Middle Santa Ana River Bacteria Synoptic Study: Draft Report

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Acronyms *(to be completed)*

Basin Plan	Water Quality Control Plan for the Santa Ana Region
°C	Degrees Celsius
CBRP	Comprehensive Bacteria Reduction Plan
CCWRP	Carbon Canyon Water Recycling Plant
CEDEN	California Environmental Data Exchange Network
cfs	cubic feet per second
CFU	Colony Forming Unit
COC	Chain of Custody
DWF	Dry weather flow
E. coli	*Escherichia coli*
EEES	Essential Environmental and Engineering Systems
ft	feet
ft^2	Feet per second
GEI	GEI Consultants, Inc.
IEUA	Inland Empire Utilities Agency
LA	Load allocation
mL	Milliliter
MPN	Most Probable Number
MS4	Municipal Separate Storm Sewer System
MSAR	Middle Santa Ana River
MSAR TMDL	Middle Santa Ana River Bacterial Indicator TMDL
MST	Microbial Source Tracking
NPDES	National Pollutant Discharge Elimination System
POTW	Publicly-owned Treatment Works
ppth	Part per thousand
Q	Flow
QAPP	Quality Assurance Project Plan
QA/QC	Quality Assurance/Quality Control
RBMP	Regional Bacteria Monitoring Program
RCFC&WCD	Riverside County Flood Control & Water Conservation District
REC1	Water Contact Recreation
REC2	Non-contact Water Recreation
Riverside RWQCP	Riverside Regional Water Quality Control Plant
RIX	Rapid Infiltration and Extraction Facility

RP1	IEUA Regional Water Recycling Plant No. 1
Santa Ana Water Board	Santa Ana Regional Water Quality Control Board
SAWPA	Santa Ana Watershed Project Authority
SBCFCD	San Bernardino County Flood Control District
State Water Board	State Water Resources Control Board
Task Force	MSAR Watershed TMDL Task Force
TMDL	Total Maximum Daily Load
USEPA	United States Environmental Protection Agency
WDR	Waste Discharge Requirement
WLA	Wasteload allocation
WQCP	Water Quality Control Plant

1. Background and Purpose

1.1 Regulatory Background

1.1.1 Middle Santa Ana River Bacterial Indicator TMDL

On August 26, 2005, the Santa Ana Regional Water Quality Control Board (Santa Ana Water Board) adopted Middle Santa Ana River (MSAR) Bacterial Indicator Total Maximum Daily Loads (TMDL) ("MSAR TMDL") for Reach 3 of the Santa Ana River, Mill Creek (in the Prado area), Reach 1 of Cucamonga Creek, Reaches 1 and 2 of Chino Creek, and the Prado Park Lakes (Resolution No. R8-2005-0001). The adopted TMDL was approved by the State Water Resources Control Board (State Water Board) on May 15, 2006 (Resolution No 2006-030) and by US Environmental Protection Agency (USEPA) Region 9 on May 16, 2007.

The MSAR TMDL established fecal coliform and *Escherichia coli* (*E. coli*) wasteload allocations (WLA) for urban Municipal Separate Storm Sewer System (MS4) and confined animal feeding operation discharges and load allocations (LAs) for agricultural and natural sources:

- Fecal coliform: 5-sample/30-day logarithmic mean (or geometric mean) less than 180 organisms/100 mL and not more than 10 percent of the samples exceed 360 organisms/100 mL for any 30-day period.[1]
- *E. coli*: 5-sample/30-day logarithmic mean (or geometric mean) less than 113 organisms/100 mL and not more than 10 percent of the samples exceed 212 organisms/100 mL for any 30-day period.

Soon after the adoption of the MSAR TMDL by the Santa Ana Water Board and prior to the effective TMDL date, the responsible parties named in the TMDL established the MSAR Watershed TMDL Task Force ("Task Force") to work collaboratively on the requirements in the TMDL's Implementation Plan. Among these requirements were the establishment of watershed-wide compliance monitoring program and development of an Urban Source Evaluation Plan (USEP) that was to include the steps needed to identify specific activities, operations, and processes in urban areas that contribute bacterial indicators to MSAR watershed waterbodies.

The USEP, which was approved in 2008 (Resolution No. R8-2008-0044), included a number of investigations to identify the most significant sources of bacterial contamination to the impaired waterbodies, including, for example, studies in Carbon Canyon Creek, Cypress Channel, lower Deer Creek subwatershed (Chris Basin), Box Springs Channel, and Chino

[1] The WLAs and LAs for fecal coliform were no longer following USEPA's 2015 approval of the 2012-adopted Basin Plan amendment to revised bacterial indicator objectives in the Santa Ana Region for inland freshwaters.

Creek. Data generated from the USEP studies was used to develop the first risk-based scoring system to help prioritize project implementation and measure progress towards improving water quality.

1.1.2 Comprehensive Bacteria Reduction Plans

On January 29, 2010, the Riverside and San Bernardino County MS4 Permits were re-authorized by the Santa Ana Water Board (R8-2010-0033 and R8-2010-0036, respectively). These permits required the development of Comprehensive Bacteria Reduction Plans (CBRP) to address urban sources of bacterial indicators during the dry season from April 1 to October 31. Similarly, in 2012, the Los Angeles County MS4 Permit required the Cities of Pomona and Claremont to prepare CBRPs for the portion of their jurisdictions within the MSAR watershed (R4-2012-0175). To address these MS4 Permit requirements, the risk-based approach, first developed for the USEP, was updated and used to form the foundation for the development of CBRP priorities. Following approval of the CBRPs for Riverside and San Bernardino County MS4 Programs (R8-2012-0015 and R8-2012-0016, respectively), the MSAR watershed MS4 permittees were required to implement the CBRPs in accordance with their MS4 Permit.

In December 2017, the Riverside and San Bernardino County MS4 Programs received notice from the Santa Ana Water Board that their respective CBRPs were being audited to evaluate compliance with the CBRP requirements. The outcome of this effort was the finding in the CBRP Audit Reports for each County that the MS4 Programs are in compliance with their respective CBRPs (Santa Ana Water Board October 2018a,b). In addition, the audits recommended revision to the CBRPs *but only* after the Water Quality Control Plan for the Santa Ana River Basin (Basin Plan) and MSAR TMDL are revised to be current with state and/or regional regulations to protect recreational uses.

1.1.3 Recreational Use Protection

In 2012, the Santa Ana Water Board adopted an amendment to the Basin Plan that revised bacterial indicator objectives in the Santa Ana Region for inland freshwaters (R8-2012-0001). That Basin Plan amendment was subsequently approved by the State Water Board in 2014 (Resolution No. 2014-0005, January 21, 2014) and by USEPA on April 8, 2015.

Recently in 2018, the State Water Board amended the Water Quality Control Plan for Inland Surface Waters to establish new statewide water quality standards for pathogen indicator bacteria (Resolution No. 2018-0038, August 7, 2018). These new standards supersede some portions of the Santa Ana Region's 2012 Basin Plan amendment. Both the 2012 Santa Ana Water Board Basin Plan amendment and 2018-adopted State Water Board statewide bacteria water quality standards provisions impact the basis for establishment of the 2005-adopted MSAR TMDL, which in turn impacts the basis for the MS4 Program CBRPs.

In March 2019, the MSAR Task Force recommended that the Santa Ana Water Board address the need to revise the Basin Plan and the MSAR TMDL by requesting that the following initiatives be included as a high priority during the Board's next triennial review planning period (March 8, 2019 letter to the Santa Ana Water Board, prepared by Risk Sciences on behalf of the MSAR Task Force):

- Revise the water quality objectives for pathogen indicator bacteria in the Santa Ana region's Basin Plan to be consistent with those recently approved by the State Water Board as amendments to the Basin Plan (State Water Board 2018):
  - The bacteria water quality objective for all waters where the salinity is equal to or less than 1 part per thousand (ppth) 95 percent or more of the time during the calendar year is: a six-week rolling geometric mean of *E. coli* not to exceed 100 colony forming units (cfu) per 100 milliliters (mL), calculated weekly, and a statistical threshold value of 320 cfu/100 mL not to be exceeded by more than 10 percent of the samples collected in a calendar month, calculated in a static manner.
- Update Basin Plan Table 5-REC2 only-FW antidegradation target methodology;
- Update the MSAR Bacteria TMDL to take into account changes to statewide water quality standards for bacterial indicators and changes to the Basin Plan to protect inland freshwaters.

These recommendations were included in the Santa Ana Water Board's final Triennial Review Priority List and Work Plan (Fiscal Years 2019-2022) (R8-2019-055).

1.2 Project Purpose

The existing MSAR TMDL requires stakeholders to submit written progress reports every three years. To date, three such reports have been prepared and delivered (CDM Smith 2010, 2013, 2016). Normally, the next Triennial Report would be due in 2019. However, an outcome of the CBRP audits was a determination that the next Triennial Report should be deferred for one year in order to provide time to undertake a MSAR Synoptic Study of bacterial sources and loads in the watershed. The new Study would update work originally completed as part of the USEP and development and implementation of the CBRPs. The findings from this Study would not only support development of the next TMDL Triennial Report (February 2020) but also provide data to support planned revisions to the MSAR Bacteria TMDL following planned updates to the Basin Plan.

1.3 Project Objectives

The MSAR Task Force has identified the following project objectives to be addressed by this Study Plan:

1) Characterize the current concentration of *E. coli*, including the associated variability, in the waterbodies named in the TMDL. This water quality monitoring effort should be

coordinated, to the maximum extent practicable, with the existing Regional Bacteria Monitoring Program (RBMP)[2] and to avoid duplication of effort and minimize redundant costs.

2) Characterize the flows and concentrations of *E. coli* being discharged into the waterbodies named in the TMDL from all major tributaries and discharges to those waterbodies.

3) Identify additional sources of data from similar fecal indicator bacteria monitoring programs conducted by other agencies or organizations (e.g., Inland Waterkeeper, United States Geological Survey, Orange County Water District, State Division of Drinking Water, county health departments, water supply agencies, etc.) and obtain copies if possible. Add all data collected during the Study to the Santa Ana Watershed Project Authority's (SAWPA) existing water quality database and upload qualified data to the California Environmental Data Exchange Network (CEDEN) when directed to do so by the Task Force.

4) Characterize any significant changes in the concentration and mass of *E. coli* that have occurred during the period of TMDL implementation. Determine if there is any discernable trend in the receiving water and discharge data for both *E. coli* and *Bacteroides* (or other human-associated DNA markers selected for the Study).

5) Use appropriate Microbial Source Tracking (MST) techniques to determine the extent to which human sources may or may not be contributing to elevated *E. coli* concentrations in the samples collected.

6) Update the Risk-Based Prioritization Score, reflected in Table 3-6 and Figure 3-8 in the 2013 TMDL Triennial Report for all sites evaluated as part of the new Synoptic Study and summarize how these scores have changed since the previous ranking was prepared in 2013 (CDM Smith 2013).

7) Evaluate and quantify the degree to which dry weather urban flows have declined in the time since the TMDL was approved in 2005. Estimate the net change in bacterial mass loads associated with the reduction in dry weather flows discharged from the stormwater conveyance system.

8) Confirm what specific areas of the MSAR watershed have been hydrologically-disconnected from the receiving streams identified in the TMDL, during dry weather conditions, and update the GIS maps accordingly.

9) Update and revise the *E. coli* mass balance analyses shown in Figures 4-9, 4-10 and 4-11 of the 2016 TMDL Triennial Report (CDM Smith 2017).

10) Determine whether the estimated bacterial load reductions described in Tables 3-2, 3-3 and 3-4 of Riverside County's CBRP and San Bernardino County's CBRP (RCFC&WCD 2011; SBCFCD 2011) have been achieved and evaluate the net effect of

[2] https://www.waterboards.ca.gov/santaana/water_issues/programs/planning/Bacteria_Monitoring_Program.html

the actual reductions achieved on receiving water quality at the primary instream compliance stations. Update the estimated load reductions required to achieve compliance with the *E. coli* targets identified in the 2005-adopted TMDL and with the new *E. coli* objectives adopted by the State Water Board in 2018 (Resolution No. 2018-0038, August 7, 2018).

1.4 Synoptic Study Project

The Synoptic Study was implemented as a collaborative effort that included the following agencies:

- Riverside County Flood Control & Water Conservation District (RCFC&WCD)
- San Bernardino Flood Control District (SBCFCD)
- City of San Bernardino Municipal Water Department
- City of Rialto
- Inland Empire Utilities Agency (IEUA)
- Riverside Regional Water Quality Control Plant (RWQCP

The Synoptic Study consisted of a comprehensive six-week data collection effort during dry weather conditions within the MSAR watershed. Sample collection began the week of July 29, 2019 and ended the week of September 3, 2019. The selection of sample locations was designed to meet the project objectives described above within areas of the MSAR watershed that are hydrologically connected. Data collection occurred at a total of 28 sample locations in the MSAR watershed (**Table 1-1** and **Figure 1-1**):

- Fourteen Tier 1 sites (defined as locations where urban sources of dry weather flow (DWF) may directly discharge to a downstream watershed-wide compliance site);
- Two Tier 2 sites (defined as sites that are tributary to a downstream Tier 1 site);
- Five Publicly-owned Treatment Works (POTW) (sample collection from fully treated effluent prior to discharge to the receiving water) (Table 1-2);
- Four MSAR watershed-wide compliance sites (existing compliance sites regularly sampled as part of MSAR TMDL implementation; and
- Three Santa Ana River Reach 3 mainstem sites (additional mainstem Santa Ana River sites that are not MSAR TMDL compliance sites).

Field measurements, including flow, were collected during all sample events. Water samples were collected for *E. coli* and *Bacteroides* analysis. The Study Plan and Quality Assurance Project Plan (QAPP) for the Synoptic Study were submitted to the MSAR Task Force in July 2019 (GEI et al. 2019 a, b).. These documents fully describe the field and laboratory methods used to collect the data needed to meet the objectives of the Study.

1.5 Synoptic Study Report

This Synoptic Study report includes the following key sections :

- *Section 2: Watershed Data Sources* – Summarizes other watershed data and studies acquired from various sources to support understanding of water quality in the watershed as it relates to fecal indicator bacteria (FIB) in general and *E. coli* and *Bacteroides* in particular.
- *Section 3: Synoptic Study Findings* – Reports the findings from the Synoptic Study from 2019 data collection activities within the context of other watershed data and studies summarized in Section 2.
- *Section 4: Conclusions and Recommendations* – Provides a summary of key conclusions and recommendations to the MSAR Task Force.

Table 1-1 Synoptic Study Sample Locations

Site Category	Site ID	Site Description	Latitude	Longitude
Mainstem Santa Ana River	64THST	SAR at 64th St	33.96884	-117.48779
	MISSION	SAR at Mission Blvd	33.99062	-117.39509
	P3-SBC1	SAR Reach 4 above S. Riverside Ave Bridge	34.02479	-117.36303
POTW	CCWRP	IEUA Carbon Canyon Water Recycling Plant effluent	33.97978	-117.69431
	Rialto WWTP	Rialto Wastewater Treatment Plant effluent	34.04816	-117.35658
	Riverside RWQCP	Riverside Regional Water Quality Control Plant effluent	33.96344	-117.46140
	RIX	Rapid Infiltration and Extraction Facility effluent	34.04159	-117.35482
	RP1	IEUA Regional Water Recycling Plant No. 1 effluent	34.02450	-117.59962
Tier 1	T1-ANZA	Anza Drain	33.96058	-117.46488
	T1-BRSC	Boys Republic South Channel	34.00208	-117.72618
	T1-BXSP	Box Springs Channel	33.97574	-117.39938
	T1-CCCH	Carbon Canyon Creek Channel	33.98620	-117.71561
	T1-CHINOCRK	Chino Creek upstream of San Antonio Channel	34.01343	-117.73057
	T1-CUCAMONGA	Cucamonga Creek at Hellman	33.94936	-117.61034
	T1-CYP	Cypress Channel	33.96821	-117.66039
	T1-DAY	Day Creek	33.96710	-117.53175
	T1-LLSC	Lake Los Serranos Channel	33.97543	-117.69107
	T1-MCSD	Magnolia Center Storm Drain	33.96570	-117.41561
	T1-PHNX	Phoenix Storm Drain	33.96368	-117.42718
	T1-SACH	San Antonio Channel	34.02442	-117.72815
	T1-SNCH	Sunnyslope Channel	33.97615	-117.42618
	T1-SSCH	San Sevaine Channel	33.97465	-117.50551
Tier 2	T2-CYP2	Cypress Channel upstream of California Institute of Men's agricultural fields	33.98583	-117.66577
	T2-HOLE	Anza Drain upstream of Hole Lake	33.94854	-117.45649
TMDL Watershed-wide Compliance Sites	WW-C7	Chino Creek at Central Ave	33.97414	-117.68911
	WW-M6	Mill-Cucamonga Creek	33.92663	-117.62484
	WW-S1	SAR at Pedley Avenue	33.96840	-117.44839
	WW-S4	SAR at MWD Crossing	33.95527	-117.53301

Table 1-2. POTWs Discharging Treated Effluent to the Santa Ana River within the Synoptic Study Project Area

Facility	Description	Waste Discharge Requirements
Rialto Wastewater Treatment Plant effluent (Rialto WWTP)	Treats wastewater from the City of Rialto. Tertiary treated recycled water effluent is discharged into SAR Reach 4. Effluent from the Rialto WWTP is one of the major components of SAR Reach 3 & 4 baseflow.	Order No. R8-2014-0010; NPDES No. CA0105295
Riverside Regional Water Quality Control Plant effluent (Riverside RWQCP)	Treats wastewater from the City of Riverside and the Community Service Districts of Edgemont, Rubidoux, and Jurupa. Tertiary treated effluent is discharged into SAR Reach 3.	Order No. R8-2013-0016; NPDES No. CA0105350
Rapid Infiltration and Extraction Facility effluent (RIX)	Receives treated wastewater from San Bernardino Municipal Water Department's Water Reclamation Plant and Colton's wastewater treatment facility. RIX provides tertiary treatment to the wastewater effluent received from those facilities and discharges into SAR Reach 4. Effluent from RIX is one of the main components of SAR Reach 3 & 4 baseflow.	Order No. R8-2013-0032; NPDES No. CA8000304
Carbon Canyon Water Recycling Plant effluent (CCWRP)	Treats wastewater from Chino, Chino Hills, Montclair, and Upland. A portion of the tertiary treated recycled water effluent is discharged into Chino Creek.	Order No. R8-2015-0036; NPDES No. CA8000409
IEUA Regional Water Recycling Plant No. 1 effluent (RP1)	Treats wastewater from Chino, Fontana, Montclair, Ontario, Rancho Cucamonga, and Upland. A portion of the tertiary treated recycled water effluent is discharged into Cucamonga Creek.	

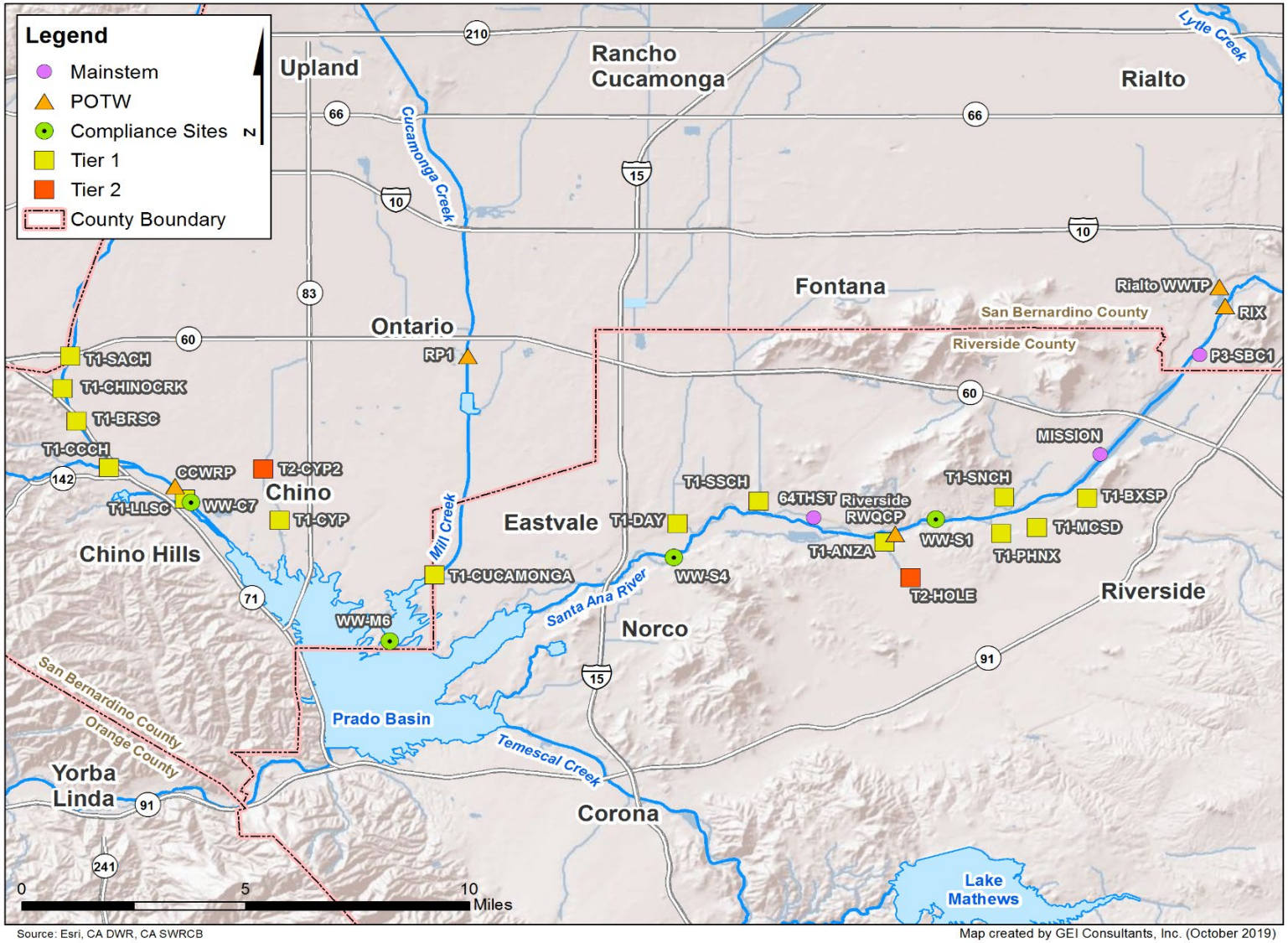

Figure 1-1. Locations of Sample Sites Included in the 2019 Synoptic Study (see Table 1-1).

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2. Watershed Data Sources

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2.1 MSAR Task Force

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2.2 Other Watershed Data

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3. Synoptic Study Findings

This section provides the findings from the 2019 Synoptic Study as they relate to the objectives of the Study. The findings are based on data collected during the 2019 six-week sample period and other studies that have been conducted in the watershed, as summarized in Section 2. Findings are presented in the following three sections:

- *Section 3.1: Characterization of Dry Weather Flow and* E. coli *in the MSAR Watershed* – This section reports on current findings and provides a comparison between 2019 study findings and previous studies in 2007 and 2012. In addition, this section updates previous *E. coli* loading analyses on a subwatershed basis and evaluates sources of bacteria, including both MS4 and non-MS4 sources.
- *Section 3.2:* Bacteroides *Analyses* – This section summarizes the findings from the analysis of all samples for the human marker HF183.
- *Section 3.3: Tier 1 Prioritization Analysis* – Based on the findings in Sections 3.1 and 3.2, this section provides the outcome of the prioritization of Tier 1 MS4 outfalls for additional work to mitigate controllable sources of *E. coli*. The resulting prioritization updates previous prioritization analyses completed for the MSAR watershed.

3.1 Characterization of Dry Weather Flow and *E. coli* in the MSAR Watershed

3.1.1 Dry Weather Flow Characterization

3.1.1.1 Sources of Flow

The primary source of DWF in impaired waters in the MSAR watershed is treated effluent from the five POTWs. This regular DWF is supplemented by numerous other non-POTW sources, including:

- Turnouts of imported water by the Metropolitan Water District;
- Well blow-offs;
- Water transfers;
- Groundwater inputs;
- Urban water waste from excess irrigation and other outdoor water uses;
- Other authorized discharges (as defined by the MS4 or Santa Ana Region General Order for De Minimis discharges (R8-2015-0004); and
- Non-permitted, prohibited discharges.

Each of these non-POTW sources of flow in the watershed has the potential to transport bacteria to or within an impaired waterbody Thus, it is important to understand the relative role of each of these categories of DWF. Additionally, some sources of bacteria are not transported to receiving waters through DWF, e.g., fecal deposition from wildlife, re-suspension of bacteria in channel bottom sediments, shedding from swimmers, or activities around transient encampments.

The 2019 Synoptic Study focused sample collection only on waterbodies that are known to contribute DWF to the impaired waters – a total of 14 sites. Areas that do not contribute DWF were excluded from the Study; these sites were identified based on findings from previous studies in the watershed (e.g., CDM Smith 2009, 2013) and the knowledge gained by MS4 permittees over time.

3.1.1.2 Hydrological Disconnection in the MSAR Watershed

The MSAR watershed covers approximately 477,000 acres. **Figure 3-1** illustrates the drainage areas upstream of Synoptic Study Tier 1 sites and the portions of the watershed that are either hydrologically disconnected or contribute only minimal flow to an impaired waterbody during dry weather conditions.

The extent of hydrologically disconnected areas has been refined over time through the implementation of source evaluation studies. For example, in 2012 the DWFs at a total of 30 Tier 1 sites were evaluated. In 2019, the number of Tier 1 outfalls with DWF was reduced to 14. The combined drainage area of these 14 sites that contribute urban flow to an impaired downstream waterbody is approximately 69,000 acres (or about 14% of the MSAR watershed). The DWF at these Tier 1 sites comprise over 99% of all DWF from urban sources in the MSAR watershed. This contributing drainage area includes a mix of urban and agricultural land uses, intersects multiple jurisdictions, and experience different non-MS4 discharges during dry weather. The remaining 86% of the MSAR watershed includes drainage areas described as follows (see Figure 3-1):

- Hydrologically disconnected during dry weather conditions (47%);
- Not tributary to an impaired waterbody (e.g., Temescal Creek) (30%); and
- Limited drainage infrastrucure or evidence of DWF connectivity (9%). These areas include riparian zones where no MS4 infrastructure is present and the agricultural area in the Chino basin (e.g., around Prado Lake).

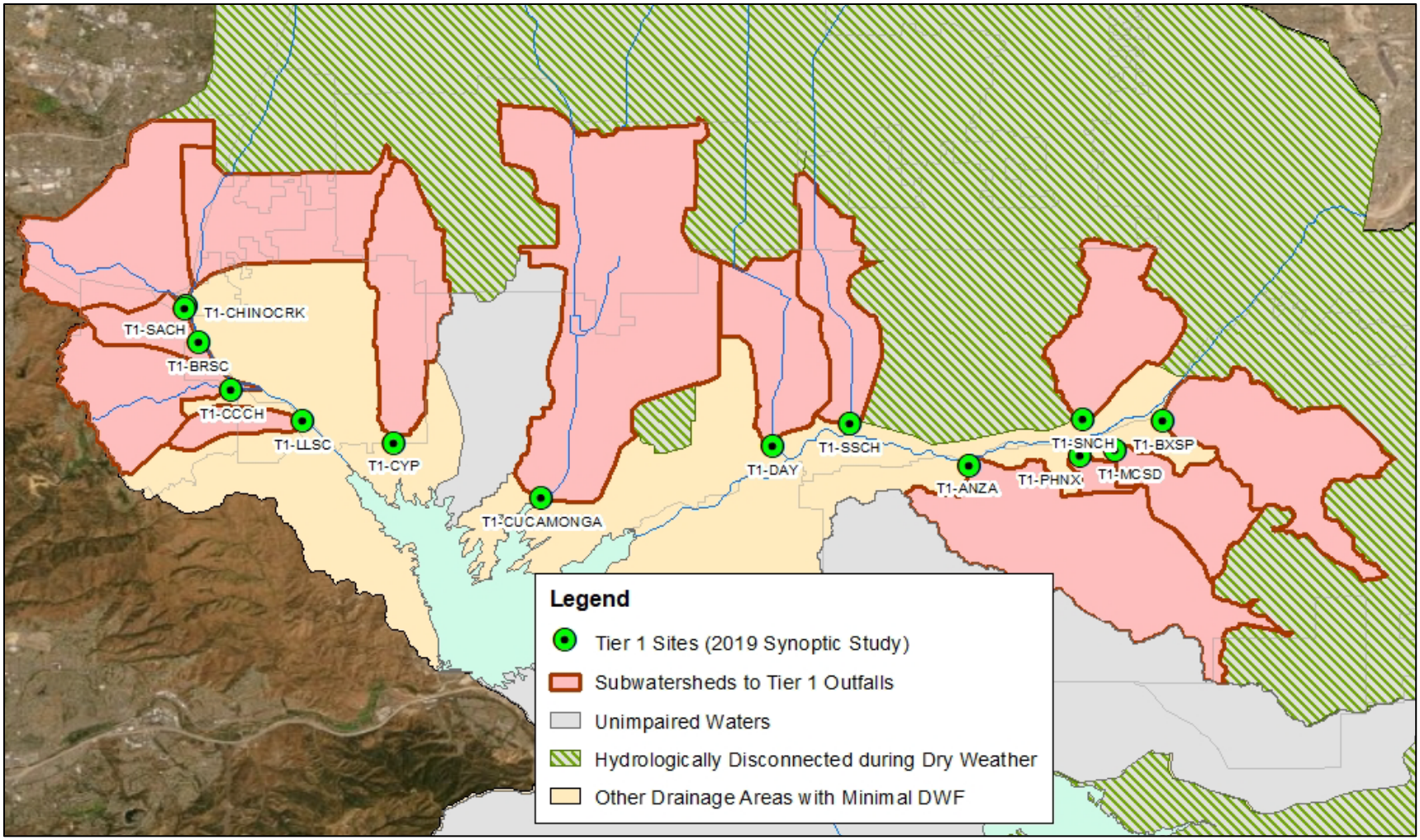

Figure 3-1. Map of Tier 1 Subwatersheds and Hydrologically Disconnected Drainage Areas during Dry Weather.

3.1.1.3 Dry Weather Flow – 2007 to 2019

Table 3-1 shows that the DWF rate (cubic feet per second, cfs) at each of the Tier 1 sites has declined since 2007. **Figure 3-2** shows that when Tier 1 sites are aggregated by the downstream compliance site, the reductions achieved exceed the targeted DWF reduction needed to demonstrate compliance with WLAs for MS4s reported in Table 3-4 of the CBRPs for San Bernardino County[3] and Riverside County[4] (**Table 3-2**). The observed decline in DWF at the Tier 1 MS4 outfalls is the result of better water management/conservation and coordination between water purveyor and stormwater agencies.

POTW effluent comprises the majority of total flow in the impaired waters and must be accounted for in the source contribution analysis. In recent years, POTW effluent discharge rates to Chino Creek, Cucamonga Creek, and the Santa Ana River have declined as a result of increased recycling of POTW effluent to serve indirect and direct non-potable reuse projects. **Figure 3-3** shows long-term trends of dry season POTW effluent at the five discharge locations upstream of MSAR TMDL compliance monitoring locations (see also Table 3-1).

Other de minimus discharges to MS4s do occur in the MSAR watershed upstream of several Tier 1 sites (e.g., see above for examples of types of de minimus discharges), but are intermittent and not reported at the daily or sub-daily timesteps needed to accommodate inclusion in the source contribution analysis. Except for one location, lack of variability in week to week flow measurements at Tier 1 sites suggests than no sources of de minimus discharges were active during the 2019 synoptic study. The one exception occurred during the final week of the study (week of September 3) when a valve to capture recycled water for groundwater recharge in the San Antonio Channel functioned improperly resulting in increased flow at Tier 1 site T1-SACH.

3.1.2 E. coli Observations

Analysis of *E. coli* concentration data from the 2019 Synoptic Study showed that bacterial water quality in DWF within impaired waters and at Tier 1 sites is highly variable, and typically exceeds the wasteload allocation (WLA) for *E. coli* of 113 MPN/100 mL (**Figure 3-4**). Some Tier 1 sites had significantly greater *E. coli* concentrations than others, e.g..T1-MSCD and T1-BRSC (see Attachment A for sample results for each site over the six-week sample period – *Note: not included in this draft*).

[3] https://www.waterboards.ca.gov/santaana/water_issues/programs/tmdl/docs/msar/cbrp/scb/Final_SBCo_CBRP_wAttachments.pdf
[4] https://www.waterboards.ca.gov/santaana/water_issues/programs/tmdl/docs/msar/cbrp/rc/Final_RivCo_CBRP_wAttachments.pdf

Table 3-1. Comparison of Average DFW Measurements at Tier 1 Sites for 2007, 2012 and 2019

Compliance Site	Tier 1 Site	Average MS4 Dry Weather Flow (cfs)		
		2007	2012	2019
Chino Creek at Central Avenue (WW-C7)	CHINOCRK	Not Measured	1.70	0.53
	T1-BRSC	Not Measured	0.44	0.13
	T1-CCCH	6.5	4.52	0.46
	T1-SACH[1]	0.7	0.01	0.01
	T1-LLSC	Not Measured	0.02	0.00
	OTHER (2007 est.)[2]	1.7	N/A	N/A
	Subtotal (WW-C7)	**9.1**	**6.69**	**1.13**
Santa Ana River at MWD Crossing (WW-S1)	T1-MCSD	No Hydro Connection	0.91	0.33
	T1-SNCH	2.0	2.42	0.39
	T1-BXSP	1.8	1.19	0.13
	T1-PHNX	No Hydro Connection	0.01	0.01
	OTHER (2007 est.)[2]	0.9	N/A	N/A
	Subtotal (WW-S1)	**4.7**	**4.53**	**0.86**
Santa Ana River at Pedley Avenue (WW-S4)	T1-ANZA	2.6	3.29	1.35
	T1-SSCH	1.3	0.50	0.36
	T1-DAY	0.5	0.22	0.19
	OTHER (2007 est.)[2]	1.0	N/A	N/A
	Subtotal (WW-S4)	**6.0**	**4.01**	**1.90**
Other Sites	T1-CYP	Not Measured	0.002	Dry
	T1-CUCAMONGA[3]	3.8	1.4	2.2
Total DWF Flow			**16.63**	**6.09**

[1] Values from the September 3, 2019 sampling event excluded from average because an upstream valve to capture recycled water for groundwater recharge was not functioning properly on this date.

[2] 2007 estimate for unmonitored areas based on an assumed DWF rate of 100 gallons/acre/day.

[3] Flow measurements were not collected at this Tier 1 site in 2012 or 2019. Values shown represent the sum of flows measured at MS4 outfalls to Cucamonga Creek in 2012 Tier 1 source evaluation (CDM Smith 2013) and from 10-week sampling program in 2016-2018 (SBCFCD 2016, 2017, and 2018).

Table 3-2. CBRP Estimate of Required DWF Reduction Compared to Observed DWF Reduction Since 2012 (RCFC&WCD 2011; SBCFCD 2011)

MSAR Watershed Compliance Site	CBRP – Estimated DWF Reduction to Comply with WLAs (cfs)	Actual DWF Reduction Since 2007 Analysis (cfs)
Santa Ana River at MWD Crossing (WW-S1)	305,000 (gal/day) (0.47 cfs)	3.84
Santa Ana River at Pedley Avenue (WW-S4)	206,000 (0.32)	4.1
Mill-Cucamonga Creek (WW-M6)	1,481,465 (2.29)	1.6
Chino Creek (WW-C7)	767,082 (1.19)	7.97

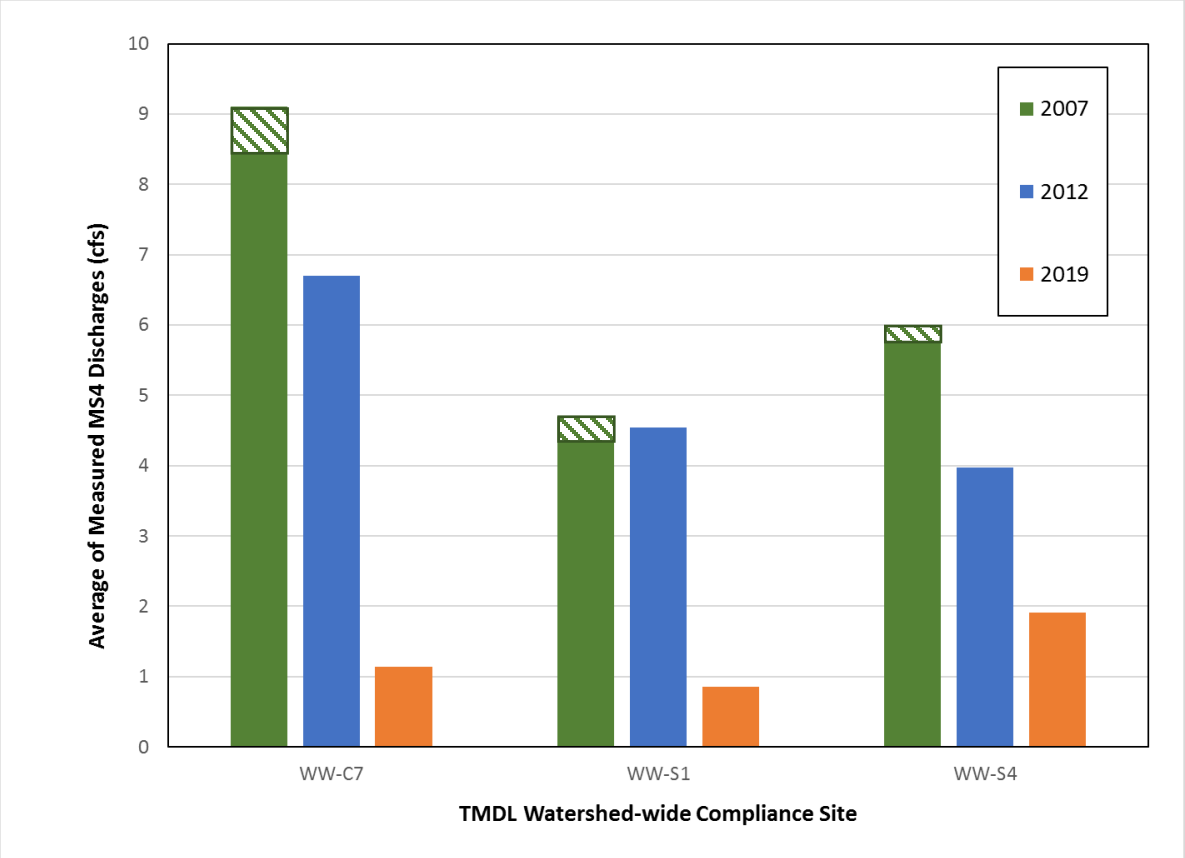

Figure 3-2. Reduction in DWF from MS4 Outfalls Upstream of the Chino Creek and the Santa Ana River Compliance Monitoring Sites. Reduction in MS4 flow from 2007 to 2019 exceeded the target DWF reduction (hatched area of 2007 bars) in CBRPs to demonstrate compliance with WLAs.

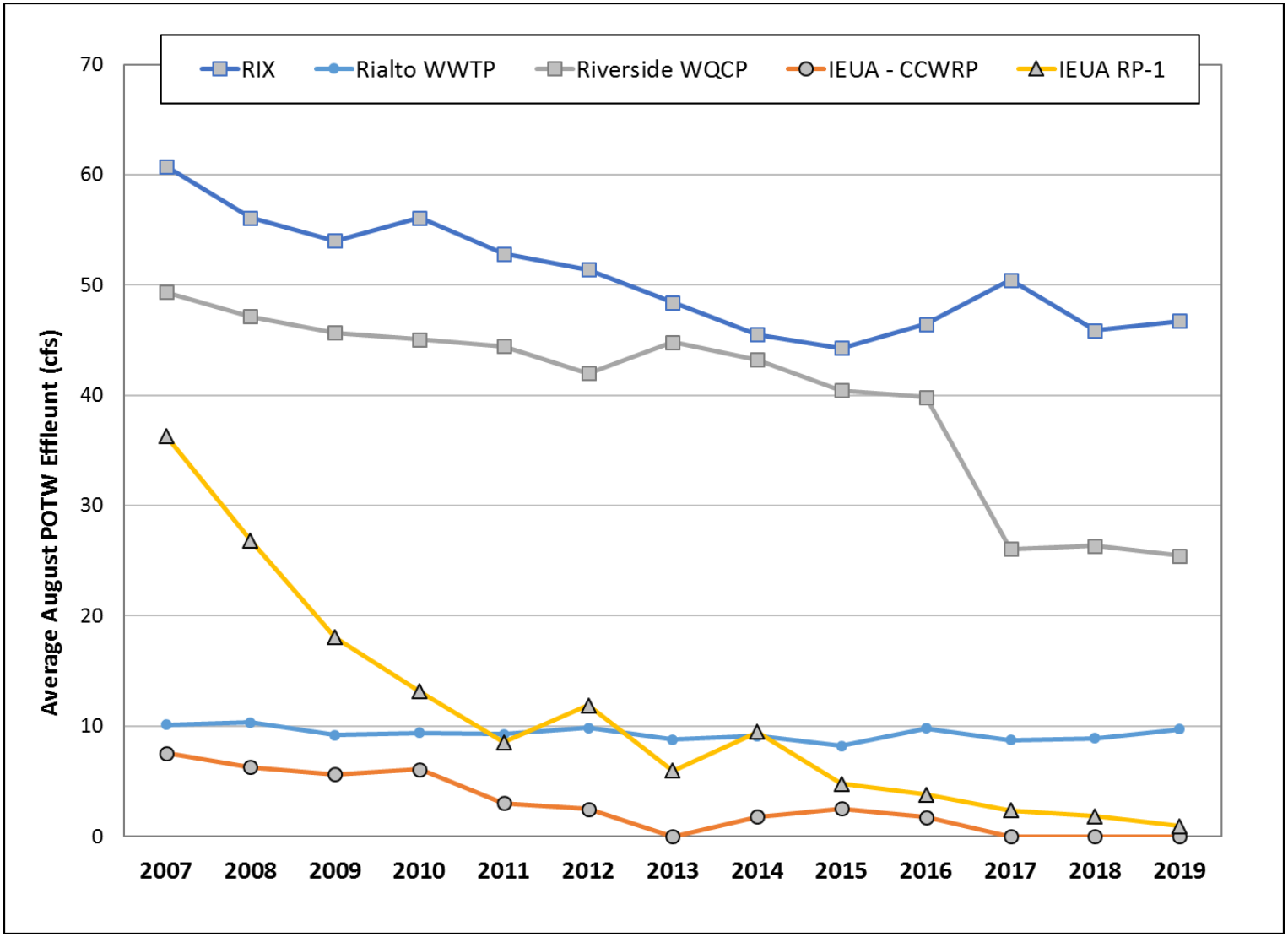

Figure 3-3. Average August POTW Effluent Flow to Impaired Waters (2007-2019)

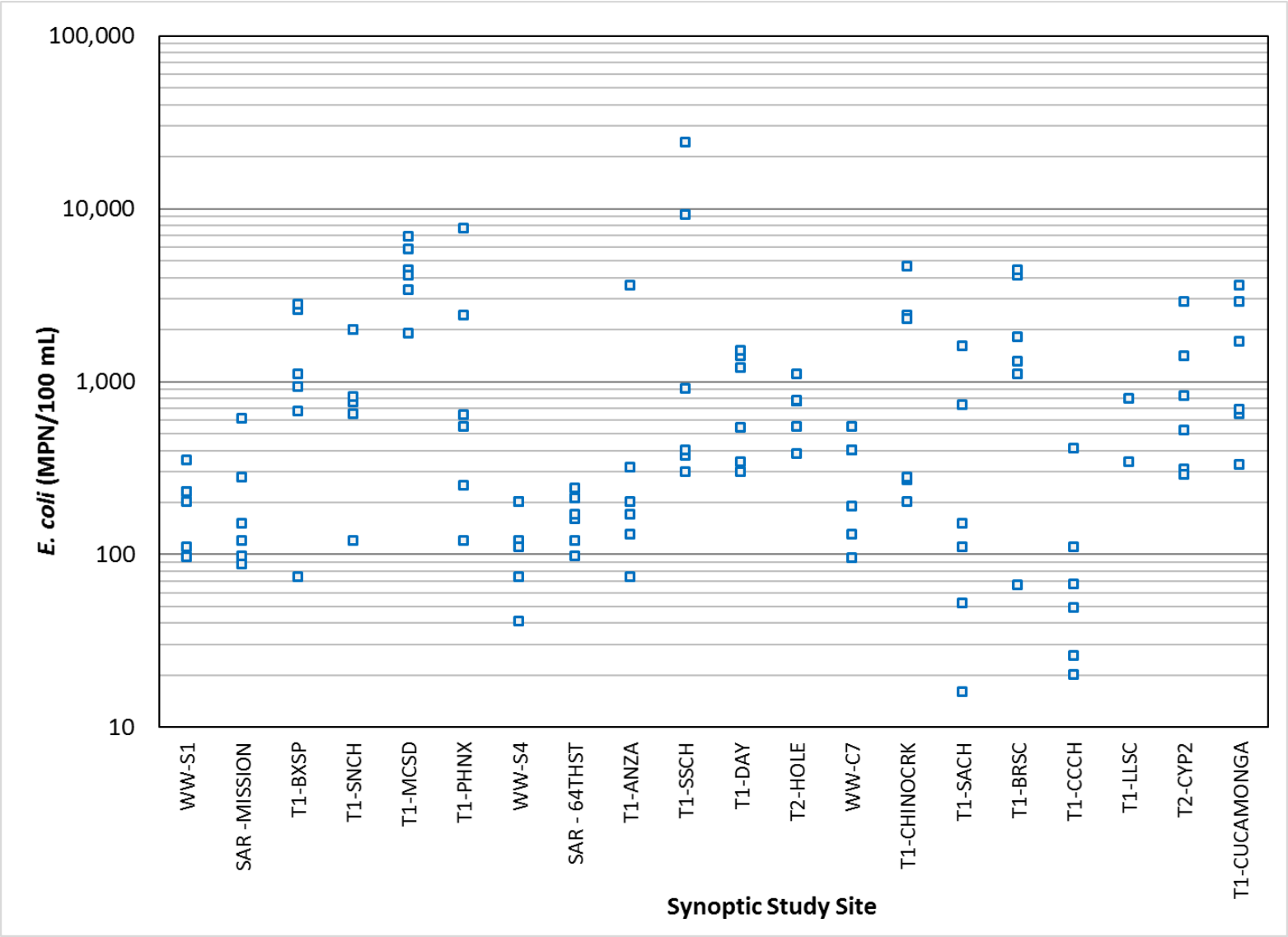

Figure 3-4. Range of *E. coli* Concentrations from all 2019 Synoptic Study Sites (Note log scale on y-axis)

Most of the Tier 1 sites had at least one sample with an *E. coli* concentration greater than 1,000 MPN/100 mL (exceptions include T1-CCCH with maximum of 410 MPN/100 mL and T1-LLSC with maximum of 800 MPN/100 mL). **Figure 3-5** shows the changes in geomean concentrations that have occurred at each Tier 1 site from 2012 to 2019. Concentrations have increased at some sites (e.g., MSCD) and decreased at others (BXSP).

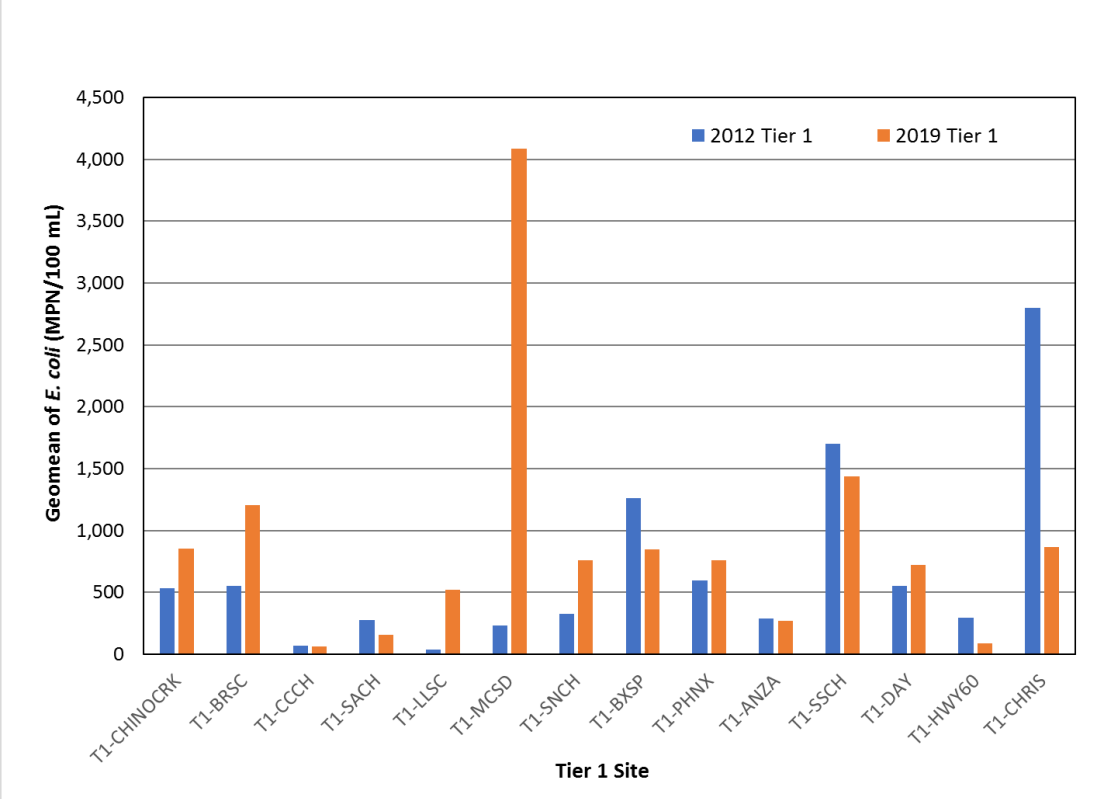

Figure 3-5. Comparison of 2019 Tier 1 Site *E. coli* Geomeans with Previous Studies (Sites T1-HWY60 and T1-CHRIS from SBCFCD (2016-2018)

3.1.3 Bacteria Load Analysis

The potential for DWF at a Tier 1 site to impact water quality at a downstream compliance site can be evaluated through a bacteria load analysis, which considers both the DWF volume and *E. coli* concentration. **Table 3-3** reports estimated loads for each Tier 1 site based on the average DWF rate and *E. coli* geomean concentration measured over the 6-week Synoptic Study in 2019 and the 10-week Tier 1 source evaluation study completed in 2012 (CDM Smith 2013)).

When taking into account changes in DWF, water quality, as measured by *E. coli* loads, has generally improved at Tier 1 sites. Reductions in *E. coli* loading to impaired waters were observed at all Tier 1 sites except two: T1-MCSD and T1-LLSC. The *E. coli* load from these two sites was much greater in 2019 versus 2012. However, when data are aggregated by

compliance site, an assessment of the total *E. coli* load from Tier 1 sites has declined in all impaired waters since 2012 (**Figure 3-6**).

Table 3-3. Comparison of Median *E. coli* Load Estimates at Tier 1 Sites in 2007, 2012 and 2019

Compliance Site	Tier 1 Site	Median *E. coli* Load (Billion MPN/Day)			
		2007	2012	2019	Change in Load 2012 to 2019
Chino Creek at Central Avenue (WW-C7)	T1-CHINOCRK	Not Measured	22.2	14.3	- 7.9
	T1-BRSC	Not Measured	6.9	4.8	- 2.1
	T1-CCCH	22.0	7.5	0.7	- 6.8
	T1-SACH	7.0	0.1	0.1	0.0
	T1-LLSC	Not Measured	0.001	0.1	+ 0.1
	OTHER (2007 est.)[2]	24.0	N/A	N/A	N/A
	Subtotal (WW-C7)	**53.0**	**36.7**	**20.0**	**- 16.7**
Santa Ana River at MWD Crossing (WW-S1)	T1-MCSD	No Hydro Connection	4.9	35.3	+ 30.4
	T1-SNCH	9.0	15.6	7.0	- 8.6
	T1-BXSP	75.0	25.5	3.1	- 22.4
	T1-PHNX	No Hydro Connection	0.0	0.0	0.0
	OTHER (2007 est.)[2]	10.0	N/A	N/A	N/A
	Subtotal (WW-S1)	**94.0**	**46.0**	**45.4**	**- 0.6**
Santa Ana River at Pedley Avenue (WW-S4)	T1-ANZA	31.0	16.9	7.3	- 9.6
	T1-SSCH	10.0	29.3	4.6	- 24.7
	T1-DAY	7.0	1.9	1.3	- 0.6
	OTHER (2007 est.)[2]	14.0	N/A	N/A	N/A
	Subtotal (WW-S4)	**62.0**	**48.1**	**13.2**	**- 34.9**
Other Sites	T1-CYP	Not Measured	11.5	Dry	- 11.5
	T1-CUCAMONGA[1]	82.0	44.7	14.3	- 30.4
Total *E. coli* Load		**--**	**317.8**	**171.5**	**-146.3**

[1] 2007 estimate for unmonitored areas based on *E. coli* concentration of 600 MPN/100 mL, which was the geomean of all MS4 outfall samples in 2007.

[2] This Tier 1 site is downstream of the RP1 discharge. Flow measurements were not collected at this Tier 1 site in 2012 or 2019. Values represent the sum of bacteria loads estimated from SBCFCD MS4 inputs only (CHRIS + HWY60) in 2012 Tier1 source evaluation and from 10-week sampling program in 2016-2018 (SBCFCD 2016, 2017, 2018).

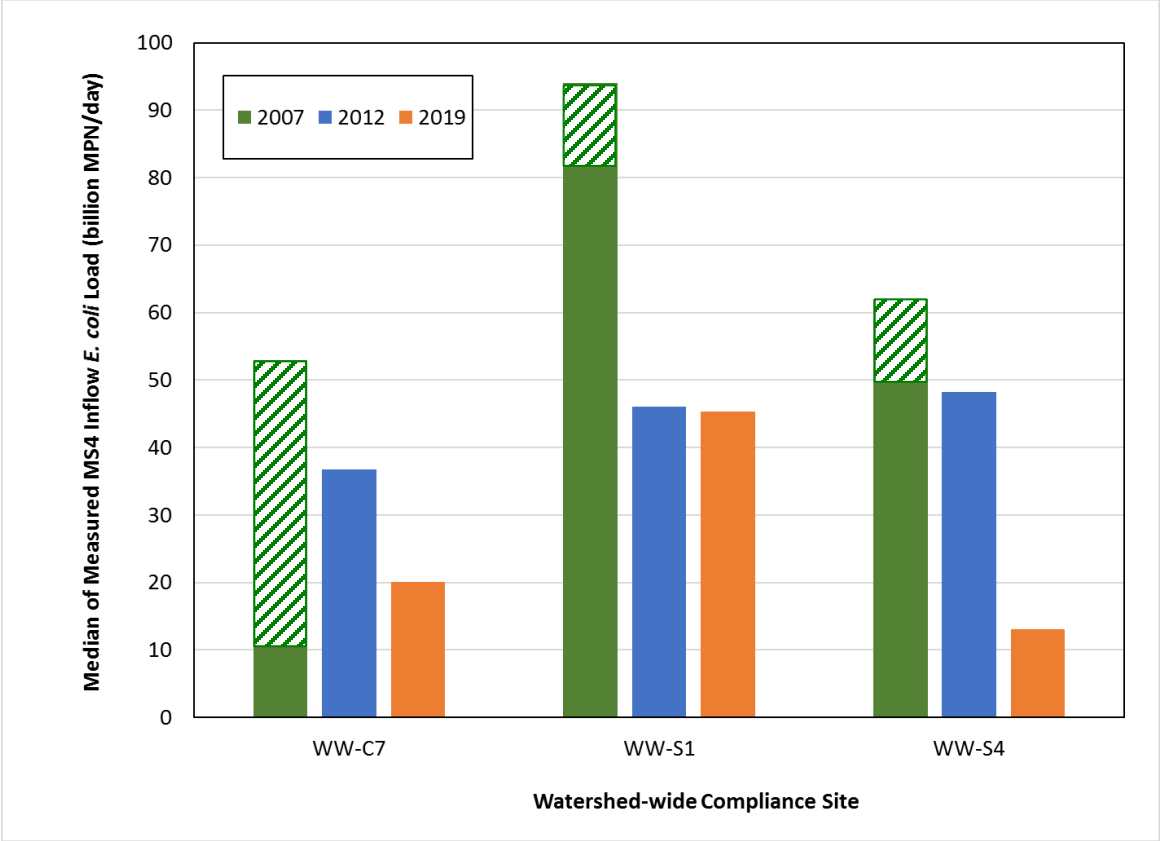

Figure 3-6. Median MS4 *E. coli* Load from Tier 1 Sites Tributary to the Chino Creek and the Santa Ana River Watershed-wide Compliance Sites (Note: Reduction in MS4 bacteria load targeted by CBRP implementation to demonstrate compliance with the WLAs Shown as hatched area of the 2007 bars).

The observed bacteria load reductions result from both reduced DWF (from better water management and coordination between water purveyor and stormwater agencies) and reduced *E. coli*, e.g., through focused deployment of Tier 2 inspections that have successfully identified and eliminated illicit connections and illegal discharges within the MS4s.

3.1.4 Source Evaluation

The 2019 Synoptic Study provides the opportunity to update previous estimates (CDM Smith 2012, 2017) of the total MS4 loading of *E. coli* to impaired waters dry weather. When dry weather flow from the MS4s is blended with tertiary treated POTW effluent (compliant with the facility's *E. coli* permit effluent limit),[5] a mass balance calculation can approximate the expected *E. coli* concentrations ($C_{MS4+POTW}$) within each impaired water (omitting any instream losses or gains).

[5] See specific Waste Discharge Requirements (WDR) for each POTW (Table 1-2 provides the WDR Order No.) and/or discussion in the Synoptic Study Plan (see Section 1.4)

The difference ($C_{Non\text{-}MS4}$) between the blended concentration and *E. coli* measurements at downstream compliance sites (C_{WW}) provides an estimate of the nature of *E. coli* losses or gains of that occur instream. Instream losses of *E. coli* may be attributed to natural degradation processes in the environment and instream gains of *E. coli* may come from new sources of bacteria, including, but not necessarily limited to shedding from swimmers, fecal deposition by wildlife, impacts from homeless encampments, and scouring of naturalized *E. coli* colonies in sediment/biofilms.

Instream sources are collectively referred to as "Non-MS4" sources in this report[6] (Note: In past reports referred to as "unaccounted-for sources" or "*e*"). The relative portion of downstream water quality associated with non-MS4 sources is thus estimated as follows:

$$C_{MS4+POTW} = \frac{[\sum_i^j (Q_{MS4} * C_{MS4})]}{(Q_{MS4} + Q_{POTW})}$$

$$C_{NonMS4} = C_{WW} - C_{MS4+POTW}$$

This source evaluation approach is equivalent to the analyses in the CBRPs (RCFC&WCD 2011; SBCFCD 2011) and subsequent TMDL Triennial Reports (CDM Smith 2013, 2017). The sub-sections below provide the source evaluation analysis results for each impaired waterbody based on the 2019 Synoptic Study. Each section contains a comparable series of figures including:

- Schematic of MS4 and POTW inflows to the waterbody, key retention facilities, and nearest downstream compliance monitoring site;
- Weekly time series plot of the MS4 + POTW blended concentration compared with concentrations measured at the downstream compliance monitoring locations; and
- Proportion of each Tier 1 MS4 drainage area that is included in the estimated blended bacterial indicator concentration.

3.1.4.1 Chino Creek Subwatershed

Figure 3-7 provides a schematic of the Chino Creek subwatershed, including sources of flow (e.g., POTWs and Tier 1 sites) and flow diversions. DWF from most of the Chino Creek subwatershed does not reach the downstream compliance site at Central Avenue (WW-C7) because of diversions. For example, DWF in San Antonio Channel, the largest tributary to Chino Creek, is diverted into a series of retention basins that span from San Antonio Dam in the upper part of the subwatershed to Brooks Basin in the City of Montclair. Downstream of the diversion to Brooks Basin, there are five MS4 outfalls to Chino Creek that comprise nearly all the DWF (see Figure 3-7).

[6] Note: In past Triennial Reports "Non-MS4" sources were referred to as "unaccounted-for sources" or "*e*" (e.g., see CDM Smith 2013)

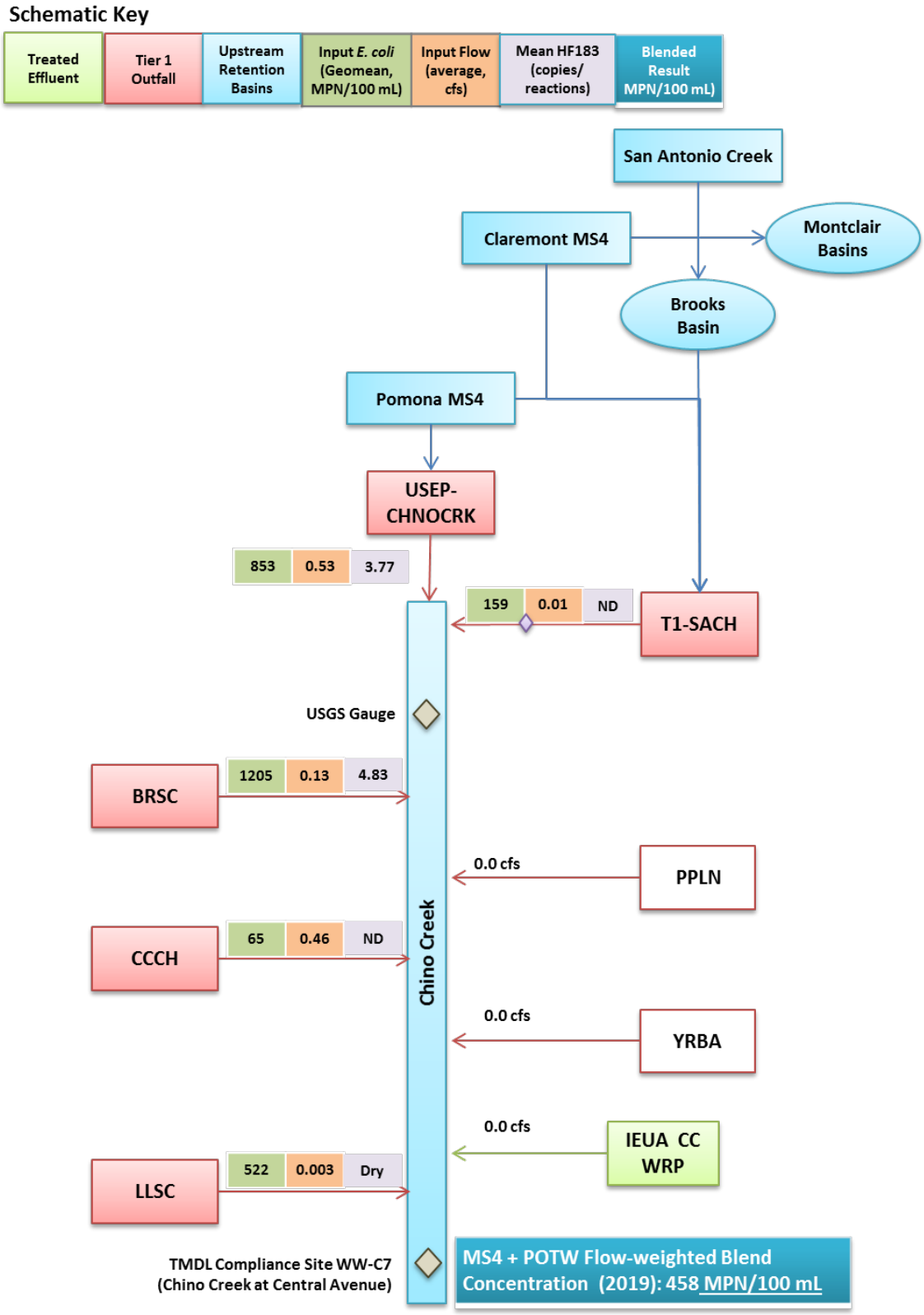

Figure 3-7. Schematic Showing Known Bacteria Inputs (*E. coli* and HF183 Gene Copies), DWF Inflows and POTW Effluent Discharges to Chino Creek in Relation to Downstream Compliance Monitoring Site

During the 2019 dry season and during the Synoptic Study, IEUA's Carbon Canyon WRP, the only source of treated effluent to Chino Creek, discharged no effluent to Chino Creek. Consequently, the source evaluation analysis for the Chino Creek watershed involves computation of a flow-weighted concentration for the five Tier 1 MS4 outfalls with DWF. The estimated blended *E. coli* concentration was found to be greater than the concentration of *E. coli* at the downstream watershed-wide compliance monitoring site at Central Avenue (**Figure 3-8**). This finding suggests that in-stream processes yield a net decay in fecal bacteria between upstream sources and the impaired portion of Chino Creek, and that non-MS4 sources of *E. coli* in Chino Creek are likely to be minimal during dry weather.

Figure 3-9 shows that significant week to week variability exists in the relative *E. coli* load to Chino Creek among Tier 1 sites. Because multiple sites contribute the majority of *E. coli* loads during some weeks, future *E. coli* mitigation activities may need to address multiple drainages within the Chino Creek subwatershed to effectively reduce the *E. coli* load to meet the MS4 WLA.

3.1.4.2 Santa Ana River Subwatershed

Figure 3-10 provides a schematic of the Santa Ana River Reach 3 subwatershed, including sources of flow to the river. The source evaluation analysis for this subwatershed involved computation of a blended *E. coli* concentration from MS4 outfalls and the three POTWs that discharge treated effluent in this subwatershed: City of Riverside's WQCP, City of Colton and San Bernardino RIX facility, and the City of Rialto WWTP. Seven Tier 1 sites accounted for all DWF and associated *E. coli* bacteria from MS4 sources.

The estimated *E. coli* concentration in the MS4 and POTW blend was compared with actual concentrations in the Santa Ana River at MWD Crossing (WW-S1, **Figure 3-11**) and Santa Ana River at Pedley Avenue (WW-S4, **Figure 3-12**). These comparisons suggest the presence of additional non-MS4 sources of *E. coli* within this subwatershed, which is consistent with findings in previous mass balance analyses (e.g., CDM Smith 2013).

Figure 3-13 shows that two MS4 drainages consistently accounted for at least 85 percent of the E. coli load from MS4s discharging to the Santa Ana River upstream of the MWD Crossing compliance site:

- T1-MCSD – DWF is mostly from the City of Riverside underground MS4 system; and
- T1–SNCH - An open channel with DWF that is believed to be a combination of urban runoff from residential areas in Jurupa Valley and potentially rising groundwater.

For the three Tier 1 sites located between WW-S1 (MWD Crossing) and WW-S4 (Pedley Avenue), the site that comprised the majority of *E. coli* load to this reach of the Santa Ana River Reach 3 varied from week to week (**Figure 3-14**).

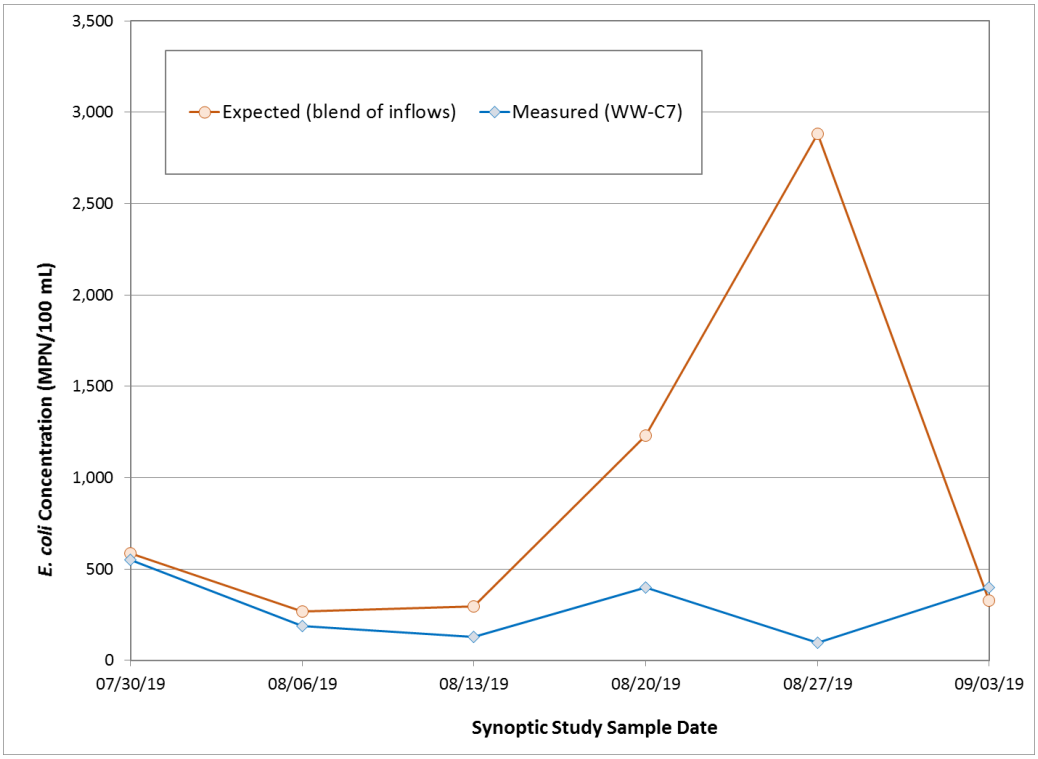

Figure 3-8. Comparison of Estimated Blended *E. coli* Concentration of MS4 Inflows with Downstream Watershed-wide Compliance Site Data for Chino Creek at Central Avenue

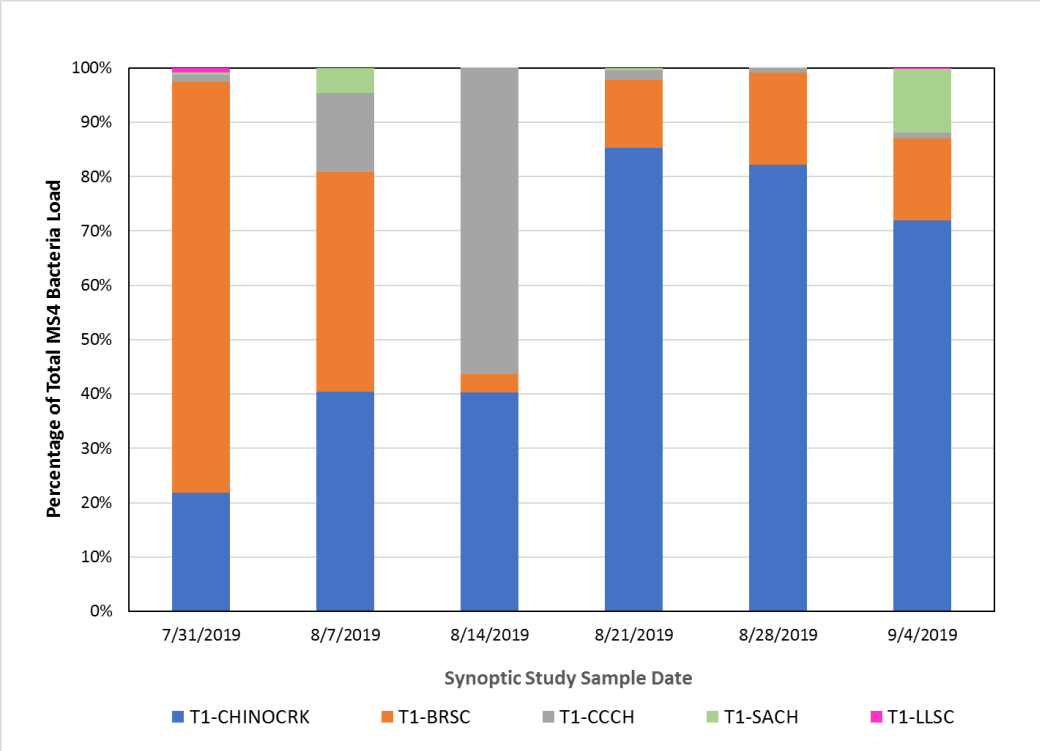

Figure 3-9. Relative Loading from Tier 1 Sites to Total MS4 *E. coli* Load to the Chino Creek at Central Avenue Compliance Site

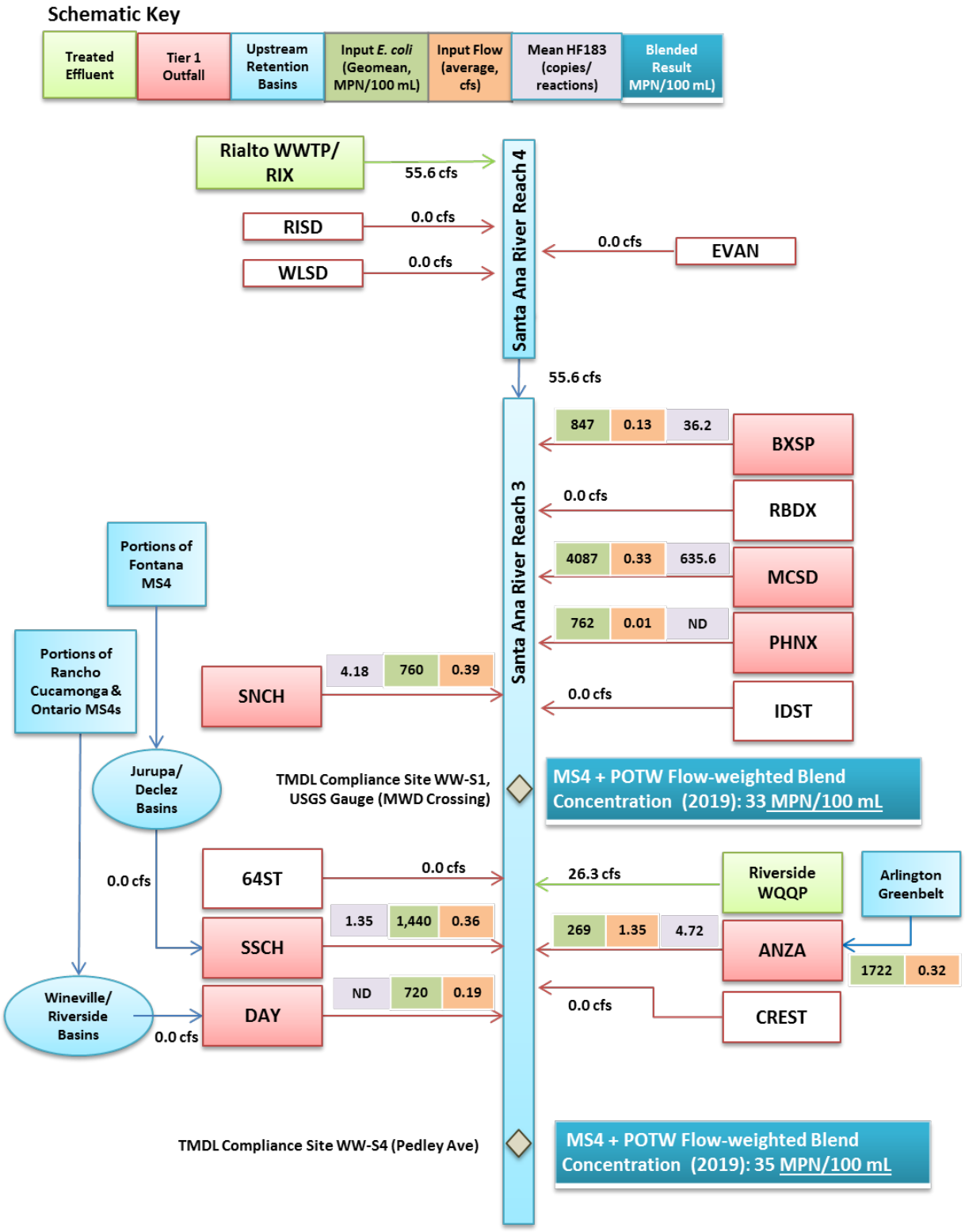

Figure 3-10. Schematic Showing Known Bacteria Inputs (*E. coli* and HF183 gene copies), DWF Inflows and POTW Effluent Discharges to the Santa Ana River in Relation to Downstream Compliance Monitoring Sites

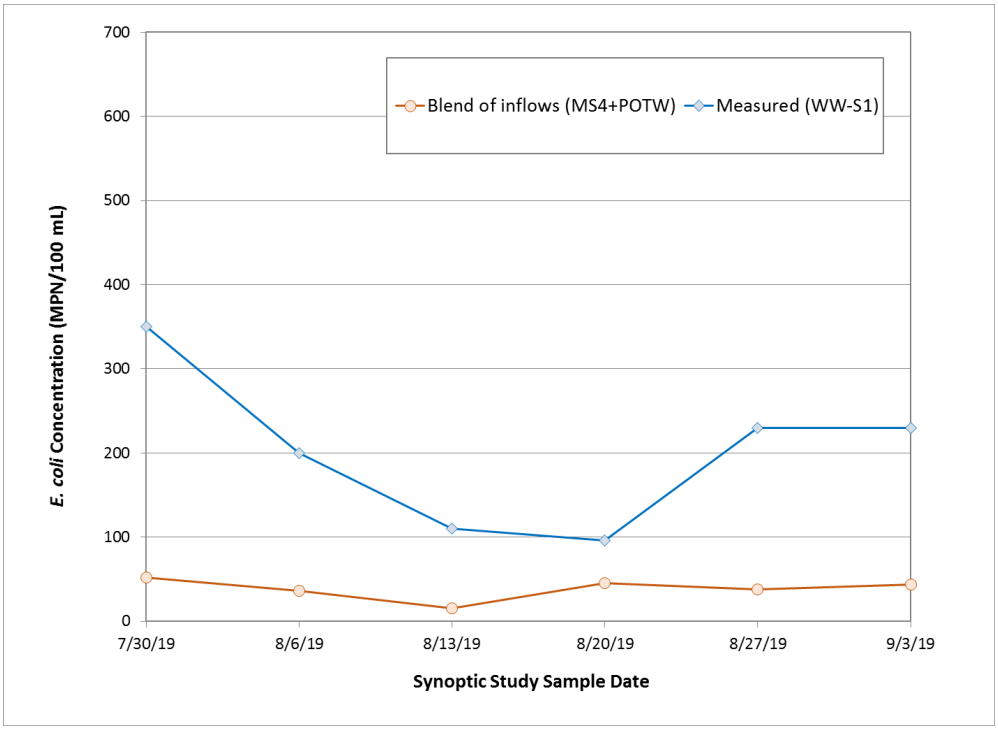

Figure 3-11. Comparison of Estimated Blended *E. coli* Concentration of MS4 Inflows with Downstream Watershed-wide Compliance Monitoring Data for Santa Ana River at MWD Crossing

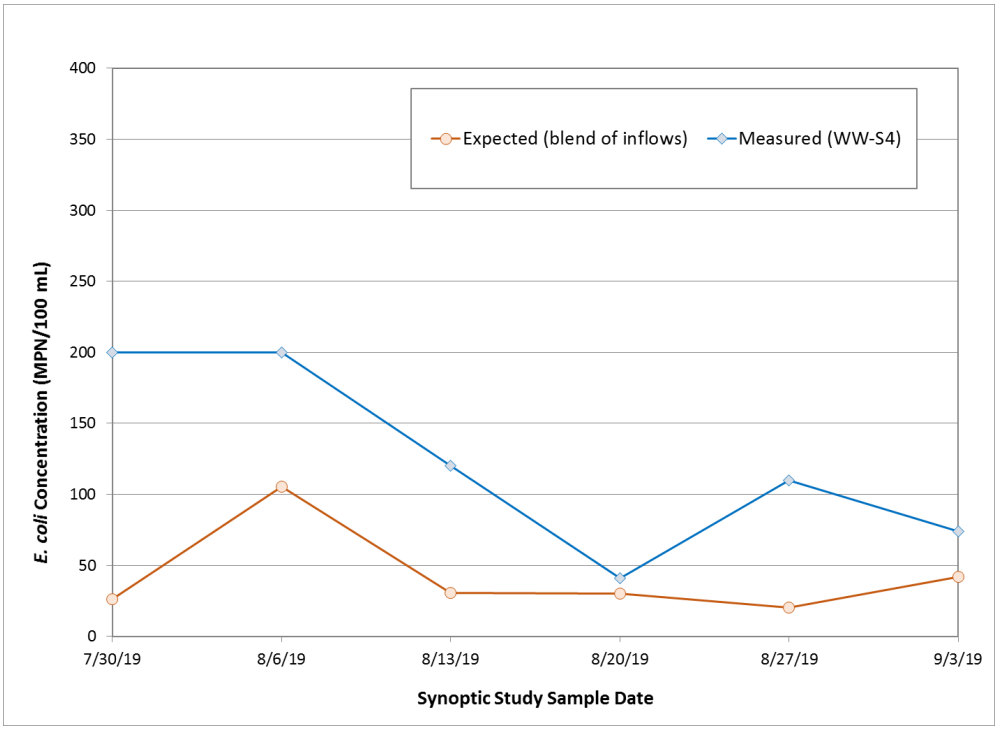

Figure 3-12. Comparison of Estimated Blended *E. coli* Concentration of MS4 Inflows with Downstream Watershed-wide Compliance Monitoring Data for Santa Ana River at Pedley Avenue

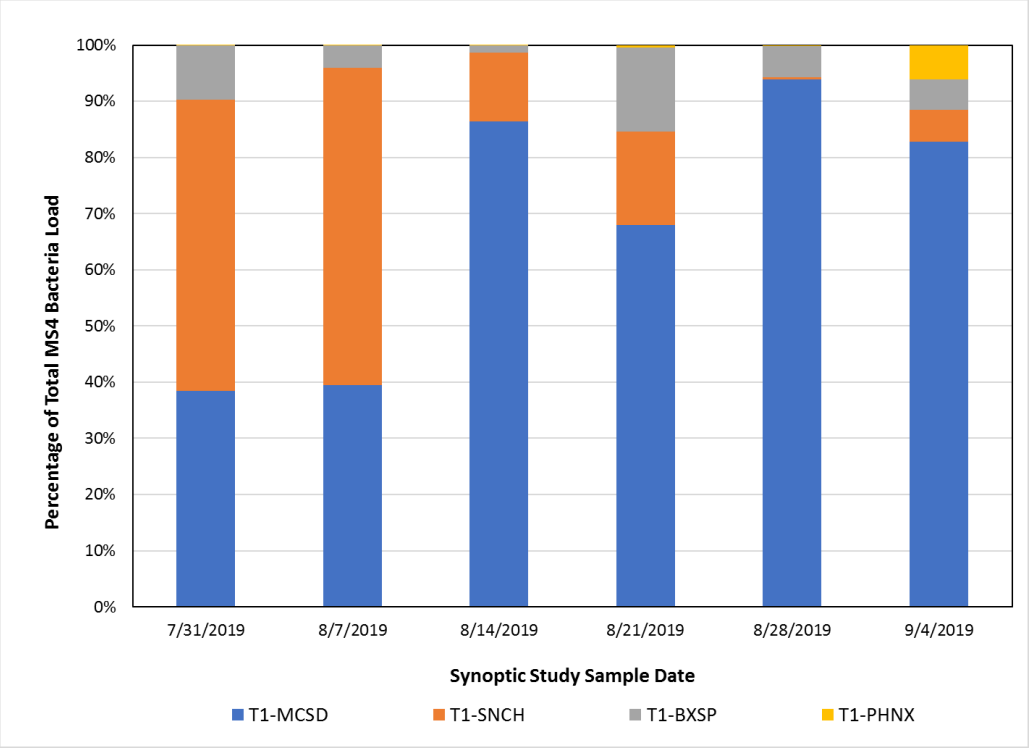

Figure 3-13. Relative Contribution from Tier 1 Sites to Total MS4 *E. coli* Load to the Santa Ana River at MWD Crossing Compliance Site

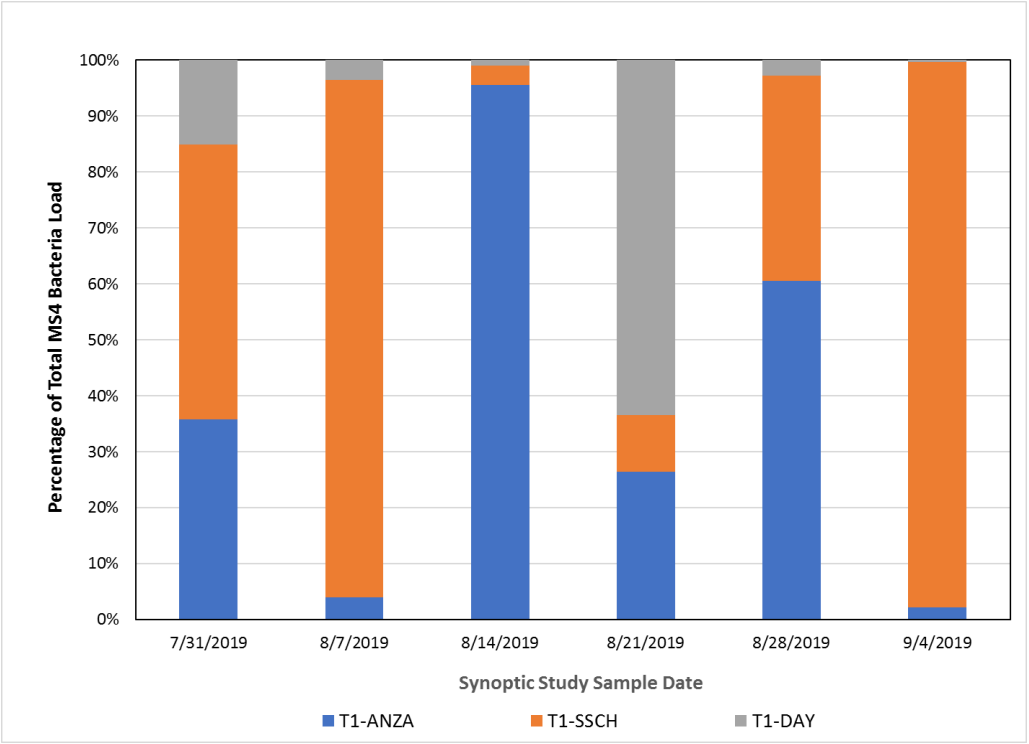

Figure 3-14. Relative Loading from Tier 1 Sites to Total MS4 *E. coli* Load at the Santa Ana River at Pedley Avenue TMDL Compliance Monitoring Location

3.1.4.3 Cucamonga Creek Subwatershed

On April 8, 2015,[7] USEPA approved the amendment to the Santa Ana Region Basin Plan to remove REC1 use from Cucamonga Creek Reach 1 based on findings from use attainability analysis for this segment.[8] Prior to this regulatory decision, numerous drainages that discharge to Cucamonga Creek Reach 1 were classified as Tier 1 sites; thus, the 2012 source contribution analysis included an evaluation of nine MS4 outfalls that discharged into Cucamonga Creek between 23rd Street in Upland at the upper end of the reach and Hellman Avenue Bridge, at the lower end of the reach. With the removal of REC1 from Cucamonga Creek Reach 1, all of these 2012 Tier 1 sites became Tier 2 and the only Tier 1 site in this subwatershed is where Cucamonga Creek Reach 1 drains into Mill-Cucamonga Creek downstream of Hellman Avenue (T1-CUCAMONGA).

Per the objectives of the Synoptic Study, flow and bacteria data from the T1-CUCAMONGA site will be used to prioritize the site along with all other Tier 1 sites in the MSAR watershed. While useful from an overall watershed standpoint, the findings from this site on their own do not provide information regarding where to prioritize future DWF/*E. coli* mitigation activities in the MS4 within the Cucamonga Creek Subwatershed. To assist with that evaluation, data collected during the dry-season over a ten week period in each of the three years (2016, 2017, and 2018) were evaluated for the purposes of this report (SBCFCD 2016, 2017, 2018).

During the 10-week sampling program from 2016 to 2018, samples of DWF at T2-SR60 had relatively low concentrations of *E. coli* (geomean of 87 MPN/100 mL). These concentrations were even lower when evaluating data from only 2017 and 2018 (geomean of 20 MPN/100 mL). As noted above, four other Tier 2 sites convey DWF from MS4 outfalls to Cucamonga Creek downstream of T2-SR60. These sites and the availability of data for this analysis include:

- T2-CHRIS - SBCFCD collected 30 samples at the Chris Basin outflow; these data show there is a persistent *E. coli* load in the Lower Deer Creek drainage area.
- T2-CLCH – No DWF was observed on any sample data during the 2016 to 2018 data collection period.
- T2-EVLA and T2-EVLB - Data was not collected by SBCFCD at either of these two Eastvale MS4 outfalls during the 10-week 2016-2018 sample program. Data from 2012 were used for the purpose of this source evaluation analysis.

Figure 3-15 provides a schematic of the portion of the Cucamonga Creek watershed that is relevant to potential contributions of DWF and bacteria to the downstream T1-CUCAMONGA site. The majority of the Cucamonga Creek watershed is hydrologically

[7] https://www.waterboards.ca.gov/santaana/water_issues/programs/basin_plan/docs/2015/Santa_Ana_Basin_UAA_Approval_Letter_040815.pdf
[8] https://www.waterboards.ca.gov/santaana/water_issues/programs/basin_plan/docs/rec_standards/UAA/Cucamonga_UAA_10-7-13_Final.pdf

disconnected during dry weather as a result of diversions for groundwater recharge at Turner and Ely Basins. Downstream of these retention basins, there are nine major MS4 outfalls to Cucamonga Creek that were key sources of DWF and *E. coli* data for the 2012 Tier 1 source evaluation (CDM Smith 2013). Four of these sites (T2-CAPT, T2-CNRW, T2-CFRN, and T2-WCUC) are upstream of the Cucamonga Creek at State Route 60 (T2-SR60) sample location; therefore, can be represented by data collected from this one monitoring location. Figure 3-15 shows how various sources of flow and *E. coli* to Cucamonga Reach 1 translate to an expected downstream *E. coli* concentration at the Tier 1 site (T1-CUCAMONGA). For example, downstream of T2-SR60 four Tier 2 sites convey DWF from the MS4 to Cucamonga Creek Reach 1: T2-CHRIS and T2-CLCH in San Bernardino County; T2-EVLA and T2-EVLB in Riverside County.

IEUA's RP1 treated effluent is an important source of DWF to Cucamonga Creek Reach 1 (Figure 3-15). During the dry seasons of 2016 to 2018 effluent flow varied from 0 to 14 cfs, with day-to-day fluctuations as great as 8.1 cfs and 1.7 cfs on average. Effluent rates were not obtained for sub-daily timesteps, but it is reasonable to assume variability over a few hours could be substantial during periods when IEUA's operations require more or less water be added to their recycled water system. Thus, the effluent rate used in the mass balance analysis may not be representative of the volume from RP1 at the time samples were collected downstream. This reality makes it difficult to design a synoptic study that accurately balances DWF volume on sampled dates. The long-term average flow shown in Figure 3-15 (3.42 cfs) may be the best estimate of relative source contribution because such extremes are averaged. On the other hand, as noted above the presence of an average condition is not typical with regard to effluent discharge from RP1.

DWF and *E. coli* data were evaluated for each of the 30 sampled dates during the 2016 to 2018 time period. **Figure 3-16** plots the expected blend of MS4 and POTW effluent against measured *E. coli* in Cucamonga Creek at Hellman Avenue (T1-CUCAMONGA. Results suggest that the MS4 inflows adequately account for the measured *E. coli* downstream on most sampled dates and that there is likely a net decay within stream between the SR60 and Hellman Avenue bridges. These results are based on MS4 inflows from Eastvale (T2-EVLA and T2-EVLB) that are assumed to be unchanged since the 2012 source evaluation. As part of future Tier 2 source evaluation efforts, it may be appropriate to collect updated data from these Eastvale Tier 2 sites to support future estimates of sources of bacteria loads to the downstream Tier1 site (T1-CUCAMONGA).

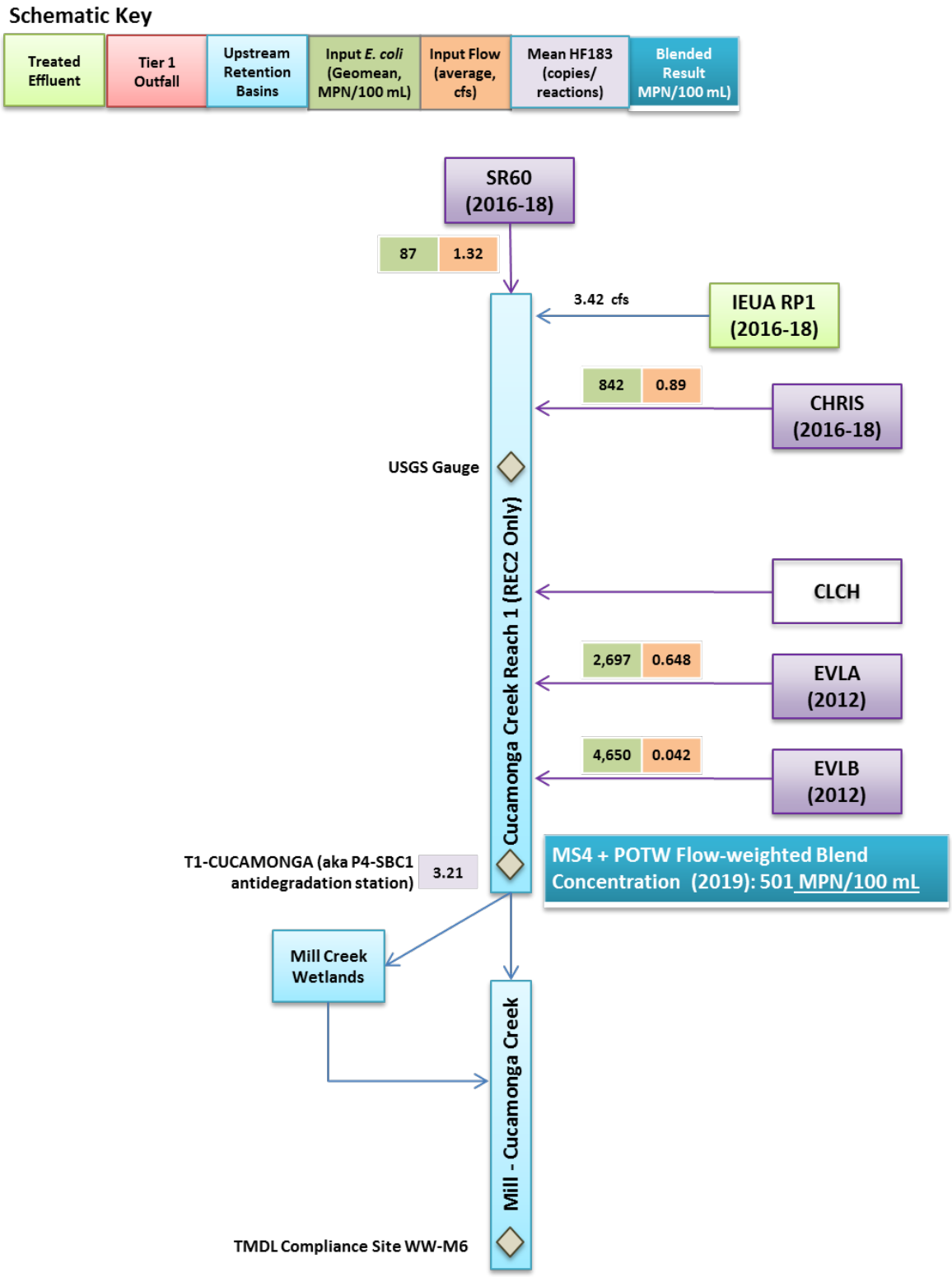

Figure 3-15. Schematic Showing Known Bacteria Inputs (*E. coli* and HF183 Gene Copies), DWF Inflows and POTW Effluent Discharges to Cucamonga Creek Reach 1 in Relation to the Downstream Compliance Monitoring Site

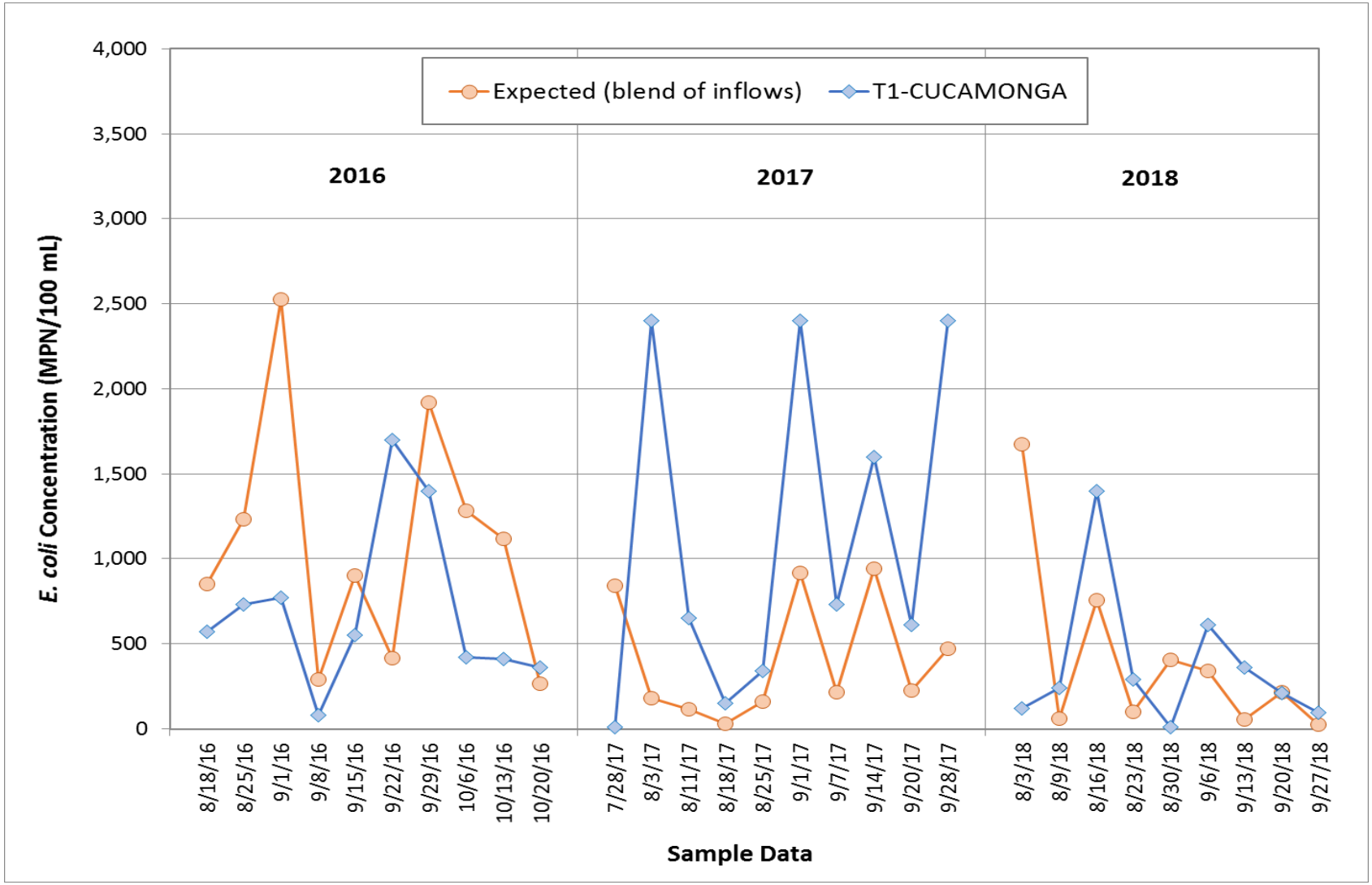

Figure 3-16. Comparison of Estimated Blended *E. coli* Concentrations in MS4 Inflows with Downstream Data from Cucamonga Creek at Hellman Avenue (T1-CUCAMONGA) (Note: Expected blend of inflows assumes 2019 DWF from Eastvale sites remains unchanged from 2012 (see text)

Downstream of Hellman Avenue, a portion of DWF is diverted to the Mill Creek Wetlands for treatment. The remainder is required to stay within Mill-Cucamonga Creek to support riparian habitat. The diversion flow restrictions are documented in a streambed alteration agreement.[9] This agreement is based on older dry weather flow records during a period when RP1 discharge rates were 5-10 times greater than current conditions. Currently, diversions to the Mill Creek Wetlands occurs on a regular basis, but no continuous metering is conducted on this flow split; therefore, it is challenging to balance upstream and downstream volumes.

Data collected to support the RBMP has shown a steady decline in *E. coli* concentrations at the downstream Mill-Cucamonga Creek watershed-wide compliance site (WW-M6). However, to date, no relationship between concentrations of *E. coli* at the upstream Tier 1 site (T1-CUCAMONGA) and *E. coli* concentrations at this compliance site has been found (**Figure 3-17**).

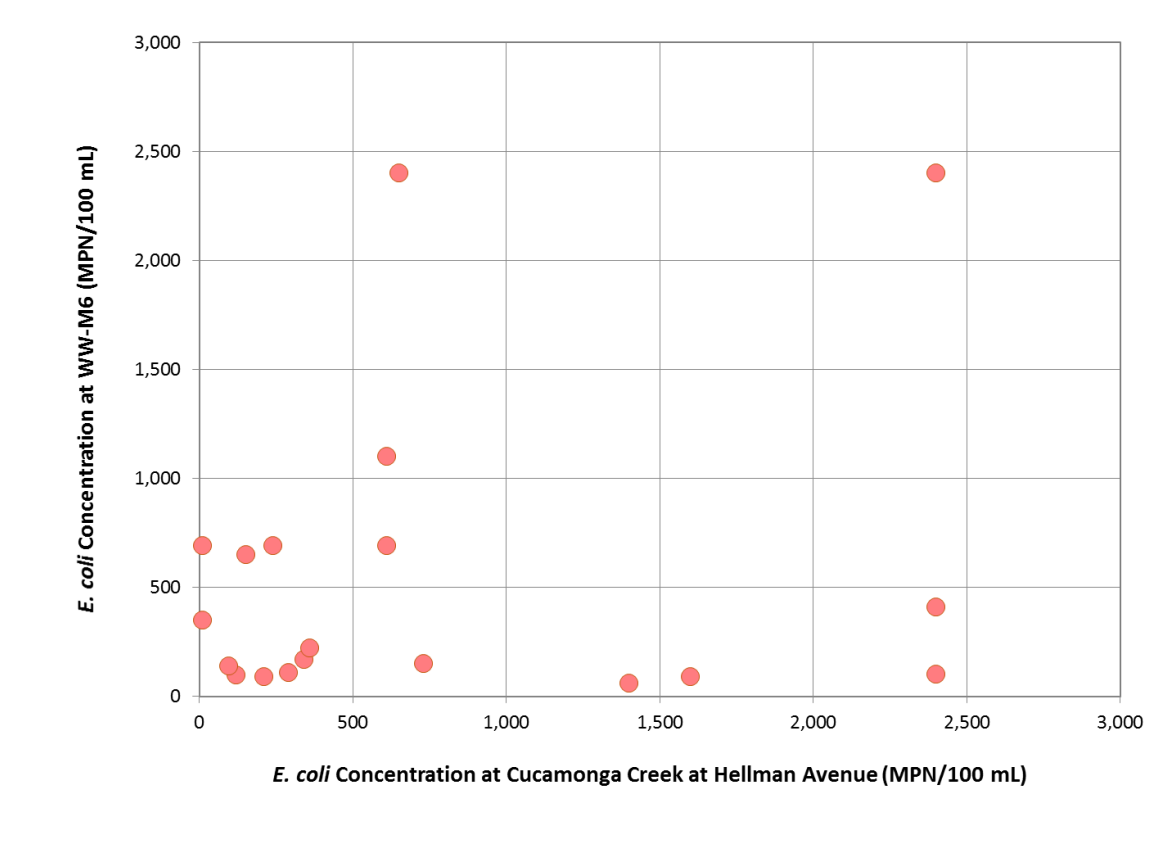

Figure 3-17. Comparison of *E. coli* Concentration at Cucamonga Creek at Hellman Avenue (T1-CUCAMONGA) (Upstream of Mill Creek Wetlands) and Mill-Cucamonga Creek Watershed-wide Compliance Site (WW-M6) (Downstream of Mill Creek Wetlands)

[9] *Reference to be determined*

3.1.5 Uncontrollable, Non-MS4 Bacteria Sources

Consistent with the many iterations of the source contribution analyses completed over a number of years, studies have shown that sources of fecal bacteria exist in the MSAR watershed that cannot be attributed solely to MS4 discharges. Historically, the basis for quantifying non-MS4 sources has involved a process of elimination, subtracting measured inflows from the MS4 from measured loads within the receiving waters.

In 2015, RCFC&WCD implemented the Uncontrollable Bacteria Sources Study (UBSS) (RCFC&WCD 2016), which evaluated the potential for uncontrollable sources of *E. coli* to influence *E. coli* concentrations in the MSAR watershed. For example, the UBSS study found that *E. coli* levels were higher in biofilm and sediment samples than levels in overlying water samples by as much as four orders of magnitude, indicating that biofilm and sediment behave as a reservoir for *E. coli*. In contrast, the outcome from investigations of other potential uncontrollable sources, e.g., bird activity, did not point to any predominant sources responsible for elevated levels of *E. coli*.

The UBSS also evaluated a segment of Santa Ana River Reach 3 to evaluate non-MS4 sources of bacteria in a reach where no MS4 outfalls are present. Given this unique characteristic, quantification of bacteria from non-MS4 sources within this Santa Ana River reach could be evaluated directly through collection of actual water quality samples rather than through estimates developed by data subtraction. This study provided an opportunity to further evaluate *E. coli* present in this Santa Ana Reach. Samples were collected from the Santa Ana River at the Riverside Avenue Bridge. This site was selected because:

- The only documented source of water to this portion of Reach 3 during dry weather conditions is tertiary treated effluent from the Rialto WWTP and RIX Facility (approximately 56 cfs). Upstream of these POTWs the Santa Ana River bed is dry.
- It is upstream of all MS4 outfalls to Santa Ana River Reach 3 and thus the MS4 cannot be causing or contributing *E. coli* bacteria to the impaired waterbody and these *E. coli* may be, for the most part, resulting from uncontrollable sources.[10]

The UBSS Study provided information regarding likely concentrations of *E. coli* that are present in the river without MS4 influence. **Figure 3-18** identifies the locations and monitoring programs that have collected *E. coli* samples from this Santa Ana River segment. Results from all of these programs were pooled to develop a rigorous estimate of *E. coli* concentration and load from non-MS4 sources in the WW-S1 subwatershed (**Figure 3-19**).[11]

[10] The Basin Plan defines "uncontrollable sources" as: wildlife activity and waste; bacterial regrowth within sediment or biofilm; resuspension from disturbed sediment; Concentrations (flocks) of semi-wild waterfowl; shedding during swimming,
[11] For this Study, it was confirmed from POTW monitoring reports that the treated effluent discharged to this Santa Ana River reach was in compliance with their *E. coli* effluent limits at the time samples were collected in the river.

Figure 3-18. Map of Monitoring Locations in Santa Ana River Segment with No MS4 Discharges

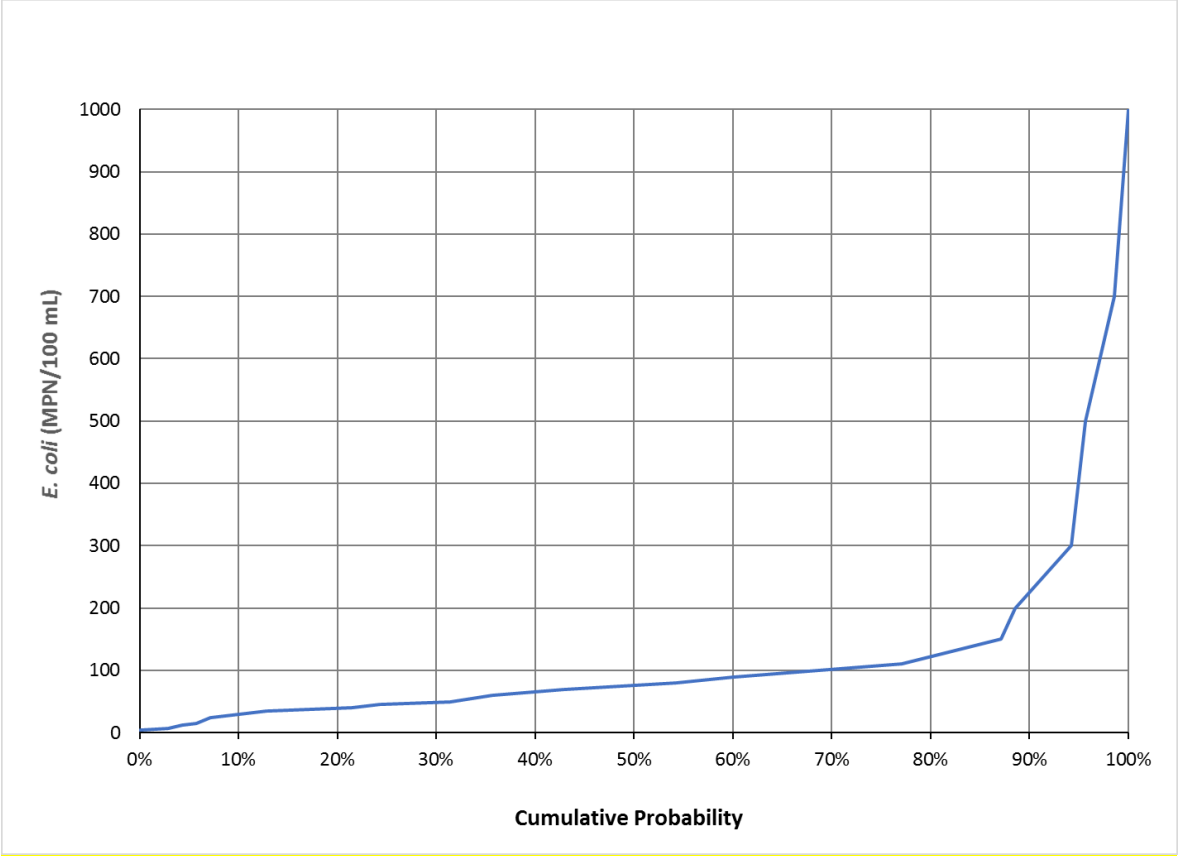

Figure 3-19. Cumulative Distribution Frequency of *E. coli* Concentrations in the Santa Ana River Segment Influenced by Non-MS4 Sources of Bacteria Only

During the six-week 2019 dry season synoptic study, *E. coli* samples were collected from the Mission Avenue Bridge, which is the most downstream site within the non-MS4 segment of the Santa Ana River. When the quantified non-MS4 load is accounted for in the source contribution analysis for WW-S1 (see Figures 3-10 and 3-11 above) over the six-week Synoptic Study, the following is apparent:

- Upstream *E. coli* sources more closely explain downstream observations;
- Majority of *E. coli* load comes from non-MS4 sources; and
- Weekly fluctuations in MS4 loads may not translate to measured differences within the Santa Ana River Reach 3 (Figure 3-20).

Non-MS4 *E. coli* loads at the Mission Avenue Bridge averaged 357 billion MPN/day (ranging from 121 to 831 billion MPN/day), which is significantly greater than the total *E. coli* load from all MS4 inflows upstream of the WW-S1 location which average 54 billion MPN/day (ranging from 22 to 73 billion MPN/day).

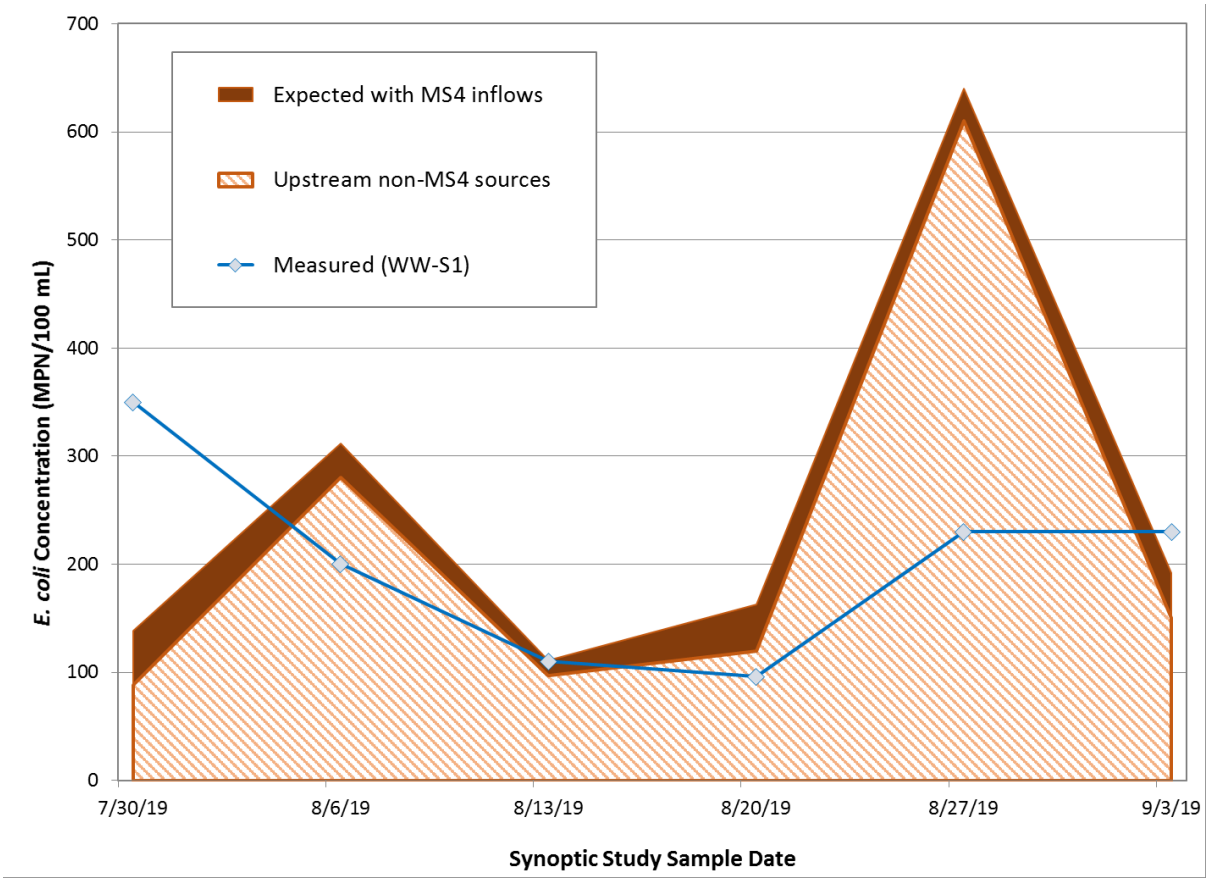

Figure 3-20. Comparison of Estimated Blended *E. coli* Concentrations of MS4 Inflows Plus Mission Bridge Non-MS4 Inflows with Downstream Watershed-wide Compliance Data at the Santa Ana River MWD Crossing Site

3.2 *Bacteroides* Analysis

Elevated levels of FIB have been identified in water bodies within the MSAR watershed, however, the sources of bacteria remain unknown. Generally, regulatory agencies commonly assess the microbial river water quality by determining the concentration of FIB using culture based assays for total coliforms, fecal coliforms and *E. coli*, because these assays are quick and economical. However, FIB measurements cannot determine whether the bacteria originate from human, animal, or natural sources (i.e., plants, sediments, etc.; Litton et al. 2010). Understanding the sources and categories of FIB is important so that the various contributions of FIB can determined and public health risks can be assessed (Soller et al. 2010, 2014).

3.2.1 Data Collection and Analysis for Bacteroides, Human Host-specific Marker (HF183)

An important objective of the Synoptic Study was to use appropriate MST techniques to determine the extent to which human sources may or may not be contributing to elevated *E.*

coli concentrations in the samples collected. To facilitate understanding of the findings from this study, the following information is provided regarding sample collection and laboratory analysis. The Study Plan and QAPP for the Synoptic Study provide additional information.

Host-associated genetic markers that allow for the identification of human gut bacteria, *Bacteroides* by quantitative real-time polymerase chain reaction (qPCR) have been widely used and recently approved by USEPA as standard method 1696. This method, which targets the HF183 16S rRNA gene cluster of *Bacteroides* (USEPA 2019), was used to determine the presence or absence, and the relative concentration of the human-host *Bacteroides* HF183 marker in water or effluent samples collected during this study. The standard curves for all qPCR reactions passed the acceptance criteria with amplification efficiencies in the range of 0.90 to 1.10 with $R^2 > 0.98$ (USEPA 2019). All qPCR reactions were run in triplicate, including controls and field blanks.

For the purposes of this study, a sample result was reported as not detected (ND) if the gene copy number was below the detection limit (DL) of the assay, 10 gene copies/2 microliters (µL); however, if the HF183 gene was amplified, then it was further quantified even when the concentration of the gene was below the DL of 10 gene copies/2 µL. This conservative approach was applied to be more protective of public health because it reasons that low concentrations of HF183 genes could warrant further investigations, and because not all qPCR replicates will amplify when the HF183 gene copy number is at the DL or lower. This same approach of reporting low concentrations of HF183 genes has been consistently applied in other studies (Cao et al. 2017). To improve the detection of the HF183 marker, a total volume of 200 mL was analyzed for all samples, except where noted in tables of results.

3.2.2 Evaluation of the HF183 Human Marker at Synoptic Study Sites

As described above, samples were collected to determine the *E. coli* concentrations and presence/absence and relative concentrations of the *Bacteroides* HF183 gene at all study sites. The frequencies and mean concentrations were reported using the following accepted approach (Cao et al. 2016):

- A positive sample is any sample in which the HF183 gene was amplified in any of the three qPCR replicates.
- A negative sample is a sample where the results are ND for the HF183 gene copies;
- The sample was below the DL but the quantified gene copies were regarded as positive (where the gene was amplified and quantifiable).

The mean concentration was calculated by summing the results (gene copies/reaction) from all positive samples and dividing by the total number of positive samples observed during the study. The following sections summarize the key findings.

3.2.2.1 *Bacteroides* HF183 Gene Concentrations in POTW Effluent Samples

The human marker HF183 gene was ND in all of the POTW effluent samples (**Table 3-4**). In these samples, the concentration of HF183 gene was too low to be amplified using the described qPCR assay.

This finding is inconsistent with another recently completed study in the MSAR watershed (Gedalanga et al. 2019). Gedalanga et al. (2019) reported that the human marker HF183 gene was detected in all Santa Ana River samples (except on 10/26/18 when an effluent sample was below detection for the HF183 gene). The findings of Gedalanga et al. (2019) are similar to those reported in previous studies by Bae and Wuertz (2009) in which *Bacteroides* gene copies were detected in untreated influent, heat-treated influent, and UV-treated effluent samples collected directly from the University of California, Davis wastewater treatment plant using a different human-host specific gene, the BacHum gene. In contrast, Litton et al. (2010) reported that HF183 was below detection in three different effluent samples collected from the Riverside RWQCP discharge location. The differences observed from effluent samples reported in the studies noted above and the findings from the Synoptic Study are likely due to:

- The effluent samples in the Synoptic Study were collected post-disinfection and chlorination, further inactivating and destabilizing the bacterial load which includes *Bacteroides*.
- Current improved plant performance result in lowering the bacterial load compared to the previous referenced studies (this interpretation is consistent with the conclusion reached by Litton et al. (2010).
- With disinfected samples, the qPCR standard method is deficient in detecting low gene copies of HF183. To address this issue, newer qPCR methods have been developed that employ droplet digital PCR (ddPCR). These methods are being used to provide greater resolution (Cao et al. 2016), especially in treated effluent samples, however, to date these methods are experimental.
- As determined in literature, the decay of host-specific makers in different environments can by influenced by a number of environmental factors and treatment processes, thus affecting their detection by qPCR (Ahmed et al. 2019). For this reason, it is more important to determine the absence or presence of the human marker at each specific site rather than focus on quantification.

Table 3-4. Detection and Quantification (Gene Copies/Reaction) of *Bacteroides* Human-host Specific Marker (HF183) in POTW Effluent Samples (ND= Below detection limit of assay, 10 gene copies/2 µL)

Site ID	7/30-7/31/19		8/06-8/07/19		8/13-8/14/19		8/20-8/21/19		8/27-8/28/19		9/03-9/04/19	
	Result	Quantity	Result	Quantity	Result	Quantity	Result	Quantity	Result	Quantity	Result	Quantity
Rialto WWTP	ND	--	ND	--	ND	--	ND	--	ND	--	ND	--
RIX	ND	--	ND	--	ND	--	ND	--	ND	--	ND	--
Riverside RWQCP	ND	--	ND	--	ND	--	ND	--	ND	--	ND	--
RP-1	ND	--	ND	--	ND	--	ND	--	ND	--	ND	--

3.2.2.2 *Bacteroides* HF183 Gene Concentrations at Watershed-wide Compliance Sites

For the watershed-wide compliance sites, *Bacteroides* HF183 was detected at a frequency of 45.6% with a range of 0.6 to 10.82 copies of HF183 genes, with a mean concentration of 3.76 HF183 gene copies detected for all of the watershed-wide compliance sites (**Table 3-5**). For the Chino Creek and Mill-Cucamonga sites, *Bacteroides* HF183 was only detected in the last sample collected from each site (week of September 3). In contrast, *Bacteroides* HF183 was detected during most sample events at the Santa Ana River Reach 3 MWD Crossing and Pedley Avenue compliance sites. Observations varied during the study period. For example, during the first sample week (week of July 29), all watershed-wide compliance samples were negative for the human marker, whereas, during the week of September 3 all samples were positive for the human marker, although at relatively low concentrations (**Figure 3-21**). The mean concentration of the human marker at the Santa Ana River at MWD Crossing site was 4.96 at a frequency of 66.7%. At the downstream site at Pedley Avenue, the frequency of human marker detection was higher (83.3% with a mean concentration of 3.52).

3.2.2.3 *Bacteroides* HF183 Gene Concentrations at Mainstem Santa Ana River Sites

The human marker was detected at all mainstem Santa Ana River sites at a frequency of 65% with a range of 0.94 to100 copies of HF183 genes, with a mean concentration of 9.70 HF183 gene copies detected **(Table 3-6**). The frequency of detections increased from the most upstream site (Santa Ana River Reach 4 above South Riverside Avenue Bridge, P3-SBC1) to the most downstream mainstem non-compliance site (Santa Ana River at 64th St, 64THST). The highest observed result was at the Santa Ana River Mission Blvd site (MISSION) during the week of August 12 (100 gene copies/reaction).

Table 3-7 combines the results from the Santa Ana River Reach 3 watershed-wide compliance site and additional Santa Ana River mainstem sites in order from upstream to downstream. Human marker HF183 was observed at all Santa Ana River sites with the highest frequencies observed the last two weeks of the Synoptic Study. The highest numbers of copies/reaction were observed the week of August 12 with two sites showing results higher than the detection limit of 10 gene copies/2 µL.

The Mission Blvd results are of particular interest in this study, given the Mission Blvd site does not receive MS4 discharge. Moreover, the human marker was not detected in any of the effluent samples analyzed from the Rialto WWTP or RIX, the sources of flow at the Mission Blvd site. Therefore, these facilities were not the source of human bacteria source at the Mission Blvd site during the week of August 12. Additionally, during this same week the human marker was not detected at the upstream Santa Ana River Reach 4 above South Riverside Avenue Bridge (P3-SBC1) site (see Table 3-7). Combined, these observations suggest the presence of an additional non-MS4 human source at the Mission Blvd site that was subsequently diluted and degraded over time but still persisted. Interestingly, at the same time *E. coli* concentrations were relatively low at this site (97 MPN/100 mL).

Table 3-5. Detection and Quantification (Gene Copies/Reaction) of *Bacteroides* Human-host Specific Marker (HF183) at Watershed-wide Compliance Sites (ND= Below detection limit of assay, 10 gene copies/2 µL)

Site ID	7/30-7/31/19		8/06-8/07/19		8/13-8/14/19		8/20-8/21/19		8/27-8/28/19		9/03-9/04/19	
	Result	Quantity	Result	Quantity	Result	Quantity	Result	Quantity	Result	Quantity	Result	Quantity
WW-C7	ND	--	ND	--	ND	--	ND	--	ND	--	ND	1.89
WW-M6	ND	--	ND	--	ND	--	ND	--	ND	--	ND	3.21
WW-S1	ND	--	ND	2.05	ND	6.87	ND	2.41	ND	1.95	ND	4.33
WW-S4	ND	--	ND	0.6	Detected	10.82	ND	--	ND	6.31	ND	0.96

Table 3-6. Detection and Quantification (Gene Copies/Reaction) of *Bacteroides* Human-host Specific Marker (HF183) at Mainstem Santa Ana River Non-compliance Sites (ND= Below detection limit of assay, 10 gene copies/2 µL)

Site ID	7/30-7/31/19		8/06-8/07/19		8/13-8/14/19		8/20-8/21/19		8/27-8/28/19		9/03-9/04/19	
	Result	Quantity	Result	Quantity	Result	Quantity	Result	Quantity	Result	Quantity	Result	Quantity
P3-SBC1	ND	--	ND	--	ND	--	ND	--	ND	0.94	ND	1.68
MISSION	ND	--	ND	2.72	Detected	100	ND	1.38	ND	5.84	ND	3.76
64THST	ND	1.08	ND	2.01 (Duplicate = 0.96)	ND	--	ND	1.02	ND	2.24	ND	2.49

Table 3.7. Detection and Quantification (Gene Copies/Reaction) of *Bacteroides* Human-host Specific Marker (HF183) at Watershed-wide Compliance Sites and Mainstem Santa Ana River Non-compliance Sites Ordered from Upstream (P3-SBC1) to Downstream (64THST) (ND= Below detection limit of assay, 10 gene copies/2 µL)

Site ID	7/30-7/31/19		8/06-8/07/19		8/13-8/14/19		8/20-8/21/19		8/27-8/28/19		9/03-9/04/19	
	Result	Quantity	Result	Quantity	Result	Quantity	Result	Quantity	Result	Quantity	Result	Quantity
P3-SBC1 (Upstream)	ND	--	ND	--	ND	--	ND	--	ND	0.94	ND	1.68
MISSION	ND	--	ND	2.72	Detected	100	ND	1.38	ND	5.84	ND	3.76
WW-S1	ND	--	ND	2.05	ND	6.87	ND	2.41	ND	1.95	ND	4.33
64THST	ND	1.08	ND	2.01 (Duplicate = 0.96)	ND	--	ND	1.02	ND	2.24	ND	2.49
WW-S4 (Downstream)	ND	--	ND	0.6	Detected	10.82	ND	--	ND	6.31	ND	0.96

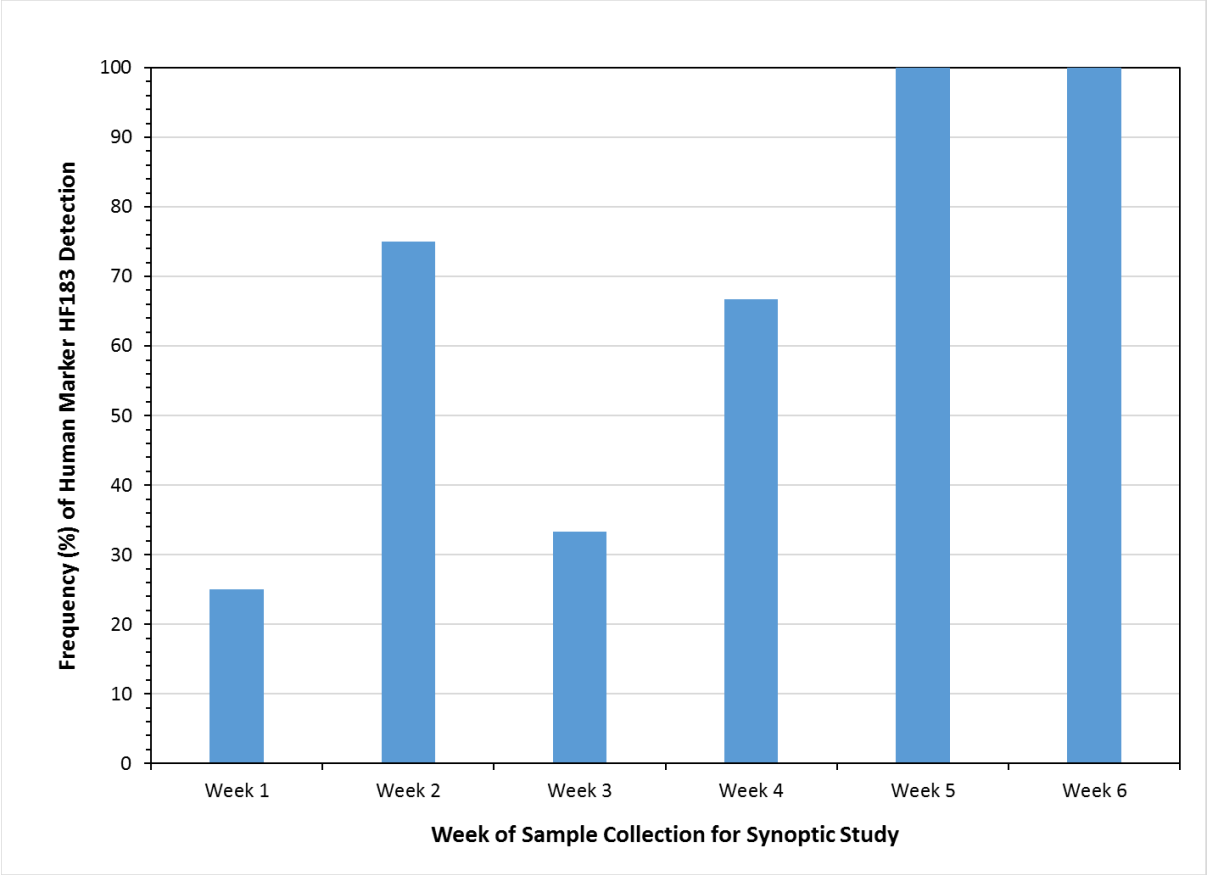

Figure 3-21. Frequency of *Bacteroides* Human-host Specific Marker (HF183) at Watershed-wide Compliance Sites (see Table 3-5).

It has been demonstrated that the persistence of *Bacteroides* in surface water depends on environmental factors, in particular temperature, ultraviolet (UV) inactivation by sunlight, and predation by other bacterial species (Kreader 1998; Bell et al. 2009; Boehm et al. 2018; Ahmed et al. 2019). It has been shown that the *Bacteroides* can persist between 1-14 days in surface water depending on environmental conditions with an average of 3-4 days. It has also been reported that the HF183 gene can decay faster than pathogens under certain environmental conditions (Ahmed et al. 2019).

3.2.2.4 *Bacteroides* HF183 Gene Concentrations at Tier 1 Sites

For Tier 1 sites the human marker was detected at a frequency of 30% with a range of 0.83-1,643.4 copies of HF183 genes. For all Tier 1 sites the mean concentration was 168.4 HF183 gene copies (**Table 3-8**). The human marker was detected often, with the highest percentage of positive samples identified during the week of August 19 (week 4) and the lowest during the week of August 5 (week 2) (**Figure 3-22**). Following is a discussion of different HF183 patterns observed that demonstrate that both the human marker and *E. coli* concentrations are variable across the Tier 1 sites during DWF:

Table 3-8. Detection and Quantification (Gene Copies/Reaction) of *Bacteroides* Human-host Specific Marker (HF183) in Tier 1 and Tier 2 Samples (ND= Below detection limit of assay, 10 gene copies/2 µL)

Site ID	7/30-7/31/19		8/06-8/07/19		8/13-8/14/19		8/20-8/21/19		8/27-8/28/19		9/03-9/04/19	
	Result	Quantity	Result	Quantity	Result	Quantity	Result	Quantity	Result	Quantity	Result	Quantity
T1-ANZA	ND (duplicate = ND)	--	ND	--	ND	4.72	ND	--	ND (duplicate = ND)	--	ND	--
T1-BRSC	ND	1.2	ND	--	ND	1.7	ND (duplicate = 2.94)[1]	--	ND	9.84	ND	0.83
T1-BXSP	ND	8.07	ND	--	Detected	131.91	ND (duplicate = 7.71)[2]	7.52	ND	1.73	Detected	31.69[3]
T1-CCCH	ND	--	ND (duplicate = ND)	--	ND	--	ND	--	ND	--	ND	--
T1-CHINOCRK	ND	3.77	ND	--	ND	--	ND	--	ND (duplicate = ND)	--	ND	--
T1-CUCAMONGA	ND	--	ND	--	ND (duplicate = ND)	--	ND	--	ND	--	ND	2.76
T1-CYP	Dry	--	Dry	--	Dry	--	Dry	--	Dry	--	Dry	--
T1-DAY	ND	--	ND	--	ND	--	ND	--	ND	--	ND (duplicate = ND)	--
T1-LLSC	ND	--[4]	Dry	--	Dry	--	Dry	--	Dry	--	Dry	--
T1-MCSD	Detect	279.19	Detect	158.43	Detect	1643.36	Detect	281.02	Detect	951.71	Detect	499.82
T1-PHNX	ND	--	ND	--	ND (duplicate = ND)	--	ND	--	ND	--	ND	--
T1-SACH	ND	--	ND	--	ND	--	ND	--	ND	--	ND	--
T1-SNCH	ND	5.63	ND	--	ND	5.24	ND	4.09	ND	1.76	ND	--
T1-SSCH	ND	--	ND	--	ND	--	ND	1.35	ND	--	ND	--

Table 3-8. Detection and Quantification (Gene Copies/Reaction) of *Bacteroides* Human-host Specific Marker (HF183) in Tier 1 and Tier 2 Samples (ND= Below detection limit of assay, 10 gene copies/2 µL)

Site ID	7/30-7/31/19		8/06-8/07/19		8/13-8/14/19		8/20-8/21/19		8/27-8/28/19		9/03-9/04/19	
	Result	Quantity	Result	Quantity	Result	Quantity	Result	Quantity	Result	Quantity	Result	Quantity
T2-CYP2	ND (duplicate = ND)[4]	--	ND	--	ND	--	ND	--	ND	--	ND (duplicate = ND)	--
T2-HOLE	ND	--	ND	--	ND	--	ND	--	ND	1	ND	--

[1] *Bacteroides* detected in blank sample = 0.97
[2] *Bacteroides* detected in blank sample = 0.37
[3] Total volume analyzed was 150 mL instead of 200 mL
[4] Total volume analyzed was 100 mL instead of 200 mL

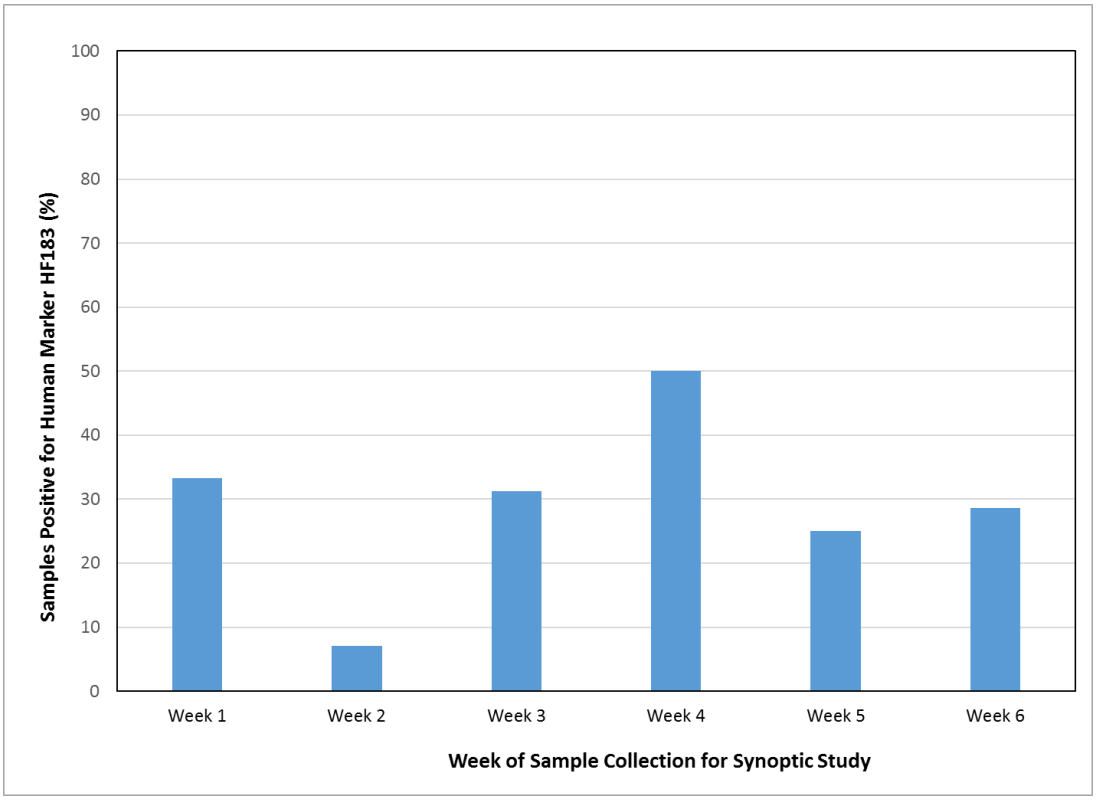

Figure 3-22. Frequency of *Bacteroides* Human-host Specific Marker (HF183) at Tier 1 Sites (see Table 3-8).

- *High Number of HF183 Gene Copies/High* E. coli *Concentrations* - Relatively high concentrations of the human marker were detected at the Magnolia Center Storm Drain site (T1-MCSD) where the mean concentration was 635.6 HF183 gene copies. High *E. coli* concentrations corresponded with the high gene copy numbers at T1-MCSD, especially during the weeks of August 12 and August 27 when *E. coli* concentrations were 1,900 and 4,100 MPN/100 mL, respectively.
- *High Number of HF183 Gene Copies/Varied* E. coli *Concentrations* - Relatively high concentrations of the human marker were also detected at the Box Springs Channel site (T-BXSP), where the mean concentration was 36.2 HF183 gene copies, respectively. The corresponding *E. coli* concentrations varied. For example, the weeks of August 12 and September 3 had the highest human marker result results (131.91 and 31.69 HF183 gene copies, respectively). The corresponding *E. coli* concentrations were 74 and 1,100 MPN/100 mL, respectively.
- *Low Number of HF183 Gene Copies/High* E. coli *Concentrations* – At the Phoenix Storm Drain site (T1-PHNX), high concentrations of *E. coli* were observed, but the human marker was consistently below detection. A similar pattern was observed at San Sevaine Channel (T1-SSCH). High concentrations of *E. coli* (> 1000 MPN/100 mL) were routinely observed but the concentration of the human marker was relatively low (mean 1.35 gene copies).

The variability in both the human marker and *E. coli* concentrations across the Tier 1 sites is consistent with previous findings by Gedalanga et al., (2019) and Litton et al. (2010). Variable results may occur because there are many different factors that may influence bacteria concentrations. As stated above, the human marker can persist in the environment on average for 3-4 days depending on the surface water conditions, which includes temperature and predation (Ahmed et al. 2019). Bell et al. (2009) demonstrated that *Bacteroides* can decay at a much slower rate at lower temperatures than at higher temperatures (i.e., 25° C).

For this study, the frequency of the human marker was compared with water temperature at Tier 1 sites (**Figure 3-23**). However, no clear discernable relationship was observed from this data set. For example, relatively low temperatures were observed at T1-MCSD and T1-SNCH but the frequency of the human marker was generally higher than other sites. At the site with the lowest observed temperature of approximately 20° C (T1-SSCH), the frequency of human marker detection was also low (16.6%). Conversely, the frequency of the human marker at T1-ANZA was very low (10%) but the temperature was relatively high (23.8° C). Therefore, the differences in the frequency of the human marker do not appear to be related to temperature. However, the dataset is limited. Moreover, because the characteristics of each site vary and are complex (e.g., variable DWF, predation, dilution, decay rates, etc.), site specific investigations would be necessary to further understand relationships between *Bacteroides* and *E. coli* in this watershed.

3.2.2.5 *Bacteroides* HF183 Gene Concentrations at Tier 2 Sites

The T2-HOLE site was included to isolate the *E. coli* load and evaluate the presence/absence of the human marker in the Hole Lake area upstream of the Tier 1 Anza Drain site (T1-ANZA). The T2-CYP2 site was included in the Synoptic Study to evaluate *E. coli* loads and human marker presence/absence in the City of Chino MS4 area downstream of the California Institute of Men agricultural fields. As shown in Table 3-8, the human marker was observed only once at T2-HOLE, the week of August 26 (corresponding *E. coli* concentration was approximately 550 MPN/100 mL). The human marker was not detected during any week of the study at the T2-CYP2 site.

3.2.3 Relationship between E. coli Concentrations and Bacteroides Detections

The relationship between human *Bacteroides* detection and *E. coli* concentration data shows the effectiveness of using the combination of bacterial indicators to assess potential health risks to recreational users. The *E. coli* data were divided into two datasets: (a) *E. coli* concentrations of sample results where no HF183 human marker was detected; and (b) *E. coli* concentrations associated with sample results where the HF183 human marker was detected. **Figure 3-24** compares these datasets for (a) all Santa Ana River sites (Watershed-wide compliance and Mainstem River sites); and (b) all MS4 sites (Tier 1 and Tier 2). The difference in the stratified datasets were shown be statistically significant (**Table 3-9**).

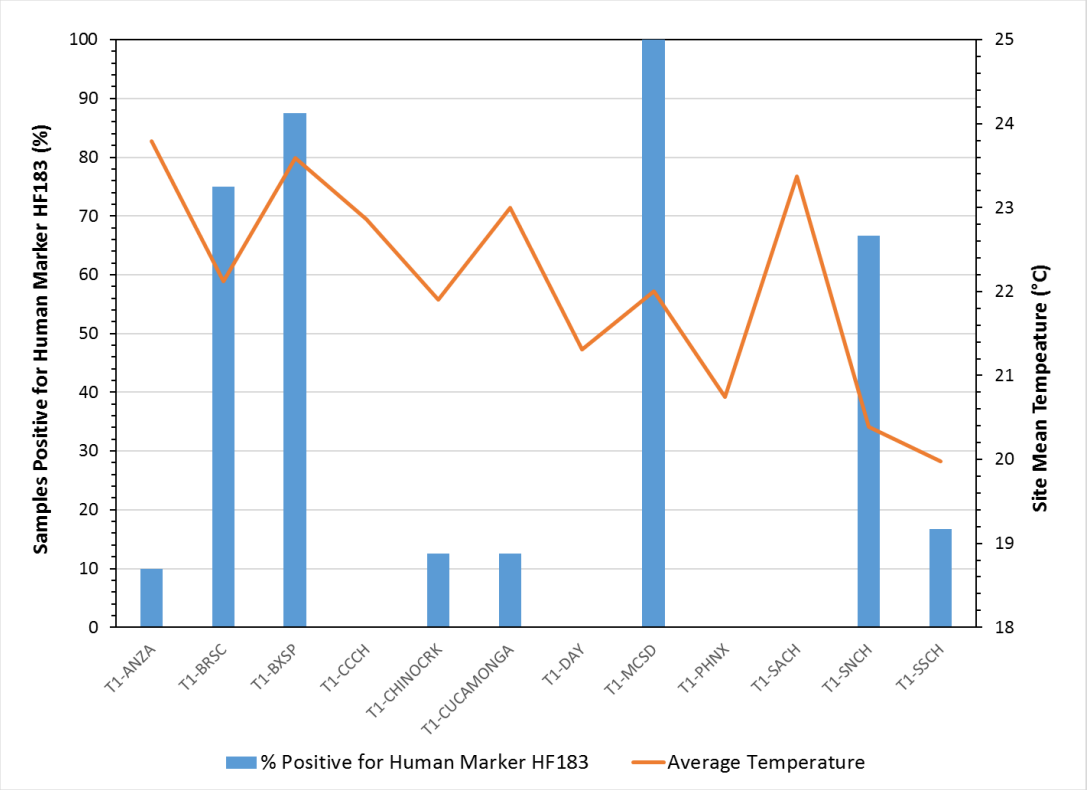

Figure 3-23. Frequency of HF183 at Tier 1 Sites Compared to the Average Water Temperature during the Synoptic Study.

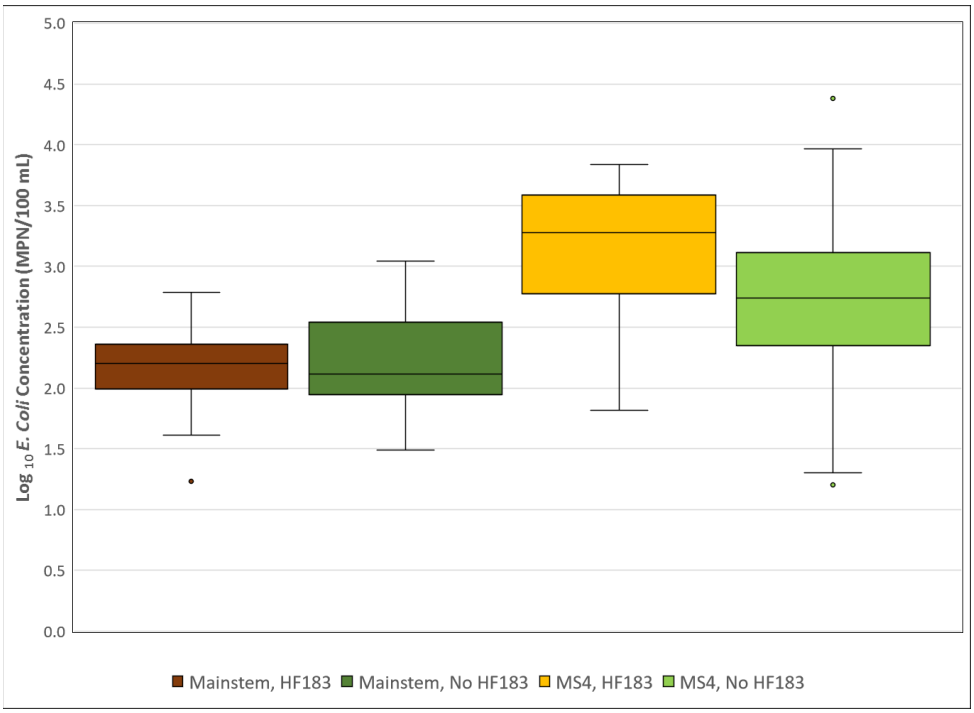

Figure 3-24. Box-Whisker Plots of *E. coli* Concentrations in Samples with/without Detection of Human Marker HF183 for all MS4 sites (Tier 1 and 2) and Santa Ana River Sites.

Table 3-9. Student T-Test Results Comparing *E. coli* Concentrations in Samples with/without Detection of Human Marker HF183 for all Tier-1 and 2 Sites

E. coli Data Set	N	*E. coli* Geomean (MPN/100 mL)	P-Value
Human Marker HF183 Detected	25	1,270	0.008
Human Marker HF183 Not Detected	61	509	

The geomean of *E. coli* concentration was approximately 250 percent greater in samples from MS4 sites where a human source was detected. Thus, the presence of human sources may be an important portion of the overall general bacterial indicator concentration at MS4 outfalls. This same analysis for samples collected from Santa Ana River sites did not show a statistically significant difference, most likely due to the diminished role of human sources of bacteria relative to the total load in the mainstem. This same relationship has been evaluated and shown to have mixed results elsewhere (e.g., Ahmed et al. 2016).

Estimates of daily loading of HF183 gene copies were developed to better understand the magnitude of human fecal waste in the MSAR watershed. Evaluation of loads rather than concentrations allow for a more sensible comparison between MS4 and mainstem Santa Ana River sites, which have 1 to 2 orders of magnitude differences in DWF rate due to POTW effluent discharges directly to the Santa Ana River.

Ahmed et al (2016) pooled data from multiple studies to relate gene copies of HF183 (and other human fecal markers) to mass of human feces (HF) and raw sewage (RS), documenting a mean of 5.8E9 gene copies/gram HF (range 1E7 to 5E10, n = 10) and 1.7E9 gene copies/liter RS (range 1E6 to 6E9, n = 16). These values for HF183 concentrations in fresh samples of HF and RS were used to approximate the mass of HF or volume of RS that could explain the results from environmental samples in the MSAR watershed during the 2019 dry season (**Figure 3-25**).

The estimates presented in Figure 3-25 assume there is limited decay of the HF183 marker prior to collection of the samples and thereby have not accounted for potential environmental degradation. Pooled studies of decay of HF183 in environmental matrices suggest a 90 percent reduction after 1-3 days in freshwaters with temperatures greater than 20° C (Ahmed et al. 2016). Longer persistence is shown in waters with cooler temperatures. During dry weather in the MSAR watershed, the hydraulic residence time is closely related to the distance upstream and generally is on the order of 0-6 hours from most source areas to downstream MS4 outfalls or mainstem Santa Ana River sites. Thus, the amount of decay from the initial fecal contamination is not likely to reveal a substantially larger source upstream responsible for downstream measurements.

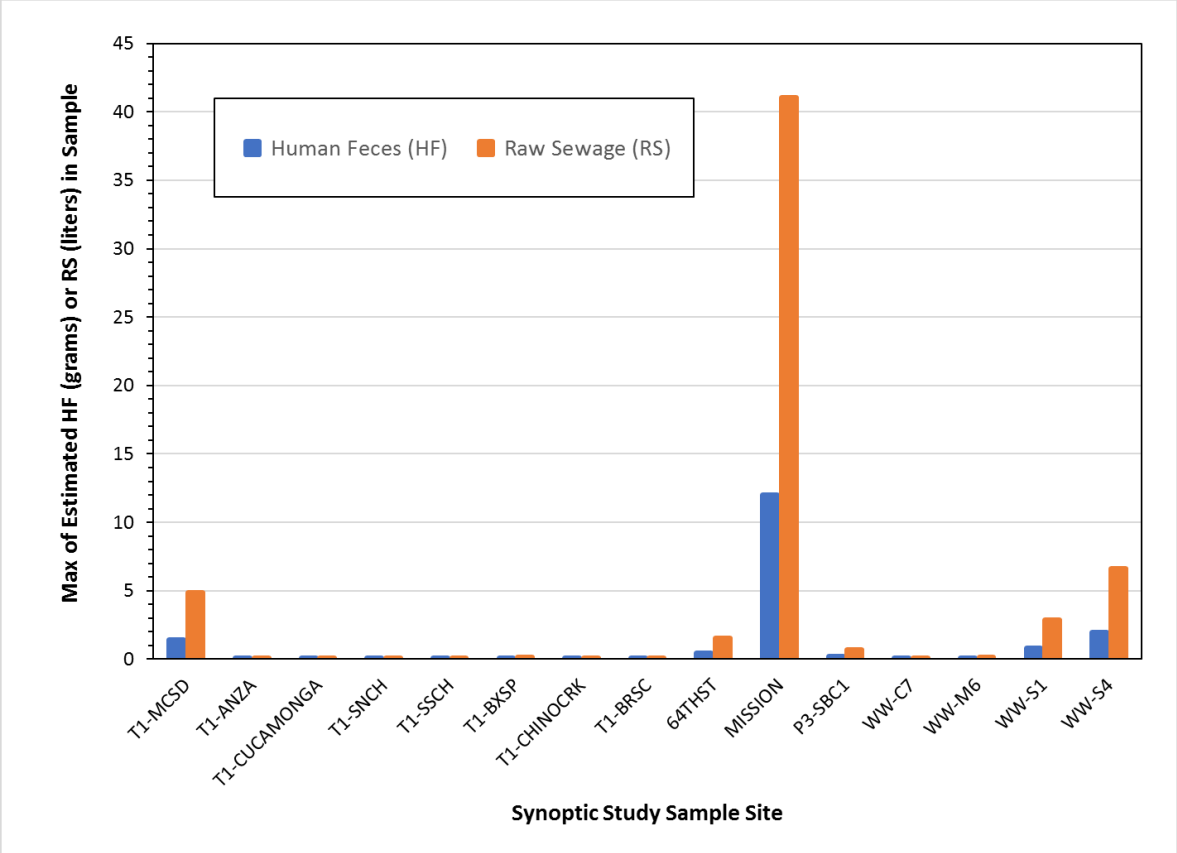

Figure 3-25. Maximum of Human Feces (HF) Mass or Volume of Raw Sewage (RS) from Each Site with HF183 Detection during the 2019 Dry Season

3.3 Tier 1 Prioritization Analysis

CBRP implementation coupled with waters conservation since 2013 has yielded significant changes in DWF rates and fecal bacteria loading from MS4 drainage areas in the MSAR watershed to the TMDL waters. The 2019 dry season Synoptic Study provides an updated data set support a new prioritization analysis of Tier 1 sites. Previous prioritizations were completed in 2009 (CDM Smith 2009) following implementation of the first MSAR watershed TMDL-related studies and in the 2012 Tier 1 source evaluation study (CDM Smith 2013).

This prioritization update was performed on the complete set of Tier 1 sites (seven sites in Bernardino County and seven sites in Riverside County). The number of sites included in this study is significantly reduced from the 2012 Tier 1 source evaluation because more information was available to exclude persistently dry outfalls and because of the change in the Cucamonga Creek Reach 1 recreational use designation.

The prioritization methodology applied to this study differs from the approach previously used (CDM Smith 2009, 2013). For this prioritization, the relative rankings from each of the following four criteria create a composite ranking for the Tier 1 MS4 outfall drainage areas:

- *Criterion 1* - Average DWF generation rate (gallons/acre/day) (previously based only on flow volume)
- *Criterion 2* - Average *E. coli* loading (MPN/day) (previously based on *E. coli* concentration)
- *Criterion 3* - Frequency of human *Bacteroides* HF183 detection (%)
- *Criterion 4* - Risk of exposure rating (low or high) with regards to recreation activity

The first three criteria are computed from data collected in the 6 weeks of consecutive monitoring at each Tier 1 site from the week of July 28, 2019 through the week of September 1, 2019. Days with no flow were included in the estimate of factors as zeros. For the risk of exposure criteria, each site was assigned either a low or high score based on the following principles:

- *Low* – Completely concrete-lined MS4 channel that outfalls to a concrete-lined receiving waterbody segment.
- *High* – Natural channel is present anywhere within MS4 channel and/or the Tier 1 outfall discharges to a natural channel segment of a receiving waterbody.

The composite bacteria prioritization score was computed through completion of the following calculation/categorization activities:

- For Criteria 1 through 3, determine the relative rank of the site among the 14 Tier 1 sites. This ranking is determined by (a) calculating the average value of the criterion at each site over the six-week sample period; and then (b) normalizing the relative rank of the range of observed average values to a range of 0 to 100. Normalization is done by applying the PERCENTRANK function in Excel to the range of average values observed at all Tier 1 sites. For example, the average DWF (gallons/acre/day) at the 14 sites ranged from 0 to 91. Applying the PERCENTRANK statistical function normalizes the range to 0 to 100 with the site with a 91 gallons/acre/day given a rank value of 100 and the dry site with 0 gallons/acre/day given a rank value of 0. Table 3-P shows the results of this computation for Criteria 1 through 3.
- For Criterion 4 (risk of exposure), sites with high risk were given a relative rank of 100 and sites with low risk are given a relative rank of 0. Table 3-10 shows how each site was ranked for this criterion.
- To calculate the composite Basin Prioritization Score (BPS) for each site, weighting factors were applied to each of the four criteria:
  - Criterion 1, dry weather flow generation rate = 0.3
  - Criterion 2, average *E. coli* load = 0.3
  - Criterion 3, frequency of human *Bacteroides* HF183 marker detection = 0.3
  - Criterion 4, risk of exposure= 0.1

The composite BPS for each Tier 1 site is computed as the sum-product of the rank value weighted for each criterion. **Table 3-10** provides the numeric results. The normalized ranked scores for each criterion are provided for each Tier 1 site. The final right hand column provides the composite BPS for each site. **Figure 3-26** categorizes the sites as high (red), moderate (yellow) or low (green) priority by generally subdividing the BPS into three equal parts.

Table 3-10. Relative Rank Results for each Prioritization Criterion and the Final Basin Prioritization Composite Score for each Tier 1 Site

Tier 1 Site	Relative Rank (0 to 100) for Prioritization Criteria				Composite BPS
	Criteria 1 DWF (gal/acre/day)	Criteria 2 *E. coli* Loading (MPN/Day)	Criteria 3 *Bacteroides* Frequency (%)	Criteria 4 Risk of Exposure	
T1-MCSD	92	85	100	100	93
T1-ANZA	100	69	17	100	82
T1-CUCAMONGA	62	92	17	100	79
T1-SNCH	69	62	67	100	75
T1-SSCH	77	100	17	0	67
T1-BXSP	31	38	83	100	58
T1-CHINOCRK	46	77	17	0	53
T1-BRSC	54	54	83	0	51
T1-DAY	38	46	0	100	35
T1-CCCH	85	31	0	0	35
T1-PHNX	23	23	0	100	24
T1-SACH	8	15	0	0	7
T1-LLSC	15	8	0	0	7
T1-CYP	0	0	0	0	0

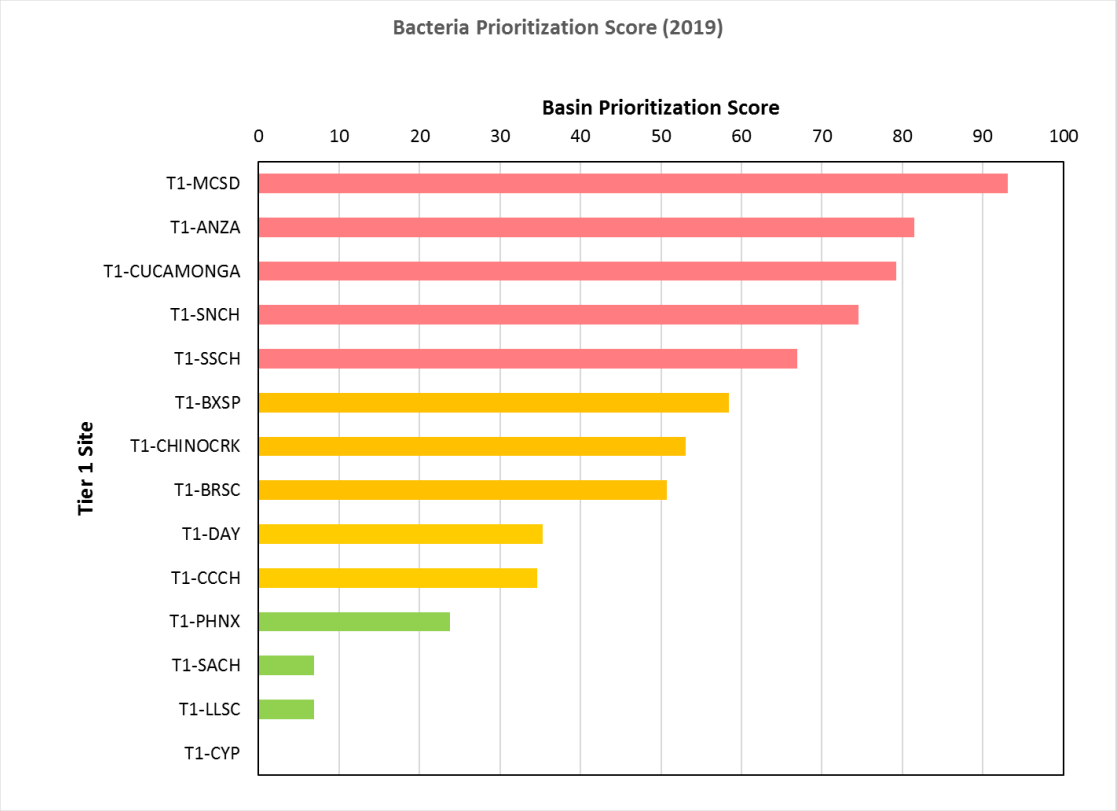

Figure 3-26. Bacteria Prioritization Score for Tier 1 MS4 Outfalls. Red – High; Yellow – Moderate; Green – Low Priority (Note: T1-CYP was dry for the entire study period).

4. Conclusions and Recommendations

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