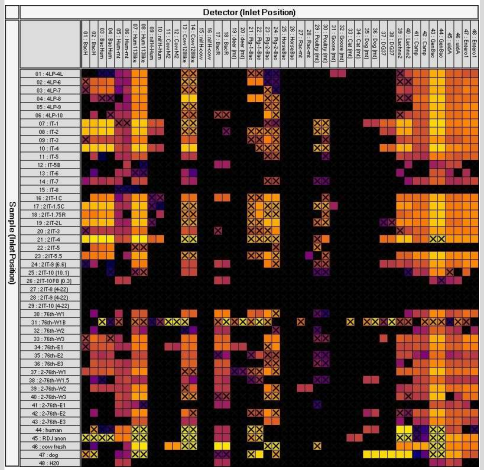

Figure 3. Heat map obtained from the qPCR run on August 22, 2016. This run included water samples from the Little Plaster, Indian Trails, and 76th Street tributaries. Brighter color indicates greater amplification. Human markers are concentrated to the left of the grid, while the large section of color on the right are positive controls.

Conclusions

- *E. coli* counts throughout the Plaster Creek watershed remain at unsafe levels.
- Every tributary that we subsampled indicates the presence of fecal contamination.
- Results confirm previous concerns of ruminant contamination in the 76th St. area – consistent with farming activities.
- Whiskey Creek appears to carry dog and goose fecal contamination.
- Human contamination seems the most widespread and remains of the greatest concern.
- DNA analysis of 76th St and Little Plaster Creek indicate substantial human contamination.
- From qPCR results, Indian Trails is the most clearly human-contaminated tributary.
- Possible sources of human contamination are leaky septic tanks or sanitary sewer lines.

Future Plans

Scent-trained Canines

In order to confirm our results and provide additional clarity as to geographic sources of fecal pollution, we plan to utilize Environmental Canine Services (ECS), a business that trains dogs to indicate when they smell human waste in water samples. Samples can be brought to the dog for quick results, or the dogs can be guided along streams giving results in real time as they go. We will be out in the field with ECS in October.

Figure 4. ECS dog Kenna working in the field.

Reference:
Michigan DEQ. Total Maximum Daily Load for E. coli for The Grand River Kent County. 2006.