Technical Memorandum

To:	Rick Whetsel, Santa Ana Watershed Project Authority Middle Santa Ana River Watershed TMDL Task Force
From:	Richard Meyerhoff, GEI Consultants Steven Wolosoff, CDM Smith Menu Leddy, Essential Environmental & Engineering Systems
Date:	October 16, 2020
Re:	Proposed Middle Santa Ana River Special Study Scope of Work - Final

The Middle Santa Ana River (MSAR) Watershed TMDL Task Force (Task Force) conducted a Bacteria Synoptic Study ("Synoptic Study") in the MSAR watershed in 2019. The final report (*Middle Santa Ana River Bacteria Synoptic Study and TMDL Triennial Report*) was submitted to the Santa Ana Regional Water Quality Control Board (Santa Ana Water Board) on February 13, 2020. A key purpose of the Synoptic Study was to develop data needed to evaluate key scientific questions posed by the Task Force in anticipation of a revision of the existing MSAR TMDL. One of the recommendations of the Synoptic Study was to conduct a special study to further evaluate sources of bacteria in a portion of the MSAR that receives no dry weather discharge from local MS4s ("non-MS4 segment"). At its May 19, 2020 meeting the Task Force requested preparation of a scope of work for the potential implementation of this recommended special study. The following sections describe the basis for the proposed special study and provide an implementation approach and estimated cost.

Special Study Background

The Synoptic Study consisted of a comprehensive six-week data collection effort during dry weather conditions from selected sites in the MSAR watershed. Sample collection began the week of July 29, 2019 and ended the week of September 3, 2019. Samples were collected over a two-day period for six weeks. The selection of sample locations was designed to meet the project objectives, which focused on areas of the MSAR watershed that remain hydrologically connected to downstream impaired waterbodies, e.g., Santa Ana River Reach 3. Data collection occurred at 28 sample locations in the watershed.

The Synoptic Study updated bacteria load analyses conducted in 2007 and 2012 to evaluate changes in the watershed resulting from ongoing watershed BMP implementation and changing hydrology in three impaired waterbodies: Santa Ana River Reach 3, Mill-Cucamonga Creek, and Chino Creek. The resulting analyses can be used to support revision to the fecal bacteria source assessment component of the TMDL.

For Reach 3 of the Santa Ana River, the Synoptic Study was able to parse inputs of fecal bacteria from MS4 and non-MS4 sources based on data collected at the Mission Avenue site (MISSION) (based on direct observations, experience and best professional judgment of Task Force members and the project team it is accepted that the MS4 contributed no dry weather flow or fecal bacteria upstream of this site during the period sampled for the Synoptic Study). Based on the findings

from the MISSION site, the Synoptic Study report included two recommendations for gathering additional data to supplement current understanding regarding sources of fecal bacteria in the Santa Ana River:

- Incorporate the Santa Ana River MISSION site into the ongoing Regional Bacteria Monitoring Program (RBMP) to be sampled as part of the MSAR TMDL compliance monitoring program. Regular sample collection from this location would provide data on bacteria loads in the river that are not derived from an MS4 source. **Figure 1** shows the location of the MISSION site. This recommendation was approved for implementation by the Task Force and the RBMP began regular sampling at this site in May 2020.
- Conduct a special study to evaluate releases of naturalized *Escherichia coli* (*E. coli*) from the Santa Ana River bottom. Naturalized fecal bacteria, including *E. coli* are bacteria released to the environment that can settle and colonize in the sediments or biofilms in channel bottom over a wide range of conditions (e.g., temperature, nutrients, etc.). The special study would be designed to collect site-specific data to assess the extent to which naturalized *E. coli* levels exist in the bottom sediments or biofilms of the Santa Ana River during different seasons. If desired, the study design could also include collection of data that may facilitate quantification of key factors that influence colonization and growth of *E. coli* (e.g., dissolved organic carbon [DOC], and temperature) and provide information regarding the processes that drive the release of *E. coli* colonies to the overlying water.

During the 2019 Synoptic Study, *E. coli* loads at the MISSION site from non-MS4 sources averaged 307 billion Most Probable Number (MPN)/day (ranging from 121 to 831 billion MPN/day), which is significantly greater than the total *E. coli* load from all sources of MS4 dry weather flows discharged to the Santa Ana River upstream of the WW-S1 TMDL compliance location (Santa Ana River at MWD Crossing). Loads from these MS4 sources average only 55 billion MPN/day (ranging from 22 to 75 billion MPN/day). Based on the source contribution analysis completed for the WW-S1 compliance site, the following findings were made:

- Upstream *E. coli* sources (MS4 + non-MS4) more closely explain downstream observations than in previous loading analyses completed in 2007 and 2012;
- Majority of the *E. coli* load comes from non-MS4 sources; and
- Weekly fluctuations in MS4 loads may not translate to measured differences in *E. coli* concentrations observed at the Santa Ana River Reach 3 MWD Crossing TMDL compliance site (**Figure 2**).

Given these important findings, it is imperative to the management of water quality in Santa Ana River Reach 3 to understand the sources of fecal bacteria in the Santa Ana River segment where there is no fecal bacteria load being contributed from MS4 sources. The 2019 Synoptic Study included use of microbial source tracking techniques, employing qPCR for human HF183 *Bacteroides* markers. Human bacteria sources were observed (HF183 was detected during one of six sample events and observed through amplification of the signal in the remaining five of six sample results) regularly at the MISSION site. This finding coupled with the results of *E. coli* analyses offered some observations that provide direction as to potential sources:

Figure 1. Map of Proposed Sampling Locations in Santa Ana River Upstream of MS4 Discharges

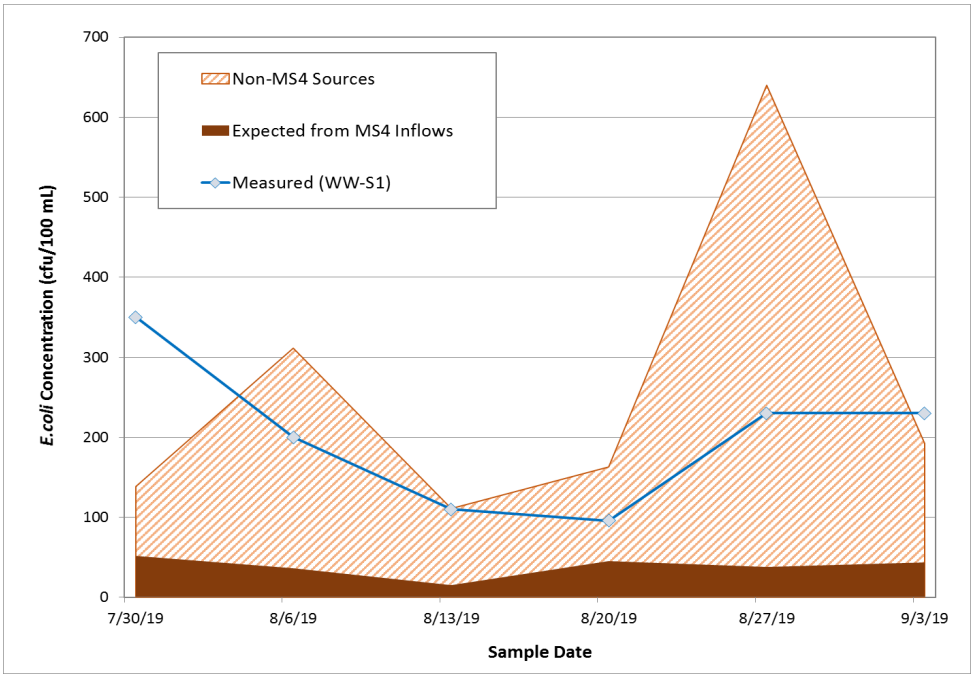

Figure 2. Comparison of Estimated Blended *E. coli* Concentrations of MS4 Inflows Plus Mission Bridge Non-MS4 Inflows with Downstream Watershed-wide Compliance Data at the Santa Ana River MWD Crossing Site (WW-S1)

- Publicly-owned Treatment Works (POTW), MS4 dry weather discharges, and agricultural runoff account for none of the human bacteria intermittently detected at the MISSION site. Other sources, such as known homeless encampments in this segment of the Santa Ana River or leaking sewer lines could explain the detection of human sources of fecal bacteria.
- The relationship between the detection of human bacteria sources and *E. coli* demonstrated that the presence of human sources is not an important factor in the total *E. coli* load within the Santa Ana River (**Table 1**). This observation suggests that non-human fecal sources are more likely to drive the *E. coli* load in the Santa Ana River than human sources.

Table 1. Student T-Test Results Comparing *E. coli* Concentrations in Samples with/without Detection/Amplification of Human Marker HF183 for Mainstem Samples

E. coli Data Set	n	*E. coli* Geomean (MPN/100 mL)	P-Value
Human Marker HF183 Detected/Amplified	23	142	0.932
Human Marker HF183 Not Detected or Amplified	19	157	

Proposed Special Study Objectives

The goal of the proposed Special Study is to improve Task Force understanding of sources of fecal bacteria in the Santa Ana River in the segment that receives no dry weather discharge from local MS4s. The objective of the proposed data collection effort is to investigate human, animal (selected) and sediment/biofilm sources of bacteria. Better understanding of these potential sources of bacteria will provide insight into their controllability in the study area. Potential sources of fecal bacteria in the non-MS4 segment may include:

- *Recent fecal deposits from humans and animals (e.g., pets, horses, or wildlife) in the river* – Until the Uncontrollable Bacteria Source was conducted in 2015-2016 (which included microbial source analyses for bird, dog, rumen and human in the non-MS4 segment), studies that employed microbial source tracking analyses to evaluate the presence of host-specific markers were conducted only within tributaries to impaired waterbodies in the MSAR watershed. The 2019 Synoptic Study collected additional human source data from the non-MS4 segment. The proposed special study would collect more samples from the Santa Ana River upstream of the MISSION site for microbial source tracking analysis to better understand and characterize the sources of bacteria in this reach (e.g., human, animal, or naturalized bacteria).
- *Releases from naturalized colonies of E. coli growing in channel bottom sediments or biofilms* - Fecal bacteria from a point source or a specific host released to the environment can settle to the channel bottom, colonize and survive within sediments or biofilms for weeks or months over a wide range of temperature, nutrients, and moisture conditions (Balzer et. al 2010). Colonization by these deposited fecal bacteria within channel bottom sediments and biofilms results in colonies, where the majority of the population is considered to be naturalized (Ishii et al. 2007; Byappanahalli et al. 2012; Ran et al. 2013). Over time these colonies evolve into an indigenous

bacterial community that is unique to the location due to the nature of the sediment and biofilms. Naturalized bacteria as a source was found to be one to five orders of magnitude higher in sediments/biofilms than in the overlying water in the 2015 MSAR Uncontrollable Bacteria Sources Study (RCFC&WCD 2016) (three sample locations: Anza Channel, Eastvale Line E, and Sunnyslope Channel). The proposed special study would build on these previous findings by collecting samples from the channel bottom in the Santa Ana River upstream of the MISSION site (see Figure 1).

Analytical methods exist that can be employed to develop quantitative information about potential fecal bacterial sources within the non-MS4 segment of the Santa Ana River. Specifically:

- Approximate the load from recent fecal deposits as a whole by quantifying a universal Bacteroidetes concentration, which measures the total fecal bacteria load. If this concentration in water at the MISSION site is correlated to the general indicator *E. coli* concentration, then it can be concluded that fresh deposits play an important role in the downstream fecal indicator bacteria loading. Conversely, if there is no correlation between universal Bacteroidetes and *E. coli* in water samples from the MISSION site, then it can be inferred that the releases from naturalized *E. coli* colonies (generally void of fresh fecal matter and associated *Bacteroides*) are the most important source for downstream fecal bacteria loading.
- Findings from the above tiered sampling approach where *E.coli* trends are evaluated to determine if *E.coli* are released from the channel bottom or are a result of recent fecal deposits, not due to releases from the channel bottom. If trends of total *Bacteroidetes* and *E. coli* correlate, then it can be concluded that the recent fecal deposits are important for the downstream fecal indicator bacteria loading. Then further analysis using host-specific *Bacteroides* can be performed to focus subsequent experimental sampling for potential sources of recent fecal deposits to the water column. Conversely, if it is determined that *E.coli* concentrations do not correlate, then additional factors that influence location, persistence, and population of naturalized *E. coli* colonies within channel bottom sediment and biofilms over the ~1.5-mile non-MS4 segment of the Santa Ana River can be assessed. For the former, both total *Bacteroidetes* and host specific *Bacteroides* markers will be used to apportion the total *Bacteroidetes* concentration into known (e.g., human and dog) and unknown natural sources (i.e., avian, pig, horses etc.). For the latter, the concentration of *E. coli* within the channel bottom will be determined at multiple sites by collecting sediment and biofilm samples. If desired, differences between sites (e.g., varying environmental conditions over different sampling events) could also be investigated to better understand the drivers for colony growth and *E. coli* release. Findings from such analyses could be used to inform future management actions, if any, that could be implemented in the watershed.

Proposed Scope of Work

Project Approach

Collection of data to support the objectives of the special study can be streamlined by leveraging the existing RBMP data collection effort and Quality Assurance Project Plan and focusing the level of effort applied to data analysis and documenting study findings. Additional analysis and

interpretation of the special study findings can be addressed as needed. The results of the special study can be used by the Task Force and/or its members to inform future actions such as development of water quality regulatory programs that are designed to address contributions of *E. coli* from controllable sources in excess of applicable standards, revisions to the existing TMDL or establishment of a new TMDL, and permit implementation provisions.

Table 2 summarizes proposed special study-related monitoring activities that could be implemented during 2020-2021 period to coincide with already ongoing RBMP monitoring events at the watershed-wide compliance sites (Santa Ana River at MWD Crossing and Santa Ana River at Pedley Avenue). Water and sediment samples will be collected from three sites on four sample dates that correspond to planned RBMP sample events. These sample dates are spread out over most of a year so that samples are collected under varying seasonal conditions during dry weather conditions.

All water and sediments samples collected during each sample event will be analyzed for Total Bacteroidetes, various specific *Bacteroides* markers and *E. coli* (Table 2). Water samples for the analysis of *E. coli* are already routinely collected at the WW-MISSION site by the RBMP. The project would require the collection of additional sediment and water samples for the analysis of each of the parameters as shown in Table 2.

Data results will be provided to the Task Force in a data summary report. This report will provide a compilation of the data, a basic analysis with appropriate tables/figures and a discussion of preliminary findings. The target for submittal of a draft summary report would be within three months of submittal of the final water and sediment samples to laboratories. A final summary report would be prepared within two weeks of receipt of comments on the draft summary report. At the end of the project the data results may be uploaded to CEDEN at the direction of the Task Force.

Cost Estimate

Table 3 provides a cost estimate to collect the additional samples, cost for all sample analyses and preparation of the summary report.

References

Balzer, M., N. Witt, H.-C. Fleming, and J. Wingender. 2010. *Faecal Indicator Bacteria in River Biofilms.* Water Science and Technology 61: 1105-1111.

Byappanahalli, M.N., T. Yan, M.J. Hamilton, S. Ishii, R.S. Fujioka, R.L. Whitman, and M.J. Sadowsky. 2012. *The Population Structure of Escherichia coli Isolated from Subtropical and Temperate soils.* Science of the Total Environment 417-418, 273–279.

Ishii S., D.L. Hansen, R.E. Hicks, M.J. Sadowsky. 2007. B*each Sand and Sediments are Temporal Sinks and Sources of Escherichia coli in Lake Superior*. Environmental Science and Technology 41: 2203-2209.

Ran, Q., B.D. Badgley, N. Dillon, G.M. Dunny and M.J. Sadowsky. 2013. *Occurrence, Genetic Diversity, and Persistence of Enterococci in a Lake Superior Watershed*. Applied and Environmental Microbiology 79: 3067-3075.

Riverside County Flood Control & Water Conservation District (RCFC&WCD). 2016. *Middle Santa Ana River Watershed: Uncontrollable Bacterial Sources Study*. Prepared by CDM Smith on behalf of RCFC&WCD. June 2016.

Table 2. Proposed Sampling Framework for Non-MS4 Segment Special Study

Matrix	Sample Date[1]	Site Name	Analyte[2, 3]
Water	• Four events during dry weather conditions • Dates selected in coordination with the Task Force • Target late spring, summer and fall	Santa Ana River Reach 3 at Mission Blvd (WW-MISSION)	Total Bacteroidetes
			Bacteroides Markers - Human, Dog, Avian, Horse, Pig
		Santa Ana River Reach 4 at Riverside Avenue (P3-SBC1)	Total Bacteroidetes
			Bacteroides Markers - Human, Dog, Avian, Horse, Pig
			E. coli
		Santa Ana River Reach 3 at Rialto Channel (SAR @ Rialto Channel)	Total Bacteroidetes
			Bacteroides Markers - Human, Dog, Avian, Horse, Pig
			E. coli
Sediment	• Four events during dry weather conditions • Dates selected in coordination with the Task Force • Target late spring, summer and fall	Santa Ana River Reach 3 at Mission Blvd (WW-MISSION)	Total Bacteroidetes
			Bacteroides Markers - Human, Dog, Avian, Horse, Pig
			E. coli
		Santa Ana River Reach 4 at Riverside Avenue (P3-SBC1)	Total Bacteroidetes
			Bacteroides Markers - Human, Dog, Avian, Horse, Pig
			E. coli
		Santa Ana River Reach 3 at Rialto Channel (SAR @ Rialto Channel)	Total Bacteroidetes
			Bacteroides Markers - Human, Dog, Avian, Horse, Pig
			E. coli

[1] Sample dates correspond with planned RBMP sample dates
[2] *E. coli* already sampled in water at WW-MISSION on each proposed sampled date
[3] Note that this proposal does not include analyzing water samples for constituents representing environmental factors that could potentially influence bacteria populations, e.g., DOC or nutrients.

Table 3. Estimate to Complete Special Study in Coordination with RBMP

Study Element	Analyte[2]	No. of Sites	No. of Events	Cost/Event or Sample	Estimated Cost
Field Labor	Collection of routine field parameters (pH, dissolved oxygen, temperature, conductivity, turbidity)	3	4	$2,500	$10,000
Water Sample Analysis[1]	*E. coli*	2	4	$35	$280
	Total Bacteroidetes	3	4	$189	$2,268
	Bacteroides Markers - Human, Dog, Avian, Horse, Pig	3	4	$400	$4,800
Sediment Sample Analysis	*E. coli*	3	4	$35	$420
	Total Bacteroidetes	3	4	$189	$2,268
	Bacteroides Markers - Human, Dog, Avian, Horse, Pig	3	4	$400	$4,800
Data Summary Report (Draft and Final Report: Data compilation and analysis, study findings, upload to CEDEN if requested)					$25,000
				Total Cost	**$49,836**

[1] Note that this proposal does not include collection or analysis of water samples for constituents representing environmental factors that could potentially influence bacteria populations, e.g., DOC or nutrients.