Passaic Valley Sewerage Commission (PVSC)
Essex County
600 Wilson Avenue
Newark, New Jersey

MICROBIAL SOURCE TRACKING (MST) PARTNERSHIP SUMMARY REPORT

Final Report
February 2021

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Section 1 Introduction

1.1 Background of PVSC's Facilities

PVSC provides wastewater treatment service to forty-eight (48) municipalities within their northeast New Jersey service area. The PVSC District covers approximately 150 square miles from Newark Bay to regions of the Passaic River Basin upstream of the Great Falls in Paterson. PVSC's main interceptor sewer begins at Prospect Street in Paterson and generally follows the alignment of the Passaic River to the PVSC Water Pollution Control Facility (WPCF) in the City of Newark. The extent of the PVSC Service District and the combined sewer areas within the study area are shown in **Figure 1-1**.

Eight (8) of the municipalities within the PVSC District have combined sewer systems and have received authorization to discharge under their respective NJPDES Permits for Combined Sewer Management. Two of the combined sewer municipalities, the Cities of Bayonne and Jersey City, own and operate their own combined sewer systems, interceptors, combined sewer overflow (CSO) control facilities, and pumping stations. In addition they jointly own the force main used to transport wastewater to the primary clarifiers at the PVSC WPCF in Newark. PVSC does not own or operate any of the combined sewer overflow control or transportation facilities which service Bayonne and Jersey City. Finally, the North Bergen MUA (Municipal Utilities Authority) connects to PVSC through the Hudson County Force Main, owns the CSOs, but does not own the collection system.

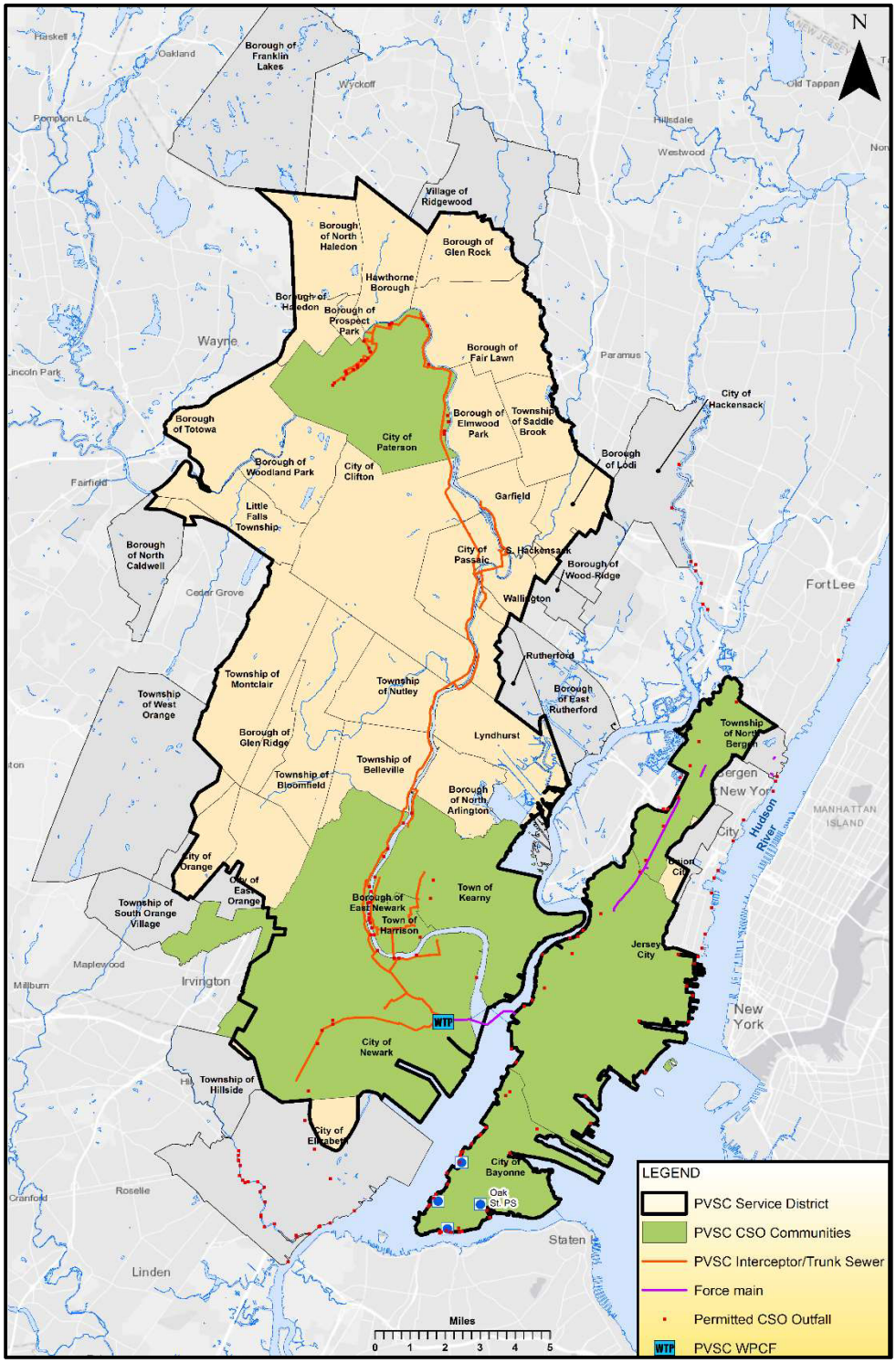
The other municipalities with combined sewer systems include the Borough of East Newark, the Towns of Harrison and Kearny, and the Cities of Newark and Paterson. All of these municipalities are tributary to the PVSC main interceptor and most of their combined sewer systems are tributary to CSO control facilities owned and/or operated by PVSC.

The Lower Passaic River basin study area is in an established urban area in northeastern New Jersey located along the 17-mile tidal section of the Passaic River from the head of tide at Dundee Dam in Clifton to Newark Bay. The land use is primarily residential and commercial. The Passaic River downstream of Dundee Dam in Clifton is designated by New Jersey Department of Environmental Protection (NJDEP) as a tidal saline (SE) water. The tributaries to the lower Passaic River downstream of Dundee Dam are designated by NJDEP as freshwater (FW2) waters. New Jersey defines these FW2 waters as not maintained in their natural state, influenced by point sources and subjected to increases in runoff from anthropogenic activities (NJDEP, 2019).

1.2 MST Partnership Purpose and Objectives

The Passaic River, Second River and Saddle River, in the highly urbanized Lower Passaic River watershed are on the New Jersey Department of Environmental Protection (NJDEP) 303(d) list of impaired waters contaminated with fecal indicator bacteria (NJDEP, 2017). Like many urban waters, the Lower Passaic is a vital resource for the region, but also susceptible to pollution from many sources. However, not enough data is available from the Third River to determine impairment. While regulation has focused on CSO control, the presence of high fecal indicator bacteria levels upstream of CSOs during dry and wet weather periods has prompted multiple investigations to identify the source of the elevated levels, which may be attributed to wildlife, domestic animals, failing septic systems, leaking sewer lines and sewer lines cross-connected to storm systems, regrowth of disinfected Wastewater Treatment Plant effluents, and other

Figure 1-1: PVSC Service District



unknown sources. As a result, microbial source tracking (MST) techniques have proven effective in identifying the source of fecal bacteria, and have been used in previous studies performed by the Passaic Valley Sewerage Commission (PVSC), the United States Environmental Protection Agency (USEPA), and the NJDEP.

Several recent studies, including those performed by the USEPA and PVSC, independent of one another, found *E. coli* cell counts in the Second and Third Rivers exceeding the New Jersey Surface Water Quality Standard (SWQS) for a 30-day geometric mean of 126 counts/100 ml and the single sample maximum of 235 counts/100 ml. Findings from an MST study completed by PVSC in 2017 indicated that several wildlife and domestic animal sources were contributing to fecal contamination in both the Second and Third Rivers, while the Second River was also severely impacted by human source fecal contamination.

As these studies support efforts by multiple community stakeholders to track down and remediate sources of microbial contamination, PVSC and the U.S. Geological Survey (USGS) formed a partnership through the Urban Waters Federal Partnership (UWFP), leading to this MST study in the Second and Third Rivers. By forming a partnership with the USGS, PVSC builds upon their previous success in performing MST in support of their CSO Long Term Control Plan. Leveraging the expertise and resources of the USGS, PVSC executed a program that provides a better understanding of distribution of bacterial contamination and pathogen sources in the water bodies. Collected data will also support one of the core goals of the Lower Passaic River Urban Waters Federal Partnership, to help reduce sources of pollution to and within the Passaic River, and other related activities, in order to meet the fishable/swimmable goal of the Clean Water Act. For the USGS, partnering with PVSC supported objectives identified in the Strategic Science Plan for the USGS Water Resources Mission Area in the advancement of monitoring networks and techniques for determining water quality and assessment of water resources and their suitability to meet human and ecosystem needs.

1.3 MST Partnership Report Purpose and Objectives

The purpose and objective of this Report is to provide a detailed description of the microbial source tracking task undertaken in accordance with the MST Partnership Quality Assurance Project Plan (Greeley and Hansen, Revised June 2020). The data gathered through sampling efforts on the Second River and the Third River has been analyzed and is discussed at length in this report. The report provides the methods used for sample collection, a summary of the sampling activities, laboratory analysis results, a summary of QAQC protocols implemented, and a discussion on the interpretation of the results.

This report also assesses the efficacy in achieving specific project goals and objectives set out in the MST Program QAPP, which included:

- Determining the E. coli, fecal coliform, caffeine, and DNA biomarker concentrations in the Second River and Third River to identify and quantify the sources of fecal contamination in the water bodies.
- Generating sufficient data for correlation analysis of E. coli, fecal coliform, caffeine and DNA biomarkers with various physical and geographical parameters; and
- Generating sufficient relevant data under wet and dry conditions.

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Section 2 Sampling and Analysis Plan Overview

2.1 MST Sampling and Analysis Plan Overview

Microbial source track-down sampling was completed at eight (8) receiving water locations. Four (4) were located on the Second River and four (4) were located on the Third River, as shown in **Figure 2-1**. The Sampling Program targeted locations upstream of the CSOs and tidal influence, and was conducted as follows:

- All eight receiving water locations were sampled for water quality parameters for at least six (6) total events. The four (4) Third River Sites were sampled an additional round for a total of seven (7) events. Grab samples and *in situ* measurements were collected at each location during each event.
- There were a total of two (2) wet weather events, defined as a sampling event that fell within 24 hours of at least 0.20 inches of precipitation.
- All data was recorded using USGS field forms and then digitally archived in USGS "Superfly" electronic field forms.
- The collected water samples were placed in coolers filled with ice packs and wet ice, and were kept with the sampling team at all times. An individual chain of custody (COC) detailing the contents of each cooler was also kept with the samples at all times. Upon completion of each sampling round, the sampling team verified that the information on the chain of custody was correct, double checked that each sample was accounted for and labeled properly, and proceeded to transport the coolers to each laboratory.

The sampling events took place every week from July 15, 2020 through August 12, 2020, with one last event taking place on September 16, 2020.

2.2 Sampling Locations

Sampling locations were selected to achieve a balance of geographic spacing, land use diversity, inclusion of sites with known high bacteria levels, and inclusion of sites with no historical data. The sampling locations are shown in **Figure 2-1** and **Table 2-1** is a summary of selected sampling locations.

Figure 2-1: MST Sampling Locations

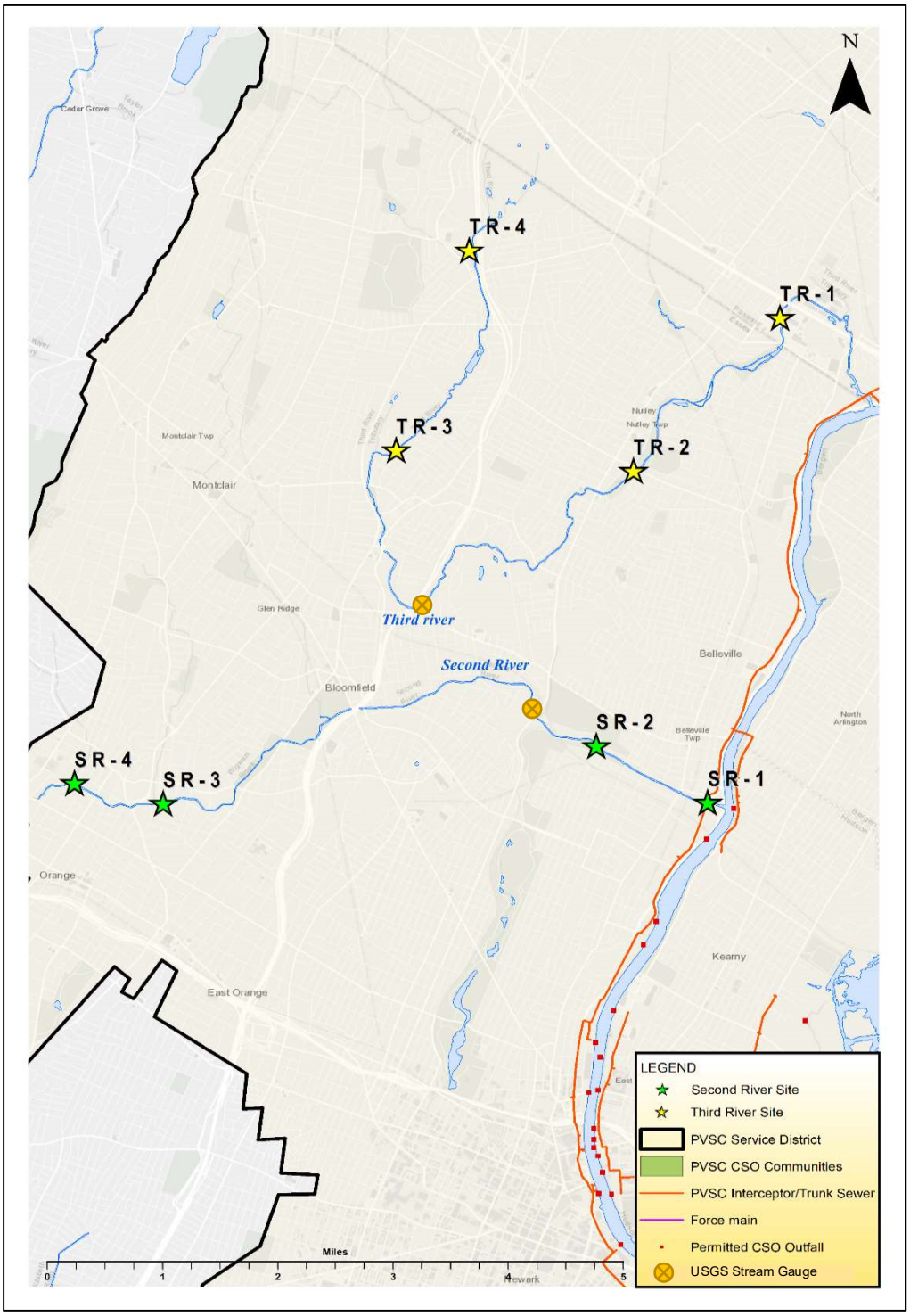


Table 2-1: Sampling Locations

Waterbody/Station		USGS Station Identification	Coordinates		Location	Location Type	Platform[1]	Number of Samples	Sampling Frequency[2]
			Latitude	Longitude					
Second River	SR-1	01392520	40.78029	-74.15095	Main Street, Belleville, NJ	Channelized Stream	Land – Main Street Bridge	4 Total *E. coli* & FC Caffeine DNA	6 Events – one every week
	SR-2	01392505	40.78603	-74.16492	Branch Brook Park Dr, Newark, NJ	Channelized Stream	Land – Overpass Bridge	4 Total *E. coli* & FC Caffeine DNA	6 Events – one every week
	SR-3	01392468	40.78020	-74.21918	123-107 N Park St, East Orange, NJ	Channelized Stream	Land – Overpass Bridge	4 Total *E. coli* & FC Caffeine DNA	6 Events – one every week
	SR-4	01392448	40.77986	-74.22336	281-291 Washington St, City of Orange, NJ	Channelized Stream	Land – Overpass Bridge	4 Total *E. coli* & FC Caffeine DNA	6 Events – one every week
Third River	TR-1	01392230	40.82611	-74.13303	River Road, Clifton, NJ	Natural Bed Stream	Land – Overpass Bridge	4 Total *E. coli* & FC Caffeine DNA	7 Events – one every week
	TR-2	01392190	40.81382	-74.15990	Centre Street, Nutley, NJ	Natural Bed Stream	Land – Centre Street Bridge	4 Total *E. coli* & FC Caffeine DNA	7 Events – one every week
	TR-3	01392157	40.81600	-74.19005	Bloomfield, NJ	Natural Bed Stream	Land – Concrete Bridge	4 Total *E. coli* & FC Caffeine DNA	7 Events – one every week
	TR-4	01392142	40.83659	-74.18019	Near Brookdale Service Area, Bloomfield, NJ	Natural Bed Stream	Stream – Wade (low flow)	4 Total *E. coli* & FC Caffeine DNA	7 Events – one every week

2.3 Analytical Parameters

Sampling and laboratory analysis were performed to determine concentrations for *E. coli*, fecal coliform, caffeine, bacterial DNA biomarkers and certain field measurements. **Table 2-2** provides a summary of the sampling parameters, field measurements, and laboratory information.

Table 2-2: Analytical and Field Parameters

Sampling Parameter	Sub-Parameter	Analysis Location	Number of Samples/Measurements
E. coli	N/A	PVSC WWTP Laboratory Newark, NJ	60 Total Samples [(8 sites + 1 blank + 1 dup per round) x 6 rounds*]
Fecal Coliform	N/A	PVSC WWTP Laboratory Newark, NJ	60 Total Samples [(8 sites + 1 blank + 1 dup per round) x 6 rounds*]
Caffeine	N/A	ALS Environmental Kelso, WA	64 Total Samples [(8 sites + 1 blank + 1 dup per round) x 6.5 rounds*]
Microbial DNA Biomarkers	• Human • Dog • Ruminant • Bird	USGS Ohio Water Microbiology Laboratory Columbus, OH	65 Total Samples [(8 sites x 6.5 rounds*] + 8 dup + 5 blank
Field Parameters	• Depth • Temperature • Dissolved Oxygen (DO) • pH • Specific Conductivity • Turbidity	In-situ	311 Total Measurements** [(8 sites x 6 parameters per round) x 6.5 rounds*]

* Samples for FIB were only valid for 6 rounds, while caffeine and microbial DNA biomarker samples were valid for all 6.5 rounds (6 rounds on the Second River, 7 rounds on the Third River).
** Meter malfunction occurred at one Third River site during 1 sampling event

2.4 Sampling Schedule

Collection of water quality samples was originally planned to take place between May and August of 2020, in an effort to capture potential seasonal changes in water quality. However, due to delays related to the COVID-19 pandemic, the sampling timeline had to be protracted to the summer of 2020. Sampling was timed to capture two (2) "wet weather" events, considered as "wet weather" when a minimum of 0.20 inches of rain fell within 24 hours prior to sample collection. The PVSC Project and Field Coordinator and USGS Field Sampling Liaison used USGS real-time gages, National Weather Service QPF and a NOAA flood

forecast gage on the Saddle River to select days to sample. The sampling collection matrix for each event is shown in **Table 2-3**.

Table 2-3: Sample Collection Matrix

Event	Parameter	Second River	Third River	Total Samples/Event
Event 1 (7/15/2020) [Dry Weather Event]	*E. coli*	0	4	*E. coli* – 6 Fecal coliform – 6 Caffeine – 5 Microbial DNA biomarkers – 5
	E. coli duplicate	0	1	
	E. coli blank	0	1	
	Fecal coliform	0	4	
	Fecal coliform duplicate	0	1	
	Fecal coliform blank	0	1	
	Caffeine	0	4	
	Caffeine duplicate	0	1	
	Caffeine blank	0	0	
	DNA biomarkers	0	4	
	DNA biomarker duplicate	0	1	
	DNA biomarker blank	0	0	
Event 2 (7/22/2020) [Dry Weather Event]	*E. coli*	4	4	*E. coli* – 12 Fecal coliform – 12 (*2 of 12 no result, flagged for interference) Caffeine – 11 Microbial DNA biomarkers – 11
	E. coli duplicate	1	1	
	E. coli blank	1	1	
	Fecal coliform	4	4*	
	Fecal coliform duplicate	1	1	
	Fecal coliform blank	1	1	
	Caffeine	4	4	
	Caffeine duplicate	0	1	
	Caffeine blank	1	1	
	DNA biomarkers	4	4	
	DNA biomarker duplicate	0	1	
	DNA biomarker blank	1	1	
Event 3 (7/23/2020) [Wet Weather Event]	*E. coli*	4	4	*E. coli* – 12 Fecal coliform – 12 (*4 of 12 no result, flagged for QC) Caffeine – 12 Microbial DNA biomarkers – 10
	E. coli duplicate	1	1	
	E. coli blank	1	1	
	Fecal coliform	4	4*	
	Fecal coliform duplicate	1	1	
	Fecal coliform blank	1	1	
	Caffeine	4	4	
	Caffeine duplicate	1	1	
	Caffeine blank	1	1	
	DNA biomarkers	4	4	
	DNA biomarker duplicate	1	1	
	DNA biomarker blank	0	0	
Event 4 (7/29/2020) [Dry Weather Event]	*E. coli*	4*	4*	*E. coli* – 12 (*12 of 12 no result, flagged for QC) Fecal coliform – 12 Caffeine – 12 Microbial DNA biomarkers – 10
	E. coli duplicate	1*	1*	
	E. coli blank	1*	1*	
	Fecal coliform	4	4	
	Fecal coliform duplicate	1	1	
	Fecal coliform blank	1	1	
	Caffeine	4	4	
	Caffeine duplicate	1	1	
	Caffeine blank	1	1	

Event	Parameter	Second River	Third River	Total Samples/Event
	DNA biomarkers	4	4	
	DNA biomarker duplicate	0	0	
	DNA biomarker blank	1	1	
Event 5 (8/5/2020) [Wet Weather Event]	*E. coli*	4	4	*E. coli* – 12 Fecal coliform – 12 Caffeine – 12 Microbial DNA biomarkers – 10
	E. coli duplicate	1	1	
	E. coli blank	1	1	
	Fecal coliform	4	4	
	Fecal coliform duplicate	1	1	
	Fecal coliform blank	1	1	
	Caffeine	4	4	
	Caffeine duplicate	1	1	
	Caffeine blank	1	1	
	DNA biomarkers	4	4	
	DNA biomarker duplicate	0	1	
	DNA biomarker blank	1	0	
Event 6 (8/12/2020) [Dry Weather Event]	*E. coli*	4	4	*E. coli* – 12 Fecal coliform – 12 Caffeine – 12 Microbial DNA biomarkers – 10
	E. coli duplicate	1	1	
	E. coli blank	1	1	
	Fecal coliform	4	4	
	Fecal coliform duplicate	1	1	
	Fecal coliform blank	1	1	
	Caffeine	4	4	
	Caffeine duplicate	1	1	
	Caffeine blank	1	1	
	DNA biomarkers	4	4	
	DNA biomarker duplicate	1	1	
	DNA biomarker blank	0	0	
Event 7 (9/16/2020) [Dry Weather Event]	*E. coli*	4	4	*E. coli* – 12 Fecal coliform – 12 Caffeine – 12 Microbial DNA biomarkers – 10
	E. coli duplicate	1	1	
	E. coli blank	1	1	
	Fecal coliform	4	4	
	Fecal coliform duplicate	1	1	
	Fecal coliform blank	1	1	
	Caffeine	4	4	
	Caffeine duplicate	1	1	
	Caffeine blank	1	1	
	DNA biomarkers	4	4	
	DNA biomarker duplicate	1	1	
	DNA biomarker blank	0	0	

2.5 Receiving Water Flows

The USGS National Water Information System (NWIS)provided current flow data and gage height information at the USGS stations listed in **Table 2-4**. Graphs of the river discharge for the Second River and Third River gage over the duration of the sampling program are shown in **Figure 2-2** and **Figure 2-3,** respectively.

Table 2-4: Gage Information

Water Body	Gage Identification	Gage Description	Latitude	Longitude	NWIS Weblink
Second River	USGS 01392500	Second River at Belleville, NJ	40°47'17"	-74°10'18"	https://nwis.waterdata.usgs.gov/nwis/inventory/?site_no=01392500
Third River	USGS 01392170	Third River at Bloomfield, NJ	40°48'00"	-74°11'16"	https://nwis.waterdata.usgs.gov/nwis/inventory/?site_no=01392170

Figure 2-2: Second River Flow, USGS Gage at Belleville, NJ

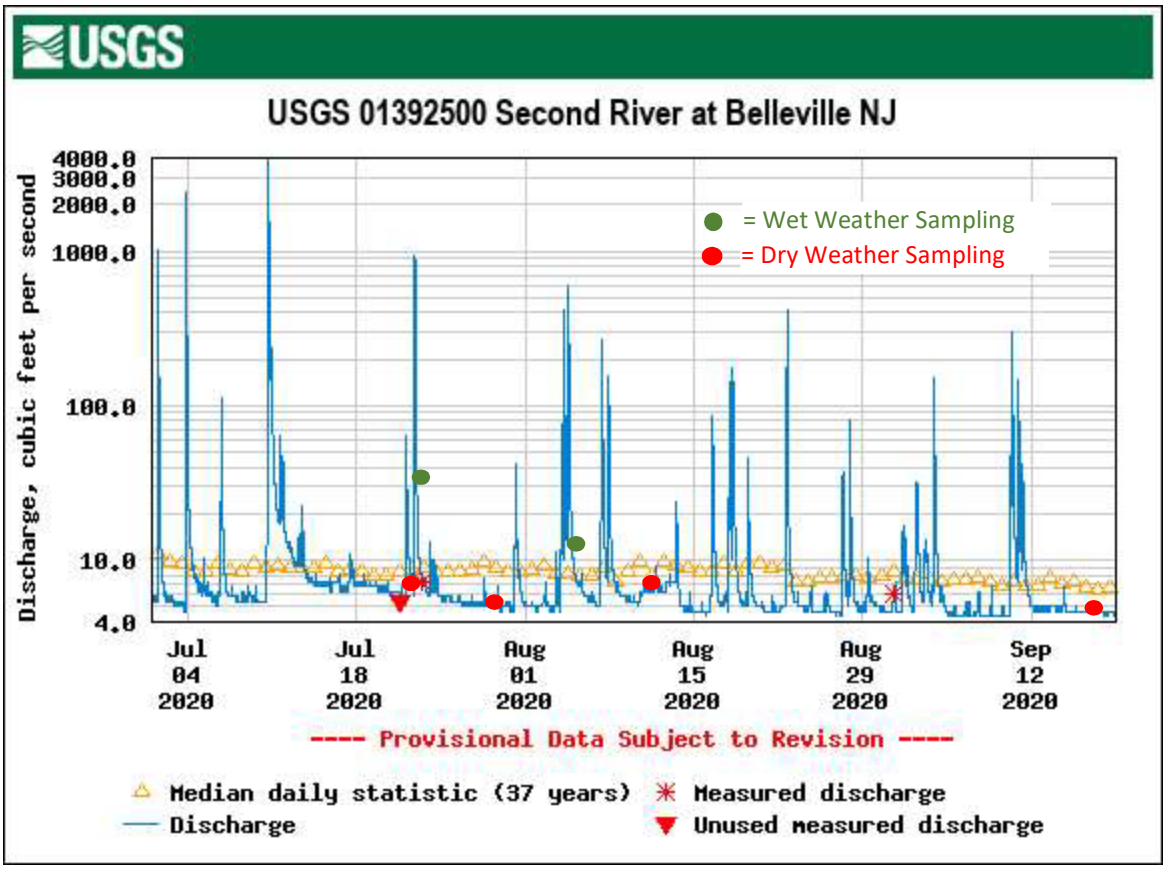
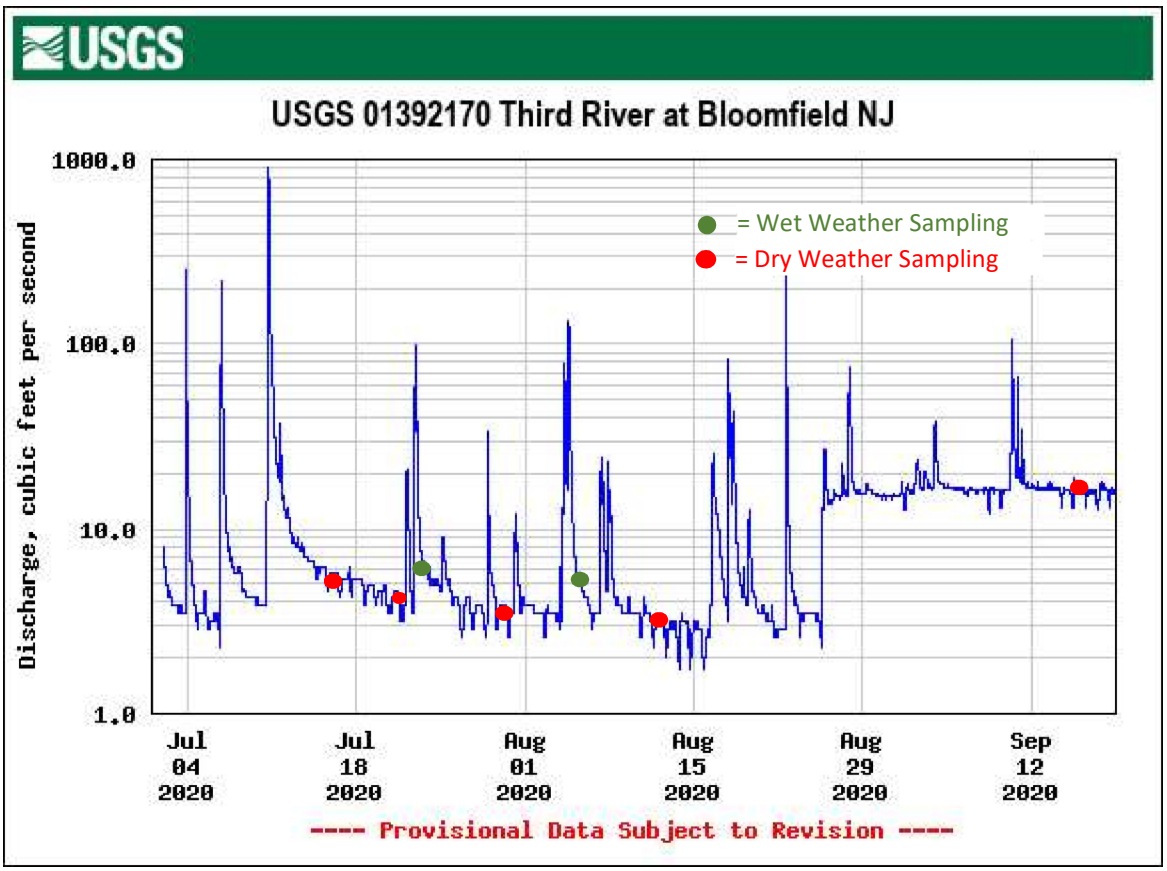


Figure 2-3: Third River Water Level, USGS Gage at Bloomfield, NJ



Section 3 MST Sampling Activities

3.1 Sampling Setup and Pre-trip Activities

The physical, microbiological, chemical, and genotypic data that was collected from the water bodies was obtained either through direct (*in situ*) measurements or through laboratory analysis of a grab water sample. The general collection procedures that were used for receiving water MST sampling were as follows.

Sampling in the Second River and Third River took place over six (6) and seven (7) weekly events, respectively. The sampling began on July 15, 2020 and ended on September 16, 2020. A large emphasis was placed on following uniform procedures during each event to introduce as little variance as possible between sampling sites, and between sampling events. Performing proper equipment setup, testing the equipment to confirm if it is working and has no safety issues, and confirming that the equipment is calibrated before sampling was essential in maintaining uniformity in sampling procedures.

3.1.1 Sampling Equipment

Prior to each sampling deployment, the field crew was provided all equipment necessary to safely and efficiently collect aliquots of water and in situ measurements. **Table 3-1** includes all of the equipment used in the MST Program. Note that sample bottles and deionized (DI) water were provided by the individual laboratories.

Table 3-1: MST Sampling Equipment

Sampling Activity	Required Equipment	
In-Situ Measurements	Tape measure	YSI Multiparameter
	Hach 2100Q Turbidimeter	Notebook/electronic recording device
	Field data sheets	Calibration standards
	Phone / camera	
Sampling	Weighted bottle/adjustable pole swing sampler	125 mL sterile specimen cups
	500 mL sample bottles	1,000 mL sample bottles
	DI water	Blank water
	Plastic bags	Labels
Transport	Coolers with ice	Chain of custody (where applicable)
	Shipping labels	Packing material
General / Safety	PPE (mask, safety glasses, etc.)	Safety vest
	Nitrile gloves	First Aid kit
	Sampling location map	PVSC sampling letter
	MST Program QAPP	Emergency Contact List
	Health and Safety Plan	Pens, pencils, and markers

3.1.2 Equipment Calibration, Pre-trip Activities and HASP

All field meters used for in-field analysis were operated, maintained and calibrated in accordance with the, "Regulations Governing the Certification of Laboratories and Environmental Measurements", N.J.A.C. 7:18. A QC of the meter readings was performed prior to each survey day by direct comparison to a second calibrated meter or by wet analysis. PVSC calibrated both the YSI Pro Plus and HACH 2100Q prior to each sampling event using certified know standards. PVSC recorded calibration results in electronic field forms. USGS performs quarterly checks on the meters to make sure they are fully functional and reading to USGS standards (check against other relevant meters). Meters were calibrated daily in the lab before field sampling. The calibration results were recorded in the paper long books and digitized by scanning.

Grab sample packaging and shipping procedures were designed to ensure that the samples would arrive at the laboratory intact and correctly labeled. All samples collected were labeled in a clear and precise way for proper identification in the field and for tracking in the laboratory. Each laboratory provided unique sample bottles, which were pre-labeled with all information except for the date and time of sample collection. The person that collected the sample then completed the label with date and time using an indelible waterproof marking pen. The Sample identification code (ID) which consisted of the site designation number and the sampling date are shown as follows:

__ __ __ - __ __ __ __ __ __ __ __

Where,
Characters 1-3: Sample Site ID (i.e. SR1)
Character 4-11: Sampling round date in yyyymmdd format (i.e. 20200715)
Field Blank: Samples labeled FB in place of Sample Site ID
Duplicates: Samples labeled DUP in place of Sample Site ID

Neither equipment failure nor unsafe weather conditions were encountered during the sampling program. However, these conditions were prepared and planned for in the event that they occurred, and field personnel were trained in response procedures contained in the USGS Health and Safety Plan (HASP). With the recent development of the COVID-19 pandemic, "go/no go" decisions were made for each event with the consideration of all available information from governmental and regulatory directives. Additionally, proper PPE was utilized and social distancing was observed by all field and laboratory personnel.

3.2 In-situ Measurements

In-situ measurements, summarized in **Table 2-2**, were taken at each sampling location to give a secondary indication of the water quality at each sampling location. All in-situ measurements, along with grab samples, were obtained by field personnel lowering equipment from bridges spanning the water bodies, or from the banks of the water bodies. The sampling crew prioritized safety at these sites, particularly from adjacent vehicular and pedestrian traffic. Fluorescent safety vests were worn at all times. One crew member was designated to observe traffic, pedestrians, and keep equipment from obstructing egress on the walkways.

Depth was the first measurement obtained at each site, measured using a 100-foot-long tape measure. The depth at each water body was measured midstream, equidistant from either bank. After the measurement

was recorded, the midstream depth of the water body was calculated as one half the measured depth. All other in-situ measurements and samples were obtained from mid-stream and the calculated mid-depth.

A YSI Pro Plus 2030 (or YSI 6920) was used to record temperature, dissolved oxygen, pH and specific conductance (salinity). The sampling crew lowered the meter probe to the proper depth, waited for readings to stabilize on the meter, and recorded results in the field data sheets. For turbidity, an aliquot was obtained from the weighted or pole sampler for analysis. After each round of in-situ measurements, the equipment was decontaminated using DI water.

3.3 Grab Sampling

For pathogen analyses in the laboratory, sample was collected from mid-depth for each indicator bacteria using a new 1 L sterile-HDPE container. Collected sample was then transferred to 125 mL sterile cups. For caffeine analysis, a 1 L sample was collected using an amber glass container. For the bacterial DNA biomarkers, a 500mL sample was collected at each sampling location using leak proof sterile bottles. Sample bottles were prepared prior to initiating sampling at each site. All sample bottles were marked with the site ID, parameter and date of collection. The pre-sterilized sample bottles were provided by the respective laboratories.

Each grab sample was collected from the middle of the river channel at mid-depth, to be representative of the most stable conditions of the water body. Either a weighted bottle sampler or adjustable pole swing sampler was utilized, depending on the particular site constraints. To avoid cross-sample contamination, the sampling apparatus was rinsed with DI water before moving on to the next site.

The desired volume of water required for E.Coli and Fecal Coliform analysis was transferred to the pre-sterilized containers for transport to the laboratory. All sampling equipment used in the field was cleaned after each sampling event using laboratory grade glassware detergent, tap water and a DI water rinse.

3.4 Sample Preservation and Transfer

All samples for bacteriological and caffeine laboratory analysis were preserved per laboratory methods and transferred to the respective laboratory for analysis under standard chain-of-custody (COC) protocol. DNA biomarker samples did not require COC forms, but did require Analytical Service Request (ASR) forms for each sample. Laboratory analysis was performed within the documented hold times for each parameter.

Collected grab samples were immediately stored on wet ice in a designated cooler. The temperature of the first sample taken by the field crew was measured upon delivery of samples to the each laboratory and recorded on the chain of custody or ASR forms. For caffeine and DNA biomarker samples, the bottles were stored in coolers with ice packs and sent to each laboratory by overnight courier to maintain viability of the sample.

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Section 4 Laboratory Results

The primary goal of the MST Program, in acquiring the volume and range of pollutant data needed to obtain a better understanding of the sources of contamination in the Second River and Third River, was largely met. A summary of the range of observed pollutant concentrations collected during the dry and wet weather sampling events is provided in **Table 4-1**.

The MST indicators analyzed in the samples collected in the MST Program include *Escherichia coliform* (*E. coli*) and Fecal Coliform as microbiological indicators; caffeine as a chemical indicator; and host specific DNA biomarkers using the Quantitative Polymerase Chain Reaction (qPCR) testing methodology as genotypic indicators. The coliform group has been used to assess sanitary quality in recreational waterways for decades (Standard Methods, 1980). *E. coli* is one member of the fecal coliform group that provides more specificity than fecal coliform as a potential indicator of pathogenic organisms (USEPA, 1986), particularly in freshwater systems (USEPA, 2012). Caffeine is an effective indicator of human fecal contamination because of its exclusivity in the human diet. It is excreted by humans after consumption, and found in highest concentrations near metropolitan areas and areas where untreated wastewater may be discharging into receiving waters, such as CSOs (Buerge et al, 2006). Finally, Quantitative Polymerase Chain Reaction techniques are used to measure the amount of DNA of an organism present in a water sample by amplifying certain host-specific genetic markers. Using this method, bacteria do not need to be grown or cultured in a lab, however, a library of genotypic markers for bacterial strains specific to host organisms was created by collecting and analyzing known source fecal samples.

For this study, each sample was analyzed to quantify human, bird, canine, and ruminant biomarkers to detect their respective presence using the following four (4) assays:

- Human-associated Bacteroides HF183/BacR287 assay
- Bird-associated GFD assay
- Canine-associated Bacteroides BacCan assay
- Ruminant-associated Bacteroides Rum-2-Bac assay

Fecal indicator bacteria (FIB) such as those that comprise the phylum *Bacteroidetes* are considered as an advantageous alternative to *E. coli* and *Fecal Coliform* because they are strict anaerobes, indicating recent fecal contamination when found in water bodies, and they are more abundant in the feces of warm-blooded animals than the traditional FIB (Scott et al, 2002). Certain genetic sequences found within the different species and strains of these bacteria are specific to the desired host (i.e. humans), allowing for identification of fecal contamination from that source. The GFD assay has found to be 100% avian specific, occurring in gulls, geese, ducks, and chicken (Hyatt et al, 2011).

This section describes the laboratory results for the individual MST indicators at each sampling site. Plots of contaminant concentrations for all locations are presented in the following sections. Additional analysis of the data, including further statistical analyses, is presented in Section 6. The quality of the data with respect to the MST Program QAPP objectives are described in additional detail in Section 5

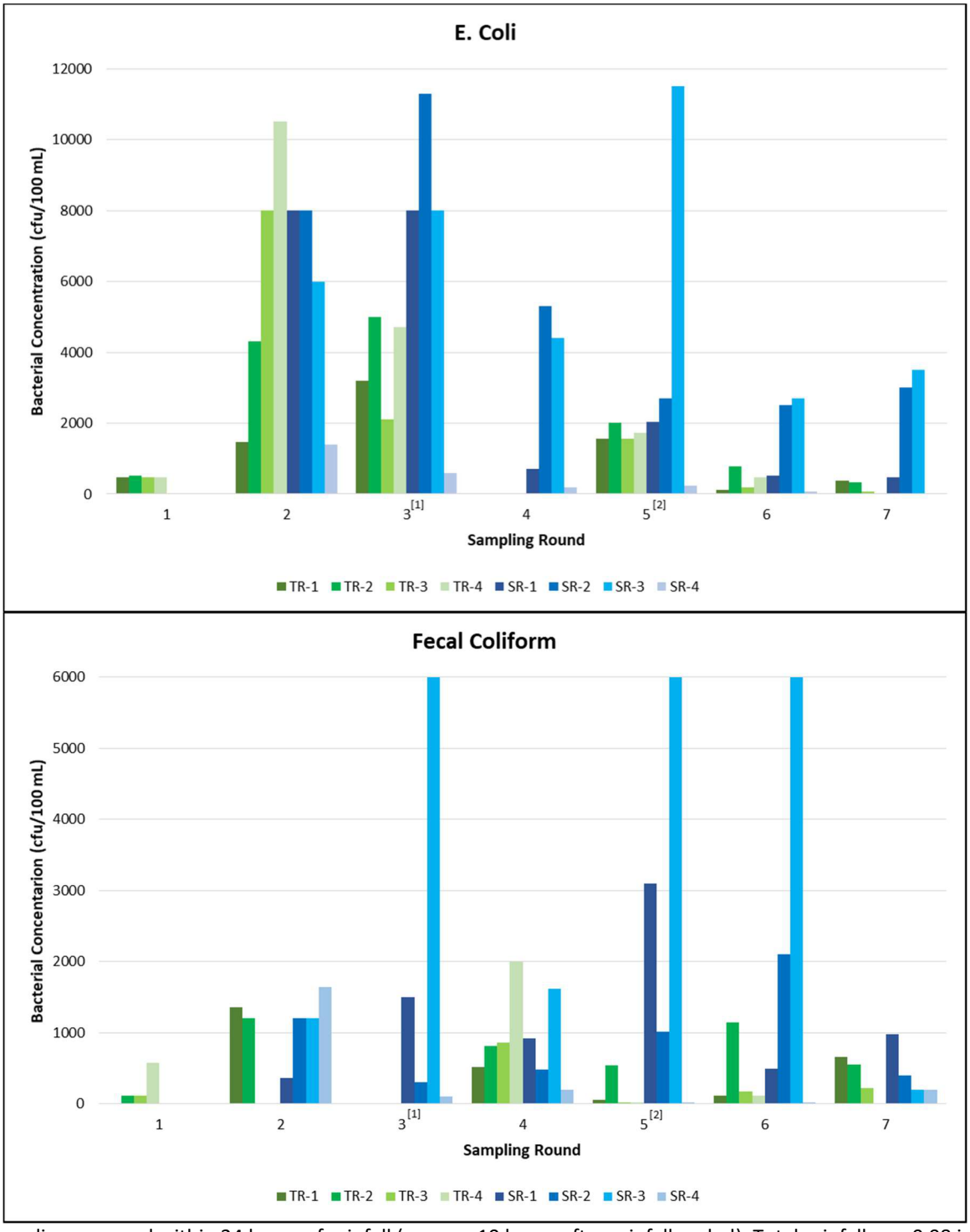
Table 4-1: Range of Observed Contaminant Concentrations

Parameter	Event Type	Location	Minimum	Maximum	Average	Count
E. coli (cfu/100 mL)	DRY	Second River	20	8,000	2,924	16
		Third River	20	10,500	1,787	16
	WET	Second River	240	11,500	5,548	8
		Third River	1,560	5,000	2,733	8
Fecal Coliform (cfu/100 mL)	DRY	Second River	20	6,000	1,126	18
		Third River	10	2,000	589	16
	WET	Second River	20	6,000	2,255	8
		Third River	20	540	160	4
Caffeine (ng/mL)	DRY	Second River	0.006	1.300	0.324	20
		Third River	0.007	1.000	0.148	16
	WET	Second River	0.007	1.300	0.230	8
		Third River	0.045	0.180	0.100	8
Human (# copies/100 mL)	DRY	Second River	280	1,100,000	264,481	20
		Third River	280	18,000	3,955	16
	WET	Second River	440	10,000,000	2,171,364	8
		Third River	790	51,000	13,686	8
Bird (# copies/100 mL)	DRY	Second River	540	2,300	829	20
		Third River	540	4,100	1,313	16
	WET	Second River	540	7,200	1,658	8
		Third River	720	2,600	1,628	8
Canine (# copies/100 mL)	DRY	Second River	6,500	330,000	69,769	20
		Third River	780	45,000	12,064	16
	WET	Second River	6,700	290,000	98,775	8
		Third River	7,500	43,000	18,250	8
Ruminant (# copies/100 mL)	DRY	Second River	1,900	3,800	2,400	20
		Third River	700	7,500	2,810	16
	WET	Second River	1,900	3,800	2,900	8
		Third River	1,400	6,300	3,875	8

4.1 Fecal Indicator Bacteria Sampling Results

Fecal indicator bacteria samples, *E. coli* and fecal coliform, were analyzed at PVSC's in-house laboratory in Newark, NJ. Plots of the FIB at each site across all events are presented in **Figure 4-1**. Overall, the concentration of FIB across all sites was high. For *E. coli*, the combined geometric mean in the Second and Third Rivers was 1,690 CFU/100 mL, 895 CFU/100 mL, respectively. For reference, the Water Quality Criteria for freshwater surface waters in New Jersey per N.J.A.C. 7:9B-1.14(d), in which all three water bodies are classified as FW2 in the area that they were sampled, specify a maximum geometric mean of 126 CFU/100 mL and a single sample maximum of 235 CFU/100 mL for *E. coli*. Higher concentrations were observed during the two wet weather events particularly in the Second River.

Figure 4-1: FIB Concentration



[1] Sampling occurred within 24 hours of rainfall (approx. 10 hours after rainfall ended). Total rainfall was 0.98 in.
[2] Sampling occurred within 24 hours of rainfall (approx. 20 hours after rainfall ended). Total rainfall was 0.52 in.

4.1.1 Second River FIB

Samples obtained during dry weather were fairly similar in concentration for each of the FIB and all of the concentrations were above values used as maximum water quality standard criteria. Factoring the results from wet weather sampling, maximum water quality standard criteria were exceeded by an even greater amount. Generally, *E. coli* concentrations were higher than fecal coliform in the Second River. It is notable that the most upstream sampling location, SR-4, contained the least amount of fecal contamination of any site. Sampling locations downstream of SR-4 had much higher levels of contamination. During wet weather sampling events especially, sampling location SR-3 (directly downstream of SR-4) had very high levels of each FIB, which further impacted the downstream sampling locations. Because the Second River appears greatly impaired, this additional bacterial loading will also impact the Lower Passaic River and its estuary, downstream of its confluence with the Passaic River.

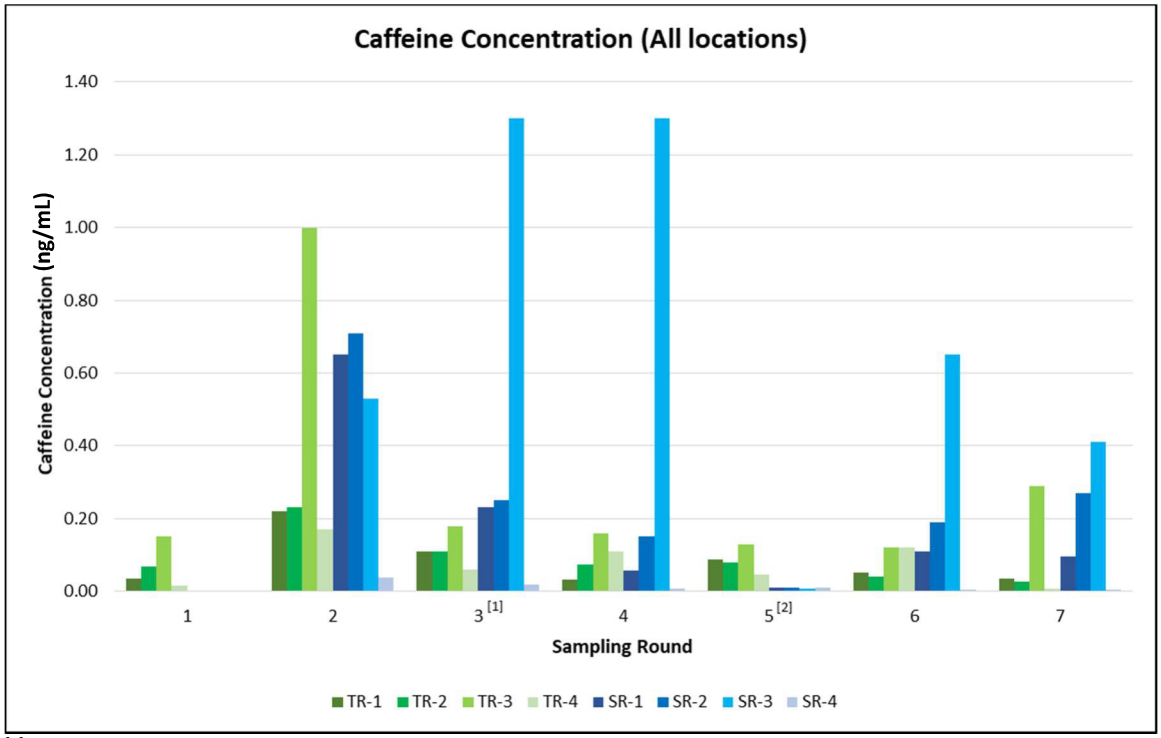
4.1.2 Third River FIB

Similar to the Second River sampling locations, samples obtained during dry weather at the Third River sampling sites exhibited uniformity for each of the FIB. The exception to this was during the second sampling event, for *E. coli*, where bacterial concentrations were unusually high. As with the Second River, pollutant concentrations were above values used as maximum water quality standard criteria, though to a lesser degree. Also of note, Third River sensitivity to impacts from precipitation appear much lower than what is observed in the Second River. Spatial differences in the water body are minimal, as all four Third River sites contribute a relatively equal amount of contamination. Despite overall lower fecal contamination in the Third River, it appears to contribute to additional bacterial loading into the Lower Passaic River and estuary, downstream of its confluence with the Passaic River.

4.2 Caffeine Sampling Results

Caffeine samples were analyzed at the ALS Environmental laboratory in Kelso, WA. Previous caffeine detection sampling was performed in the Second and Third Rivers in 2017, so reference caffeine concentrations do exist for the water bodies. However, there are no WQS for caffeine in NJ. During the 2017 PVSC Source Sampling study, caffeine concentration ranged between 0.05 ng/mL to 0.95 ng/mL in the two waterbodies, with concentration averaging highest in the Second River (Greeley and Hansen, 2018). Considering the historical data, the concentration of caffeine across all sites was consistent with some level of human fecal contamination, particularly in the Second River. The geometric mean of caffeine in the Second River and Third River was 0.088 ng/mL, 0.083 ng/mL, respectively. A plot of caffeine concentration at each site across all events can be seen in **Figure 4-2**. Higher caffeine concentrations overall were observed in the Second River, suggesting that human fecal contamination was elevated in the waterbody. Higher caffeine concentrations were observed during the two wet weather events, with the largest spike in concentration occurring during the more intense and immediate sixth sampling event.

Figure 4-2: Caffeine Indicator Concentration



[1] Sampling occurred within 24 hours of rainfall (approx. 10 hours after rainfall ended). Total rainfall was 0.98 in.
[2] Sampling occurred within 24 hours of rainfall (approx. 20 hours after rainfall ended). Total rainfall was 0.52 in.

4.2.1 Second River Caffeine

Caffeine samples obtained during dry weather at the Second River sampling locations ranged from 0.006 ng/mL to 1.300 ng/mL, suggesting that the higher levels of FIB in the Second River are from human sources. Like the FIB results, caffeine levels were at peak levels at sampling location SR-3, further indicating a potential issue with human fecal contamination there. Examining the results from wet weather sampling, a significant spike in concentration was seen during the third event, but not on the fifth event. It is unclear why the fifth event did not produce a similar spike at SR-3, considering both FIB were at maximum levels during the fifth event at that location. Caffeine concentration was lowest at sampling location SR-4, again pointing to a potential issue at SR-3, directly downstream.

4.2.2 Third River Caffeine

Dry weather caffeine sampling results at the Third River ranged from 0.007 ng/mL to 1.000 ng/mL, somewhat lower than in the Second River. Additionally, neither wet weather event produced a noticeable spike in caffeine concentration at the Third River. This indicates a low sensitivity from impacts due to precipitation for human fecal contamination in the Third River. It also indicates a higher presence of fecal contamination from non-human sources in the Third River. However, the correlation between caffeine, human fecal contamination, and FIB can only be inferred through further statistical analysis, which is discussed in Section 6. Caffeine concentration was lowest at sampling location TR-4, while at a relative maximum at TR-3.

4.3 qPCR Sampling Results

Microbial DNA biomarkers were analyzed using qPCR methodology at the USGS Water Science Center laboratory in Columbus, OH. Each sample was analyzed to quantify human, bird, canine, and ruminant biomarkers. Results are reported in #copies/100mL, which refers to the copies of DNA found within the sample volume for a particular biomarker, amplified by the qPCR methodology for detection. The relative abundance of detected sources is thus reported as a DNA concentration, but it should be emphasized that the qPCR methodology is limited in definitively identifying the relative quantification of bacteria among multiple sources. This limitation is discussed in further detail in Section 6 . The results using qPCR are useful in confirming presence/absence of a particular source and to gain a qualitative comprehension of the relative abundance of contaminant sources.

Microbial DNA biomarkers for each of the targeted source-identifiers were detected across all sampling locations and events, meaning there was *some* level of fecal contamination from every source, though the exact amount diverged greatly between sites. The results from the human-associated assay were mostly found in abundance in the Second River sampling sites, and to a much lower degree at Third River sites. The concentration of human-associated biomarkers was two to three orders of magnitude larger in the Second River than in the Third. This is consistent with the findings from the caffeine analysis in these water bodies, and points to high levels of human-associated fecal contamination in the Second River and low levels of human-associated fecal contamination in the Third River. It is also consistent with the findings from the 2017 PVSC Source Sampling Program Report (Greeley and Hansen, 2018), in which the degree of human-associated bacteria in each waterbody is well documented.

As for non-human source DNA, varying levels of wild and domesticated animal fecal contamination were observed at each site. In general, wild animal-associated (i.e. from bird and ruminant) biomarkers were found in slightly higher concentrations in the Third River than in the Second River, while canine-associated biomarkers were present in higher concentrations in the Second River locations than in those of the Third River. Plots of each of the four microbial DNA biomarker results are presented in **Figure 4-3** through **Figure 4-6**. For the qPCR results, the Y-axis is plotted on a logarithmic scale for visibility purposes.

Figure 4-3: Human: HF183/BacR287 Assay

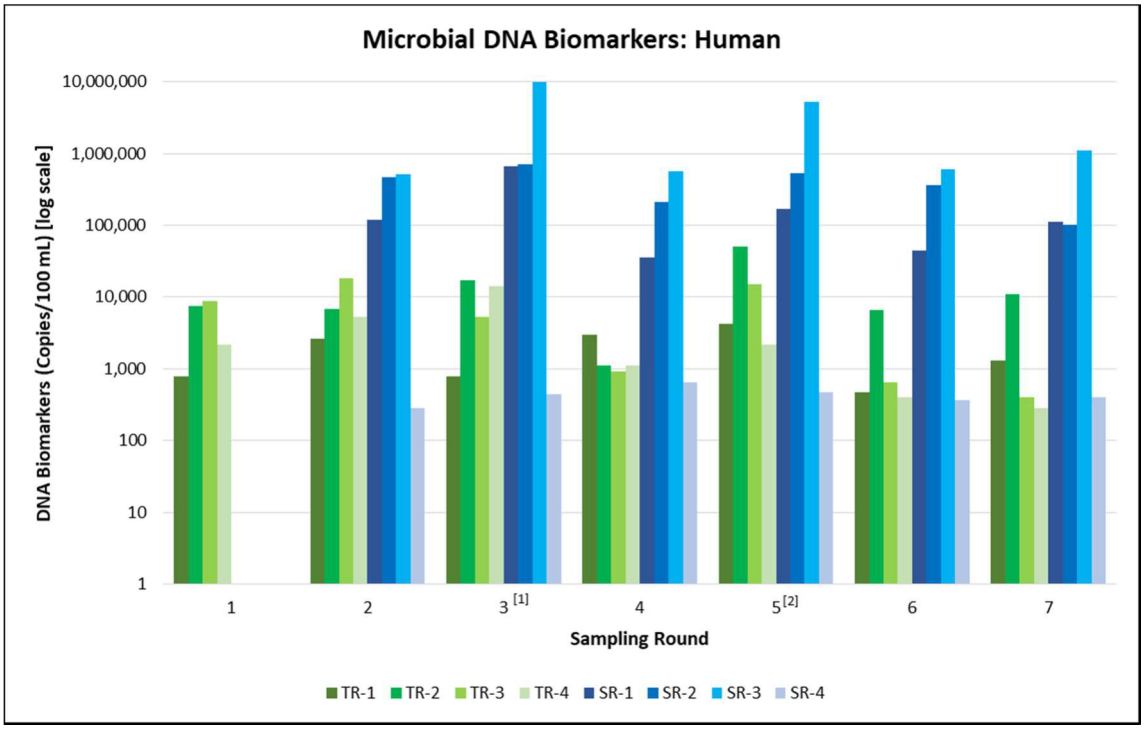


Figure 4-4: Waterfowl: GFD Assay

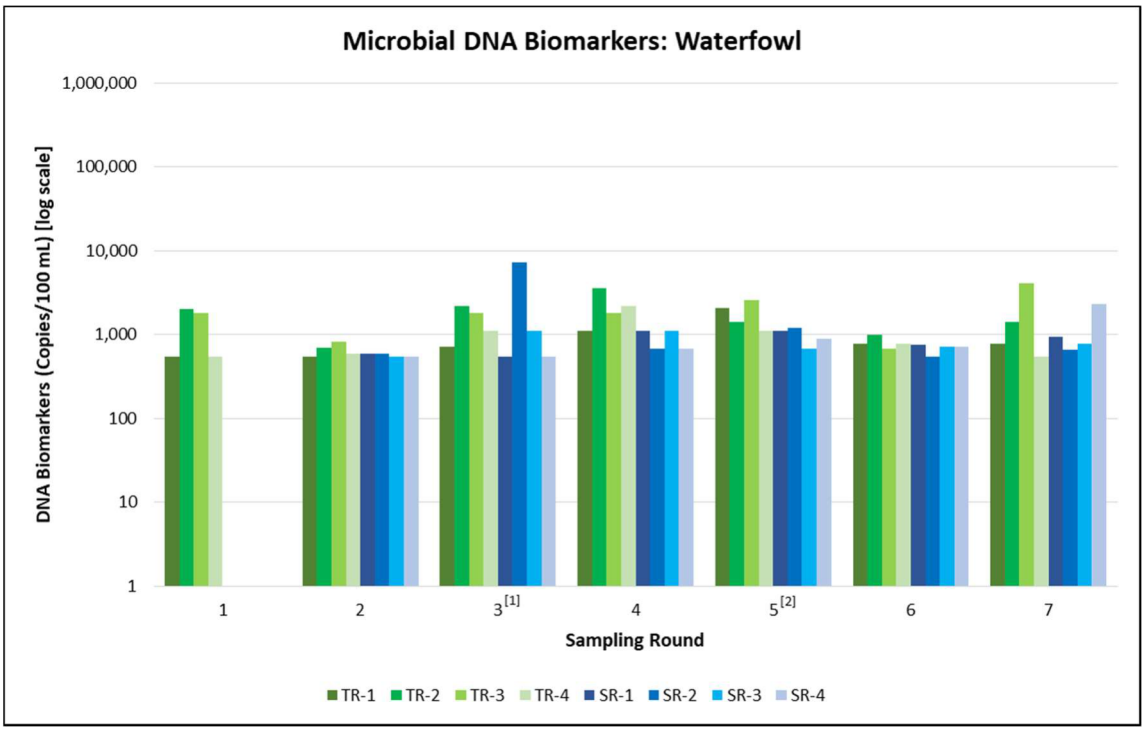


Figure 4-5: Canine BacCan Assay

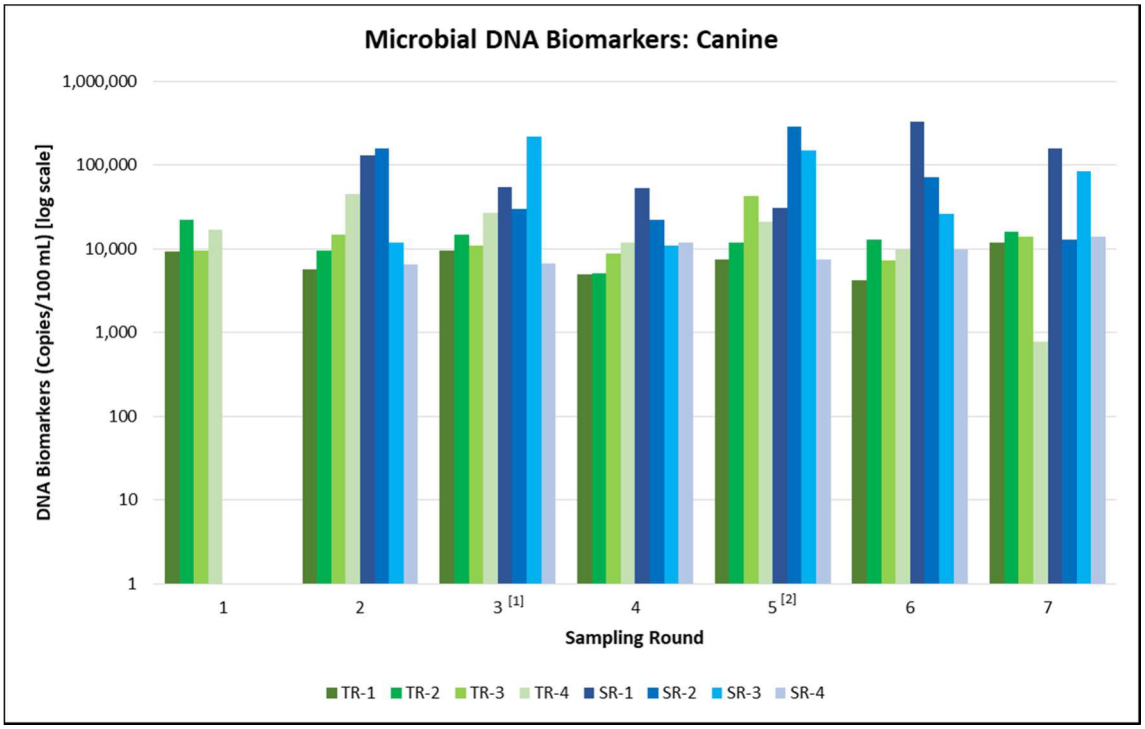
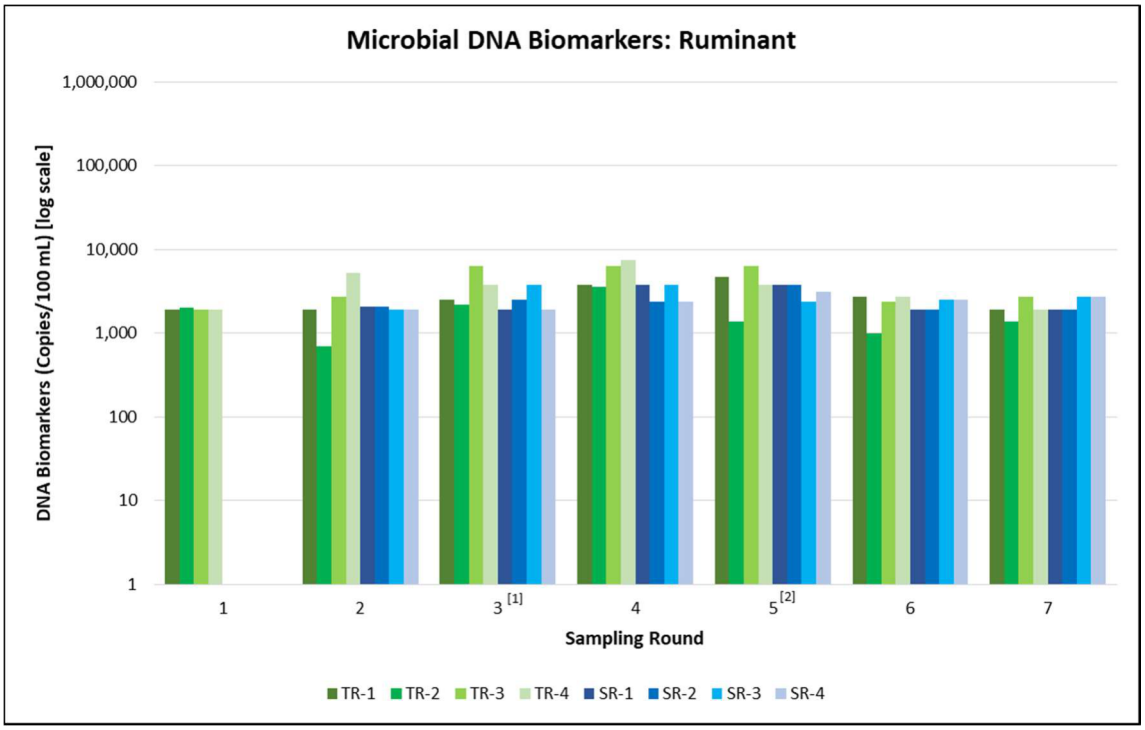


Figure 4-6: Ruminant Rum-2-Bac Assay



4.3.1 Second River DNA Biomarkers

Human-associated DNA biomarkers were found in high number in the Second River. Concentrations for the human-associated biomarkers ranged from 280 copies/100 mL to 1,100,000 copies/mL in the waterbody during dry weather. Following precipitation, a spike in concentration was observed for both wet weather events, where human-assay biomarker concentrations rose to 10,000,000 copies/100 mL in the third sampling event. Once again, sampling location SR-3 exhibited the clearest indication of human-associated fecal contamination, while the lowest levels were observed at upstream location SR-4. Sampling locations SR-2 and SR-1, downstream of SR-3, also contained high quantities of human-associated biomarkers.

Non-human associated DNA biomarkers observed in the Second River were limited to mostly canine specific *Bacteroidetes*. The canine assay yielded the highest concentrations among all sites, ranging from 6,500 copies/100 mL to 330,000 copies/100 mL. Interestingly, the downstream sites SR-1 and SR-2, both adjacent to park and pedestrian areas, presented with the highest canine-associated biomarker concentrations. Concentrations for the bird-associated assay were present at generally low concentrations, from 540 copies/100 mL to 7,200 copies/100 mL. Ruminant-associated *Bacteroidetes* concentrations ranged from 1,900 copies/100 mL to 3,800 copies/100 mL. Overall, the impact from wet weather in the Second River relating to non-human associated biomarkers was much less significant than as with human-associated biomarkers.

4.3.2 Third River DNA Biomarkers

Human-associated DNA biomarkers were found in much lower quantities in the Third River, in comparison with the Second River. The human assay concentrations ranged from 280 copies/100mL to 18,000 copies/100 mL during dry weather. A minor spike in concentration during wet weather sampling was observed during event five, where human-associated biomarkers increased to 51,000 copies/100 mL at sampling location TR-2. All sampling locations were relatively uniform in exhibiting low concentrations of human-associated biomarkers.

While non-human associated DNA biomarkers were observed in the Third River consistently, the quantities were only slightly elevated when compared with the Second River sampling locations. Bird assay concentrations ranged from 540 to 4,100 copies/100 mL. Canine-associated *Bacteroidetes* present in moderate numbers: from 780 copies/100 mL to 45,000 copies/100 mL. Ruminant-associated *Bacteroidetes* were also present, ranging from 700 copies/100 mL to 7,500 copies/100 mL.

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Section 5 QAPP Adherence and Control

The MST Program execution was consistent with the QAPP objectives. This section presents a summary of the quality of the data relative to the QAPP objectives. Out of a total of 265 analyses, twelve (12) were discarded due to QAPP nonconformance, seven (7) discarded due to contaminant interference, and an additional nine (9) required data qualifications are as a result of failed QC (89.4%). Several additional analyses were flagged for minor QC infringements. However, these results have been qualified as minimal impact on the use of these data is anticipated due to the similarity in concentrations between results measured within and outside the analytical hold time, and the small degree to which the sample temperature deviated from the specified preservation temperature. Further uncertainty exists within the analytical methodology and field practices (e.g. obtaining a homogenous, representative sample, etc.).

5.1 QAPP Requirements

Section A.8 of the QAPP provided data quality objectives for the following areas:

- Accuracy
- Precision
- Sensitivity
- Completeness
- Representativeness
- Comparability

Accuracy, precision and sensitivity requirements for field and laboratory activities are summarized in **Table 5-1**. The completeness objective for the field measurements, sample collection and laboratory extraction was 90%. Representativeness and comparability objectives were qualitative and are presented in the context of the MST Program comparison in Section 5.2.

Table 5-1: Data Quality Objectives

Parameter	Data Accuracy Objectives (% Recovery)		Data Precision Objectives				Sensitivity	
			Field Precision (RPD) [1]		Analytical Precision (RPD)			
	Estimated By	Objective	Estimated By	Objective	Estimated By	Objective	Reference Method	RL [2]
Escherichia coliform (E. coli)	Laboratory Fortified Blanks / Matrix Spikes	80% - 120% Recovery	Field Duplicates	RPD < 40%	Lab Replicates	RPD < 40%	EPA 1603	1,2,4,10 / 100 ml
Fecal Coliform	Laboratory Fortified Blanks / Matrix Spikes	80% - 120% Recovery	Field Duplicates	RPD < 40%	Lab Replicates	RPD < 40%	EPA 600	1,2,4,10/ 100 ml
Caffeine	Laboratory Fortified Blanks / Matrix Spikes	63% - 145% Recovery	Field Duplicates	RPD < 40%	Lab Replicates	RPD < 30%	EPA 1694	2 ng/L
Microbial DNA [3]	Pos – Neg control / Extraction Blanks	Pos: detection Neg: No detection for 3 C_T units above sample values	Field Duplicates	RPD < 40%	qPCR Duplicates	± 1 Standard Deviation unless C_T value ≥ 33	qPCR detection of host associated DNA and quantification	Detection Limit NA

[1] RPD – Relative Percent Difference. RPD values are non-representative when (a) both the original and duplicate results are less than 5x the reporting limit or not detected at the reporting limit or (b) either result is estimated, rejected, or suspected of contamination.

[2] RL - Reporting Limit. Results for qPCR are reported as Not Detected (ND), Detected below level of quantification (DNQ). The limit of Quantification varies for each sample and is dependent on C_T values.

[3] There is currently no certification for microbial source tracking methods. Microbial source tracking is a rapidly evolving technology and USEPA is in the process of finalizing their approved method details. Source Molecular Corporation has obtained accreditation from the American Association for Laboratory Accreditation under ISO17025 standards.

5.2 Comparison of Results to QAPP

This section presents a high level summary of the comparison of the QAPP objectives to the results from the MST Program.

5.2.1 Accuracy

Accuracy, or the closeness of a result to the true value, was assessed by the laboratory through the analysis of matrix spikes, positive and negative controls and laboratory blanks. Matrix spike samples were generated by the respective laboratory to assess matrix interference effects on method accuracy, while method blank samples were generated by the laboratories and used to assess contamination resulting from laboratory procedures. Overall, the accuracy of the MST Program was good.

For the FIB *E. coli* and fecal coliform, eight (8) of the samples were associated with failed matrix spikes, failing to meet the 80 to 120 percent recovery accuracy objective (94.1% effective). For caffeine, 130 out of 131 (99.2%) matrix spike samples met the 63 to 145 percent recovery accuracy objective. For microbial DNA, at least one positive and three negative control samples were run per test, per sampling round (130 minimum). There were no false positives or false negatives observed in the controls. Extraction blanks, used to evaluate contamination during DNA extraction occurred once every week samples were extracted. No contamination was found from the extraction blanks.

5.2.2 Precision

Precision is a measure of agreement between two or more measurements. The precision test objective is shown in **Table 5-1**. Field duplicates and laboratory replicates were taken for a portion of the samples. As noted in the QAPP, the precision test (i.e.: comparison between two or more samples) is applied if the average result of the duplicate/replicate samples is greater than five times the analysis detection limit. If the average result of the duplicate/replicate samples is less than five times the analysis detection limit, the precision test was not utilized. Overall, the MST Program exhibited success at meeting the QAPP objectives for precision. The issues impacting field precision were largely due to factors outside the control of sampling personnel as well as by the inherent challenges in generating reproducible results analytically for these parameters.

A total of 43 field duplicates were collected during the MST Sampling Program. Each field duplicate was analyzed for a specific analyte (FIB, caffeine, and four types of microbial DNA biomarker) providing up to 67 measures of field precision. **Table 5-2** provides a summary of the field duplicate precision, calculated as the relative percent difference (% RPD).

Table 5-2: Field Precision % RPD Summary

Sampling Round	Location	E. coli % RPD	Fecal Coliform % RPD	Caffeine % RPD	Human % RPD	Bird %RPD	Canine %RPD	Ruminant %RPD
1	TR-3	19.23	**133.33**	6.90	**43.36**	**88.72**	18.18	<5 x DL
	SR: N/A							
2	TR-1	12.22	14.17	4.44				
	SR-1	0.00						
3	TR-4	4.17		8.85	13.33	20.00	<5 x DL	<5 x DL
	SR-3	0.00	**198.67**	32.26	0.00	25.64	<5 x DL	<5 x DL
4	TR-4		28.57	0.00				
	SR-3	4.65	17.45	26.67				
5	TR-4	1.72	**191.30**	4.55	**58.82**	21.05	<5 x DL	<5 x DL
	SR-3	11.48	**182.17**	4.38				
6	TR-2	5.00	7.27	7.23	4.44	0.00	1.01	<5 x DL
	SR-4	28.57	0.00	19.05	<5 x DL	24.00	<5 x DL	<5 x DL
7	TR-2	**55.32**	20.90	0.00	9.52	11.76	<5 x DL	<5 x DL
	SR-1	25.45	4.00	1.04	16.67	11.76	6.52	<5 x DL

Fourteen (14) of the 67 RPDs could not be calculated because of the low pollutant concentrations in the samples and the field duplicates. For *E. coli*, 11 of the 12 calculable field duplicates met the 40% precision objective. For fecal coliform, 7 of the 11 calculable field duplicates met the 40% precision objective. For caffeine, all 12 calculable field duplicates met the 40% precision objective. For microbial DNA, 15 of 18 calculable field duplicates met the 40% precision objective.

Several lab replicates were produced during the MST Sampling Program at each of the individual laboratories. For the purposes of this report, only caffeine, lab replicates results were examined for QC. In this case, all 11 lab replicates met the 40% precision objective.

All of the microbial DNA results were generated from two replicate reactions per test per sample, and a 1:10 dilution was also analyzed for each test, sample, and event. Reaction duplicates were within the precision objective of plus/minus one standard deviation for all reported results.

5.2.3 Sensitivity

All required detection limits were met in the MST Sampling Program. All standard curves generated for qPCR analysis fell within the following criteria:

- R^2 value ≥ 0.98
- Efficiency between 80% to 100%
- Slope between -3.0 to -4.0

5.2.4 Completeness

Completeness is a measure of the amount of valid data obtained from the monitoring program compared to the amount of data that were expected. Events that may contribute to reduction in measurement completeness include sample container breakage, inaccessibility to desired sampling locations, sampling apparatus failure, and laboratory equipment failures.

Field completeness is determined by the number of measurements collected versus the number of measurements planned for collection. As noted in the QAPP, the completeness criterion for all measurements and sample collection is 90 percent. Laboratory completeness is a measure of the amount of valid measurements obtained from all samples submitted for each sampling activity. The completeness criterion for each laboratory is 90 percent.

The overall field completeness for measurements and sample collection was close to 100%, as there were no serious issues in the field except a USGS meter failure at TR1 for one sampling event. Two of the laboratories obtained a completeness measure of 100%. The PVSC laboratory responsible for analyzing FIB obtained a completeness measure of 86%, due to sample disqualification for incubator temperature and interference.

5.2.5 Representativeness

Representativeness is the degree to which data accurately and precisely represents a characteristic of a population, parameter variations at a sampling point, a process condition, or an environmental condition. Representative data of dry weather and wet weather conditions are required to support the evaluation sampling results.

For sample collection, representativeness is followed by adhering to the QAPP (Greeley and Hansen, Revised June 2020) and applying proper collection techniques including the proper sample sizes and volumes, sampling times, and sampling locations. In the laboratory, representativeness is followed by using the appropriate sample preparation techniques, by following appropriate analytical procedures, and by meeting the recommended sample holding times. Additionally, equipment blanks, field blanks and laboratory method blanks are used to verify that no contamination is occurring from outside sources, and that the sample is representative of actual conditions at the sampling sites.

Representativeness was achieved in the MST Program by sampling five dry weather events under a range of flow conditions, and by sampling two wet weather events under a range of environmental conditions, with rainfall ranging from 0.52 inches to 0.98 inches. There were no instances where samples lacked

sufficient volume for analysis. All field apparatus inspections, calibrations, and documentations occurred prior to each sampling round.

In the laboratory, representativeness was fair. All samples were analyzed using accepted methods, and within appropriate holding times. However, 2 of the FIB samples exceeded the hold time slightly. These results have been qualified but minimal impact on the use of the data is anticipated due to the similarity in concentrations between results measured within and outside the hold time, and the minimal time that the samples were outside of this range.

A total of 43 field blanks were collected during the MST Program. Each blank sample was analyzed for a specific analyte (FIB, caffeine, and four microbial DNA biomarkers) providing up to 58 measures of field representativeness. None of the FIB equipment or field blank results had a measurement higher than the analytical reporting limit. For caffeine, only two (2) of the 12 field blanks were within the reporting limit. However, those outside of the reporting limit were all over by an amount deemed to be inconsequential. These blank concentrations were inspected for potential evidence of contamination in their associated samples, but it was found that the blank concentrations were much lower than the corresponding sample concentrations, so the apparent impact on the sample result was nominal. The microbial DNA equipment and field blanks were performed on a non-detect / detect basis.

5.2.6 Comparability

The objective for data comparability is to generate data for each parameter that related water quality conditions between sampling locations and over time. Data comparability is promoted by:

1. Using standard USEPA approved methods, where possible.
2. Consistently following the sampling methods detailed in the QAPP.
3. Consistently following the analytical methods detailed in the QAPP.
4. Achieving the required detection limits detailed in the QAPP.

All sample collection and analytical methods were specified, and any deviations from the methods were documented. All results were reported in the standard units as outlined in the MST Program QAPP. All field and laboratory calibrations were performed using standards traceable to National Institute of Science and Technology (NIST) or other USEPA approved sources.

The field crews and laboratory were consistent in collecting and analyzing the samples from the MST Program in a manner that allows the data to be compared between events. In the laboratory, the same EPA-approved analytical method was used for all applicable parameters across all sampling events and samples were consistently analyzed at the dilutions specified in the QAPP. The MST Program successfully met this objective of the QAPP.

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Section 6 Interpretation of Results

Section 4 presented an overview of the dry and wet weather datasets. The following observations can be made with respect to the data:

- FIB concentrations were high at all sampling locations, and greater in the Second River than in the Third River.
- Caffeine concentrations were also greater in the Second River, suggesting the largest amount of human fecal contamination is occurring there. Within the Second River, highest caffeine concentrations were observed at sampling location SR-3.
- Human-associated DNA biomarker concentration was greatest at the Second River, at sampling location SR-3.
- The Third River location exhibited slightly elevated levels of wildlife (i.e. bird and ruminant) DNA biomarkers, while the Second River location had high levels of canine-associated DNA biomarkers. These non-human sources appear to be contributing to fecal contamination in both waterbodies.
- Spatial variability and precipitation appear to have the greatest impact on caffeine and human-associated biomarkers. Both have less of an impact/no impact on non-human biomarkers.

6.1 Statistical Analysis

There are several limitations in using MST techniques to predict the presence of pathogenic organisms in a given water body. For example, many pathogens may exist in water that have the potential to cause harm, such as cysts, spores, bacteria, and viruses. Because of the vast diversity of pathogens, and because they are often present in numbers that are difficult to detect, fecal indicator bacteria that are positively associated with more harmful pathogens and that can be detected in abundance are commonly used. However, using *E. coli* and fecal coliform can be somewhat limiting in predicting human fecal contamination because they are found in the intestines of many animals. Additionally, because they are facultative anaerobes, they may thrive in benthic sediment and in other aquatic environments long after contamination has occurred, which might not be indicative of recent human fecal pollution.

Caffeine is particularly useful as an MST tool, as it should be absent from receiving waters that have no human fecal contamination, making it an ideal fecal indicator. However, as observed in the data presented in Section 4, caffeine levels in receiving waters are significantly dilute. Fate and transport of caffeine in environmental waters is still not completely understood, which can contribute to analytical results that lack uniformity.

Finally, qPCR methods lack the accuracy required for quantifying all fecal bacteria sources. The sensitivity and specificity of the assays used in analysis can vary dramatically, leading to easy detection of certain organisms, and ambiguity about the detection of others. Additionally, qPCR results may capture only a subset of sources active at the time of sampling and the number of copies of DNA will diverge considerably depending on the spatial and temporal proximity of the sample from the actual source of contamination. Because of such limitations, it is not practical to attribute a "percentage" of contamination to a source based on the number of DNA copies found from that source.

These limitations highlight the need to use several MST indicators for analysis, which the MST Program succeeded in accomplishing. To provide a better understanding of the results, log concentration one-to-one plots are provided in **Figure 6-1** through **Figure 6-7**.

As expected, the culture-based FIB showed a positive relationship as demonstrated in the regression plot in **Figure 6-1**. These statistical results suggest that for every increase in colony forming units of fecal coliform from a particular sample, a similar increase is anticipated for *E. coli*. A positive correlation also exists between FIB and caffeine, as exhibited in **Figure 6-2**, with a determination coefficient that is higher for *E. coli* than for fecal coliform (R^2 = 0.36, 0.14 respectively). This points toward human-associated sources having a positive correlation with *E. coli*, which is supported by the plot in **Figure 6-3** (R^2 = 0.45). **Figure 6-4** and **Figure 6-6** show minimal correlation between FIB and the wildlife biomarkers, while **Figure 6-5** illustrates a more positive correlation between FIB and canine-associated sources. When compared to the human-associated DNA biomarkers (**Figure 6-7**), there is a positive correlation with caffeine (R^2 = 0.21), but flat trend lines in relation to the non-human biomarkers. This data adds confidence in using caffeine as a chemical indicator for human fecal contamination, though the relationship is somewhat weaker than anticipated, due to anomalies with the detection of caffeine.

For the human-associated microbial DNA assay, a positive correlation with caffeine also gives credibility for its use as an indicator of human-associated contamination. When compared to the fecal indicator bacteria, the positive trend line with human and canine-associated assays is in line with those sources having more of an impact on *E. coli* reporting, especially in the Second River. Because bird and ruminant trend lines were so flat, these assays are not a good predictor of FIB contamination, by themselves. This is most likely because the bird, and especially the ruminant assays were not found in significant numbers at every site. In summary, human and canine-associated assays are strong predictors of *E. coli* and fecal coliform in the Second and Third Rivers for the purposes of this study.

Figure 6-1: Log Fecal Coliform vs. Log *E. coli*

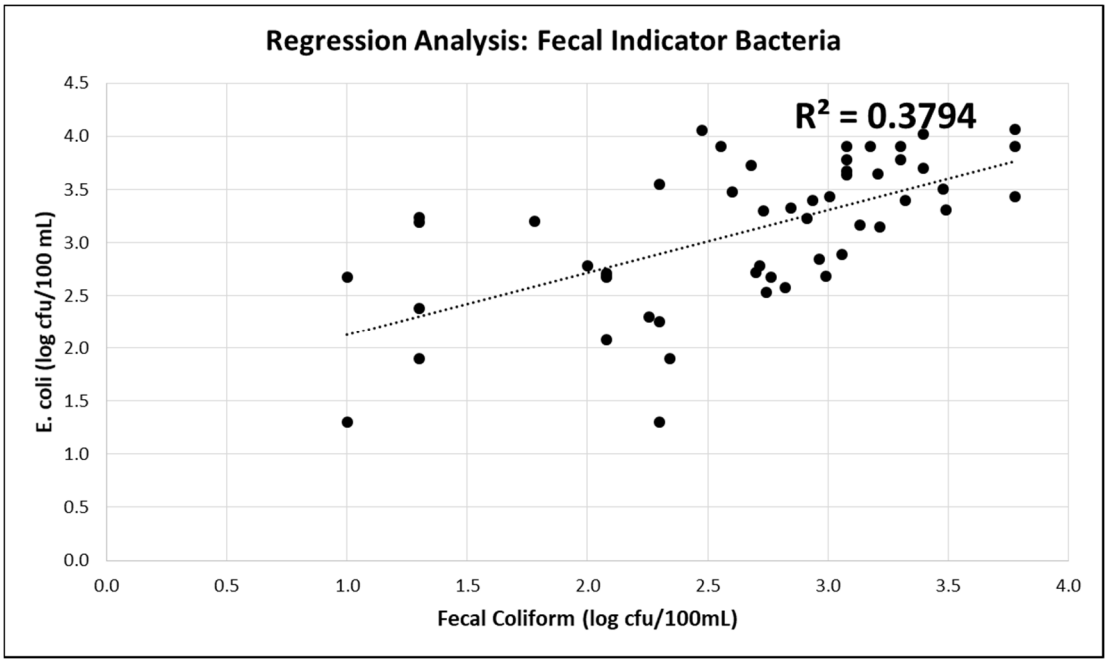


Figure 6-2: Log FIB vs. Log Caffeine

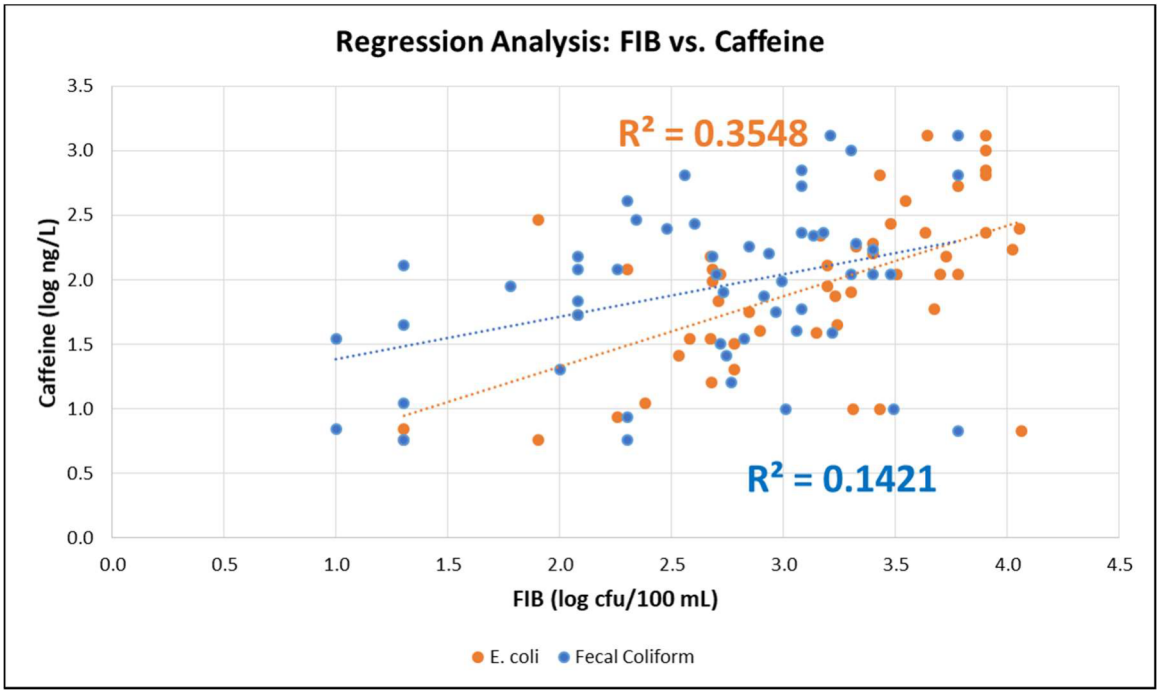


Figure 6-3: Log FIB vs. Log Human DNA

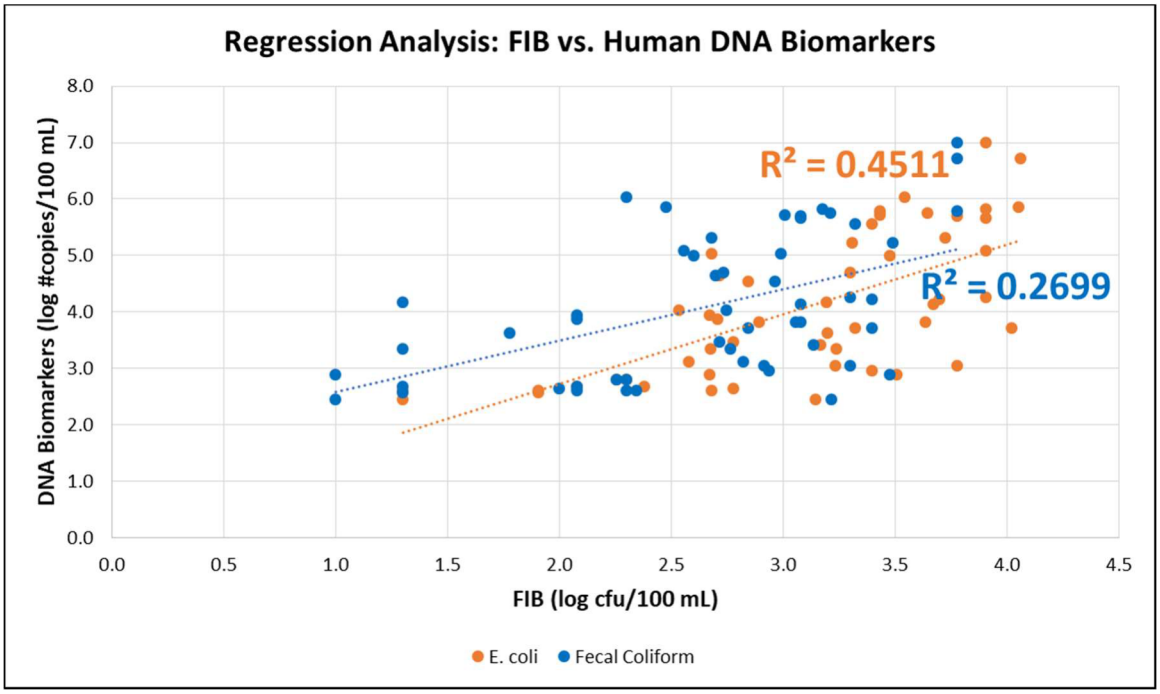


Figure 6-4: Log FIB vs. Log Bird DNA

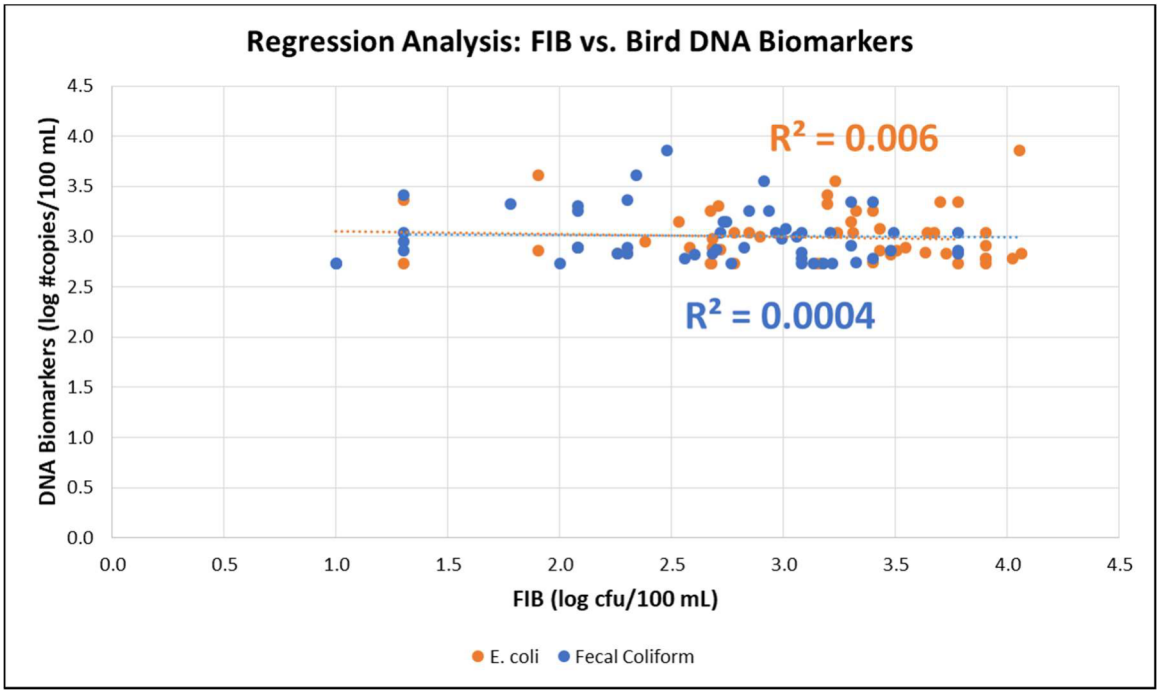


Figure 6-5: Log FIB vs. Log Dog DNA

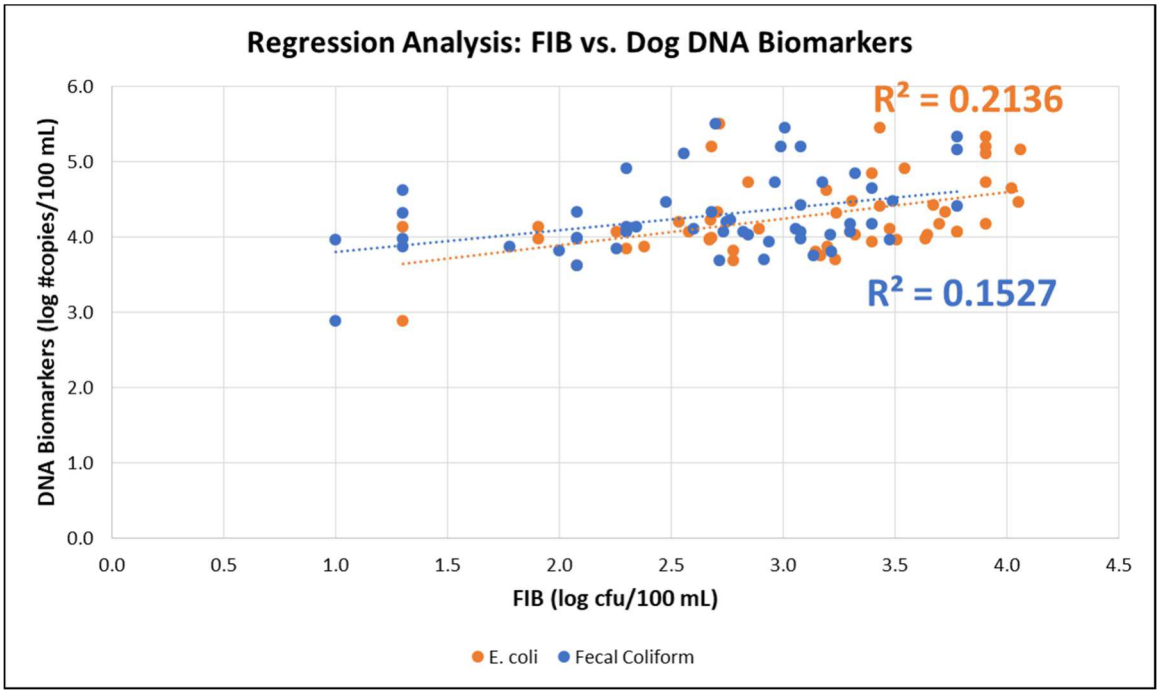


Figure 6-6: Log FIB vs. Log Ruminant Biomarkers

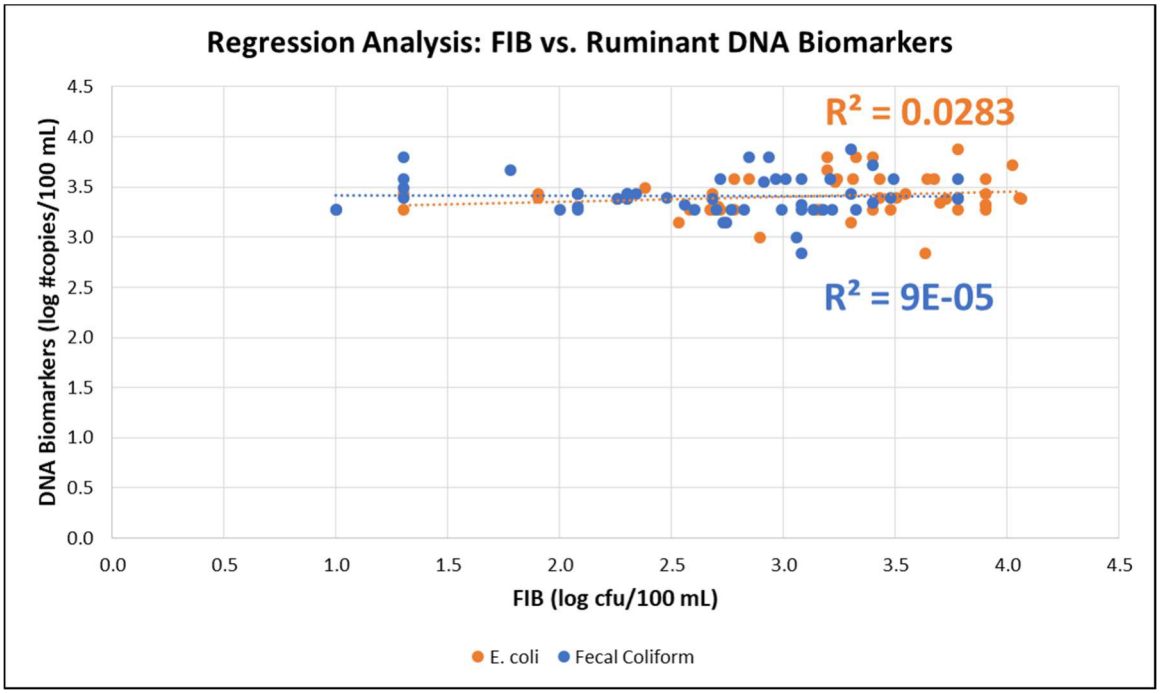
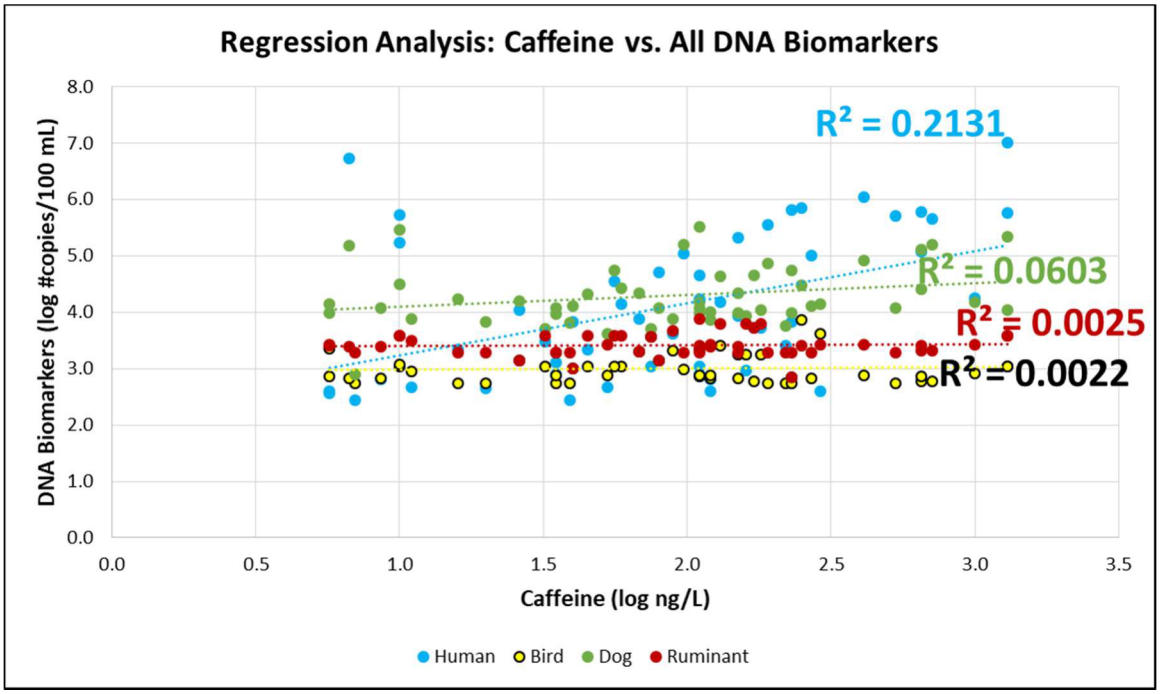


Figure 6-7: Log Caffeine vs. Log DNA Biomarkers



6.2 Remediation Efforts on the Second River

Previous sampling efforts conducted by the USEPA, NJDEP, and PVSC on the Second River have documented the impairment of the waterbody due to high levels of FIB. Consequently, remedial and investigative efforts were facilitated by NJDEP and EPA compliance and enforcement personnel working together with individual municipalities to address these concerns beginning in 2017, and continuing through the present time. While some sewer rehabilitation work has been completed, other work is ongoing. Some of this work will be managed through the NJ MS4 permit program. Corrective actions and investigations to date have focused on the following four (4) sites:

- USEPA Site SR-06, Meadowbrook Storm Sewer Outfall, Newark
  The City of Newark has televised the entire Meadowbrook Storm Sewer system. Two illicit sewer connections were removed in 2018. Internal sampling in the sewer system has revealed other potential sources. Investigation was halted for some time but is still active and will continue.

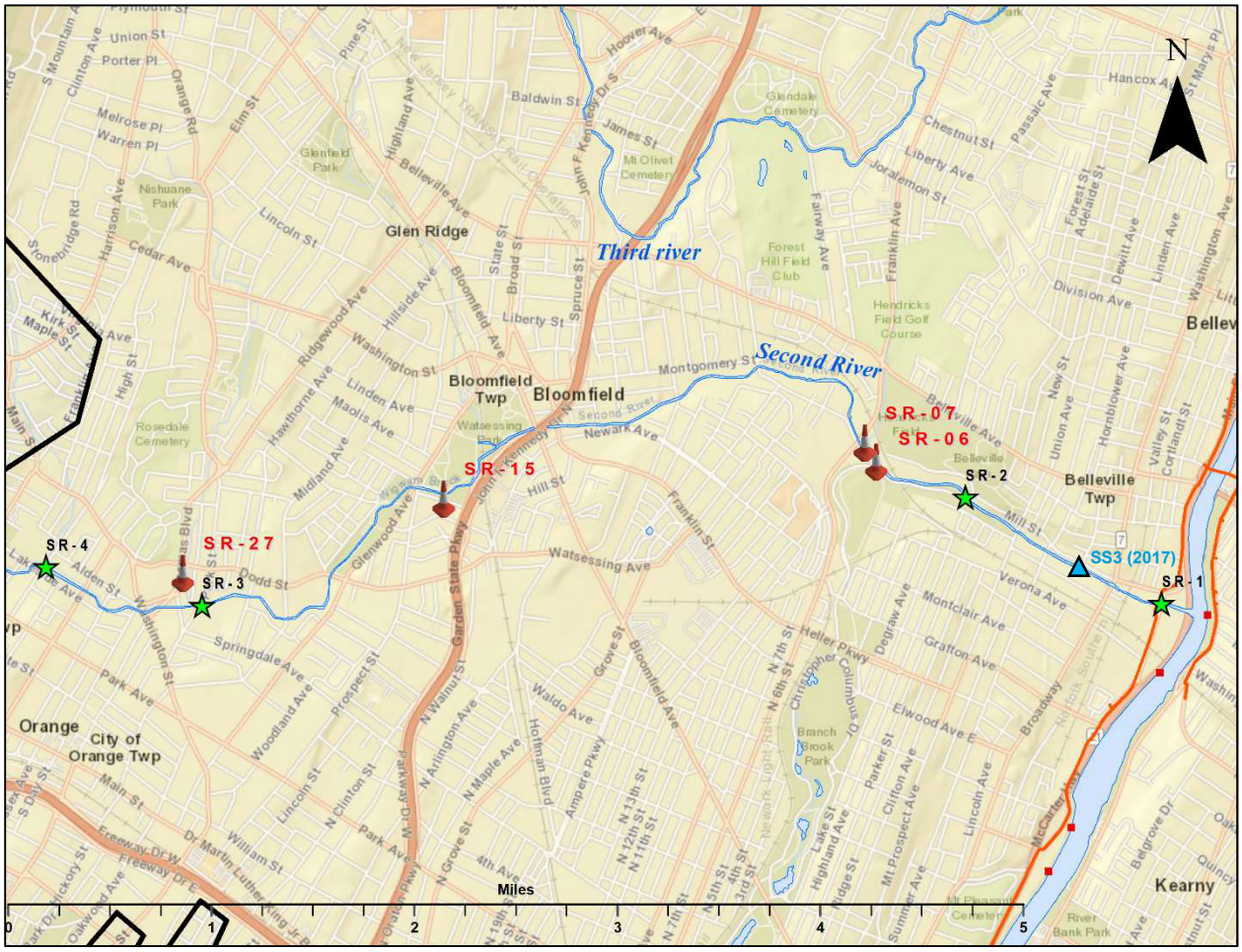
- USEPA Site SR-07, Tributary Outfall, Belleville
  Dye testing of large trunk sewers in 2017 was negative indicating no discharge to the Second River tributary. The storm sewer crossing the tributary and its connecting sewer lines were televised in 2018. Storm sewer connections were subsequently plugged and sealed in February of 2020.

- USEPA Site SR-15, Tributary into Second River, Bloomfield
  A large underground storm sewer daylights as a tributary flowing into the Second River in Bloomfield. An apartment complex on the Bloomfield-East Orange border was suspected of contributing sewage to the storm sewer system. Two sets of cross-connected sewers were identified in the facility's garage and were corrected in 2019. However, there are likely additional sources that need to be remediated in the tributary storm sewer system which flows from East Orange into Bloomfield.

- USEPA Site SR-27, Tributary into Second River, Orange
  The sewer system in the vicinity of SR-27, located underneath an overpass by Dodd St and Thomas Blvd. in the City of Orange, was investigated and a portion of the sewer system was replaced in 2019.

A map of these four sites on the Second River, along with the four sampling locations from the MST Sampling Program and the Second River sampling site from the 2017 PVSC Source Sampling Program is provided in **Figure 6-8**. Note that USEPA sites SR-06 and SR-07 are directly upstream of MST sampling location SR-2; USEPA site SR-15 is located in between MST sampling locations SR-2 and SR-3; USEPA site SR-27 is on an upstream tributary flow to MST sampling location SR-2; and the Second River 2017 Source Sampling location SS3 is located in between SR-1 and SR-2.

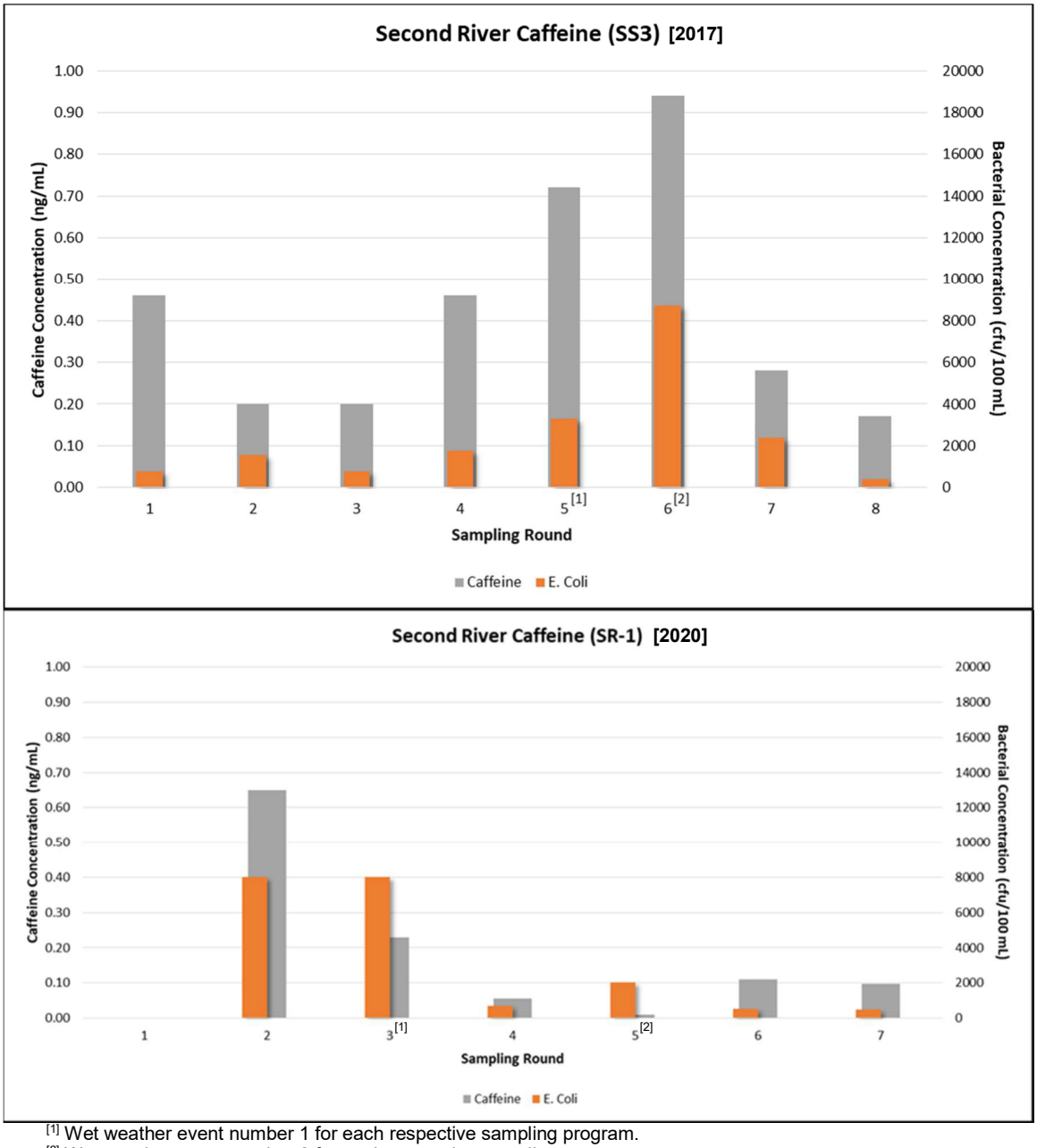
Figure 6-8: USEPA Remediation Sites on the Second River



A comparison of the MST Program results for FIB and caffeine with those from the 2017 PVSC Source Sampling Program (**Figure 6-9**) shows that the restorative efforts at the USEPA sites has contributed to an incremental improvement in water quality. The average dry-weather *E. coli* concentration decreased from 1280 cfu/100 mL to 925 cfu/100 mL, while the average wet-weather concentration for *E. coli* decreased from 5990 cfu/100 mL to 5020 cfu/100 mL. For caffeine, average dry-weather concentration reduced from 0.295 ng/mL to 0.228 ng/mL and average wet-weather concentration reduced from 0.830 ng/mL to 0.120 ng/mL. Comparisons of results obtained from qPCR methodology could not be provided because of the differences in assays used in the analysis between the two sampling programs. The assay used for the MST Program appears to have a greater sensitivity in these water bodies, perhaps due to the compiling of known source samples for this study. Future studies should employ the same assay during analysis for direct comparison. If possible, the same laboratory should be used for qPCR analysis, as standardization between laboratories for qPCR methodology has yet to occur.

Although improvements were noted at the downstream sampling locations, the area surrounding SR-3 remains a "hot spot" for human-associated fecal contamination. Future efforts should focus on this area to identify the contributing contamination source and remedy the problem through collaboration with local municipalities and property owners.

Figure 6-9: Comparison of FIB and Caffeine Results – 2017 to 2020



[1] Wet weather event number 1 for each respective sampling program.
[2] Wet weather event number 2 for each respective sampling program.

6.3 Conclusion

The goal of the MST Program was to identify sources of high fecal contamination in the Second River and Third River, over a range of environmental conditions. The program succeeded in achieving its objectives through coordinated sampling efforts of the Second River and the Third River for *E. coli* and fecal coliform - the two fecal indicator bacterial parameters most often used to protect recreational water quality and identified as Pollutants of Concern in the PVSC CSO LTCP, caffeine - an established chemical indicator of human fecal contamination, and microbial DNA biomarkers - an effective technology for identification among multiple sources of fecal contamination. The data collected was sufficient to characterize these sources as background sources contributing to the overall impairment of these water bodies. The MST Program also fully achieved the quality objectives set out in the MST Program QAPP.

Finally, the MST Program was effective in documenting changes in water quality due to remediation efforts along the Second River. Incremental improvement to the water quality in the Second River demonstrates the effectiveness of microbial source tracking from programs such as the MST Program. Future efforts on the Second River should prioritize the area surrounding SR-3 to identify the causes of established high human-associated fecal contamination, which significantly contributes to waterbody impairment downstream and into the Lower Passaic River.

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Greeley and Hansen LLC
1700 Market Street, Suite 2130
Philadelphia, PA 19103
(800) 837-9779
www.greeley-hansen.com

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