USING MICROBIAL SOURCE TRACKING TO IDENTIFY FECAL CONTAMINATION SOURCES IN BOZEMAN CREEK

BOZEMAN, MONTANA

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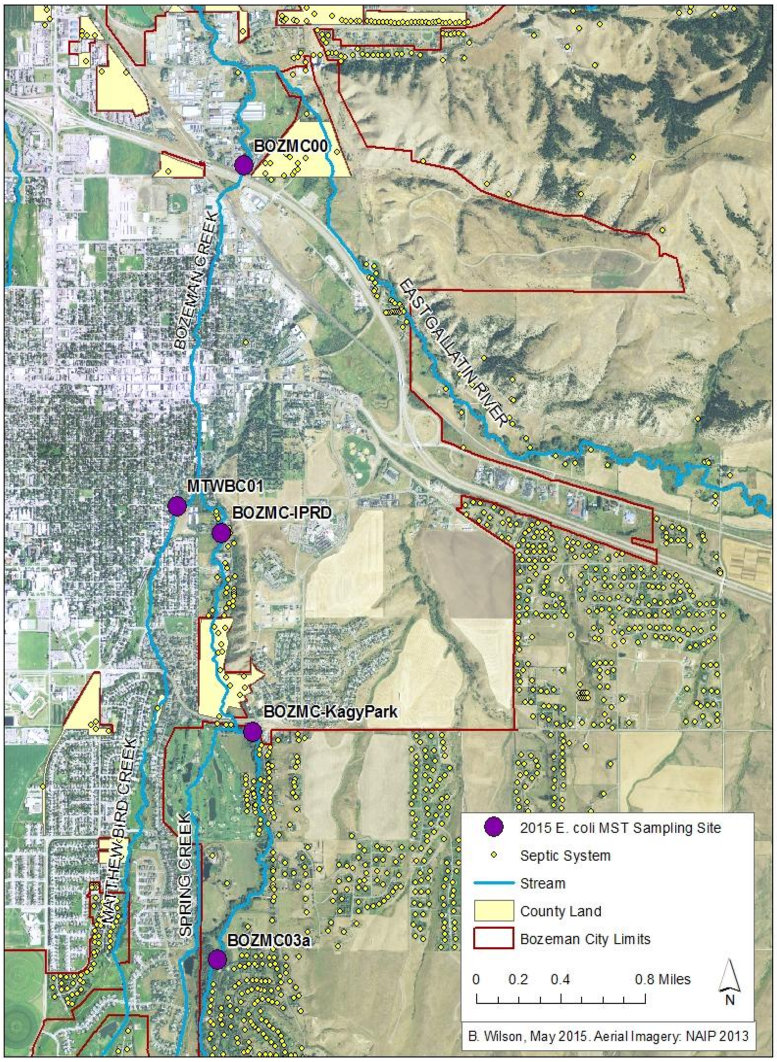
1.0 INTRODUCTION and BACKGROUND

Urbanization along streams can have detrimental effects on stream health and water quality, and Bozeman Creek is no exception. Bozeman Creek originates in the mountains south of the City of Bozeman in Gallatin County, Montana (**Figure 1**). Gallatin County is in a semi-arid climate with 16 inches of average annual precipitation in Bozeman and up to 50 inches in the mountains. Bozeman Creek serves as a drinking water source for the City of Bozeman drinking water supply. Flowing north through a rural area of larger parcels, the creek crosses the city limits into the urban area of Bozeman where it merges with Matthew Bird Creek, and finally flows into the East Gallatin River at the Story Mill Community Park north of town. Although the creek is flanked in spots by parks and open space, many stretches of the creek are adjacent to homes with aging septic systems, or urbanized areas where the creek's meander is constricted. The creek is subject to the effects of urbanization, eroded banks, and untreated stormwater runoff.



B. Wilson, May 2015. Aerial Imagery: NAIP 2013

Figure 1. Study sites on Bozeman and Matthew Bird Creeks. Flow direction in Bozeman Creek and Matthew Bird Creek is south to north.

Bozeman Creek is on the Montana Department of Environmental Quality (DEQ) 303(d) list of impaired streams for excess nutrients, sedimentation, and *E. coli* bacteria. *E. coli* is of particular concern because its presence in streams indicates fecal contamination, and suggests that pathogenic microorganisms might also be present. Sources of fecal contamination to surface waters include septic systems, domestic and wild animal manure, and storm runoff.

In 2008-2010 *E. coli* samples were collected on Bozeman Creek by the Montana DEQ where ten percent of the samples exceeded the state water quality standard of 126 colony forming units (cfu)/100 mL. In 2012, Montana State University Extension Water Quality (MSUEWQ) conducted *E. coli* monitoring at 17 sites along Bozeman Creek and its tributaries. This study found higher concentrations of *E. coli* in Bozeman Creek within city limits and in Matthew Bird Creek just above its confluence with Bozeman Creek.

In September 2013, the Gallatin Local Water Quality District (GLWQD) collected samples from four locations on Bozeman Creek (**Table 1**) to discern human-sourced fecal contamination from dog-sourced fecal contamination using microbial source tracking (MST) methods.

Table 1. *E. coli* and MST sampling results from GLWQD's September 2013 collection event

Site ID	**Month**	***E. coli* Geometric Mean (MPN/100 mL)**	**Human MST**		**Dog MST**	
			Fecal Biomarker	**Approx. Contribution of Fecal Pollution**	**Fecal Biomarker**	**Approx. Contribution of Fecal Pollution**
BOZMC03	July	268.75				
	August	157.26				
	September	110.99	Absent	Negative	Absent	Negative
BOZMC02a	July	271.99				
	August	242.59				
	September	234.95	2 of 3 present – high levels	Major Contributor	Present	Minor Contributor
BOZMC01	July	286.66				
	August	206.74				
	September	197.92	2 of 3 present – high levels	Major Contributor	Absent	Negative
BOZMC00	July	255.62				
	August	194.37				
	September	265.09	3 of 3 present – high levels	Major Contributor	Present	Minor Contributor

Results indicated human-sourced fecal contamination was a major contributor at three of the four sample sites, while dog-sourced fecal contamination was a minor contributor at two of the four sample sites. This was a single sampling event, yet it raised concern about human-sourced fecal contamination as a major contributor of *E. coli* bacteria in Bozeman Creek, as well as questions about its origins.

2.0 PROJECT GOALS, OBJECTIVES, and TASKS

The goal of this project was to collect data that will aid in reducing the concentration and loading of *E. coli* bacteria to Bozeman Creek and differentiate sources of fecal contamination to Bozeman Creek. To achieve this goal, the following objectives and associated tasks were conducted:

1. Utilize GIS files that exhibit septic systems along the streams.
    - Use for sample location selection to bracket human influence.
2. Identify the major contributing sources of fecal contamination to Bozeman Creek and Matthew Bird Creek.
    - Use microbial source tracking methods to identify the source of fecal contamination.
    - Test for wastewater tracers such as fluoride and chloride in order to indicate any municipal or septic system wastewater influence.

3. Coordinate with the City of Bozeman and Gallatin City-County Health Department to inform the public of the potential health risks associated with recreating in water bodies that are impacted by levels of *E. coli* bacteria that exceed state water quality standards.

3.0 METHODS

3.1 Sample Site Selection

This study was conducted during the summer of 2015, utilizing *E. coli* enumeration and MST to identify the sources of fecal contamination in Bozeman and Matthew Bird Creeks. Four sample sites along Bozeman Creek were chosen to bracket areas of dense septic system development that correlated with the positive MST results for human fecal biomarkers in the 2013 study (**Figure 1**). Matthew Bird Creek was included so its inputs to Bozeman Creek could be assessed.

3.2 Microbial Source Tracking Methods

MST is a set of methods using genetic biomarkers from several strains of *Bacteroidetes* bacteria to identify the animal sources of fecal contamination. Like *E. coli* bacteria, *Bacteroidetes* are found in the intestines of warm-blooded animals and are used as indicators of possible fecal contamination in surface waters. The presence of *Bacteroidetes* in water is an indicator of recent fecal contamination. The laboratory used for this analysis, Source Molecular Corporation, utilizes one dog biomarker and four human biomarkers. Source Molecular Corporation recommends that at least two human biomarkers be used to increase confidence in human sourced fecal contamination results. Human biomarkers used for this study were, *B. stercoris* and *B. dorei*. It is important to note that MST analysis and *E. coli* analysis focus on different bacteria strains, yet both help identify the presence of fecal contamination.

3.3 Sample Collection

Samples were collected in the late mornings on July 15th, August 11th, and September 9th, 2015. The MST, *E. coli*, fluoride, and chloride grab samples were collected synoptically. Field parameters and stream discharge were collected shortly after grab samples were collected. Sampling occurred during non-storm events to capture ambient base flow conditions that minimized the potential for pollutant dilution. Stream discharge was measured at each sampling site to calculate bacterial loading. Fluoride samples were collected to help identify city sewer influence; and chloride samples, which could indicate septic system or sewer influence. Field parameters (pH, specific conductivity, dissolved oxygen, and water temperature) were measured to look for differences in water quality conditions between sites that could support identification of areas of incoming wastewater contamination.

3.4 Quality Assurance/Quality Control

Field meters were calibrated at the beginning of each day using standard reference solutions to assure the meters were working properly. This information was recorded in calibration logbooks maintained for each instrument to document calibrations and meter checks.

Quality control samples consisted of one duplicate and one blank sample collected at random from one of the sampling sites for *E. coli* bacteria analysis, chloride, and fluoride. Because of the cost associated with MST analysis, duplicate and blank samples were not collected. Quality control criteria for duplicate and field blank samples are:

- Relative Percent Difference (RPD) of duplicates = <25%
- All field blank results must be < Reporting Limit (RL)

All samples were submitted to certified laboratories for analysis. MST samples were sent to Source Molecular Corporation in Miami, Florida. The *E. coli* bacteria, chloride and fluoride samples were sent to Energy Laboratories in Billings, Montana. Laboratory analytical methods and reporting limits are listed in **Table 2**.

Table 2. Analytical methods and reporting limits

Analyte	Method	Reporting Limit
E. coli bacteria	A9223 B	1 CFU/100 mL
MST • Human *Bacteroides dorei* DNA • Human *Bacteroides stercoris* DNA • Dog Bacteroidetes	Real-Time Quantitative PCR DNA (qPCR)	Presence/Absence and Percent Quantification
Chloride	E300.0/A 4500 CL B	1 mg/L
Fluoride	4500F-C	0.1 mg/L

4.0 RESULTS

4.1 Field Parameters

The peak water temperature of 15.71°C was found in July at site BOZMC-IPRD. The lowest water temperature (7.00°C) occurred at BOZMC-KagyPark in September. pH ranged from 7.39 to 8.74. SC ranged from 248.0-341.0 µS/cm with one anomaly of 640.6 µS/cm at BOZMC-IPRD during the September sampling event. This also correlated with the lowest DO reading of 79.5% among all project measurements (**Table 3**). This higher SC and low DO measurement could potentially indicate incoming water with some degree of wastewater influence, but more sampling at this site is needed before this can be confirmed.

Table 3. Field parameter measurements

Site ID	Month	Water Temperature (Celsius)	pH	Specific Conductivity (µS/cm)	Dissolved Oxygen (% Saturation)
BOZMC03a	July	12.18	7.97	279.0	87.9
	August	11.86	7.94	248.0	85.2
	September	9.48	7.90	311.0	82.0
BOZMC-KagyPark	July	12.39	7.81*	264.0	99.1
	August	12.43	7.39	272.0	93.4
	September	7.00	7.99	341.0	90.1
BOZMC-IPRD	July	15.71	8.39	285.0	95.5
	August	13.19	7.71	285.0	89.5
	September	10.90	8.17	640.6	79.5
MTWBC01	July	11.57	7.74	319.0	91.0
	August	11.32	8.74	315.0	86.6
	September	10.41	7.53	272.0	91.2
BOZMC00	July	15.00	8.26	321.7	97.9
	August	14.20	8.19	292.6	91.5
	September	10.68	8.07	320.0	91.2
*pH measured, but YSI field meter would not stabilize reading					

4.2 Wastewater Tracers: Fluoride and Chloride

Grab samples were collected for fluoride and chloride analysis at all sites. Blank and duplicate samples were collected in July at BOZMC-KagyPark. The relative percent difference for both fluoride and chloride duplicate samples was 0%, and fluoride and chloride were not detected in the field blank samples. Fluoride levels ranged from no detection to 0.1 mg/L (**Table 4**). Chloride levels ranged from no detection to 4 mg/L. Matthew Bird Creek had the highest chloride levels of all sites sampled at 4 mg/L for all sampling events.

Table 4. Fluoride and chloride results.

Site ID	Month	Fluoride (mg/L)	Chloride (mg/L)
BOZMC03a	July	ND	ND
	August	0.1	ND
	September	ND	1
BOZMC-KagyPark	July	0.1	2
	July- Duplicate	0.1	2
	July-Blank	ND	ND
	August	0.1	2
	September	ND	3
BOZMC-IPRD	July	0.1	2
	August	0.1	2
	September	ND	3
MTWBC01	July	0.1	4
	August	0.1	4
	September	ND	4
BOZMC00	July	0.1	3
	August	0.1	3
	September	ND	3
ND = Not Detected			

4.3 *E. coli* Bacteria

Grab samples were collected for *E. coli* bacteria analysis at all sites (**Table 5**). Blank and duplicate samples were collected in July at site BOZMC-KagyPark. The relative percent difference for the field duplicates was 0% and the field blank sample was less than the detection limit of 1 cfu/100 mL.

Table 5. *E. coli* enumeration results

Site ID	**Month**	***Escherichia coli*** **(cfu/100mL)**
BOZMC03a	July	200*
	August	78
	September	110
BOZMC-KagyPark	July	>2400*
	July- Duplicate	>2400
	July-Blank	<1
	August	220*
	September	190*
BOZMC-IPRD	July	2400*
	August	93
	September	120
MTWBC01	July	460*
	August	1100*
	September	820*
BOZMC00	July	440*
	August	86
	September	150*
*Exceeds MT DEQ Water Quality Standard of 126 cfu/100 mL.		

E. coli values ranged from 78 to >2400 cfu/100 mL (**Figure 2**). *E. coli* levels were highest in July at BOZMC-KagyPark and BOZMC-IPRD, with levels greater than 2400 cfu/100 mL. The lowest *E.coli* level was 78 cfu/100 mL in August at the upstream site BOZMC03a. The State water quality standard for *E. coli* in summer months is 126 cfu/100 mL. All samples collected in July exceeded the water quality standard of 126 cfu/100 mL. BOZMC-KagyPark and MTWBC01 exceeded the water quality standard during all sampling events. BOZMC00 exceeded the water quality standard for two of the three sampling events. Overall, 67% of samples exceeded the 126 cfu/100 mL water quality standard.

The MT DEQ state water quality standard for *E. coli* bacteria loading were calculated using the measured discharges and compared to daily loads calculated using the collected data (**Equation 1**). All sites exceeded the calculated water quality standard target value for the July sampling event (**Figure 3**). MTWBC01 and BOZMC-KagyPark exceeded the calculated water quality standard target values for all sampling events. Overall, 60% of the samples collected exceeded the *E. coli* daily load target values.

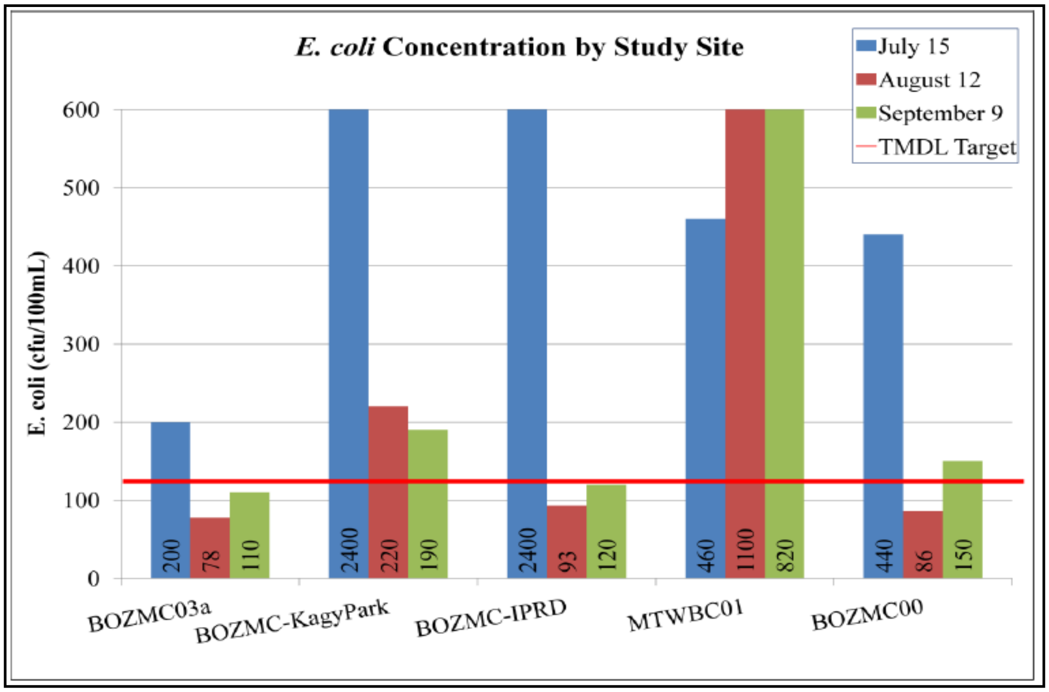


Figure 2. *E. coli* concentrations from all sampling events at each study site. Sites are listed from upstream (left) to downstream (right).The red line represents the MT DEQ water quality standard for *E. coli* bacteria in summer months. Sixty-seven percent of all samples collected exceed the MT DEQ water quality standard of 126 cfu/100 ml.

Equation 1:

Water quality standard target value calculation for loading:

Target Value = (126 cfu/100 mL)(discharge in cfs)(2.44E+7)

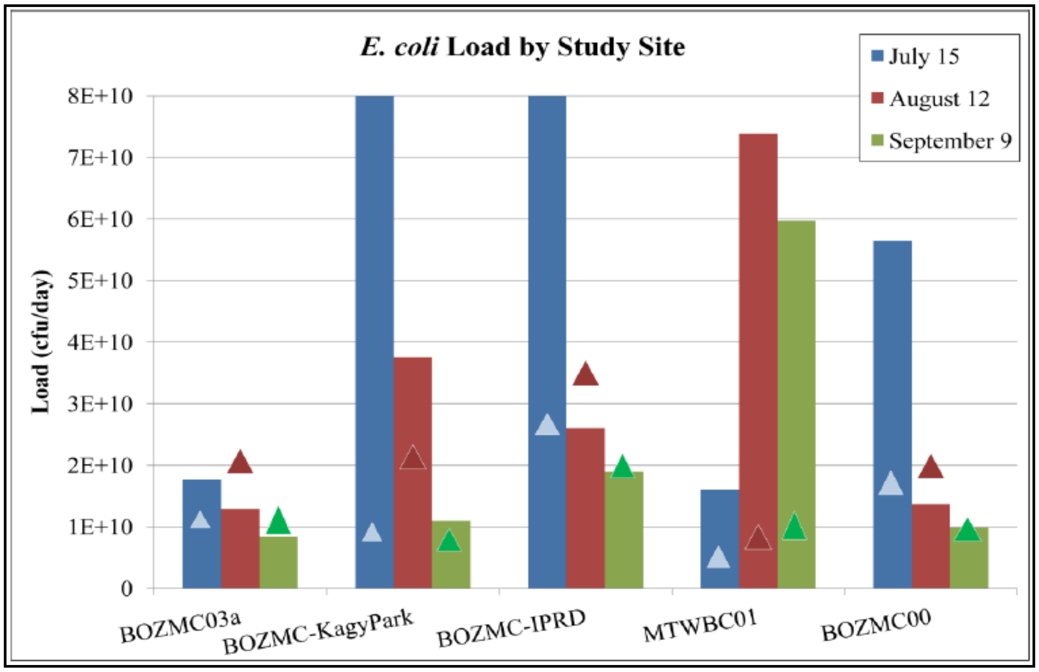


Figure 3. Calculated *E. coli* loading from all sampling events at each study site. Sites are listed from upstream (left) to downstream (right). Water quality standard target loads for all *E. coli* sources are indicated by triangles on the graph. Sixty percent of samples collected exceeded MT DEQ water quality standard *E. coli* daily load target values.

4.4 Microbial Source Tracking

Grab samples were collected at all sites for MST analysis. The September samples were not received by Source Molecular Corporation within the recommended holding time (24 hours), and therefore the September data was flagged. Qualitative descriptors (negative, trace, minor, moderate, and major) for the contribution of human and dog fecal contamination were provided by the analytical laboratory (Source Molecular Corporation) (**Table 6**). Each gene biomarker was also quantified and reported in copy numbers/100 mL of water.

Human sourced fecal contamination was greater in the July sampling event; it was a major source at four sites, and a moderate source at the most upstream site, BOZMC03a. At MTWBC01, human sourced fecal contamination was a major or moderate source of fecal contamination for each sampling event. Dog sourced fecal contamination ranged from trace to major for the sampling period, with the major contribution occurring in July at sites BOZMC-IPRD and BOZMC-KagyPark.

Table 6. MST results for human and dog sourced fecal contamination for each sampling event

Site ID	**Month**	**Approx. Contribution of Dog Fecal Pollution in Water Sample**	**Dog Bacteroidetes (copy #/100 mL of Water)**	**Approx. Contribution of Human Fecal Pollution in Water Sample**	***B. dorei* (copy #/100 mL of water)**	***B. stercoris* (copy #/100 mL of water)**
BOZMC03a	July	Minor	204	Moderate	2,060	Absent
	August	Minor	385	Negative	ND	ND
	September	Minor	210*	Negative	ND*	ND*
BOZMC-KagyPark	July	**Major**	238,000	**Major**	2,030	8,150
	August	Minor	215	Negative	ND	ND
	September	Minor	184*	Negative	ND*	ND*
BOZMC-IPRD	July	**Major**	81,000	**Major**	1,230	7,400
	August	Minor	170	Trace	ND	<LOQ
	September	Minor	315*	Negative	ND*	ND*
MTWBC01	July	Moderate	8,210	**Major**	4,630	167
	August	Minor	925	**Major**	16,400	13,600
	September	Minor	823*	Moderate	2610*	285*
BOZMC00	July	Minor	643	**Major**	1,850	Present (Trace)
	August	Minor	178	Minor	392	<LOQ
	September	Trace	<LOQ*	Trace	<LOQ*	<LOQ*

LOQ = Level of quantification. ND = Not detected.
*Results flagged, holding time exceeded

5.0 DISCUSSION

It was initially thought that chloride and fluoride would help determine the sources of wastewater contributing to fecal contamination in Bozeman and Matthew Bird Creeks, with higher chloride concentrations indicating wastewater influence and higher fluoride levels indicating contributions from a municipal water or sewer source. However, concentrations of these wastewater tracers were not sufficiently elevated above native levels to pinpoint either as a potential source (**Table 4**). Field parameter data (specifically dissolved oxygen and specific conductivity) did not correlate with high *E. coli* concentrations that would support identification of a wastewater influence (**Table 3**).

BOZMC03a (upstream site) had consistently lower concentrations than sites downstream, indicating fecal contamination is occurring as Bozeman Creek flows through more developed and urban areas (**Figure 2**). BOZMC-KagyPark had higher *E. coli* concentrations than upstream BOZMC03a, possibly due to the area of high-density septic systems located between these two sites.

The highest daily loads of *E. coli* for Bozeman Creek occurred in July with all samples from all sites exceeding water quality standard target loads **(Figure 3**). The highest daily loads for August and September occurred in Matthew Bird Creek. While it may be a major contributor to Bozeman Creek's *E. coli* load, the trend is not reflected downstream. This is likely due to a dilution effect between the Matthew Bird Creek confluence and BOZMC00. Daily loading of *E. coli* decreased in Bozeman Creek during the August and September sampling events. The peak of *E. coli* loading in July may indicate a connection with high spring flows and elevated groundwater that dissipates by mid to late summer.

This study focused on human and dog fecal contamination. The relative qualitative contribution levels of human-sourced fecal contamination generally correlate with *E. coli* concentrations (**Table 6**). However, this is not seen in all samples. Rural runoff in the upper Bozeman Creek watershed and native wildlife are likely natural sources of *E. coli* to Bozeman and Matthew Bird Creeks that were not analyzed in this study. Data from other potential sources (wildlife, livestock) may help paint a clearer picture of overall fecal contaminant sources in Bozeman and Matthew Bird Creeks.

6.0 FUTURE STUDY

Collecting data year round with greater frequency could help identify seasonal trends in *E. coli* levels and contributions of fecal contamination. Focusing sampling efforts during spring and early summer could provide insight on possible septic system contributions when groundwater levels are higher. Collection of total suspended solid (TSS) samples to examine if there is a correlation with high fecal contamination could be undertaken to determine if it is sediment-borne. Sampling during and after storm events, targeted around stormwater outfalls, could help identify the extent of stormwater contributions, if any. Additional sites along the stream continuum would help hone in on origins and isolate specific areas of incoming fecal pollution. The use of additional human biomarkers could increase confidence in the identification of humans as a contributor to fecal contamination. The use of other animal biomarkers could shed light on other potential sources of fecal contamination, but this would be low priority due to cost and the difficulty of reducing these contributions.

7.0 ACKNOWLEDGEMENTS

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