

Final Report

Plaster Creek Bacterial Monitoring and Source Tracking Project

March 27, 2015 – March 26, 2017

Dr. Randall DeJong

Calvin College and Plaster Creek Stewards

DEQ Tracking code #2015-0504

Introduction

The objective of this project was to identify important sources of bacterial loading in Plaster Creek (Kent County). The following goals were set to reach this objective: 1) the relative contributions of bacterial loading for 10 major tributaries will be assessed and the five most egregious contributors will be prioritized; 2) molecular markers will be used to try to identify the primary taxonomic sources, and 3) to pinpoint bacterial loading locations using molecular markers and scent-trained canines in sub-watersheds.

Background

Plaster Creek drains a 58 square mile area within the Lower Grand River watershed in south-central Kent County, Michigan. Plaster Creek and its tributaries receive runoff from much of southern Grand Rapids, parts of the suburbs of Kentwood and Caledonia, and rural southern lands.

Neither the precise geographic locations nor the sources of the fecal contamination are known. Levels of *E. coli* are easily detected by culture-based methods (Figure 2), and in past studies of Plaster Creek these have been as much as 25X higher than that considered safe for partial body contact (1000 cfu/100 mL). However, culture-based tests for *E. coli* do not allow for differentiation among animal sources (cow vs. human vs. dog, for instance). We utilized a DNA-based technique called quantitative PCR (qPCR) and genetic markers that have been developed for other common gut bacteria – various *Bacteroides* spp. that are specific to different animal guts. Our project also used scent-trained canines to identify and source track human wastewater contamination.

Methods and Results

Year 1 sampling (Summer 2015)

Ten tributaries of Plaster Creek were selected and numbered (Figure 1). Water samples were taken upstream of the mouth of each tributary, weekly from late May through June, including both dry and wet (following significant rain events) conditions. At most locations, three 250mL bottles were filled from the creek at each location using an extension pole. (QAPP can be consulted for more details on sampling technique and protocols). Two samples from each location were used for *Coliscan Easygel* assays, while at least one other was vacuumed filtered and used for DNA extraction and subsequent qPCR tests. Field blanks of distilled water and field duplicates were conducted as recommended and documented in the QAPP.

Figure 1

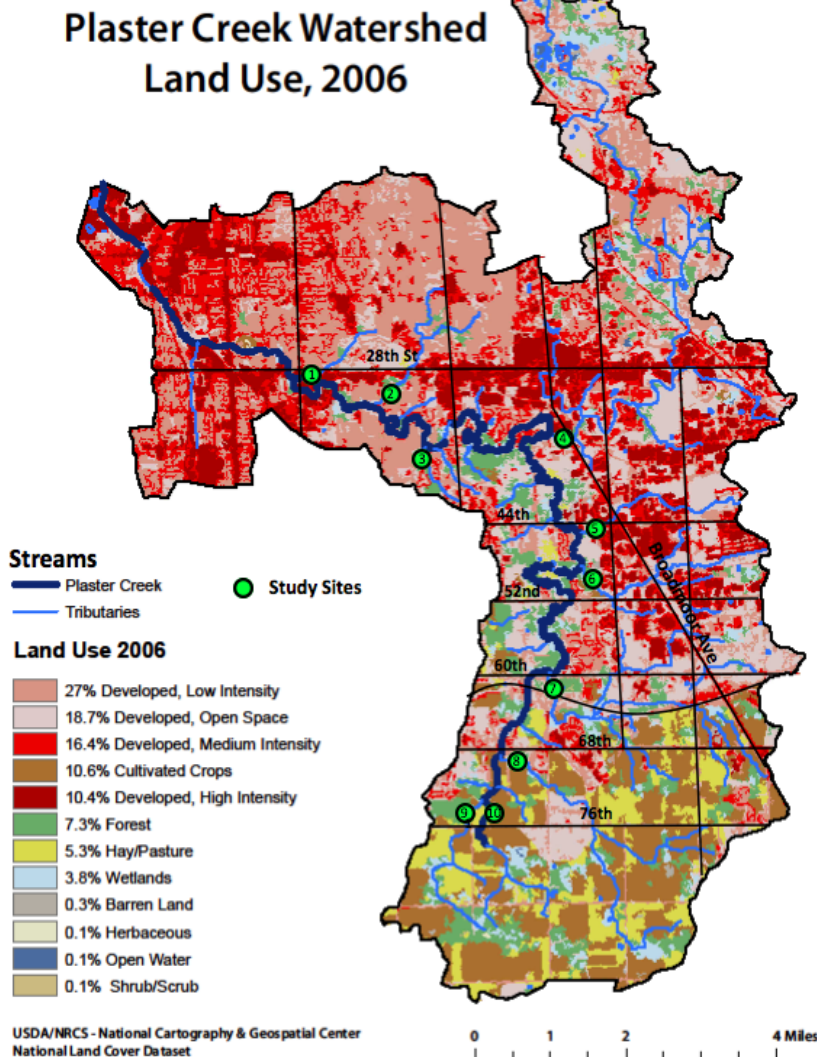


Figure 1. Land use map of Plaster Creek watershed, highlighting 10 tributaries sampled in first year of project.

Table 1. *E. coli* levels (cfu/100mL) at 10 tributaries, measured by Coliscan Easygel plates, 5/19/2015-8/20/15 (year 1).

Site	Mean	Maximum count	Minimum count
1	2795	6613	140
2	3138	9600	160
3	2471	6507	967
4	2965	10000	130
5	5627	20587	107
6	3016	13180	53
7	4622	16690	173
8	2119	8560	213
9	3375	12480	250
10	4214	24240	26

Based on *Coliscan Easygel* data (Table 1), the six most contaminated tributaries were then determined and sampled bi-weekly through August. Three of those tributaries were also subsampled in order to investigate the geographical source of contamination.

The genetic markers used in qPCR were obtained from the literature, each developed to detect specific animal-associated *Bacteroides* species, and used to identify taxonomic sources of contamination. Each test was done in duplicate. Results were scored as positive or negative for each test. Positive controls were performed by isolating DNA from animal fecal samples and conducting qPCR with the animal-associated *Bacteroides* probes.

E. coli counts varied considerably, especially temporally. The minimum at each site was 6-900X lower than the maximum (Table 1). However, in most samples taken, wet or dry, the levels of *E. coli* measured in the creek were much higher than the maximum allowed levels for partial (1000 cfu/100 mL) or full body (300 cfu/100 mL) contact. In fact, mean values for each of the ten sites were considerably above these recommendations (2119-5627 cfu/100 mL). **These *Coliscan Easygel* results suggest that all ten tributaries have at least one significant source of *E. coli* contamination.** Quantitative PCR (qPCR) results confirmed the *Easygel* results: nearly every sample was positive for a general *Bacteroides* test, indicating some kind of fecal contamination (data not shown).

qPCR tests for animal-specific *Bacterioides* included tests for human, ruminant, cow, dog, Canada goose, pig, and muskrat. Most tests were negative for these markers, but some samples were positive. Since the number of positives was small out of a large number of tests, it is not efficient to show the results in tables and they are instead summarized below. Notably, several tributaries gave strong indication of contamination by specific sources.

- **Tributary 2 (drains Indian Trails golf course, and Indian Village and Ridgemoor neighborhoods) was positive for human bacterial markers at multiple sites, and some sites were positive more than once.**
- **Tributary 4 (Broadmoor and 32nd, Whiskey Creek and Little Plaster drainages) was positive for human markers at 4 sites in the Little Plaster drainage, while the Whiskey Creek drainage was not tested until later.**
- **Tributaries 7 (60th and Hanna Lake Rd; drains area east of Dutton), and 9 and 10 (Breton and 76th, drain areas south of 76th street) were positive for ruminant and human markers multiple times at multiple sites.** Direct access by cattle to the creek was noted for at least three locations in these tributaries.

Based on the *Easygel* and qPCR results above, in 2016 we selected tributaries – tributaries 2, 4, 9, and 10, for further investigation. This investigation began by sending samples to Environmental Canine Services LLC (ECS) in March 2016 and again in April 2016. ECS offers a service to receive collected water samples and subject them to canine scent testing (a program they call Ship n' Sniff!). Duplicate samples were subjected to qPCR analysis. Tributary 7 was not included on the basis of accessibility to sites upstream of the original sampling point.

Scent-trained canines alert to the presence of human wastewater with a trained behavior, such as a sit, down, or a bark, when smelling containers of collected water samples or at field

investigation sites. It is important to understand that the dogs are trained to human wastewater, and while fecal material is one component they may be alerting to, they also can detect detergents and fats/oil/grease. Even if feces is not present in the wastewater, such as in the case of laundry or restaurant grease discharge, the dogs may still recognize it as human wastewater and alert to its presence. It should be noted that the canine protocols also make ample use of field blanks of distilled water and field duplicates to guard against false positives. In addition, all samples are presented to two dogs, which differ in their sensitivity as well as which characteristics of human wastewater they alert to.

The results of ECS Ship n' Sniff tests, using canines Sable and Logan, confirmed the qPCR tests:

- **Tributary 2 had multiple sites where samples contained human wastewater. In fact, for the more sensitive dog used (Logan), 7 out of 8 locations were positive.**
- **Tributary 4 contained human wastewater at 2 of 8 locations.**
- **Tributaries 9 and 10 had 4 of 5 sites positive for a human wastewater component.**

Year 2 sampling (Summer, Fall 2016)

Work in the summer of Year 2 focused on sub-sampling the 4 tributaries identified in Year 1, and preparing to have ECS investigate at least two areas in detail by bringing dogs to the sites. The sub-sampling scheme was more intensive geographically, in hopes of getting closer to pinpointing sources, but with less frequent sampling over time. Easygel and qPCR sampling were conducted on these samples. As part of this preparation, we plotted relevant data onto maps of these sub-watersheds.

Easygel results in 2016 were a little lower than the previous year in Tributaries 9 and 10, but were still mostly (12 of 18 samples) above the level allowed for partial body contact, with a range of 150-3350 cfu/100 mL at 7 different sites. In Tributaries 2 and 4, most samples were lower than in Year 2, with only 6 of 52 samples above the partial body contact limit, and a range of 0-2000 cfu/100 mL.

qPCR results in 2016 were consistent with those in 2015, though more samples were positive for dog:

- **Tributary 2 had 7 sites positive for human bacterial markers, 1 for ruminant, and 1 for dog. It should be noted that this is a suburban area, so the positive ruminant marker may indicate deer as a source (we verified this by collecting deer scat in the area and it was positive for the ruminant marker).**
- **Tributary 4 had 4 sites positive for human bacterial markers, 4 for Canada goose, and 4 for dog.**
- **Tributary 9 and 10 had 6 sites positive for human bacterial markers, 4 positive for ruminant, and 1 for dog.**

We also obtained and examined in detail maps of storm water, sewer line, and known septic tanks from the various counties and municipalities. These were cross-referenced, by hand, with the maps we created of sampling sites, qPCR data, and scent-trained canine results. For Tributaries 2, 4, 9, and 10, the data from Years 1 and 2 are summarized on the attached maps. These maps contain sampling sites, qPCR results (human only), and canine results:

1. Indian Trails (Tributary 2).pdf
2. Whiskey Creek and Little Plaster (Tributary 4).pdf
3. 76th and Breton (Tributaries 9 and 10).pdf

The following attachments are the maps of storm water, sewer line, and known septic tanks:

4. Indian_Trails_Sanitary_only.pdf
5. Indian_Trails_Storm_only2.pdf
6. Sanitary_Kentwood.pdf
7. Stormwater_Kentwood.pdf
8. Whiskey_Beltline&28th_GR_Storm.pdf
9. Cascade_Township_sanitary.pdf
10. Gaines_Township_Stormwater.pdf

In addition, a excel spreadsheet containing all Coliscan Easygel *E. coli* results is forthcoming:

11. Ecoli counts 2015 and 2016

In October 2016, ECS brought two canines, Abby and Kenna, to our location. Our hope was that we might better pinpoint sources of human wastewater contamination, and we planned meticulously for this event. With these dogs and their handlers, we surveyed two tributaries, Tributary 2 (Indian Hills) and Tributary 4 (Whiskey Creek/Little Plaster), both of which are extensive and complicated. We chose these two suburban tributaries because of the human bacterial signature in the samples, accessibility was high, and also it seemed more likely that we would be able to pinpoint the source to a sump-pump discharge (possibly with an illegal laundry discharge) or to one of a relatively low number of septic tanks near the tributary.

In two days of sampling, much was accomplished, confirming some of the results of the Ship n' Sniff, and expanding to additional sites. However, since a large number of samples were indicated as positive by the dogs, specific sources did not become easily apparent. A few possible sources will be detailed in the results and recommendations below, but it is clear that there are likely multiple sources even within one tributary, and it is difficult if not impossible to identify them all at once. Elimination of these sources, rather, has to occur one by one, followed by subsequent testing – this is the only way to verify a putative source.

Having the dogs in the field does not instantly enable easy answers. In the case of Plaster Creek, the high number of positive samples and low number of negative ones makes it difficult to pinpoint samples. In addition, the handling policy for the dogs had changed in the two years since we had written the grant. Dogs are not allowed to enter brush or debris, and dogs are no longer allowed to wade any part of the water body due to safety and humane concerns. Fortunately, we had learned this during the summer when we had the opportunity to observe

the dogs in action on another municipality's project. This foreknowledge enabled us to plan well, and these restrictions probably had little affect on our results.

Since clear answers did not arise during the ECS field visit, Karen Reynolds of ECS offered to look at the data and give her recommendations. She was provided all of the data and all of the maps above, of which she was very complimentary and found very useful. Her report, complete with her own set of Google Earth maps, is attached as:

12. ECS Plaster Creek Investigation Results.pdf

Readers are welcome to read through her report. However, the following section incorporates her analysis and conclusions alongside our own perspective and recommendations.

Analysis and Conclusions

Each of the four tributaries investigated in detail in Year 2 is presented here with relevant data, summary comments, and recommendations. Highlighted are the canine and qPCR data, as these are specific to human wastewater.

Important information on the qPCR and canine response data tables below. The number of positive tests out of the number of tests done is listed (e.g., 2/4 = 2 positive tests out of 4 total tests), with 6 being the maximum number of tests at any one site (if it was sampled in March, April, and October 2016). For the canine data, the dogs that gave positive alerts are listed in parentheses, and if a sample was examined by dogs, but it was negative, these dogs are listed in the Notes column. This is valuable information, as in each team of dogs, one is more sensitive than the other (Logan is more sensitive than Sable, and Kenna is more sensitive than Abby). The Ship and Sniff samples in March and April 2016 were examined by Sable and Logan, and the on-site samples were examined by Abby and Kenna. Where relevant, positive qPCR results for non-human bacterial markers are added (ruminant, dog, Canada goose). Sites are listed, as much as possible, upstream to downstream in the data tables. Counts of *E. coli* are mentioned in the comments where relevant, but since most collections were quite high, we chose not to list them in the tables, as they do not usually add much to the analysis.

Tributary 2 (Indian Trails)

The Indian Trails tributary has two branches, one that drains the Indian Village neighborhood, and another that drains part of the Ridgemoor (or Barfield) neighborhood. The two branches merge a little north of the east corner of Indian Trails golf course and then flow southwest to the east end of Ken-O-She park, near Kalamazoo and 32nd Street. Tributary 2 had multiple sites that were positive by qPCR for human bacterial markers and/or were identified as having human wastewater contamination by scent-trained canines.

Relevant documents:

1 Indian Trails (Tributary 2).pdf

4 Indian Trails Sanitary only.pdf

5 Indian Trails Storm only.pdf

11 ECS Plaster Creek Investigation Results.pdf

Sample Site IT6, north of Burton, small stream in front of house with statue collection

Site	Canine (dogs alerting)	qPCR (human)	Notes, including dogs that did not alert
IT6	2/4 (Logan)	1/1	Sable negative

Because ECS canine Logan (very sensitive – often detects even if other dogs do not) indicated presence of human wastewater in 2 of 2 Ship And Sniff samples, and because there are sanitary and stormwater lines in the area, it is possible that there is a small sewage leak into the soil that flows to the creek and contributes to the positive canine and qPCR findings at ITS 5.5. We note that there has not been much investigation upstream of this location, as it’s not clear where the water comes from and how much open stream there is. The homeowner has been resistant to access to this location, so inquiries should perhaps be made with Breton Village Green apartments for access in the rear yard. From maps, it seems likely that this stream has been covered upstream.

Recommendation:

- Continue to monitor, and investigate stream morphology upstream of this site.

Sample Sites IT5.5, behind the Grand Rapids Christian Middle School to ITS3, just north of Okemos Rd – the west branch of the stream

Site	Canine (dogs alerting)	qPCR (human)	Notes
IT5.5	2/2 (Abby, Kenna)	1/1	
IT5	0/2	1/2	Sable, Logan neg.
IT4.8	2/2 (Abby, Kenna)	-	
IT4.5	2/2 (Abby, Kenna)	-	
IT3	5/6 (Sable, Logan, Abby, Kenna)	1/2	

This tributary has a strong signal for human wastewater contamination, with multiple indicators at multiple sites. There could be some input from IT6, but the stronger signals suggest further input at ITS5.5 and/or downstream. There are sanitary and stormwater lines along the bank of the creek east of the school, and there is a stormwater outfall on the south side of the school, flowing directly into this branch of the creek. There is a strong possibility that a leaking sewer line is contaminating the nearby stormwater system which discharges directly to the creek.

Recommendation:

- The sewer system along the west bank of the creek, on the east side of the school, should be investigated for possible leaks that may be seeping directly through the

soil and/or entering the stormwater outfall. This request for investigation will be put forward to the City of Grand Rapids.

Sample Sites IT9, in the Ridgemoor neighborhood to PC-2.19 – the east branch of the stream

Site	Canine (dogs alerting)	qPCR (human)	Notes
IT9	1/2 (Kenna)	0/2	Abby negative
IT8	3/6 (Logan, Kenna)	1/2	Sable, Abby neg.
IT7.5	1/2 (Kenna)	-	Abby negative
IT7	3/4 (Sable, Logan, Kenna)	0/1	Abby negative
IT4	4/6 (Logan, Abby, Kenna)	1/2	Sable negative
PC-2.19	-	1/1	

ECS canine Kenna indicated human wastewater contamination in all of the samples sent in March 2016 from this branch, and an important note is that all of these samples had low counts of *E. coli* (0-80 cfu/100mL). ECS canine Logan also indicated human wastewater contamination in all the samples from this area the following fall. Both of these canines are able to alert to low levels of wastewater scent. Less frequent qPCR positive results may indicate a dilution effect and/or primarily detergents. On-site observations by several members of our team have noted that there are many discharge pipes coming from the homes on the banks of the ravine. These are likely sump pump discharges, and it is possible that one or more homes has a laundry machine or utility sink discharging to the sump pump. At the same time, septic contamination cannot be ruled out, as high *E. coli* counts (>1000 cfu/100 mL) were found at upstream sites (IT8 and IT9) on other occasions, and the sanitary line is frequently in the stream bed between IT7 and IT8. In this location, in fact, there was a faint sewage odor, though it is difficult to know if this was due directly to leakage or perhaps also due to stagnant water that appeared to be pooling in front of the Breton St. culvert.

At sites IT4 and PC-2.19, just before this east branch meets the western branch, the situation may be more complex. Site IT4 had high *E. coli* counts (2800, 6520). This branch crosses two more sanitary lines just upstream of IT4. These two lines and/or the line in the stream bed upstream (IT7-IT8) could be leaking and contributing to the bacterial load.

Recommendations:

It is difficult here to know whether there is detergent or septic contamination, or both. However, it seems to us wisest to check the sewer lines in this area on the basis that there was a smell, they are very near and in the stream bed, the ECS canine results and the very strong indication of human wastewater downstream (see next section). Therefore,

- The sewer line in the creek between IT7 and IT8 (east of Breton St.) should be checked for leaks. It may be the case that this line was checked recently (~2014), at least according to the city engineer who joined us for the on-site canine investigation.
- The sewer lines that are crossed to the west of Breton street should be checked for leaks.
- Determine if the culvert under Breton St could/should be dredged, and if that would help with flow in this area.

Sample sites IT2 (before stream enters golf course), downstream to IT1/PC-2.1 (before it joins Plaster Creek main channel)

Site	Canine (dogs alerting)	qPCR (human)	Notes
IT2	3/6 (Logan, Kenna)	2/2	Sable, Abby neg.
IT1.75	0/4	1/1	Abby, Kenna neg.
IT1.7	1/2 (Abby)	-	Kenna negative
IT1.6	2/2 (Abby, Kenna)	-	
IT1.5	2/2 (Abby, Kenna)	1/1	
PC-2.7	-	1/1	
PC-2.3	-	1/1	
IT1/PC-2.1	2/4 (Logan)	3/3	Sable negative

Consistently positive qPCR tests for human bacterial markers suggests that there are human wastewater sources in this stretch and/or upstream. These sites tended to have high *E. coli* counts (967-6714) as well. All of these results could be the result of possible upstream contamination noted above, but there are also possible sources within this downstream portion. Maps show and field investigations confirm that the sanitary system runs in the creek for much of this stretch, and that stormwater systems are nearby as well. In addition, there is an additional stormwater line that runs through the Indian Trails Golf Course and connects just south of site IT1.6. It appears to be the line that drains the Georgetown Apartments north of the GRCHS football stadium. Maps show that the sanitary lines and stormwater lines run very near each other throughout the apartment complex, and if the sanitary lines leaked into the stormwater system it would be carried directly to the stream.

Recommendations:

- Option 1: If upstream sources are identified and eliminated first, then this portion should be monitored to see if there is improvement.
- Option 2: Have the sanitary line checked for leaks between IT2 and IT1 at the same time that upstream sources are being investigated.

- The stormwater system draining Georgetown Apartments should be accessed at the manhole just north of the railroad tracks and sampled by Coliscan Easygel, qPCR, and canine testing. These measures could quickly rule out or implicate this system as a source.

Tributary 4 (Whiskey Creek and Little Plaster)

Little Plaster Creek drains an area east of East Paris and north to Cascade Road, and merges with Whiskey Creek south of 32nd street, east of Broadmoor. Whiskey Creek originates in the Ecosystem Preserve on the Calvin University campus, flows west onto the main campus and then south through the Calvin Seminary campus, and then drains south through suburban and commercial areas. It is highly modified, flowing underground in many locations south of Calvin and through many retention ponds such as at Calvin Seminary and Lake Eastbrook near Centerpointe Mall. Shortly after merging with Whiskey Creek, the streams join the main channel of Plaster Creek just west of Broadmoor and north of 36th street.

Relevant documents:

2 Whiskey Creek and Little Plaster (Tributary 4).pdf

6 Sanitary Kentwood.pdf

7 Stormwater Map Kentwood.pdf

8 Whiskey Beltline&28th GR Storm.pdf

9 Cascade Township sanitary.pdf

11 ECS Plaster Creek Investigation Results.pdf

Sample sites LP6 downstream to LP0 and PC4.G – Little Plaster creek

Site	Canine (dogs alerting)	qPCR (human)	Notes
LP6	2/2 (Sable, Logan)	1/2	
LP5	0/2	0/1	Sable, Logan neg.
LP4	4/6 (Sable, Logan)	0/4	Sable, Logan neg.
LP3	0/2	1/3	Sable, Logan neg.
LP2	0/2	1/3	Sable, Logan neg.
LP1.5	-	0/1	
LP1	0/2	1/2	Sable, Logan neg.
LP0	0/2	0/1	Sable, Logan neg.

Interestingly, the strongest indication of human wastewater occurs at the upstream sampling locations, while most downstream locations were negative for canine tests.. ECS analysis suggested continuing to monitor sites LP6 and LP4, and to investigate the two ditches or North Branch 1 and 2 upstream of LP6, as it is possible there is a septic discharge somewhere in these areas. A contributing source that far upstream may be consistent with a dilution effect causing the lack of positive qPCR tests at LP4 and the

infrequent positive qPCR results at all the other downstream sites. Unfortunately, at the time, the sanitary line information available to us ended just west of this area, so ECS was not able to incorporate that into the analysis. Noticing that North Branch 1 may originate just east of Spaulding Rd, ECS suggested checking whether this small ditch crosses a sanitary line. Since that time, we were able to obtain a sanitary system map from Cascade township, and it confirmed that there is a single sanitary line that is crossed by this short portion of North Branch 1.

Recommendations:

- Continue to monitor LP4 and LP6.
- Test upstream in North Branches 1 and 2.
- If there are indicators of human wastewater contamination by qPCR and/or canine tests, conduct further on-site investigations, and request the septic line just east of Spaulding Rd to be tested. (According to the Cascade township map, this request will have to be made to Ada township, as this line is actually connected to and serviced by Ada).

All WH sample sites – Whiskey Creek

Site	Canine (dogs alerting)	qPCR (human)	Notes
WHA/WH2	1/2 (Sable)	0/1	Logan negative
WHB	0/2	0/2	Abby, Kenna neg.
WHCR (West)	0/2	0/2	Abby, Kenna neg.
Manhole (Vitamin Shoppe)	2/2 (Abby, Kenna)	-	
Manhole (Motherhood)	2/2 (Abby, Kenna)	-	
WHCL (East)	2/2 (Abby, Kenna)	0/2	
WHD	2/2 (Abby, Kenna)	0/2	
WHF	2/2 (Abby, Kenna)	0/2	
WHE	0/2	0/2	Abby, Kenna neg.
WH1B	2/2 (Sable, Logan)	0/1	
WH1A	2/2 (Sable, Logan)	0/1	
WH1	3/4 (Sable, Logan)	0/2	Logan neg.
PC-4.9	-	1/2	

All of Whiskey Creek samples, except PC4.9, which lies just before it merges with Little Plaster, were negative by qPCR for human bacterial markers. The positive canine results mostly occur after the paired culverts underneath 28th St. (WHCL, WHD, WHF,

WH1B, WH1A, WH1). We believe that the WHCL (West) is fed by lines coming underneath Centerpoint Mall, and we sampled two manholes north and east in the parking lot, one in front of the Motherhood store, the other in front The Vitamin Shoppe – both were positive with ECS canines Abby and Kenna. The frequent detection by the canines and consistently negative qPCR results strongly suggest that the canines are detecting detergents (such as from a laundry and floor mopping discharge into the stormwater system) or fats/oils/grease discharges (such as from restaurant food/trash) into the stormwater system. ECS indicates that this is a common occurrence in their experience.

Some sources for the modest *E. coli* counts that are found in Whiskey Creek were identified through qPCR. Two sites were positive for dog, two sites were positive for Canada goose, and two sites were positive for both dog and goose. Given that Lake Eastbrook is in a high-density residential area frequented by pet owners, and Canada geese are frequently present and known to breed there, this is not surprising.

Recommendations:

- Request the City of Grand Rapids to investigate the stormwater line servicing the east side of the mall for detergent or fats discharge.
- Exclude Whiskey Creek from future monitoring efforts. While *E. coli* is frequently detected and can have counts above contact limits, most readings are lower (0-680), and almost all qPCR tests were negative for human bacterial markers.

Sample sites LP6 downstream to LP0 and PC4.G – Little Plaster creek

Site	Canine	qPCR (human)	Notes
WH/LP1	0/2	2/3	Sable, Logan neg.
PC-4C	-	1/1	

In addition to the possible sources upstream in Little Plaster and Whiskey Creek, possible sources exist near these sample sites that could be contributing. The septic systems at the residences along 32nd St, especially the one at the corner of 32nd and Lake Eastbrook Blvd could be possible sources. Also, there are septic lines in and near the creek at these locations that could be leaking.

Recommendations:

- Investigate the condition of the residential septic systems along 32nd St. It is possible that these homes are now on city septic and the old systems have been neglected.
- Request the City of Grand Rapids and the City of Kentwood test the septic lines in and nearby the creek. (Maps show that the waterway goes in and out of both municipalities in this area).

Tributaries 9 and 10 (Plaster Creek headwaters, 76th Street area)

The two most southern tributaries (9 and 10) of Plaster Creek begin south of 84th Street, each comprised of two first-order streams draining small wetlands located in rural farmland. The two tributaries join just north of 76th street. In the ECS report, the terminology used is a little different – they refer to Tributary 9 as the ‘west branch of Plaster Creek’ and Tributary 10 as the ‘east branch’.

Relevant documents:

3 76th Street and Breton (Tributaries 9 and 10).pdf

10 Gaines Township Stormwater.pdf

11 ECS Plaster Creek Investigation Results.pdf

Sample sites W1-W3, PC9.6 – Tributary 9

Site	Canine (dogs alerting)	qPCR (human)	Notes	qPCR (ruminant)
W3	0/2	1/2	Sable, Logan neg.	1/3
PC-9.6	-	1/1		-
W2	3/4 (Sable, Logan)	1/2	Logan neg.	0/3
W1	2/2 (Sable, Logan)	1/2		2/3

The canine and qPCR indicators of human wastewater at sites PC-9.6, W2, and W1, are likely from sources upstream from the two first-order streams that form Tributary 9. Counts of *E. coli* were also high at these three sites (1550-3350), but not at W3 (150). ECS recommended investigating the three septic systems along 84th St. to Breton Ave., and the septic systems on Breton Avenue. In addition, there are four septic systems to the west, 1 of which is near the first-order stream to the west, which went unsampled because of accessibility.

Recommendations:

- Request the three septic tanks along 84th St. to be investigated.
- Request the three septic tanks on Breton Ave. to be investigated.
- If the above are not identified as sources, request the 4 septic tanks to the west to be investigated.
- Based on the frequency of positive qPCR tests for ruminant bacterial markers, a careful study of the location of farms, their lagoons and cattle fields (including access to stream) should be conducted. Appropriate ways to communicate with farmers and cooperate with appropriate agencies should be incorporated into Plaster Creek Stewards’ activities. The DEQ (now EGLE) should be consulted as to their recommendations and practices.

Sample sites E1-E3 – Tributary 10

Site	Canine (dogs alerting)	qPCR (human)	Notes	qPCR (ruminant)
E3	2/2 (Sable, Logan)	1/2		1/3
E2	2/4 (Logan)	0/2	Sable negative	0/3
E1	2/2 (Sable, Logan)	1/2		1/3

ECS pointed to detection at E2 by canine Logan, who can detect very low levels, along with no positive qPCR at E2, as suggesting that one of the multiple septic systems upstream of E2 might have a minor leak. There are multiple newer homes on an unnamed road running north from 84th St SE (note: Google labels this small north-south road the same as the real east-west 84th St SE).

ECS also suggested that the septic systems of houses upstream of E3 be considered as possible sources. They also suggested that there might be runoff from the farm near the corner of 92nd street and Hanna Lake Avenue, into the pond that likely is the headwaters of this tributary. This and other possible farm-based sources may be sources of the ruminant bacteria seen in the qPCR results.

Recommendations:

- Request septic tanks near west headwater stream be investigated.
- As for Tributary 9, a careful study of the location of farms, their lagoons and cattle fields (including access to stream) should be conducted. Appropriate ways to communicate with farmers and cooperate with appropriate agencies should be incorporated into Plaster Creek Stewards' activities. The DEQ (now EGLE) should be consulted as to their recommendations and practices for approaching property owners in these areas. If the above are not identified as sources, request the 4 septic tanks to the west to be investigated.

Acknowledgements

Calvin College students were essential in this project, and are sincerely thanked for their excellent observations, data collection, and analysis:

2015

YeaEun Lee

Thuy-Tien Nguyen

Andre Otte

2016

Harry Ervin

Luke Tilma

In addition, many others were important contributors and supporters

Calvin University

Mike Ryskamp, Plaster Creek Stewards coordinator 2014-2016

Dave Warners, Plaster Creek Stewards and Professor of Biology

Gail Heffner, Plaster Creek Stewards and Director of Community Outreach

Jason VanHorn, Professor of Geography

Environmental Canine Services, LLC

Karen Reynolds, President

Laura Symonds, Canine Handler

Dan Ringel, Canine Handler

Sable, Logan, Abby, and Kenna, amazing canines

City of Grand Rapids

Numerous property owners who allowed access to their property