A Study of Bacteria in Honey Creek and Mill Creek, Tributaries to the Huron River

2012-2013

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Study Background and Purpose

Project Description
The main purpose of the investigation was to determine bacteria levels and detect patterns of contamination in two neighboring tributary watersheds to the Huron River. The first watershed, Honey Creek, is a 23 square mile, mixed land use watershed that is listed as impaired for pathogen contamination. The second watershed, Mill Creek, is much larger (130 square miles), more heavily agricultural, and also exhibiting high bacteria levels. The study included volunteer sampling for Escherichia coli (E. coli) to identify likely sources and genetic Bacteria Source Tracking (BST) to identify the likely sources of bacteria. The result will be used to supplement aerial imagery analysis to determine if failing septic systems could be detected. Honey Creek was sampled in 2012 and 2013 as it was part of a concurrent study to develop a watershed management plan (WMP). Mill Creek was only sampled in 2013.

Water Quality Concerns
Portions of the middle Huron River watershed, including Honey and Mill Creeks, fail to meet minimum water quality standards or provide designated uses. In 1996, the middle Huron became Michigan’s first nutrient TMDL and was listed as impaired due to excess phosphorus. Ford and Belleville Lakes, reservoirs of the Huron River, are impaired waterbodies on Michigan’s Impaired Waters list due to restricted recreational use caused by the presence of nuisance algal blooms. According to the Ann Arbor-Ypsilanti Metropolitan Area Watershed Management Plan, high nutrient loading and pathogens are primary pollutants. That WMP was developed primarily to address the phosphorus impairments. As a result, the forthcoming plan for Honey Creek will not focus on phosphorus. However, some activities related to pet waste developed to address E. coli may also serve to reduce phosphorus.

Table 1. State-listed impaired waterbodies that include Honey and Mill Creek watersheds –

<table>
<thead>
<tr>
<th>AUID</th>
<th>Waterbody</th>
<th>Parameter</th>
<th>TMDL Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>040900050403-02</td>
<td>Ford and Belleville</td>
<td>Phosphorus (Total); Excess Algal</td>
<td>1996, rev. 2010</td>
</tr>
<tr>
<td></td>
<td>Lake</td>
<td>Growth</td>
<td></td>
</tr>
<tr>
<td>040900050309-05</td>
<td>Honey Creek</td>
<td>Escherichia coli</td>
<td>2009</td>
</tr>
</tbody>
</table>

In 2009, the Michigan Department of Environmental Quality (DEQ) developed a TMDL for Honey Creek due to body contact impairments as a result of elevated bacteria levels. Specifically, high E. coli counts were recorded at a sampling location near the Honey Creek outflow. Also, the Huron River Watershed Council (HRWC) has been monitoring E. coli levels in the creek since 2006. Under HRWC’s program, single samples were collected once per month, May through September in 2006, and twice per month from 2008 through 2013. The mean E. coli count was 675 per 100 ml, with a maximum count of 12,000...
per 100 ml (August, 2010), above the single event water quality standard of 300 E. coli per 100 ml for total body contact (TBC), but with a median of 205 per 100 ml.

Michigan DEQ collected additional E. coli data in 2007 at four sites (see Figure 1) along Honey Creek to develop the TMDL. All sites exceeded the TBC standards, and many events exceeded the partial body contact standard. The highest counts were found at the most upstream location, where the Sample Period geometric mean exceeded 1,000 E. coli per 100 ml for the entire sampling period (dry and wet weather). E. coli counts were progressively lower at each downstream location for most sampling events, which suggested a source near or upstream of sampling station 1. One sample from station 1 was analyzed for human biomarkers, yet none were found, suggesting animal sources. Potential sources in the area of station 1 identified in the TMDL include livestock manure, horse pastures, pet waste and urban/suburban wildlife. One high-density housing development, Scio Farms, was identified with high pet ownership and stormwater runoff that may enter Honey Creek upstream of station 1.

Limited E. coli data from Mill Creek makes a characterization of water quality difficult. HRWC has sampled Mill Creek for E. coli since 2006, but MDEQ has yet to list the creek as impaired or engage a study of bacteria levels. Long-term Mill Creek bacteria levels are discussed in the results section.

**Study Goals**

The goals and objectives of this project will complement ongoing water quality projects within Honey Creek, Mill Creek, and the broader middle Huron including the Middle Huron TMDL Initiative, Middle Huron River Watershed Stormwater Group, and the Washtenaw County Illicit Discharge and Elimination Program (IDEP).

Results will be used to identify potential bacteria sources including failing septic systems to compare with the goals of the full project. Sampling was conducted at tributary confluences to identify geographical differences in bacteria levels. Samples were analyzed with bacterial source tracking (BST) techniques to identify human or animal sources. Results will be compared to remote sensing identification of failing septic systems to evaluate consistency.

Monitoring results will be provided to DEQ and the project advisory team along with recommendations for future remedial actions to address potential bacteria sources. For example, high bacteria counts combined with positive identification of human source markers will suggest potential septic failures or illicit connections in the upstream catchment. The study results were also used to develop a Watershed Management Plan for Honey Creek that will foster activities to reduce the quantity of bacteria entering the creek and, eventually, meet water quality standards.
Study Methods

Detailed methods and procedures are documented in the project’s Quality Assurance Project Plan, which is a companion to this report.

Site Selection

Initial sampling sites (see Figures 1 and 2) were selected in consultation with MDEQ and advisory teams. Long-term sites (MH02 and MH03) have been sampled as part of HRWC's Water Quality Monitoring Program and were sampled through the course of the project.

Primary sites in both watersheds (six in Honey Creek and three in Mill) were identified based on their location proximate to the end of tributaries to the main creek channels. This placement divides the catchments into sub-catchments in an effort to identify geographic differences in bacteria loading. These primary sites were thoroughly sampled weekly for five weeks. Sampling five weeks allows for the computation of a 30-day geometric mean (for the total body contact standard). Additional sites were selected upstream of primary sites following initial results to further subdivide high-bacteria count catchments.

Basic methods

Stream sampling was conducted once per week for five weeks for each sampling period. For Honey Creek, sites were sampled over three periods, while Mill Creek sites were sampled over one period. The sampling periods were:

- June 5 to July 2, 2012 (Honey Creek only)
- October 9 to 31, 2012 (Honey Creek only)
- July 9 to August 8, 2013 (Honey and Mill Creeks)

Samples were collected during both wet and dry conditions over the course of each sample period. The sampling teams, consisting of at least two individuals, traveled to a site after picking up equipment at the HRWC offices, completed a field data sheet that documents the location, date, time, team members, and weather conditions and then collected samples from the site. A strict sample labeling protocol was used to identify samples. Each site was sampled in triplicate, following state standard methods, with an additional sample for Bacteria Source Tracking (BST) analysis, and randomly generated field duplicates and blanks produced for quality control.

Each team then traveled to additional sites to collect additional samples. Each team was scheduled to sample two to three sites per day. Upon completion of the fieldwork, the monitoring team delivered half of the grab samples to the Ann Arbor Water Treatment Plant laboratory for analysis and then returned equipment and forms to the HRWC office. HRWC staff collected the second set of sample containers and packaged for pick-up by Helix Labs for BST analysis. A ‘chain-of-custody’ form was completed and submitted to each lab to follow the water samples.

In addition to water samples, stream flow was measured relatively near the mouth of each creek. Continuous water level and discharge measuring instruments were in place at long-term stations MH02 (managed by USGS) and MH03 (managed by HRWC). Additionally, climatic conditions including precipitation and temperature were tracked via Weather Underground stations.
Figure 1. The Honey Creek watershed and potential sampling sites for bacterial study.
Sample Analysis

Each standard sample was processed in the lab within six hours to begin incubation of bacteria colonies following standard procedures. Total colony forming units (cfu) per 100 ml of sample volume were identified by the lab for each sample and reported to HRWC.

Quality of analytical results was assured by two metrics: comparisons (by relative percent difference) of duplicate field samples and field blanks (sample vessels filled with distilled water). The blanks test field sample handling and laboratory processing methods for sterilization. Duplicates provide a measure of field sampling variability (i.e., precision). Duplicates and blanks were run on 6% of the total samples, and sample identification was established to blind the lab from quality control samples. Targets of 0 cfu for blanks and 25% difference between duplicates were pre-established as quality standards.

Lab results indicated that all blank samples were assessed to have no *E. coli* cfu. The relative percent difference between field duplicate samples was 26.7%. Thus, the bacterial concentration results reported in this study can be considered accurate to ±27% of the value reported. Since the sampling methods were proven to be sterile, the duplicate result suggests that the variability in bacterial concentration at any given site is relatively high.

To account for this variability, sampling was done in triplicate at each site. Samples were drawn from three representative locations at each sampling site location, usually near each stream bank and the middle of the channel. The resulting three bacteria concentrations were then averaged using their
geomean, following state standard procedures. Each geomean of three samples was recorded as the site’s *E. coli* concentration result.

**Bacteria Source Tracking (BST)**

All samples were collected in replicate to allow for BST analysis. One set of samples was sent to the Ann Arbor Water Treatment Plant laboratory for *E. coli* concentration, and the replicates were sent to Helix Biological Laboratory (Helix) for BST analysis. A subset of samples that were determined to have high bacteria counts were selected for BST analysis. Bacteria counts were considered "high" if the samples exceeded the single event sample standard of 300 cfu per 100 ml. From that set, a list of sites was selected such that each site would be analyzed for BST, and consistently high-count sites would be assessed multiple times.

Target species for BST analysis were human, canine (dog), equine (horse), bovine (cow), and goose. In 2012, BST analysis included the presence or absence of the five species markers only. In 2013, the lab was able to provide the relative predominance of each marker that was positively identified.
Results and Discussion

**E. coli counts and distribution**

HRWC has been monitoring Honey Creek and Mill Creek for *E. coli* during the April-September recreational season since 2006, as part of the Middle Huron Water Quality Monitoring Program. That program has ten long-term sites starting with a site on the Huron River upstream of Dexter down to tributary stations in Ann Arbor and Ypsilanti. In comparing all long-term stations in this program, only the Fleming Creek tributary station and the Huron River station have lower mean and median *E. coli* counts (Figure 1) than the Honey Creek site. That site has a geomean *E. coli* count of 211 per 100 ml, -below the single-sample TBC standard, but not the 30-day standard. In comparison to Honey Creek, Mill Creek has a geomean *E. coli* count of 391 per 100 ml, above the single-sample and 30-day TBC standards, but not the single-sample PBC standard.

![Figure 3. E. coli concentrations for the ten long-term sites in the Middle Huron Water Quality Monitoring Program. Figures illustrate the range of data collected from 2006 to 2013. Shown are interquartile range with median, mean (yellow diamond), 3xIQR (whiskers) and extremes. Single sample standards are shown as well (TBC=orange, PBC=red).](image)

Mill Creek sampling only took place at the downstream-most station, however, and does not represent conditions upstream or in smaller branches. Sampling under that program also is based on single grab samples at each site for each sampling event. *E. coli* counts or concentrations can be quite variable. To account for this variability, the State of Michigan uses a standard that composites samples from three representative locations at each sampling site into a single geomean of the sampling event. The entire sets of sample results from these studies are included in tables in Appendix A.
These results can also be viewed geographically in relation to the calculated geomeans for each site. Figure 4 shows the sample site locations for Honey Creek, along with sample period geomeans for the three sampled seasons sequentially (June/July 2012, October 2012, and July/August 2013). Figure 6 shows the five different Mill Creek sites, along with corresponding geomeans, for the sampled period (June – August 2013). Note that not all sites were sampled each period and some sites on the map were never sampled at all, but are shown for reference.

**Honey Creek**

Sites 2 and 3 were original DEQ sampling sites and were never intended to be resampled. Site 6 was deemed to be redundant with sites 7 and 8. Site 10 was not sampled because the same branch draining to site 8 only achieved flow during rain events, so obtaining a clean sample from 10 would likely have been very difficult. Site 13 was not sampled due to low concentrations from sites downstream. Finally, site 19 was not sampled as it was difficult to access, did not achieve much flow, and was not far upstream of site 18.
Figure 4. Geomeans of bacteria (E. coli) counts per 100 ml by sampling period at each site sampled.
The downstream site 4 was only sampled in the initial period to establish downstream conditions for comparison. A number of upstream sites were only sampled during or following rain events, as there was otherwise too little flow from which to obtain a sample. Other Honey Creek sites were only sampled during the first period (June/July 2012) because they showed relatively low bacteria concentrations.

The Honey Creek spring/summer 2012 geomean near the mouth was 425, exceeding the TBC standards for single sample and 30-day mean. Sample results at two branches were below concern levels and were not subsequently sampled in October. Those branches are represented by sampling sites HC12 and HC14. All other branches had bacteria concentrations well above TBC standards as well as the level at the downstream station HC04. Branches represented by sampling stations HC01 and HC17 had geomeans for June/July sampling that exceeded the PBC standard. Sampling upstream of these two stations did not result in levels that were substantially lower. Therefore, potential sources upstream are candidates for further investigation.

Several interesting observations emerged from October sampling. First, bacteria concentrations were generally lower, which primarily may be the result of lower temperatures. The second observation is that the levels at upstream station HC09 (671 cfu) were significantly higher than those downstream at HC01 (180 cfu) as well as the levels further upstream at station HC11 (182 cfu). These levels suggest that there may be a source that is actively contributing bacteria to the creek during the fall season between stations HC11 and HC9, but the population is reduced by the time it enters the main creek. October sampling also indicated a runoff source above site HC08.

Follow-up late summer sampling in 2013 confirmed the patterns from the previous summer. A particular strong source appears to be located between sites HC09 and HC01, though sources appear to also exist upstream of HC09.

The results depicted in Figure 4 combine bacteria levels detected in both wet and dry periods. Looking at the hydrologic state provides an understanding of the relative contribution of consistent inputs or point sources (which should be detectable in dry conditions, but less so in wet conditions) and runoff sources (which should be absent in dry conditions and high in wet conditions). To look at this dynamic, one can compare results to the preceding rainfall conditions.

Figures 3 through 5 show the bacteria concentrations from regional groups of sample stations along with the preceding 48-hours of rainfall. Downstream station HC04 is shown to be above the TBC standard in dry conditions, but then in excess of the PBC standard following a significant rain event. This increase in bacteria concentration suggests that the Honey Creek system has a combination of point sources and runoff sources. Likewise, upstream branches contributing to sites HC07, HC01, HC09 and HC17 show a similar pattern. Mean concentrations at all of those sites are higher than HC04 under all conditions and increase with rain events.

In contrast, Figure 3 shows that the stream contributing to HC15 has the opposite trend. Bacteria concentrations are high during dry conditions, but seem to get diluted following rain events. HC11 shows a similar trend, which suggests that there may be a consistent point source upstream of each of those sites.
The trend for HC12 appears similar to most of the other sites in that it increases with stormwater runoff. However, *E. coli* concentrations remain below the TBC standard in all but the largest rain events, which suggests that areas contributing to HC12 appear to be less problematic than other areas of the watershed.

Figure 3. Relationship between precipitation 48 hours before sample collection and the *E. coli* concentration at three Honey Creek sites. Single-sample standards for total (dashed) and partial (solid) body contact are shown as well as best-fit curves.

Figure 4. Relationship between precipitation 48 hours before sample collection and the *E. coli* concentration at four Honey Creek sites. Single-sample standards for total (dashed) and partial (solid) body contact are shown as well as best-fit curves.
Mill Creek

Daily geomeans for Mill Creek sites consistently exceeded the minimum TBC standards for single sample and 30-day mean at all of the sites. The branch represented by the sampling site 7 was the only site to exceed both the TBC and PBC standards per 100 ml. This site had the highest calculated geomean from any of the five sites sampled. Of all of sampled sites, site 4 had the lowest calculated geomean (Table 3, Figure 6).

Several interesting observations were a result of this sample period. First, levels at the upstream sites 6 (941 cfu) and site 7 (1175 cfu) were higher than those at downstream site 3 (680 cfu) (Figure 6). These results suggest that during the sampled period, there may be a source actively contributing bacteria to the creek, although the bacteria population is reduced as it continues downstream into the main branch.

Figures 7 and 8 show the bacteria concentrations from regional groups of sample stations along with the preceding 48-hours of rainfall. In Figure 7, downstream sites 2 and 4 are shown to be below the TBC standard in dry conditions. However, following significant rainfall, site 2 surpasses both the TBC and PBC standards, while site 4 only exceeds the TBC standard. These results suggest that drainage to site 2 from storm runoff contains much more bacteria than the other two branches. Conversely, in Figure 8, the branch leading to site 7 shows a pattern suggesting a point source, while the branch leading to site 6 does not. However, there are very few data points from these branches from which to draw any solid conclusions.
Figure 6. Geomeans of bacteria (E. coli) counts per 100 ml by sampling period at each site sampled.

Figure 7. Relationship between precipitation 48 hours before sample collection and the E. coli concentration at three Mill Creek sites. Single-sample standards for total (dashed) and partial (solid) body contact are shown as well as best-fit curves.
Figure 8. Relationship between precipitation 48 hours before sample collection and the E. coli concentration at three Mill Creek sites. Single-sample standards for total (dashed) and partial (solid) body contact are shown as well as best-fit curves.

Bacteria Source Tracking (BST)

As described in the methods section, a subset of samples from sites that exceeded the TBC standard were set to a lab to conduct Molecular Source Tracking (MST) analysis of bacteriodes cells extracted from the samples. Bacteriodes is a species of bacteria that, like E. coli, exist in the guts of mammals. DNA material was assessed for the presence of five markers for which positive references were established: human (Hu), bovine or cow (B), canine or dog (C), Equine or horse (Eq) and goose (G).

Honey Creek BST

Results of this analysis conducted at the 10 Honey Creek sites are summarized in Tables 4 and 5. The downstream station was positive for all five markers in each of the three tests run. HC01 and the two stations upstream of that site were also positive for all five markers in 2/3 of the tests or more. Stations HC17 and HC12 were also positive for human sources suggesting that there may be septic system issues in these drainages. Bovine sources were positively identified at all sites tested. Since there are no active cattle or dairy operations in the Honey Creek watershed, the source may be active bacteria in manure or compost applications throughout the watershed. Canine sources were identified at all but one site indicating that pet waste is a source of bacteria throughout the watershed. Geese were positively identified as a genetic source for bacteria in all the upstream branches, as were horses.
Table 4. Bacteria Source Tracking Results for Honey Creek.
Count of tests showing positive for *bacteroides* with DNA markers

<table>
<thead>
<tr>
<th>SiteID</th>
<th>Test count</th>
<th>DNA Markers</th>
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<tbody>
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<td></td>
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<td>Human</td>
</tr>
<tr>
<td>HC01</td>
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<td>HC04</td>
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</tr>
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<td>HC05</td>
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<td>HC07</td>
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<td>HC12</td>
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<td>2</td>
</tr>
<tr>
<td>HC16</td>
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</tr>
<tr>
<td>HC17</td>
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<td>5</td>
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</tbody>
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Table 5. Percent of BST tests showing positive for DNA markers by type for Honey Creek.

<table>
<thead>
<tr>
<th>Site ID</th>
<th>Human</th>
<th>Bovine</th>
<th>Canine</th>
<th>Equine</th>
<th>Goose</th>
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<td>HC01</td>
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<td>HC04</td>
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<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
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<td>100%</td>
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<tr>
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<td>100%</td>
<td>60%</td>
<td>60%</td>
</tr>
</tbody>
</table>

Percentages exceeding 50% positive are highlighted

Mill Creek BST
The Mill Creek analysis results for the five different sites are summarized in Tables 6 and 7. Only one test was run for each of the sites, and three sites (Mill04, 05, and 06) tested positive for all of the DNA markers. The remaining two sites (Mill02, and 03) each tested positive for four out of five DNA markers. Downstream site Mill03 tested negative for the canine marker and Mill02 was the only site that tested negative to the equine DNA marker.
The DNA markers at Mill03 were dominated by goose markers with much smaller proportions of human, bovine, and equine markers. Other downstream sites showed relatively no predominance by any of the markers. Upstream site Mill06 showed a predominance of bovine marker, while Mill07 showed a predominance by bovine, canine and equine over smaller amounts of goose and human. Human markers did not predominate in any of the Mill Creek samples.

**Table 6. Bacteria Source Tracking Results for Mill Creek.**
Count of tests showing positive for *bacteriodes* with DNA markers

<table>
<thead>
<tr>
<th>SiteID</th>
<th>Test count</th>
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<th>Bovine</th>
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<th>Equine</th>
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</table>

Percentages exceeding 50% positive are highlighted

**Table 8. Quantification means of BST tests showing positive for DNA markers by type for Mill Creek.**

<table>
<thead>
<tr>
<th>SiteID</th>
<th>Human</th>
<th>Bovine</th>
<th>Canine</th>
<th>Equine</th>
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Conclusions

Although geographically separated, both Honey Creek and Mill Creek contain high concentrations of bacteria that impair potential uses of these streams. Sampled sites at both creeks have upstream conditions that greatly exceed PBC standards indicating potential source locations. Both creeks show widespread bacterial contamination rather than narrow “hot spots” or geographically specific bacteria sources. Honey Creek watershed does have some branches with much lower bacteria concentrations and, thus, reduction efforts can be focused on several critical areas, or branches where concentrations are substantially higher. Mill Creek branches, in contrast, all exceeded the TBC standard, though the easternmost branch (draining to site 4) was somewhat lower and often below the standard. Overall, considering bacteria concentrations, both creeks should be considered impaired, and further study is needed to define critical areas for Mill Creek.

All five of the markers tested for genetic markers or bacteria source tracking (BST) analysis were present at all Mill Creek and most Honey Creek sites. This indicates broad contamination from a variety of mammalian bacteria hosts including humans, pets, livestock, and wildlife. While all of the DNA markers tested were prevalent in both watersheds, the bovine markers were the most broadly identified. Bovine markers were positively identified more than 50% of the time at every sampled site in both watersheds. The absence of active cattle and dairy farms proximate to these sites indicates that there may be active bovine bacteria applications occurring in the forms of manure or compost. To a lesser degree, the remaining four genetic markers also tested positive at many sites throughout the watersheds, demonstrating a need to develop a multi-faceted action plan to greatly reduce bacteria entering these watersheds.

The diversity and quantity of bacteria, as measured by *E. coli*, entering these watersheds are driven by rainfall. After significant rainfall events, bacteria levels increased at a majority of the sites within both watersheds, signifying that the bacteria source is largely driven by stormwater runoff. Only three sites showed a pattern indicating a dilution of bacteria concentration with additional rainfall. In these instances, upstream sources of bacteria are likely constant point sources.

Evidence of a point source and human BST markers in one Honey Creek tributary suggest the existence of an illicit connection to that branch or significant failures in septic treatment in the area draining to the sampling point on that branch. That location was the lone significant finding of potential septic failure across the two-year study of the two creeks. While human markers were identified in samples from other sites and could suggest low levels of septic failure, human sources were not identified as the predominant source in these other streams.

Enough data has been captured to inform a strategic plan to reduce bacteria sources for Honey Creek. Additional sampling of Mill Creek is needed to gain a better understanding of geographic patterns and understand seasonal and yearly variations in the bacteria regime and sources of contamination.
## Appendix A. Data Tables.

### Table 1. Honey Creek *E. coli* results by site. Counts per 100 ml.

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Honey and Mill Creek Bacteria Study
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**Sample Period Geomean** n=5 169
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Sample Period Geomean  n=4  42

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Sample Period Geomean  n=5  670

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Sample Period Geomean  n=5  516

Honey and Mill Creek Bacteria Study
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### Table 2. Mill Creek *E. coli* results by site. Counts per 100 ml

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