Middle Santa Ana River Watershed Uncontrollable Bacterial Sources Study

Final Report

Riverside County Flood Control
and Water Conservation District
1995 Market Street
Riverside, CA 92501

June 2016

CDM Smith

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Acronyms

Basin Plan	Water Quality Control Plan for the Santa Ana River Basin
BMP	best management practice
BPA	Basin Plan Amendment
cfu	colony forming unit
DOC	dissolved organic carbon
E. coli	*Escherichia coli*
EPA	Environmental Protection Agency
FIB	fecal indicator bacteria
ft	feet
mL	milliliters
mg	milligrams
MOS	margin of safety
MPN	most probable number
MS4	Municipal Separate Storm Sewer System
MSAR	Middle Santa Ana River
MSAR Bacteria TMDL	MSAR Bacterial Indicator TMDL
MST	microbial source tracking
POTW	publicly-owned treatment works
qPCR	quantitative real-time polymerase chain reaction
RCFC&WCD	Riverside County Flood Control and Water Conservation District
REC1	water contact recreation
Regional Board	Santa Ana Regional Water Quality Control Board
RIX	Regional Tertiary Treatment Rapid Infiltration and Extraction Facility
SAR	Santa Ana River
Santa Ana Water Board	Santa Ana Regional Water Quality Control Board
SAWPA	Santa Ana Watershed Protection Authority
SSV	Single Sample Value
State Water Board	State Water Resources Control Board
SWQSTF	Stormwater Quality Standards Task Force
TMDL	Total Maximum Daily Load
TSS	total suspended solids
UBSS	Uncontrollable Bacterial Sources Study
VNS	Visited Not Sampled
WLA	wasteload allocation
WQO	water quality objective

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Section 1

Introduction

1.1 Background

Various waterbodies in the Middle Santa Ana River (MSAR) watershed are listed on the state 303(d) list of impaired waters due to high levels of fecal coliform bacterial indicators. Previous source evaluation efforts have focused on identifying and mitigating controllable sources in the MS4 during the dry season. These efforts suggest that uncontrollable sources are likely a large component of fecal bacteria indicator (FIB) concentrations in receiving waters of the MSAR watershed. The Recreational Use Standards Basin Plan Amendment (BPA), which has been adopted by the Santa Ana Regional Water Quality Control Board (Regional Board) and approved by the State Water Resources Control Board (State Water Board) and the Environmental Protection Agency (EPA), lists uncontrollable bacteria sources that may be present in the MSAR watershed as:

- Wildlife activity and waste
- Bacterial regrowth within sediment or biofilm
- Resuspension from disturbed sediment
- Concentration (flocks) of semi-wild waterfowl
- Shedding during swimming

To expand on source evaluation efforts to include uncontrollable sources, six site-specific technical pilot studies were conducted as part of the Uncontrollable Bacterial Sources Study (UBSS) for the MSAR watershed to evaluate to the extent possible what portion of bacterial indicators can be attributed to specific uncontrollable sources. While the UBSS was not intended to be exhaustive in nature, each of the pilot studies was designed to provide information that increases understanding regarding the different types of potential uncontrollable sources of bacterial indicators in the MSAR watershed. The uncontrollable sources studies in this UBSS target human, i.e. from swimming, and non-human sources, including wildlife and sediment and/or biofilm resuspension and regrowth. These specialized pilot studies were conducted to help understand the relative importance of various potential uncontrollable sources of bacterial indicators to exceedances of MSAR Bacterial Indicator TMDL targets in the MSAR watershed.

1.2 Regulatory Framework

Due to exceedances of the fecal coliform objective established to protect REC1 use, the Santa Ana Water Board added multiple waterbodies in the MSAR watershed to the state 303(d) List of impaired waters in 1994 and 1998. Subsequently, the Santa Ana Water Board adopted the MSAR Bacteria TMDL for freshwaters in the Santa Ana River Watershed in 2005[1], which was approved by the EPA on May

[1] Santa Ana Water Board Resolution: R8-2005-0001, August 26, 2005

16, 2007. The TMDL established compliance targets for both fecal coliform and *Escherichia coli* (*E. coli*) as follows:

- Fecal coliform: 5-sample/30-day logarithmic 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.
- *E. coli*: 5-sample/30-day logarithmic 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.

Per the TMDL, the above compliance targets for fecal coliform became ineffective upon EPA approval of the BPA[2]. The concentration based wasteload allocation (WLA) for MS4 Permittees for *E. coli* of 113 cfu/100mL is equal to the numeric water quality objective (WQO) (126 cfu/100mL), established for a geomean based on 5 samples within a 30-day period, minus a ten percent margin of safety (MOS). Although the 5-sample WQO is the preferred method for assessing compliance, the Basin Plan relies on the Single Sample Value (SSV) in cases where the criteria for using the 5-sample geomean target is not met.[3] The SSV of 235 MPN/100 ml, as defined in the Basin Plan, is used as a measure of water quality for the purposes of five of the six pilot studies because the frequency of sampling does not comply with the geomean criteria.

On June 15, 2012, the Regional Board adopted the BPA to Revise Recreation Standards for Inland Freshwaters in the Santa Ana Region[4]. The BPA also indicated that water quality objectives pertain to controllable sources that cause or contribute to impairment of beneficial uses. Uncontrollable sources are defined by the BPA as "contributions of bacteria within the watershed from nonpoint sources that are not readily managed through technological or natural mechanisms or through source control and that may result in exceedances of water quality objectives for indicator bacteria."[5]

Santa Ana Water Board staff developed this BPA in collaboration with the Stormwater Quality Standards Task Force (SWQSTF), comprised of representatives from various stakeholder interests, including the Santa Ana Watershed Protection Authority (SAWPA); the counties of Orange, Riverside, and San Bernardino; Orange County Coastkeeper; Inland Empire Waterkeeper; and the EPA Region 9. The BPA was approved by the State Water Board on January 21, 2014[6] and the California Office of Administrative Law on July 2, 2014.[7] The EPA issued its letter of approval/disapproval on April 8, 2015 and provided a letter of clarification on August 3, 2015.

As required by the TMDL, compliance monitoring is conducted within the receiving waterbody, where multiple sources of flow and bacteria may cause or contribute to any impairments. Several of these potential sources have been determined to be uncontrollable with the adoption of the BPA, as described above. Accordingly, where a source is identified as uncontrollable, it is not the responsibility of MS4 Permittees to reduce *E. coli* from such a source. This goal of this pilot study is to evaluate

[2] Attachment A to Santa Ana Water Board Resolution R8-2005-0001
[3] Santa Ana Region Basin Plan
[4] Santa Ana Water Board Resolution: R8-2012-0001
[5] Santa Ana Water Board Resolution: R8-2012-0001: Amendments to the Water Quality Control Plan for the Santa Ana River Basin, May 15, 2015
[6] State Water Board Resolution: 2014-0005, January 21, 2014
[7] Office of Administrative Law: #2014-0520 -02 S; July 2, 2014

whether uncontrollable sources of bacteria, as defined in the BPA, are significant contributors to downstream *E. coli* concentrations observed in the MSAR watershed.

1.3 Literature Review

To determine the current scientific understanding for each of the fecal bacteria sources under investigation in the pilot studies, a preliminary literature review was conducted on a selection of relevant studies. The literature review is categorized by the type of uncontrollable bacteria source as follows:

- Direct inputs from wildlife
- Resuspension from sediment and/or biofilm
- Shedding during swimming
- Equestrian recreational use

A technical memorandum was prepared in July, 2015 to summarize methods and pertinent findings in studies related to the uncontrollable sources being investigated by this Program (Appendix A). While this literature review is not meant to be comprehensive, it summarizes some conclusions observed by existing and past investigations. This section will review findings from the technical memorandum.

1.3.1 Direct inputs from wildlife

Six studies from 2004 through 2011 pertaining to the impacts of wildlife on bacterial water quality were reviewed. The study by Byappanahalli et al (2015) detected both gull markers and elevated FIB in water samples, they concluded that no relationship between the two could be established. Other studies (Edge et al, 2007; Jiang et al, 2007; Sejkora et al, 2011; Wither et al, 2005) suggest that bacteria levels are influenced by bird activity and other nonhuman sources including cows and rabbits. The study by Sejkora et al specifically compared *E. coli* concentrations upstream and downstream of a bridge where cliff swallows nest and inhabit. Their study showed a significant increase in bacteria levels at downstream sites in dry weather with greatest differences in upstream and downstream levels during the nesting period (approximately 45 days). Other factors considered to influence FIB concentrations in water samples were water temperature and shading (Tiefenthaler et al, 2008).

1.3.2 Resuspension from sediment and biofilm

Seven studies from 2000 through 2012 pertaining to FIB survival and growth in sediment, biofilms, and overlying water were reviewed. Results from all of the studies showed that FIB levels are much higher in sediment and biofilms than in overlaying water. In all water and biofilm samples in the study by Balzer et al (2007), differences in *E. coli* were at least one order of magnitude and the difference in geometric means was four orders of magnitude. Ksoll et al (2007) also showed that the predominant source of *E. coli* in periphyton samples from a shoreline was waterfowl and sources in the overlying water included waterfowl, naturalized colonies found in periphyton samples, and sewage. This result suggests that naturalized bacteria attached to periphyton communities may be released into overlying water. Studies also showed FIB levels were higher at sites downstream of bacteria-free discharges, such as publicly owned treatment works (POTW) effluent, (Skinner et al, 2010; Surbeck et al, 2010) and increased from potable to order of magnitude over recreational use WQOs as water moved downstream within street gutters (Skinner et al, 2010). Other factors considered to influence FIB concentrations in biofilm include dissolved organic carbon levels, shading, tides, drying and wetting periods, and seasons.

1.3.3 Shedding during swimming

Studies regarding bacteria contribution from shedding during swimming provide inconsistent results. While two of the five studies reviewed conclude that swimming and shedding is not a source of FIB in waterbodies (Jian et al, 2002; Zhu et al, 2011), other studies suggest that shedding during the first thirty minutes of water contact can account for over 16,000 viruses and 5.5 x 10^5 cfu/100 mL of *Enterococci* (Elmir et al, 2007; Gerba et al, 2000). Results from Elmir et al (2007) indicated that shedding continued to occur for multiple immersions by bathers and found that bacteria associated with sand contact was low relative to shedding from bathers. A literature review by Gerba et al (2000) also found that Rose et al (1991) reported bathwater from young children contained substantially higher fecal coliform concentrations compared to bathwater from adults (children: 10^5 MPN/100 ml; adults: 10^1 to 10^2 MPN/100 ml).

1.3.4 Equestrian recreational use

Similar to shedding studies, studies investigating the impact of horse recreation on water quality provide conflicting results. Tiefenthaler et al (2011) and Long et al (2004) both found highest FIB concentrations at or downstream of horse-related land use sites compare to other land uses (i.e. commercial, residential, industrial). At the horse farm site in the study by Long et al (2004), the fecal coliform concentration (1,200 cfu/100 ml) was more than five times the average fecal coliform concentration from other land uses (233 cfu/100 ml). Additionally, the microbial source tracking indicator for grazing animal manure was detected above the threshold only at the horse farm site, suggesting the source of bacteria at this site was from horse manure. However, Airaksinan et al (2007) found no difference in bacteria levels in cleaned and uncleaned horse paddocks with active horses in each paddock.

1.4 Study Framework

The purpose of the UBSS is to better understand and quantify the influence of uncontrollable sources on bacterial indicator concentrations in waterbodies in the MSAR watershed. Six specialized studies were developed to test the following hypotheses at a pilot study level:

- Natural sources study: This study evaluates the potential for natural (wildlife) sources of bacteria, including birds, rumen, and dogs, to contribute to *E. coli* concentrations in the MSAR watershed. In MSAR areas without MS4 discharges, elevated *E. coli* concentrations will be correlated to wildlife sources.
- Bird study: This study evaluates the impacts of bird nesting under bridges on FIB in the MSAR watershed. In MSAR areas with high levels of bird population and activity, *E. coli* concentrations will be higher downstream of the bird activity than upstream of bird activity.
- Stormwater channel study: Sediment and biofilm are reservoirs for bacterial indicators and watersheds with high biofilm growth and sediment presence will have higher levels of *E. coli* than overlying water.
- Non-MS4 flow study: Non-MS4 discharges can mobilize bacteria from sediments or biofilms in stormwater channels in the MSAR watershed. In waterbodies with sediment and biofilm

presence, *E. coli* loads will be higher downstream of non-MS4 discharges than upstream of discharges.

- Human recreation study: This study evaluates potential impact of human recreation on bacteria levels at a popular swimming hole in the SAR. In areas that are popular recreational sites, shedding from swimming in the waterbodies will elevate *E. coli* concentrations downstream of swimming recreation, particularly during a holiday weekend when potential for recreation will be higher.
- Horse recreation study: Equestrian uses exist within the SAR riparian area and may impact bacteria levels in SAR. This study evaluates whether feces from horses deposited along trails or directly into the river is a contributor to downstream FIB concentration. In MSAR areas that are near horse trails and equestrian activity, *E. coli* concentrations in waterbodies will be higher on a holiday weekend, when there will likely be more horse activity than a non-holiday weekend.

The remainder of this report includes the following sections:

- **Section 2 – Design of Pilot Studies** describes the sampling plan including goals, monitoring locations, sampling frequency, and laboratory analysis, for each study.
- **Section 3 – Results** presents the results for each pilot study.
- **Section 4 – Discussion and Conclusions** presents a discussion of the findings based on results from the pilot studies.
- **Section 5 – References** contains a list of references cited in the document.

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Section 2

Design of Pilot Studies

This section describes the sampling plan for each of the pilot studies. Monitoring activities were conducted only during dry weather conditions and included collection of water quality samples, water quality parameters measurements, and digital photographs during each sampling event. *E. coli* levels were quantified in water and sediment or biofilm samples while water quality parameters were measured using a multi-parameter water quality probe. Molecular analyses used for microbial source-tracking (MST) were utilized to identify uncontrollable source contributors to bacterial indicator levels in the watershed. MST analyses involved quantitative real-time polymerase chain reaction (qPCR) methods with genetic markers specific to different human and non-human sources. As each pilot study targets different uncontrollable sources, the bacterial hosts analyzed vary between each technical study (Table 2-1) and is further described below.

Table 2-1 Uncontrollable Sources Monitoring Locations

Study	Study Location	Sample Frequency	Analysis
Natural	SAR downstream of RIX	Seasonal (3 times/year)	*E. coli*, human, dog, bird, rumen
Bird	Cucamonga Creek at Schleisman Road Bridge	Peak bird season (5 consecutive weeks)	*E. coli*, bird
Bird	SAR at Mission Boulevard Bridge	Peak bird season (5 consecutive weeks)	*E. coli*, bird
Sediment & Biofilm	Sunnyslope Channel	Seasonal (4 times/year)	*E. coli*, human, dog, bird, rumen
Sediment & Biofilm	Eastvale Line E	Seasonal (4 times/year)	*E. coli*, human, dog, bird, rumen
Sediment & Biofilm	John Bryant Park	Seasonal (4 times/year)	*E. coli*, human, dog, bird, rumen
Non-MS4	San Sevaine Creek	Summer (3 times/year)	*E. coli*
Non-MS4	Day Creek	Summer (3 times/year)	*E. coli*
Human (Swim)	SAR at Martha Mclean Anza Narrows Park	2 weekends	*E. coli*, human, dog
Horse	SAR at 66th Street & Etiwanda Avenue	2 weekends	*E. coli*, horse
Horse	SAR at Mary Tyo Equestrian Center	2 weekends	*E. coli*, horse
Horse	SAR at Downey Street & 64th Street	2 weekends	*E. coli*, horse

Each of the specific studies is presented separately. None of the pilot studies is meant to be exhaustive; instead they are intended to identify and quantify the relative potential for a defined uncontrollable bacterial indicator source to cause an exceedance of water quality objectives in receiving waters. The following terminology is employed in this report:

- Study Location – A specific waterbody reach where the study is conducted.
- Monitoring Site – Specific location(s) within a Study Location where water and/or sediment samples are collected. Multiple monitoring sites were planned at some study locations to capture spatial variability in data results.

- Sample Event – Specific time period when a study is implemented at a Study Location. Multiple sample events were planned for each study to capture potential temporal variability in data results.

2.1 Natural Sources Study

The natural sources study investigated bacterial contributions from natural sources by measuring bacterial indicators in a natural channel where there are no MS4 discharges or other anthropogenic sources of bacteria.

2.1.1 Locations

The study location is the MSAR reach between the Regional Tertiary Treatment Rapid Infiltration and Extraction Facility (RIX) discharge location and Riverside Drive Bridge crossing (Figure 2-1). This reach of SAR is not under the influence of MS4 discharges and potential for wildlife activity is high due to the riparian habitat. Eight monitoring sites were selected across four transects within this study location as described in Table 2-2.

Figure 2-1 Natural Sources Study Location

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Table 2-2 Monitoring Sites for the Natural Sources Study

Site	Description	Latitude	Longitude
Natural01A	Transition from concrete to natural channel on the east bank	34° 2'53.39"N	117°21'23.68"W
Natural01B	Transition from concrete to natural channel on the west bank	34° 2'53.33"N	117°21'23.80"W
Natural02A	500 ft downstream of RIX on the east bank	34° 2'23.70"N	117°21'16.98"W
Natural02B	500 ft downstream of RIX on the west bank	34° 2'23.81"N	117°21'17.40"W
Natural03A	4000 ft downstream of RIX on the east bank	34° 1'52.95"N	117°21'29.28"W
Natural03B	4000 ft downstream of RIX on the west bank	34° 1'52.98"N	117°21'29.46"W
Natural04A	6400 ft downstream of RIX on the east bank	34° 1'31.53"N	117°21'45.10"W
Natural04B	6400 ft downstream of RIX on the west bank	34° 1'31.60"N	117°21'45.31"W

2.1.2 Frequency and Schedule

To document seasonal variability of bacterial indicators, three sample events were conducted during different seasons throughout the year as follows:

- April 28, 2015
- June 11, 2015
- August 4, 2015

During each sample event, two water samples were collected along a transect at each monitoring site to allow characterization of sample variability (Table 2-3).

Table 2-3 Monitoring Plan for the Natural Sources Study

Number of Study Locations	1
Monitoring Sites per Location	8 (2 sites per transect)
Sample Events per Study	3
Water Samples per Monitoring Site	1
Sediment Samples per Monitoring Site	0
Sampling Period	seasonally year-round

2.1.3 Field and Laboratory Constituents

The following constituents were analyzed in water samples collected at each site on each sample date:

- Field Measurements – temperature, pH, turbidity, conductivity, dissolved oxygen
- Laboratory Water Quality Analysis – *E. coli*
- Laboratory Molecular Analysis – bacterial indicator sources (human, canine, bird, and rumen)

This list of constituents was developed to represent the key pollutants of concern relevant to identifying uncontrollable sources of bacteria.

2.2 Bird Study

As flocks of birds are present throughout MSAR waterbodies, it is important to determine the potential for birds to influence bacterial levels in the MSAR watershed. Birds are suggested to be contributors of bacterial indicators in other environments (Sejkora et al, 2011) and may also be a contributor in MSAR watershed waters at bridge structures used for nesting activity.

2.2.1 Locations

Study locations were selected based on evidence of notable bird nesting and presence so that the potential for bird impacts on water quality is high (Figure 2-2). The first study location is in Cucamonga Creek in Eastvale on Schleismann Road (Bird01), where more than forty swallow nests were observed underneath the Schleismann Road bridge crossing and multiple flocks of birds were observed along Cucamonga Creek in this area. Two monitoring sites were selected along a transect upstream and two monitoring sites were selected along a transect downstream of Schleismann Road bridge crossing to represent sites un-impacted and impacted by birds nesting under the bridge, respectively (Table 2-4).

The second location of the bird location is in the Santa Ana River (SAR) in Riverside on Mission Boulevard (Bird02). Several swallow nests were observed underneath the Mission Boulevard bridge crossing and multiple flocks of birds were observed along Santa Ana River in this area. Two monitoring sites were selected along a transect upstream and two monitoring sites were selected along a transect downstream of Mission Boulevard bridge crossing to represent sites un-impacted and impacted by birds nesting under the bridge, respectively (Table 2-4).

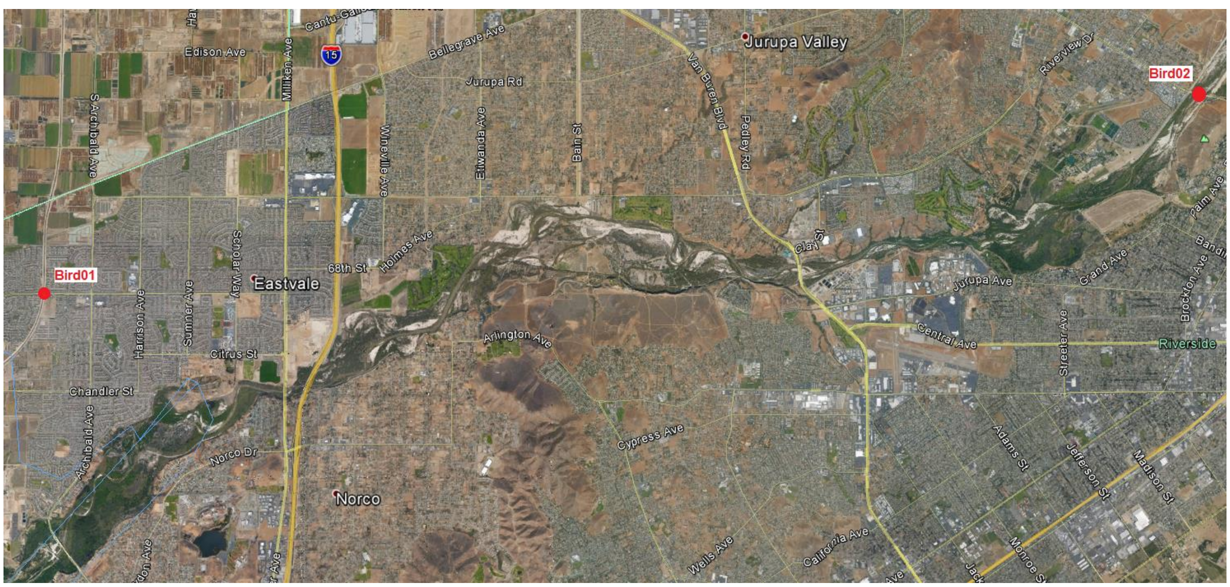

Figure 2-2 Bird Study Locations

Table 2-4 Monitoring Sites for the Bird Study

Site	Description	Latitude	Longitude
Bird01A	Upstream of Schleismann Road bird activity on the east bank	33°57'41.61"N	117°36'06.47"W
Bird01B	Upstream of Schleismann Road bird activity on the west bank	33°57'41.61"N	117°36'06.62"W
Bird01C	Downstream of Schleismann Road bird activity on the east bank	33°57'37.56"N	117°36'06.78"W
Bird01D	Downstream of Schleismann Road bird activity on the west bank	33°57'37.56"N	117°36'07.88"W
Bird02A	Upstream of Mission Boulevard bird activity on the east bank	33°59'29.07"N	117°23'38.07"W
Bird02B	Upstream of Mission Boulevard bird activity on the west bank	33°59'29.08"N	117°23’38.12”W
Bird02C	Downstream of Mission Boulevard bird activity on the east bank	33°59'26.52"N	117°23'41.72"W
Bird02D	Downstream of Mission Boulevard bird activity on the west bank	33°59'26.57"N	117°23’41.83”W

2.2.2 Frequency and Schedule

Five sample events were conducted within a five week period targeting peak bird activity in late April through May. Two events occurred prior to peak activity, one event during, and two events after peak bird activity as follows:

- April 29, 2015 before peak activity
- May 7, 2015 before peak activity
- May 14, 2015 during peak activity
- May 21, 2015 after peak activity
- May 27, 2015 after peak activity

During each sample event, two water samples were collected along a transect at each monitoring site to allow characterization of sample variability (Table 2-5).

Table 2-5 Monitoring Plan for the Bird Study

Number of Sample Locations	2
Monitoring Sites per Location	4 (2 sites per transect)
Sample Events per Study	5
Water Samples per Monitoring Site	1
Sediment Samples per Monitoring Site	0
Sampling Period	Peak activity season

2.2.3 Field and Laboratory Constituents

The following constituents were analyzed in water samples collected at each site on each sample date:

- Field Measurements – temperature, pH, turbidity, conductivity, dissolved oxygen
- Laboratory Water Quality Analysis – *E. coli*
- Laboratory Molecular Analysis – bacterial indicator sources (bird)

This list of constituents was developed to represent the key pollutants of concern relevant to identifying uncontrollable sources of bacteria. In addition to water quality monitoring, biological evaluations of the study locations were conducted by a biologist to identify bird species commonly observed in the area and other relevant information. This includes nesting requirements, densities, feeding habits, life history attributes and potential habitats.

2.3 Stormwater Channel Study

The potential for sediment and biofilms to serve as a reservoir for bacterial indicators is high in any given waterbody. The goal of this study is to evaluate sediments and biofilms in selected stormwater channels to determine the extent to which bacterial indicators are associated with them in comparison to bacterial indicators found in the water column.

2.3.1 Locations

Three study locations with two monitoring sites each were planned, where two study locations are concrete-lined channels and the third is a natural-bottomed channels (Figure 2-3). Study locations are described as follows:

- John Bryant Park (Resuspension01) – Anza Drain along the west side of John Bryant Park in Riverside is a concrete-lined channel with sediment, biofilms, and vegetation mats. Low flow is regularly observed in this section of the channel.
- Eastvale Line E (Resuspension02) – Eastvale Line E is a concrete-lined channel that becomes a natural-bottom channel in the downstream section. Samples will be collected from the channel where it daylights, and will be comprised of mostly biofilm.
- Sunnyslope Channel (Resuspension03) – Sunnyslope Channel on the east side of the Louis Rubidoux Nature Center is a natural-bottom channel surrounded by vegetation. Samples will be collected in the natural section of the channel, and will be comprised mostly of sediment.

Figure 2-3 Stormwater Channel Study Locations

At each study location, wet sediment deposits and/or biofilms are commonly present. Water and sediment/biofilm samples were collected at two monitoring sites along a transect at each study location (Table 2-6).

Table 2-6 Monitoring Sites for the Stormwater Channel Study

Site	Description	Latitude	Longitude
Resusp01A	John Bryant Park site on the north bank	33°56’07.19"N	117°27’36.95"W
Resusp01B	John Bryant Park site on the south bank	33°56’07.08"N	117°27’37.04"W
Resusp02A	Eastvale Line E site on the east bank	33°57'0.89"N	117°33'12.39"W
Resusp02B	Eastvale Line E site on the west bank	33°57'0.93"N	117°33'12.57"W
Resusp03A	Sunnyslope Channel site on east bank	33°58'31.26"N	117°25'34.68"W
Resusp03B	Sunnyslope Channel site on the west bank	33°58'31.34"N	117°25'34.92"W

2.3.2 Frequency and Schedule

Four sample events were conducted throughout the year to observe seasonal variability. The four events occurred as follows:

- May 13, 2015 before high summer temperatures occur
- July 9, 2015 during peak summer season
- October 13, 2015 under cooler conditions
- January 6, 2016 under winter dry conditions

During each sample event, sediment/biofilm samples were collected along a transect at each monitoring site to allow characterization of sample variability. If dry weather flow is present, water samples were collected from water overlying the sediment samples collected along the transect (Table 2-7).

Table 2-7 Monitoring Plan for the Stormwater Channel Study

Number of Study Locations	3
Monitoring Sites per Location	1
Sample Events per Study	4
Water Samples per Monitoring Site	2 (along transect)
Sediment Samples per Monitoring Site	2 (along transect)
Sampling Period	Dec/Jan, April/May, Jul/Aug, Oct/Nov

2.3.3 Field and Laboratory Constituents

The following constituents were analyzed in water samples collected at each site on each sample date:

- Field Measurements – temperature, pH, turbidity, conductivity, dissolved oxygen

- Laboratory Water Quality Analysis – *E. coli*
- Laboratory Molecular Analysis – bacterial indicator sources (human, canine, bird, rumen, and swine)
- Flow – the flow likely required to shear sediment or biofilm material and mobilize bacteria will be estimated based on channel characteristics. This will be compared with field measurements.

Sediment samples were analyzed for *E. coli* and bacterial indicator sources. This list of constituents was developed to represent the key pollutants of concern relevant to identifying uncontrollable sources of bacteria.

2.4 Non-MS4 Flow Study

To specifically address non-stormwater flows that could resuspend or shear bacteria present in sediment and biofilms, this study targeted stormwater channels that are often used to convey non-MS4 discharges.

2.4.1 Locations

Study locations were selected based on knowledge of when and what types of dry weather flows occur in the channels. These channels should primarily receive non-MS4 flows during summer months. Study locations are as follows (Figure 2-4):

- San Sevaine Channel (Scour01) – This is a concrete-lined channel bounded by the 60 freeway and Van Buren Boulevard and is located adjacent to a well blow-off facility. This treatment plant regularly releases well blow-off that results in small levels of flow in the channel.
- Day Creek (Scour02) – This is a concrete-lined channel bounded by Harrell Street and Riverside Drive. Sources of non-MS4 flows are predominantly publicly owned treatment works discharge.

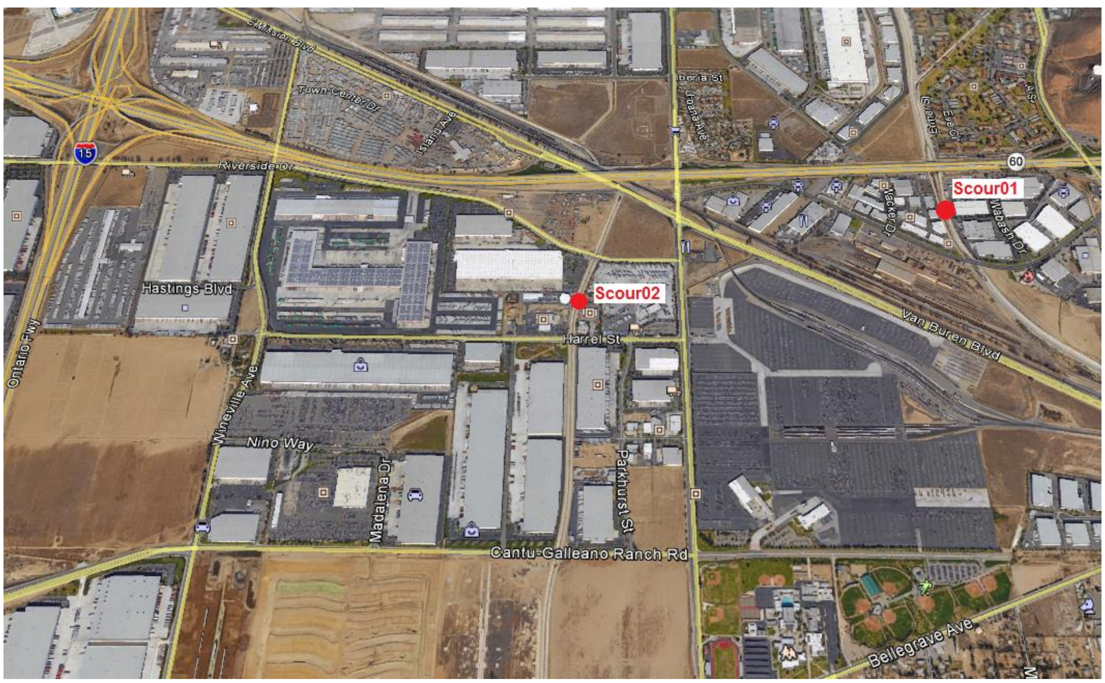

Figure 2-4 Non-MS4 Flow Study Locations

Sampling was planned at three monitoring sites located along different parts of the channel to reflect areas that are impacted by non-MS4 discharge to varying degrees (e.g., a site at or upstream of the point of non-MS4 discharge, a site immediately downstream of the non-MS4 discharge and another site further downstream of non-MS4 discharge) (Table 2-8). Only one of the planned study locations was intended to be sampled during each sample event, depending on which study location exhibited flow. Field staff performed reconnaissance at both study locations during each sample event but did not observe non-MS4 discharges at either location.

Table 2-8 Monitoring Sites for the Non-MS4 Flow Study

Site	Description	Latitude	Longitude
Scour01A	Site at point of non-MS4 discharge in San Sevaine Channel	34°01’07.95"N	117°30’49.00"W
Scour01B	300 feet downstream of non-MS4 in San Sevaine Channel	34°01’04.94"N	117°30’48.16"W
Scour01C	1900 feet downstream of non-MS4 in San Sevaine Channel	34°00’49.93"N	117°30’44.09"W
Scour02A	Site at point of non-MS4 discharge in Day Creek	34°00’47.30"N	117°31’42.83"W
Scour02B	240 feet downstream of non-MS4 discharge in Day Creek	34°00’45.01"N	117°31’43.49"W
Scour02C	575 feet downstream of non-MS4 discharge in Day Creek	34°00’41.62"N	117°31’43.80"W

2.4.2 Frequency and Schedule

Two sample events were conducted during following dry summer months:

- June 17, 2015
- July 9, 2015

Although one water sample was intended to be collected from each monitoring site at the flowing study location, none were collected during either sampling event as field staff did not observe flow. Additional coordination is ongoing with Jurupa Community Services District to collect samples during an upcoming scheduled discharge in April or May, 2016. Prior to the future sample event, field teams will be notified two days in advance of scheduled flow release times and be on site prior to anticipated non-MS4 discharge to collect one baseline water quality sample and estimate baseline flowrate at the point of discharge. After the discharge has begun, one water sample will be collected from each of the two sites downstream of the discharge to allow characterization of sample variability and discharge impacts (Table 2-9).

Table 2-9 Monitoring Plan for the Non-MS4 Flow Study

Number of Study Locations	1 (2 options provided)
Monitoring Sites per Location	3
Sample Events per Study	3
Water Samples per Monitoring Site	1
Sediment Samples per Monitoring Site	0
Sampling Period	Dry season

2.4.3 Field and Laboratory Constituents

The following constituents will be analyzed in water samples collected at each site on each sample date:

- Field Measurements – temperature, pH, turbidity, conductivity, dissolved oxygen
- Laboratory Water Quality Analysis – *E. coli*, total suspended solids (TSS)
- Flow – the flow likely required to shear sediment or biofilm material and mobilize bacteria will be estimated based on channel characteristics. This will be compared with field measurements.

This list of constituents was developed to represent the key pollutants of concern relevant to identifying uncontrollable sources of bacteria.

2.5 Human Recreation Study

Recreational activities in the Santa Ana River are a potential source of uncontrollable bacterial indicators especially in the summer months when people tend to vacation and recreate outdoors more frequently. While the TMDL is intended to protect swimmers from potentially harmful pathogens, it is possible that the act of swimming could release FIB to the receiving water. This study will evaluate humans as an uncontrollable source of bacterial indicators by comparing bacteria levels upstream and downstream of a popular swimming hole.

2.5.1 Locations

The human recreation (swim) study location is the Santa Ana River Reach 3 area adjacent to Martha Mclean-Anza Narrows Park (Figure 2-5). Its easy access and park area makes it a popular location for recreational activity. Water levels in this section are shallow, more suitable to wading and sitting in the water than swimming. However, the shallow water depth makes it a popular location for families that have younger children. It appeared to be popular for dog-walkers as both humans and canine have been observed to be wading in the river. Two monitoring sites, one upstream and one downstream of the recreational area, are selected to capture samples reflecting both un-impacted and impacted conditions, respectively (Table 2-10). Prior to collecting a sample, reconnaissance was conducted to verify that the study location adjacent to Martha Mclean-Anza Narrows Park is a popular recreational area, as expected.

Figure 2-5 Study Location for the Human Recreation Study (Martha Mclean – Anza Narrows Park)

Table 2-10 Monitoring Sites for the Human Recreation Flow Study

Site	Description	Latitude	Longitude
Swim01A	Site upstream of human recreation in SAR at Anza Narrows Park	33°58'07.14"N	117°25'57.15"W
Swim01B	Site downstream of human recreation in SAR at Anza Narrows Park	33°58'06.66"N	117°26'07.10"W

Note: These coordinates are approximate and monitoring sites at the study locations will be determined in the field to assess areas impacted and unimpacted by human recreation.

2.5.2 Frequency and Schedule

Two sample events were conducted on weekends during the summer recreational season when the amount of swimming and other recreational activities was high. One event occurred during a holiday weekend (e.g., Labor Day) where more people are likely to be contributing to bacteria levels and the second event occurred during a non-holiday weekend.

- Holiday event – July 2, 2015 through July 6, 2015
- Non-holiday event – August 13, 2015 through August 17, 2015

During both sample events, water samples were collected from each monitoring site daily from Thursday through Monday to capture sample variability and bracket peak times for recreational activity around the targeted weekend (Table 2-11).

Table 2-11 Monitoring Plan for the Human Recreation Study

Number of Study Locations	1
Monitoring Sites per Location	2
Sample Events per Study	2 (5 days per event)
Water Samples per Monitoring Site	1 (2 per day, 10 total over 5 days)
Sediment Samples per Monitoring Site	0
Sampling Period	Summer weekend (Thursday-Monday)

2.5.3 Field and Laboratory Constituents

The following constituents were analyzed in water samples collected at each site on each sample date:

- Field Measurements – temperature, pH, turbidity, conductivity, dissolved oxygen
- Laboratory Water Quality Analysis – *E. coli*
- Laboratory Molecular Analysis – bacterial indicator sources (human and dog)

This list of constituents was developed to represent the key pollutants of concern relevant to identifying uncontrollable sources of bacteria.

2.6 Horse Recreation Study

Horseback riding is a popular recreational activity in Riverside County, particularly during warmer summer months. It is possible that the presence of horses in and around the Santa Ana River can contribute bacteria to receiving waters. This study focuses on the potential for horses as a source of bacterial contamination in SAR Reach 3.

2.6.1 Locations

Three study locations with two monitoring sites along a transect at each location were selected for this study (Figure 2-6, Table 2-12). Due to the diffuse nature of horseback riding, additional field reconnaissance was necessary to determine areas in and around SAR Reach 3 that receive substantial horse activities. The study locations are as follows:

- Santa Ana River at 66th Street and Etiwanda Avenue (Horse01) – Equestrian activities occur regularly at a sandy area by SAR upstream of this site.
- Santa Ana River adjacent to Mary Tyo Trailhead Equestrian Staging Area (Horse02) – This location is adjacent to a parking lot area designed to load and unload horses for recreating along the Santa Ana River
- Santa Ana River southeast of Downey St. and 64th St. (Horse03) – Equestrian activities occur near this SAR site

Figure 2-6 Horse Recreation Study Locations

Table 2-12 Monitoring Sites for the Horse Recreation Flow Study

Site	Description	Latitude	Longitude
Horse01A	Site at the north bank of SAR at Etiwanda Avenue	33°58’02.57"N	117°31’19.78"W
Horse01B	Site at the south bank of SAR at Etiwanda Avenue	33°58’02.30"N	117°31’19.71"W
Horse02A	Site at the east bank of SAR at Mary Tyo Equestrian Area	33°58’13.22"N	117°30’51.51"W
Horse02B	Site at the west bank of SAR at Mary Tyo Equestrian Area	33°58’13.33"N	117°30’41.88"W
Horse03A	Site at the north bank of SAR at Downey St. and 64th St.	33°58'12.43"N	117°29'18.41"W
Horse03B	Site at the south bank of SAR at Downey St. and 64th St	33°58'12.00"N	117°29'18.43"W

2.6.2 Frequency and Schedule

Two sample events were conducted on Saturdays in the summer recreational season. One event was conducted during a holiday weekend, during which the potential for horse activities are greater, and the second event was conducted during a non-holiday weekend.

- Holiday event – July 4, 2015
- Non-holiday event – August 15, 2015

For each sample event, two water samples and two sediment samples were collected along a transect from each study location to develop an understanding of sample variability (Table 2-12).

Table 2-13 Monitoring Plan for the Horse Recreation Study

Number of Study Locations	3
Monitoring Sites per Location	2
Sample Events per Study	2
Water Samples per Monitoring Site	1
Sediment Samples per Monitoring Site	1
Sampling Period	Summer weekend days (Saturday)

2.6.3 Field and Laboratory Constituents

The following constituents were analyzed in water samples collected at each site on each sample date:

- Field Measurements – temperature, pH, turbidity, conductivity, dissolved oxygen
- Laboratory Water Quality Analysis – *E. coli*
- Laboratory Molecular Analysis – bacterial indicator sources (horse)

Sediment samples were analyzed for *E. coli* and bacterial indicator sources as listed above. This list of constituents was developed to represent the key pollutants of concern relevant to identifying uncontrollable sources of bacteria.

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Section 3
Results

This section presents summary results from biological and water quality monitoring for the six pilot studies. Water quality data, including *E. coli* concentrations, molecular analyses results, and water quality parameters in tabular form are included in Appendix C.

3.1 Natural Sources Study

In the natural sources studies, samples were collected from 4 sites not influenced by MS4 discharges to investigate the potential effects of natural sources on bacteria levels in SAR. Samples were collected three times during 2015 to observe possible seasonal effects.

3.1.1 Biological Assessment

A biological assessment was conducted on May 21, 2015 for the study reach, extending from RIX to the Riverside Avenue Bridge (Appendix B). The study reach consists of a wide sandy wash and well-vegetated riparian community. Prevalent horse tracks indicate that the reach is used for equestrian activities, which likely involves domestic dog as well. Other tracks indicated raccoon and rabbit presence and a feral dog and a number of bird species were observed. Vehicle tracks and evidence of homeless presence were also observed. This riparian and in-channel habitat likely supports reptiles, amphibians, possums, coyotes, and other small mammals.

3.1.2 Water Quality

In the natural sources study, *E. coli* concentrations were quantified in water samples collected along SAR seasonally in April, June, and August (Table 3-1, Figure 3-1). Although *E. coli* levels are relatively low overall (less than 100 MPN/100 ml in all samples), there is an increasing trend with distance from RIX in April and August samples as follows:

- Average *E. coli* concentrations in April 28th samples increase from 10 MPN/100 ml at the transition sites to 91 MPN/100 ml at the most downstream sites. *E. coli* concentrations from this sampling event may have been influenced by canyon flows from the April 26 rain event. Canyon flows from upstream mountains can lead to temporary environmental conditions (e.g., altered moisture or flow levels from typical dry weather conditions) that allows for prolonged bacteria survival or growth during dry weather.

- Average *E. coli* concentrations in August 4th west bank samples increase from 10 MPN/100 ml at the transition site to 52 MPN/100 ml at the most downstream site.

However, bacteria levels in other samples do not display the increasing trend:

- Average *E. coli* concentrations on June 11th remain less than 15 MPN/100 ml at all sites.

- August 4th east bank samples show a decrease in *E. coli* levels at the downstream sites.

The frequency of this study does not comply with the 30-day 5-sample geomean criteria. As a result, samples in this study are compared to the SSM of 235 MPN/100 ml and no sample in this section of the river exceeds the SSV in this study.

The increasing trend in *E. coli* concentrations with distance from RIX suggests there is the potential for bacteria levels to exceed WQO at further downstream locations. Using the April data as a basis, *E. coli* growth rate of approximately 13 MPN/100 ml for every 1000 feet was observed at this study location. If this growth rate were to continue for the entire 8 mile section, *E. coli* levels would be 535 MPN/100 ml at the end of the 8 miles, which exceeds the WQO for *E. coli* as well as typical ranges from downstream compliance monitoring sites. Applying a lower growth rate of 3 MPN/100 ml for every 1000 feet, as observed in the August data, the *E. coli* concentration would be 139 MPN/100 ml. This shows that natural sources may account for a majority of downstream bacteria.

Table 3-1 *E. coli* Concentrations Observed in the Natural Sources Study (MPN/100 ml)

Study Location	Monitoring Site	April 28	June 11	August 4	Frequency of Bird Detection[B]
Transition from Concrete Lined to Natural Bottom	East Bank	10	20 [A]	10 [A]	67%
	West Bank	10	10	10	0%
	Average	10	15	10	33%
500 ft Downstream of RIX	East Bank	31	10 [A]	41 [A]	67%
	West Bank	10	20	20	0%
	Average	20.5	15	30.5	33%
4000 ft Downstream of RIX	East Bank	74	10	20 [A]	33%
	West Bank	63 [A]	10 [A]	52 [A]	100%
	Average	68.5	10	36	67%
6400 ft Downstream of RIX	East Bank	96	20 [A]	10 [A]	67%
	West Bank	86	10 [A]	52 [A]	67%
	Average	91	15	31	67%

[A] DNA analysis showed presence of birds
[B] DNA analysis did not show presence of humans, canines, or rumen in any sample

Samples collected in this study were analyzed for human and wildlife (birds, canine, and rumen) as potential uncontrollable sources of bacteria. Bird DNA was detected by MST analyses in 50 percent of all samples collected in this study (Table 3-1). Results indicate that birds were detected more frequently during warmer months (June and August), as described below:

- Only one of the April 28th samples (13%) showed bird presence, at 4000 ft downstream of RIX.
- 63 percent of June 11th samples showed bird presence.
- 75 percent of August 4th samples showed bird presence.

Spatial trends indicate that bird DNA was detected more frequently from the two most downstream monitoring sites, where 67% of the samples that showed bird DNA were collected. The downstream sites are more riparian than the upstream sites where SAR transitions from a concrete-lined channel to a natural, soft-bottomed channel. Birds may prefer the more riparian habitat, particularly during warmer months with increased bird activity. However, samples in which birds were detected did not correspond to higher *E. coli* concentrations (8 out of the 12 samples with birds had bacteria concentrations of 20 MPN/100 ml or less). Humans, canines, and rumen were not detected at any site in this study, which may reflect limited activity by these potential hosts. Also, the absence of detection may reflect the challenge in capturing samples where specific hosts can be identified due to the limitations of MST methods.

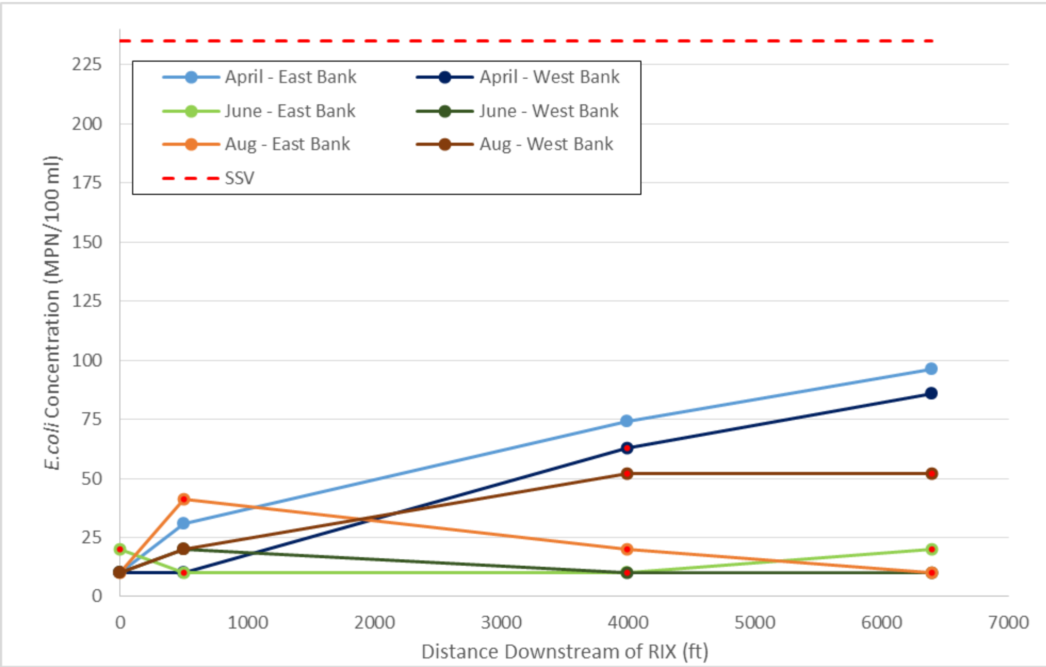

Figure 3-1 *E. coli* Concentrations Observed in the Natural Study

(Circles with red fill indicate bird detected by molecular analysis; The applicable SSV to evaluate the bacteria concentration is 235 MPN/ml. This is used where there is insufficient data to calculate a 30-day 5-sample geomean.)

Figure 3-2 Santa Ana River at transition site (Natural01)

Figure 3-3 Santa Ana River 4000 ft downstream of RIX (Natural03)

Figure 3-4 Santa Ana River 6400 ft downstream of RIX (Natural04)

3.2 Bird Study

In the bird study, water quality samples were collected weekly for five weeks at two study locations where bird activity is prominent. The five weeks target periods of peak bird nesting and activity. Additionally, a biological survey was conducted on May 21, 2015 to assess bird nesting and wildlife habitat in conjunction with water quality monitoring at the Schleisman Road and Mission Boulevard Bridges. The assessment is included in its entirety in Appendix B.

3.2.1 Schleisman Road Bridge

3.2.1.1 Biological Assessment

At Schleisman Road Bridge over Cucamonga Creek, wire netting covered over half of the underside of the bridge, installed to deter bird nesting. Approximately 60 cliff swallows, 26 active nests, and a number of nestlings were observed at Schleisman Road Bridge. However, a total of 293 nests, including inactive nests, were observed. Two thirds of active nests were located over water and the remaining were located over dry parts of the channel. Adult birds visited the nests every few minutes and fecal waste was observed to be accumulating in the dry parts of the channel under the nests. Flow spanned 40 feet of the channel width. Other birds at the this study location included barn swallows, black phoebes, Brewer’s blackbirds, American crows, turkey vultures, and merlins.

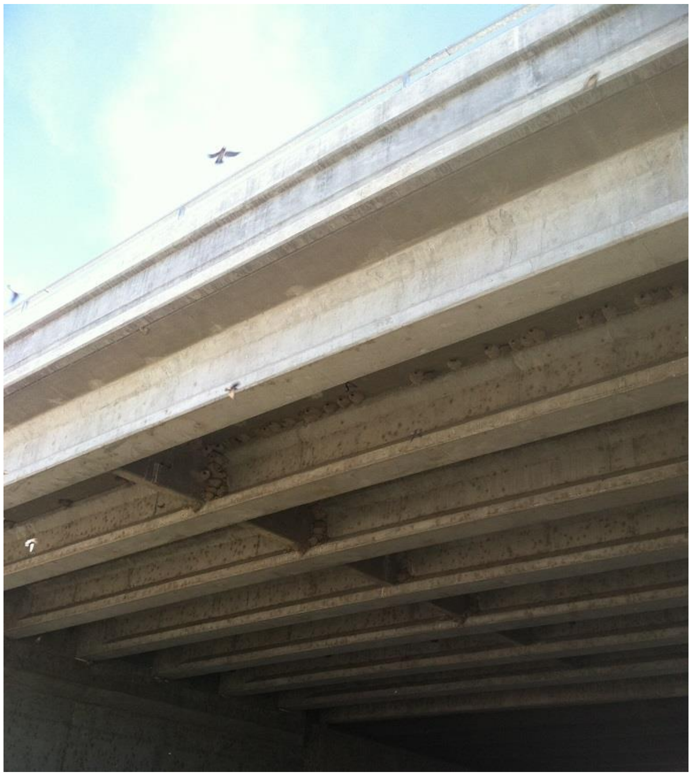

Figure 3-5 Swallow nests under Schleisman Road Bridge

3.2.1.2 Water Quality

All four monitoring sites have *E. coli* concentrations in similar ranges during each of the five weeks of monitoring (Table 3-2, Figure 3-6). However, the following observations regarding temporal and spatial variability were made based on results:

- *E. coli* levels are consistently highest on April 29 at all sites (average 3,923 MPN/100 ml) while bacteria levels during the following four weeks were substantially lower (average 758 MPN/100 ml). The elevated *E. coli* levels may have been influenced by the April 26 canyon flows, potentially resulting in more favorable environmental conditions for bacteria survival as previously described (see Section 3.1.2).

- The initial decrease (over 80%) in *E. coli* levels at all sites is followed by a generally increasing trend over the remaining four weeks. As mid-May was predicted to have peak activity during the bird season, the trend could reflect the increase in bird activity during later weeks.

- Average upstream and downstream *E. coli* levels showed differences on a weekly basis, with upstream concentrations greater than downstream concentrations two of the weeks sampled. This suggests that the upstream site may not be far enough upstream to reflect an un-impacted monitoring site and that bird activity near the bridge may extend farther upstream than anticipated.

- During the final week of monitoring, average levels at both upstream and downstream sites were similar (RPD less than 0.5 percent).

Molecular analyses detected the presence of birds in 70 percent of the samples collected. Similar to *E. coli* concentrations, detections varied temporally and spatially and are described as follows (Table 3-2):

- 9 out of 10 samples from upstream monitoring sites showed presence of bird DNA markers, which also suggests that the upstream site is not representative of un-impacted sites.
- 5 out of 10 samples from downstream monitoring sites showed presence of bird DNA markers.
- At both upstream and downstream sites, the third week of monitoring resulted in the lowest frequency (25%) of bird detection relative to other weeks.
- Although the first week of monitoring showed the highest *E. coli* concentrations, the second and fifth week had the highest frequency of bird detection (100% during both weeks).

In this study, elevated levels of *E. coli* were observed during the five week monitoring period. All twenty samples exceeded the Basin Plan single sample value of 235 MPN/100 ml (Figure 3-6). Elevated *E. coli* concentrations in conjunction with the high frequency of bird detection in the samples may reflect bird contributions to bacteria in areas where birds nest. Biofilms were also observed at this study location and could potentially increase bacteria levels in overlying water when disturbed.

Table 3-2 *E. coli* Concentrations Observed at Schleisman Avenue Bridge in the Bird Study (MPN/100 ml)

Date	Upstream Left Bank	Upstream Right Bank	Upstream Average	Downstream Left Bank	Downstream Right Bank	Downstream Average	Frequency of Bird Detection
04/29/15	4,884 [A]	4,106 [A]	4,495	3,255	3,448 [A]	3,352	75%
05/07/15	373 [A]	345 [A]	359	602 [A]	473 [A]	538	100%
05/14/15	471 [A]	836	654	464	545	505	25%
05/21/15	738 [A]	586 [A]	662	860	565	713	50%
05/27/15	1,722 [A]	906 [A]	1,314	1,624 [A]	1,017 [A]	1,321	100%
Geomean	**1,017**	**911**	**983**	**1,049**	**874**	**969**	--
Frequency of Bird Detection	**100%**	**80%**	**90%**	**40%**	**60%**	**50%**	--

[A] DNA analysis showed presence of birds

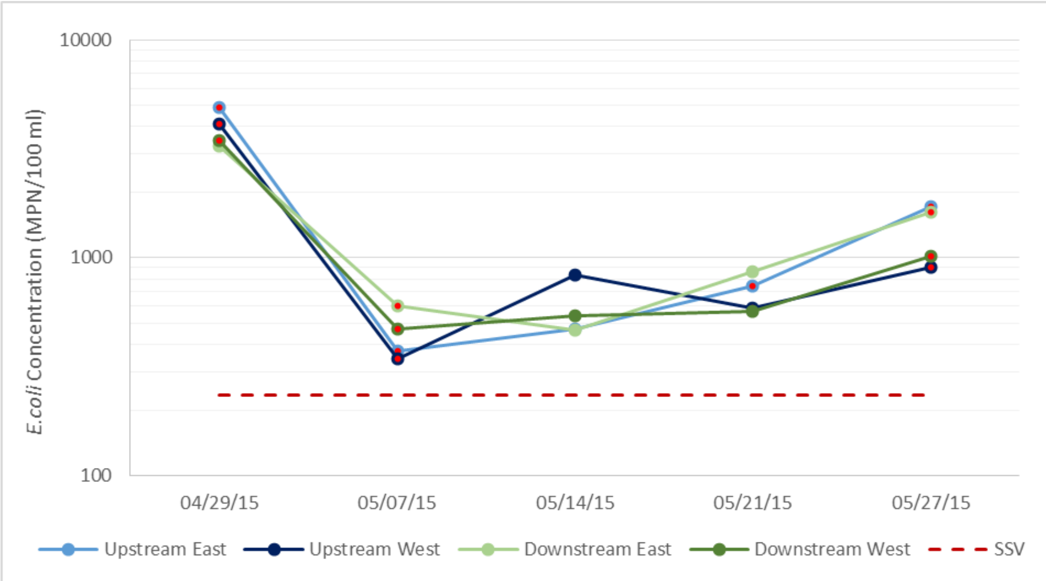

Figure 3-6 *E. coli* Concentrations Observed at Schleisman Avenue Bridge in the Bird Study
(Circles with red fill indicate bird detected by molecular analysis; The applicable SSV to evaluate the bacteria concentration is 235 MPN/ml. This is used where there is insufficient data to calculate a 30-day 5-sample geomean.)

Figure 3-7 Cucamonga Creek at Schleisman Road Bridge on April 29, 2015

3.2.2 Mission Boulevard Bridge

3.2.2.1 Biological Survey

At Mission Boulevard Bridge over Santa Ana River, water flowed approximately one foot deep across the western half of the river. Approximately 45 cliff swallows, 30 active nests, and nestlings were observed at this study location. However, a total of 128 active and inactive nests were observed under the western half of the bridge. Due to the depth of flow, the eastern half of the bridge was not surveyed due to safety concerns. Approximately two thirds of the active nests at this location were also located over the water. Rock doves (pigeons), who appeared to exhibit courtship and territorial behavior despite the lack of nests, were observed at the bridge also. Other wildlife include black phoebes, house wrens, yellow warblers, common yellowthroats, Wilson's warblers, bushtits, Anna's hummingbirds, house finches, and ground squirrels.

3.2.2.2 Water Quality

E. coli levels at Mission Boulevard Bridge are much lower than levels at Schleisman Road Bridge by more than an order of magnitude and showed no discernible temporal or spatial trends. All four monitoring sites have similar ranges of *E. coli* concentrations during the five weeks of monitoring, ranging from 20 to 130 MPN/100 ml (Table 3-3, Figure 3-8). The following observations regarding temporal and spatial variability were made based on results:

- *E. coli* concentrations varied temporally during the monitoring period with peak concentrations observed during different weeks for each monitoring site.
- Average upstream *E. coli* levels are higher than downstream levels during 4 out of 5 monitoring weeks, which may suggest that the upstream site does not represent an un-impacted site and/or that the presence of birds is ubiquitous in the area. Identifying a site without bird impacts in this region may not be possible. There may also be additional sources that contribute bacteria at these upstream sites, which could obscure impacts by birds.
- *E. coli* levels at both upstream and downstream west bank monitoring sites show an initial decrease followed by an increasing trend in later weeks. However, *E. coli* levels in east bank sites have no discernible trend. This suggests that bacteria levels vary spatially even across transects where monitoring sites are relatively close to one another.
    - The similarity in bacteria levels among the western monitoring sites may reflect the significant presence of birds (over 100 active and inactive nests) under the western half of Mission Boulevard Bridge observed by the biologist. The increasing trend in later weeks at these sites may be indicative of increased bird activity as peak bird season was estimated to occur around mid-May.

Molecular analyses detected the presence of birds in 50 percent of the samples collected. Similar to *E. coli* concentrations, molecular analyses resulted in temporal and spatial variations as follows:

- 7 out of 10 samples from upstream monitoring sites showed presence of bird DNA markers, which again suggests that the presence of birds is ubiquitous in this study location.
- 3 out of 10 samples from downstream monitoring sites showed presence of bird DNA markers.
- At both upstream and downstream sites, the fourth week of monitoring resulted in the lowest frequency (0 percent) of bird detection relative to other weeks.

Although active bird nests and birds were observed at Mission Boulevard Bridge, *E. coli* levels in all twenty samples did not exceed the Basin Plan SSV (Figure 3-8). It is unclear why bacteria levels at this study location are substantially lower than levels observed at the Schleisman Road Bridge study location, however, water is much deeper at these monitoring sites (1 foot minimum) than at Schleisman Road Bridge monitoring sites (6 inches) and the potential for spatial variability and dilution is increased. The biologist also observed that nests were more dispersed at Mission Boulevard

Bridge than at Schleisman Road Bridge, which may contribute to the lower frequency of bird detection at this study location as well as the potential for *E. coli* spatial variability.

Table 3-3 *E. coli* Concentrations Observed in the Bird Study at Mission Avenue Bridge (MPN/100 ml)

Date	Upstream Left Bank	Upstream Right Bank	Upstream Average	Downstream Left Bank	Downstream Right Bank	Downstream Average	Frequency of Brid Detection
04/29/15	97 [A]	132 [A]	115	98 [A]	85	92	75%
05/07/15	121 [A]	31 [A]	76	41	41 [A]	41	75%
05/14/15	20 [A]	109	65	122	52 [A]	87	50%
05/21/15	121	122	122	74	110	92	0%
05/27/15	85 [A]	121 [A]	103	52	98	75	50%
Geomean	**75**	**92**	**84**	**72**	**72**	**72**	--
Frequency of Bird Detection	**80%**	**60%**	**70%**	**20%**	**40%**	**30%**	--

[A] DNA analysis showed presence of birds

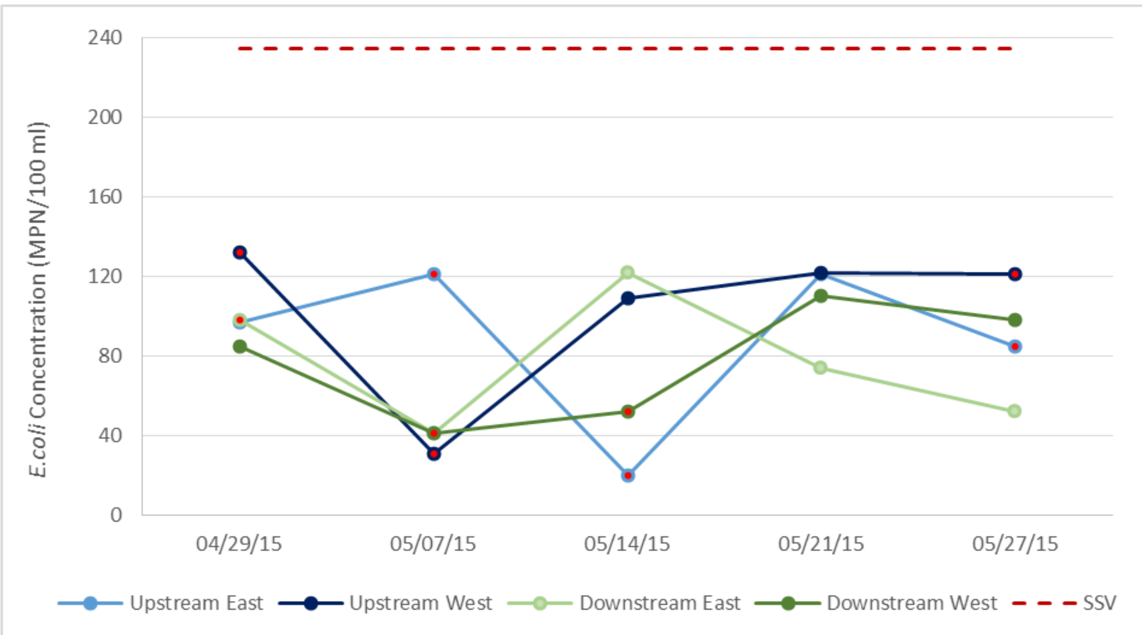

Figure 3-8 *E. coli* Concentrations Observed at Mission Avenue Bridge in the Bird Study

(Circles with red fill indicate bird detected by molecular analysis; The applicable SSV to evaluate the bacteria concentration is 235 MPN/ml. This is used where there is insufficient data to calculate a 30-day 5-sample geomean.)

Figure 3-9 Santa Ana River at Mission Boulevard Bridge on April 29, 2015

3.3 Stormwater Channel Study

In the stormwater channel study, water quality samples are collected seasonally from both the water column and sediment and biofilm to compare the extent to which *E. coli* is associated with water and sediment or periphyton. Wildlife has also been observed at both concrete-lined and soft-bottom channels and may be another potential uncontrollable sources of bacteria.

3.3.1 *E. coli* in Water Samples

E. coli concentrations in water samples (Table 3-4, Figure 3-10) have varying trends at each site:

- At John Bryant park, *E. coli* levels decreased after the first sampling event in May, where the average concentration across a transect was 370 MPN/100 ml to 9 MPN/100 ml in October. Bacteria levels increase in the final event in January with an average concentration across the transect of 260 MPN/100 ml.

- Average *E. coli* concentrations from a transect in Sunnyslope Channel are relatively stable throughout the four events with average concentrations ranging from 135 to 205 MPN/100 ml.

- At Eastvale Line E, average *E. coli* levels oscillate throughout the four events with average concentrations exceeding 2,000 MPN/100 ml during July 2015 and January, 2016, but are 240 MPN/100 ml during May and October, 2015. Concentrations were particularly high in October and January events.

3.3.2 *E. coli* in Sediment and Biofilm Samples

E. coli concentrations in sediment and biofilm samples (Table 3-4, Figure 3-11) have similar trends at John Bryant Park and Eastvale Line E, where concentrations are generally higher in the two latest events while concentrations observed at Sunnyslope Channel are higher only in October.

- At John Bryant Park, average *E. coli* levels decreased in July by more than an order of magnitude (3,200 MPN/100 g) but increased significantly in following events by approximately two orders of magnitude (210,000 MPN/100 g)

- At Sunnyslope Channel, average bacteria levels increased in October by approximately two orders of magnitude (190,000 MPN/100 g) but decreased in January by three orders of magnitude (1,950 MPN/100 g).

- At Eastvale Line E, average *E. coli* concentrations oscillate throughout the four events similar to average concentrations observed in Eastvale Line E water samples. Average concentrations decrease or increase by more than an order of magnitude during each sampling event. However, events when average concentrations in water samples decrease, average concentrations in biofilm and sediment samples increase and vice versa. This may be due to bacteria settling into sediment, moving downstream, and attaching to particles for transport (Walters et al, 2014; Curtis and Trapp, 2014) and resuspension into the water column (Jamieson et al, 2005; McDaniel et al, 2013), although other factors and mechanisms are likely occurring as well.
    - Additionally, average *E. coli* concentrations at Eastvale Line E are also generally more than an order of magnitude or more greater than average concentrations observed at the other two study locations, with particularly high levels observed in October.

E. coli concentrations in biofilm samples (average: 202,000 MPN/100 g) showed no apparent trend of being greater or lesser than concentrations in sediment samples (220,000 MPN/100 g). Observations based on the type of sample (biofilm or sediment) are as follows:

- *E. coli* concentrations at John Bryant Park reflect biofilm samples in May and January events (range: 4,800 to 210,000 MPN/100 g, average: 85,000 MPN/100 g) and sediment samples in July and October events (range: 2,400 to 190,000 MPN/100 g, average: 54,000 MPN/100 g).

- *E. coli* concentrations at Eastvale Line E reflect biofilm samples only in the May east bank sample (670,000 MPN/100 g) and sediment samples in all other samples (range: 7,800 to 2,400,000 MPN/100 g, average: 505,000 MPN/100 g).

- *E. coli* concentrations at Sunnyslope Channel range from 90 to 270,000 MPN/100 g and are generally lower than concentrations observed at the other study locations.

The single sample SSV for water samples was exceeded most frequently at Eastvale Line E (100%) and less frequently at John Bryant Park (50%) and Sunnyslope Channel (13%). Bacteria concentrations in both water and sediment and biofilm samples from Eastvale Line E are higher than concentrations at other study locations. Although it is uncertain what is causing the high presence of *E. coli* at Eastvale Line E, these results are consistent with data from prior monitoring activities, including the Tier 2 Source Evaluation where Eastvale Line E was identified as a priority MS4 drainage area[8]. Sunnyslope Channel exhibited the lowest *E. coli* concentrations in both water and sediment samples. It is possible the canopy provided by surrounding trees reduces the ambient temperature in the study location and reduces bacterial growth rates as a result, however, it is likely a number of factors are influencing bacteria levels.

[8] Triennial Middle Santa Ana River Bacterial Indicator TMDL Implementation Final Report, February 2016

In this study, *E. coli* levels are 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 are a reservoir for *E. coli.* At all study locations, biofilms and sediment also exhibit generally low *E. coli* concentrations in the July sampling event, during which higher temperatures and UV exposure may have impacted bacteria growth. The range of *E. coli* levels observed in biofilm and sediment also appear to be similar, implying that one site does not harbor *E. coli* more so than the other.

3.3.2 Source Tracking

Molecular analyses detected birds and canines in this study, however, humans and rumens were not detected in any sample. As these study locations are not popular areas for water recreation, it is not unexpected that humans were not detected. Rumens are also not likely to be present in the concrete-lined study location, which are located by housing communities. However, ruminant animals, specifically cattle, were observed at properties within 0.5 miles of the Sunnyslope Channel study location during initial site visits.

- At John Bryant Park, birds were detected in 38 percent of water samples and 13% of sediment and biofilm samples. Detection was observed only during the October and January events. Canine was also detected in one biofilm sample in January.
- At Sunnyslope Channel, birds were detected in 75 percent of the water samples and 25 percent of the sediment and biofilm samples. Spatially, bird DNA was observed more frequently at the west bank site than the east bank site. The higher frequency of bird detection at Sunnyslope Channel may be influenced by its location in the Louis Rubidoux Nature Center and the abundance of trees as potential bird habitats.
- At Eastvale Line E, birds were detected in 25 percent of the water samples but not in any sediment or biofilm samples. Detection was observed only during the October event. This study location is a concrete-lined channel adjacent to undeveloped land and housing communities and does not appear to be a good habitat for wildlife. The lack of source detection at Eastvale Line E, particularly in relation to the highest *E. coli* concentrations observed, may suggest that elevated bacteria levels are less influenced by direct wildlife inputs and more so by other sources.

Table 3-4 *E. coli* Concentrations Observed in the Stormwater Channel Study

Matrix	Date	John Bryant Park			Eastvale Line E			Sunnyslope Channel		
		North Bank	South Bank	Average	East Bank	West Bank	Average	East Bank	West Bank	Average
Water (CFU/100 ml)	May	360	380	**370**	580	240	**410**	120	150 [A]	**85**
	July	360	140	**250**	940	3,600	**2,270**	170	150 [A]	**160**
	Oct	9 [A]	9	**9**	830 [A]	240 [A]	**535**	290 [A]	120 [A]	**205**
	Jan	460 [A]	60 [A]	**260**	2,800	3,000	**2,900**	210 [A]	140 [A]	**175**
Sediment & Biofilm (CFU/100 g)	May	6.7E4	5.8E4	**6.3E4**	6.7E5	2.2E5	**4.5E5**	4.0E2	1.9E3 [A]	**1.2E3**
	July	2.4E3	3.2E3	**2.8E3**	7.8E3	7.8E3	**7.8E3**	9.2E3	4.0E2 [A]	**4.8E3**
	Oct	1.9E5	2.2E4	**1.1E5**	2.5E5	2.4E6	**1.33E6**	1.1E5	2.7E5	**1.9E5**
	Jan	4.8E3	2.1E5 [B]	**1.1E5**	3.8E5	2.7E5	**3.3E5**	3.0E2	9.0E1	**2.0E2**

[A] DNA analysis showed presence of birds
[B] DNA analysis showed presence of birds and canines

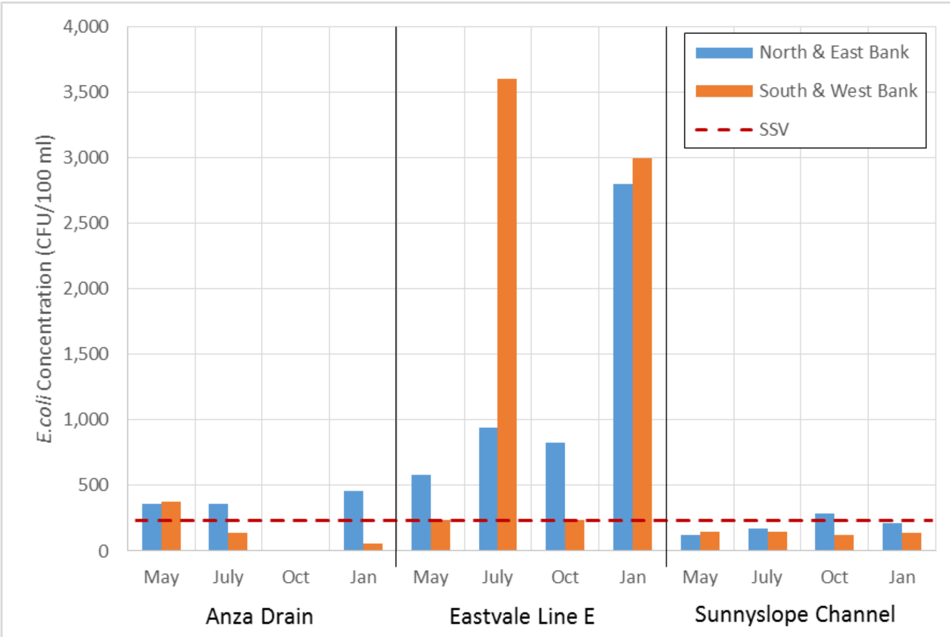

Figure 3-10 *E. coli* Concentrations Observed in Water Samples in the Stormwater Channel Study
(The applicable SSV to evaluate the bacteria concentration is 235 MPN/ml. This is used where there is insufficient data to calculate a 30-day 5-sample geomean.)

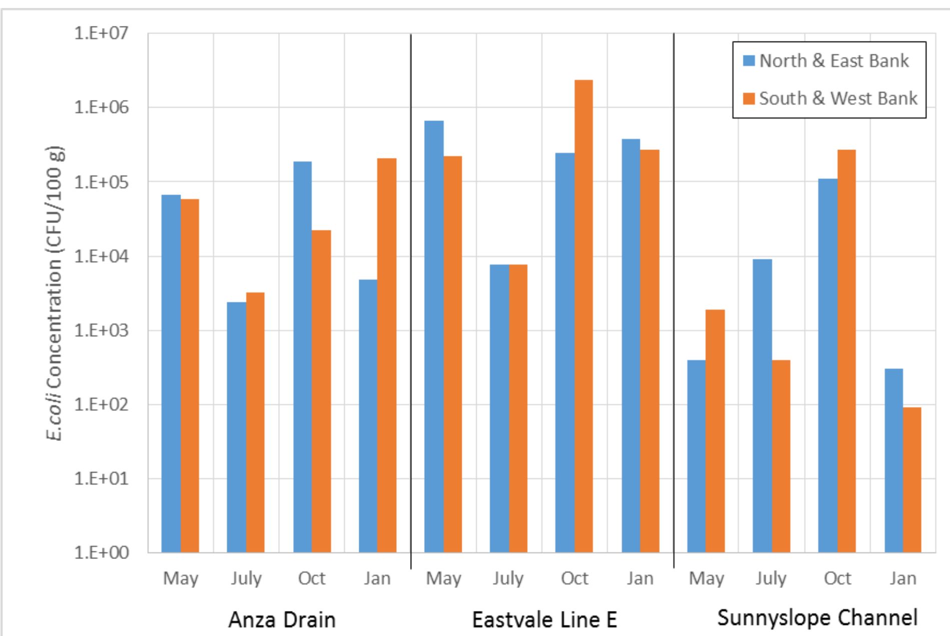

Figure 3-11 *E. coli* Concentrations Observed in Sediment and Biofilm Samples in the Stormwater Channel Study

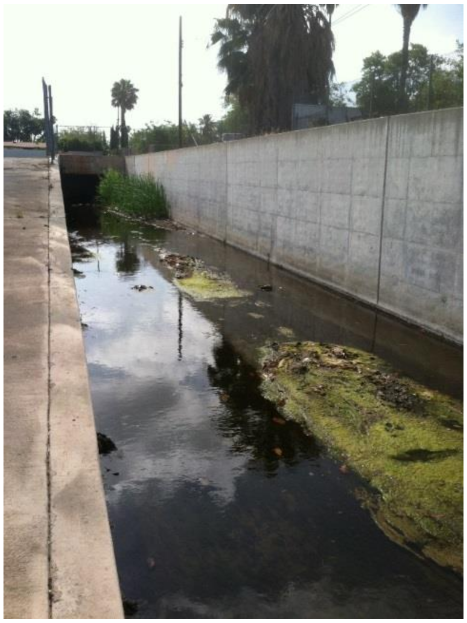

Figure 3-12 Anza Drain at John Bryant Park on May 13, 2015

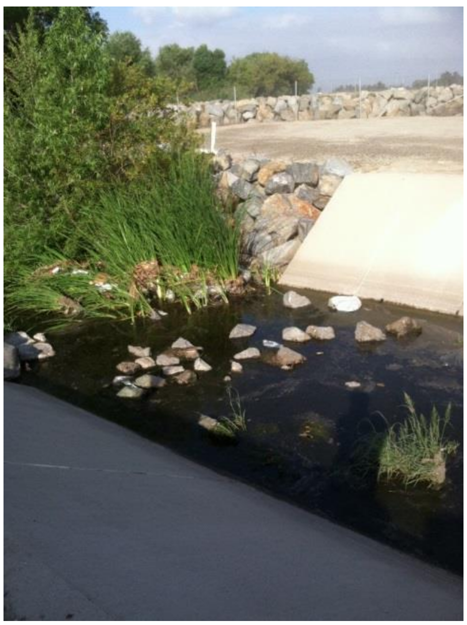

Figure 3-13 Eastvale Line E on May 13, 2015

Figure 3-14 Sunnyslope Channel on May 13, 2015

3.4 Non-MS4 Flow Study

The non-MS4 flow study intended to identify if non-stormwater flows could resuspend or shear bacteria present in sediment and biofilms by comparing *E. coli* levels in the water column and biofilm and sediment from sites upstream and downstream of non-MS4 flow discharge.

3.4.1 Monitoring Activities

Field staff were deployed on June 17 and July 22, 2015 to collect water quality samples as part of the non-MS4 flow study. Field staff were on site for an hour at each of the study locations during both sampling events. However, non-MS4 flow discharges at irregular times and field staff did not observe any flow from Jurupa Community Services District outfalls to either Day Creek (Figure 3-15) or San Sevaine Channel (Figure 3-16). As a result, no samples were collected for this study. Samples may be collected during upcoming, coordinated Jurupa Community Services District discharges in the summer of 2016.

While the study intended to test the hypothesis that non-MS4 discharges mobilize bacteria, samples were unable to be collected due to unpredictable discharge times. However, a review of water level data collected over a period of 2 months in 2014 from Day Creek and San Sevaine well blowoffs shows that water level varies up to 0.7 and 2.3 feet, respectively. This suggests that non-MS4 discharges result in highly variable flow in channels and has the potential to mobilize bacteria. As the supply for dry weather flow from tertiary effluent has decreased in recent years (Section 3.4.2), the role of non-MS4 discharges, such as *de minimus* discharges could potentially become more important.

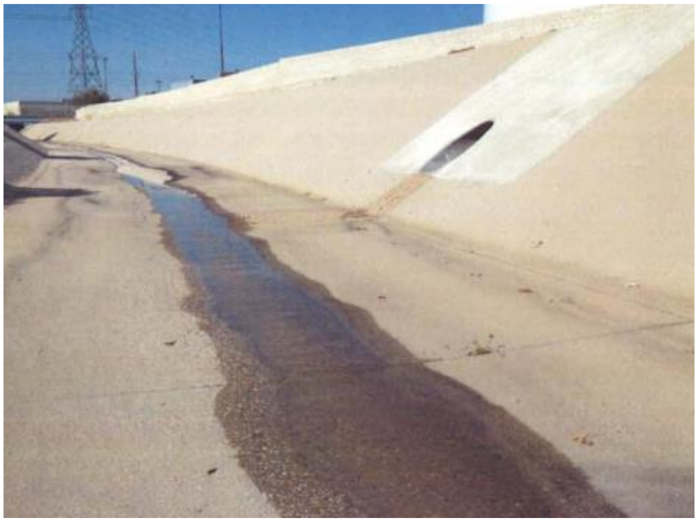

Figure 3-15 Day Creek on June 17, 2015

Figure 3-16 San Sevaine Channel on July 22, 2015

3.4.2 Historical Flow Record

Through the implementation of monitoring required to meet the Riverside County MS4 NPDES Permit for the Santa Ana River basin, RCFC&WCD and the co-permittees have assessed dry weather flow within MS4 facilities since 1990. As a result of drought conditions, economic concerns, and water conservation efforts, dry weather flow within MS4s have reduced during the last decade. Figure 3-17 shows that samples have become increasingly unable to be collected due to insufficient flow (noted as VNS in the figure) since 1996. To investigate the potential for dry weather flows to shear or resuspend bacteria from biofilms and sediment and how to address this mechanism as a source for bacteria, it is important to understand the sources and discharge rates of dry weather flow.

The predominant source of dry weather flow within the impaired waterbodies in from tertiary treated POTW effluent; however, rates have steadily declined since 2004 (Figure 3-18). This decline is largely due to implementation of projects to reuse wastewater and reduce demand on groundwater basins or imported water sources. Reduced flow rates and velocity would reduce shear stress on sediment and biofilm in the bottom of the impaired waters. On the other hand, the dilution of FIB in receiving waters provided by addition of tertiary treated effluent is diminishing (see Triennial Review Report for dynamic analysis of this condition).

Changes in water level and DWF may be attributed to factors such as diurnal water demand patterns or sporadic non-MS4 *de minimus* discharges. Water level records from San Sevaine Channel and Day Creek showed that *de minimus* discharges are highly sporadic and unpredictable (Figure 3-19), and can rapidly increase flow depths in channels. Perhaps most important to the potential for shearing and resuspension is a condition of extreme fluctuations in effluent discharge rates that is caused by operation of recycled water systems. The sharp increase or decrease in dry weather discharge to channels may facilitate deposition and scour processes that could allow for colonization and resuspension of FIB. This condition is most notable for Inland Empire Utility Agency RP1 discharge to Cucamonga Creek (Figure 3-20).

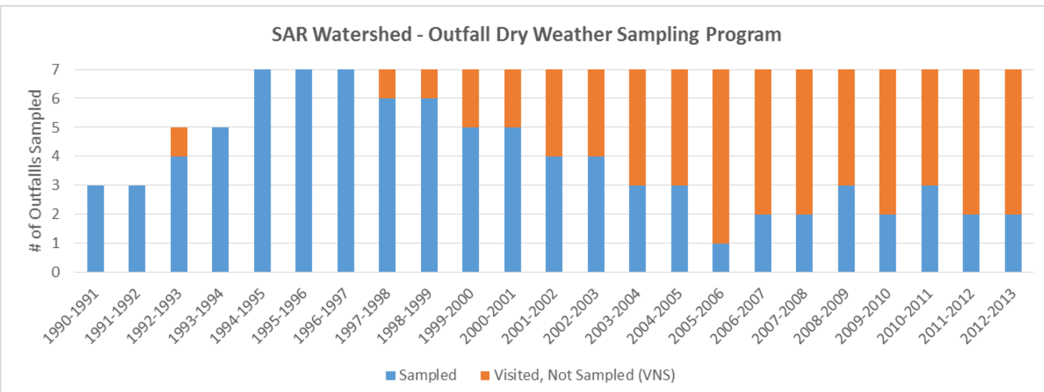

Figure 3-17 Changes in the Ratio of Outfalls "Visited Not Sampled" (VNS) and Outfalls Sampled During a Visit from 1990 through 2013[9]

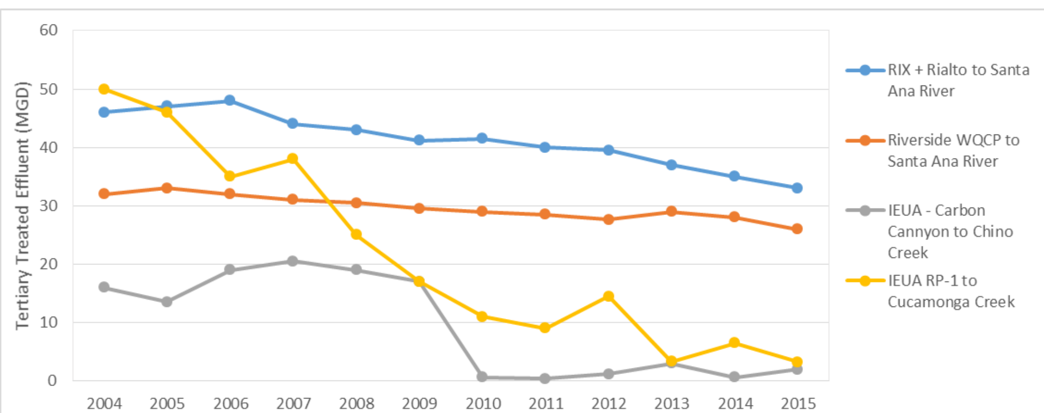

Figure 3-18 Average Daily POTW Effluent in August/September to be Impaired Waters in the MSAR Watershed from 2004 through 2005[10]

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[9] Riverside County Stormwater Program Report of Waste Discharge

[10] http://www.sawpa.org/wp-content/uploads/2012/05/20150615-Reach-3-TDS-Investigation-II-final.pdf

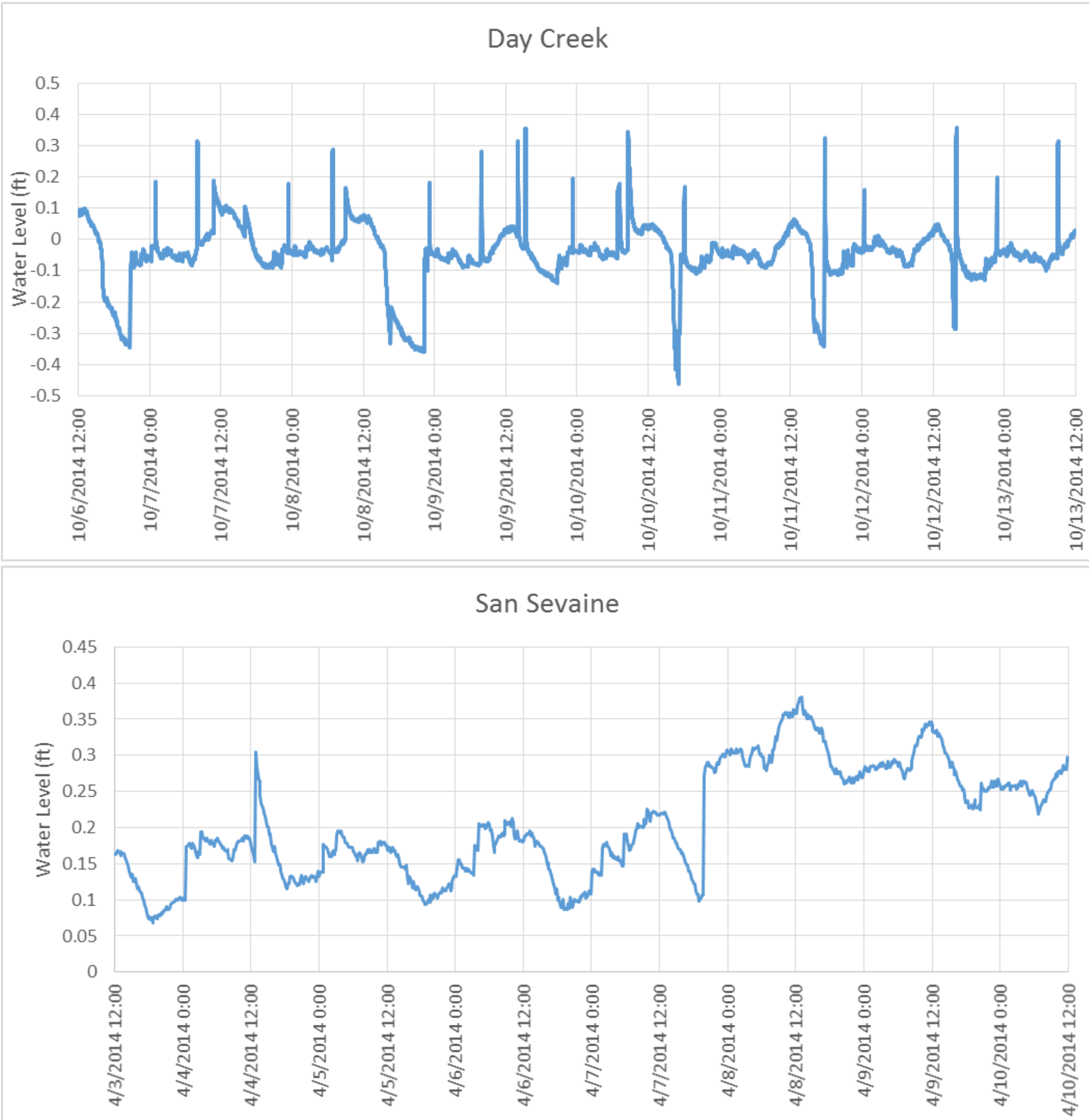

Figure 3-19 Water Level from *De Minimus* Discharges at (A) Day Creek and (B) San Sevaine Channel over a One Week Period

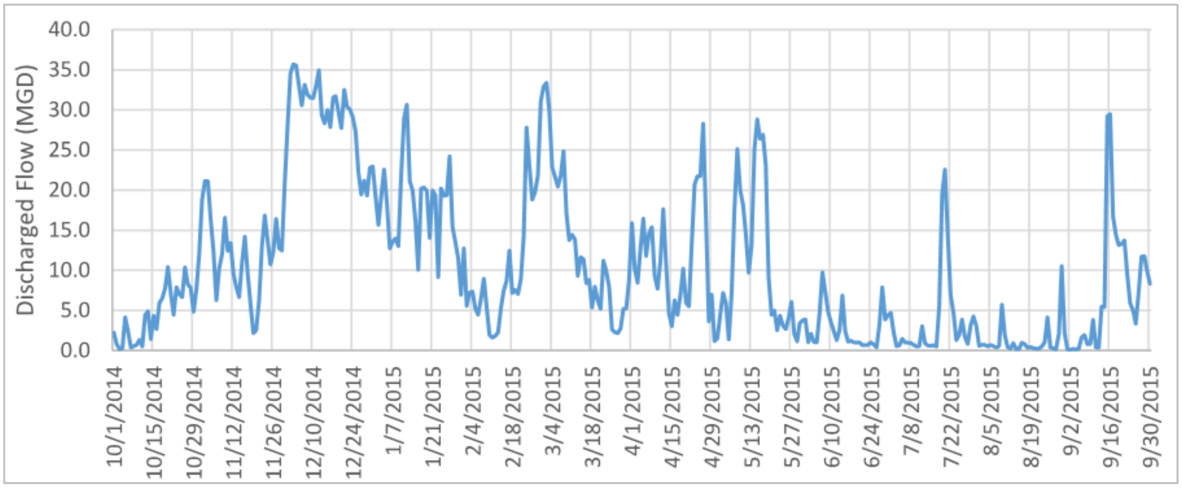

Figure 3-20 Daily Discharge of POTW Effluent to Cucamonga Creek over a One Year Period in 2014-2015

3.5 Human Recreation Study

In the human recreation study, water quality samples were collected daily for two five-day periods from the water column to observe effects of human recreation on bacteria levels. The two five-day periods target a holiday and a non-holiday weekend to compare effects of more human recreation during a holiday with that from less human recreation during a typical weekend.

3.5.1 Visual Observations

Based on visual observations (Figure 3-21 through 3-23), there were approximately a dozen or more people recreating in the river between the upstream and downstream sites on Thursday and Friday of the holiday weekend. On Saturday, July 4, more than a hundred people were in the river spanning the region upstream of the upstream site, between the two study sites, and downstream of the downstream site. On Sunday, only two people were observed in the river between the upstream and downstream sites and on Monday, nobody was in the river.

During the non-holiday weekend, three people were in the river between the two study sites on Thursday. One person was seen walking a dog in the river upstream of the upstream study site on Friday. Like the Saturday of the holiday weekend, people were observed in the river spanning the region upstream of the upstream site through downstream of the downstream site but in much fewer numbers (approximately a dozen people). People were also observed upstream of the upstream site on Saturday along with a person with a dog between the two study sites and dog feces on the sandbar in the river. Again, no person was observed in the river on Monday of the non-holiday weekend, however, a high volume of trash was present in the area.

Figure 3-21 SAR by Martha Mclean Anza Narrows Park on July 4, 2015

Figure 3-22 SAR by Martha Mclean Anza Narrows Park on July 4, 2015

Figure 3-23 SAR by Martha Mclean Anza Narrows Park on August, 2015

3.5.2 Water Quality

E. coli concentrations were analyzed in water samples collected daily over five day periods during two weekends during the summer of 2015 at Martha Mclean Anza Narrows Park (Table 3-5, Figure 3-24 and Figure 3-25). This included a holiday weekend, Independence Day, and a non-holiday weekend. During the holiday weekend, there is a generally increasing trend in *E. coli* concentrations through Sunday followed by a decrease on Monday, which is a similar trend to number of people recreating in the river throughout the weekend. This observation suggests that the presence of human recreation impacted bacteria levels in SAR at this study location. *E. coli* levels were higher at the downstream site than the upstream site on Thursday, Friday, and Monday by as much as 45%. However, *E. coli* levels were 36% and 22% higher at the upstream site on Saturday, July 4th, and Sunday, respectively. Elevated upstream *E. coli* concentrations on Saturday and Sunday may be influenced by the large number of people recreating on July 4th, extending farther upstream than the upstream monitoring site. The highest *E. coli* concentration over the holiday weekend was observed on Sunday at both upstream and downstream sites with 780 and 610 MPN/100 ml, respectively. These concentrations are much higher than typical ranges measured at the compliance monitoring sites.

Molecular analyses did not detect presence of humans or dogs on any day during the holiday weekend. Although MST analyses did not detect humans, *E. coli* concentrations may be impacted by the presence of humans. Human recreation in the river may lead to direct fecal deposition but even more so, it may result in sediment resuspension that leads to increased bacteria levels in the water column. As grab samples are not collected comprehensively in the study location, detecting sources of bacteria, which can be highly variable spatially, is challenging. Additionally, source-specific markers can degrade rapidly (within one day), making sources difficult to detect (Bae and Wuertz, 2015). Studies have suggested that detection of source-specific markers may be evidence of recent fecal deposition but associated bacteria contribution can persist longer (Balleste and Blanch, 2010).

E. coli levels at the upstream site during the non-holiday weekend remained relatively low from Thursday through Sunday (average: 288 MPN/100 ml) but increased significantly on Monday (1,200

MPN/100 ml), whereas levels at the downstream site during the non-holiday peaked on Saturday and remained relatively low otherwise. *E. coli* levels at the downstream site were higher than the upstream site on Thursday, Saturday, and Sunday by nearly as much as three-fold. These observations support a finding that bacteria levels are affected by human recreation and result in higher bacteria levels downstream of human recreation. Conversely, *E. coli* levels were higher at the upstream site on Friday and Monday by as much as 63%. Peak *E. coli* concentrations were observed on Monday at the upstream site and on Saturday at the downstream site, with 1,200 and 820 MPN/100 ml, respectively. This is more the three times and nearly twice the next highest *E. coli* levels observed at those sites.

During the non-holiday weekend, molecular analyses detected the presence of dog on Monday at the upstream site, which corresponds with elevated *E. coli* concentration. All other molecular analyses did not detect presence of humans or dogs otherwise. The lack of detection may reflect challenges described in the previous paragraph and does not necessarily eliminate humans as a source of bacteria. The presence of a few people may be enough to resuspend bacteria into the water column. The detection of dog in the sample with particularly high *E. coli* levels could reflect effects of direct deposition into the river or recent fecal deposition, as described by Balleste and Blanch (2010), given that dog feces were observed in the river.

The single sample SSV was exceeded by all but one samples and the 5-sample WQO was exceeded by geomeans calculated at each site (Figure 3-26).

Table 3-5 *E. coli* Concentrations Observed in the Swim Study (MPN/100 ml)

	Date		Swim01A (Upstream)	Swim01B (Downstream)	Average
Holiday Weekend	Thursday	7/2/2015	300	330	315
	Friday	7/3/2015	250	290	270
	Saturday	7/4/2015	440	280	360
	Sunday	7/5/2015	780	610	695
	Monday	7/6/2015	330	480	405
	Average		**420**	**398**	**409**
Non-Holiday Weekend	Thursday	8/13/2015	310	460	385
	Friday	8/14/2015	360	330	345
	Saturday	8/15/2015	220	820	520
	Sunday	8/16/2015	260	330	295
	Monday	8/17/2015	1,200 [A]	450	825
	Average		**470**	**478**	**474**

[A] Canine was detected

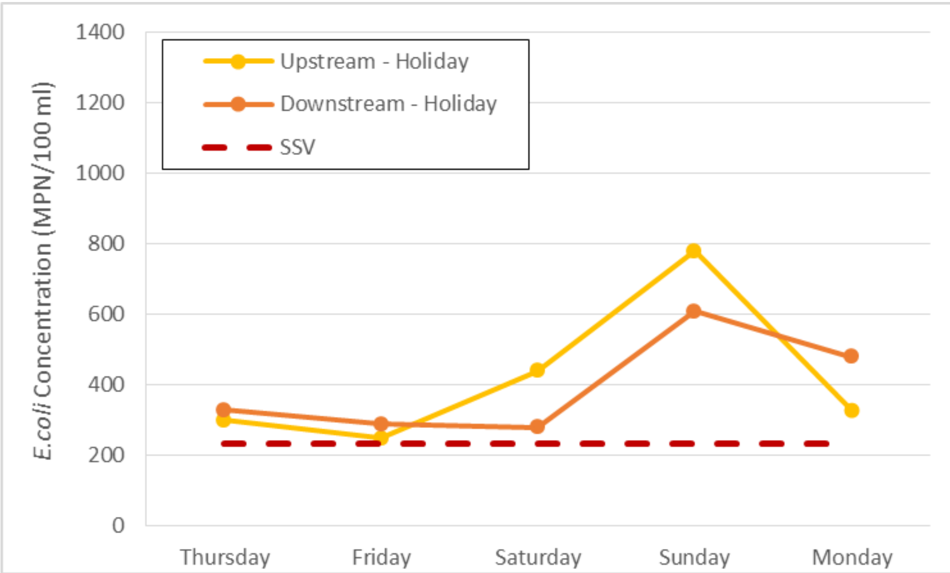

Figure 3-24 *E. coli* Concentrations Observed During a Holiday Weekend in the Swim Study
(Circles with red fill indicate birds detected in molecular analyses; The applicable SSV to evaluate the bacteria concentration is 235 MPN/ml. This is used where there is insufficient data to calculate a 30-day 5-sample geomean.)

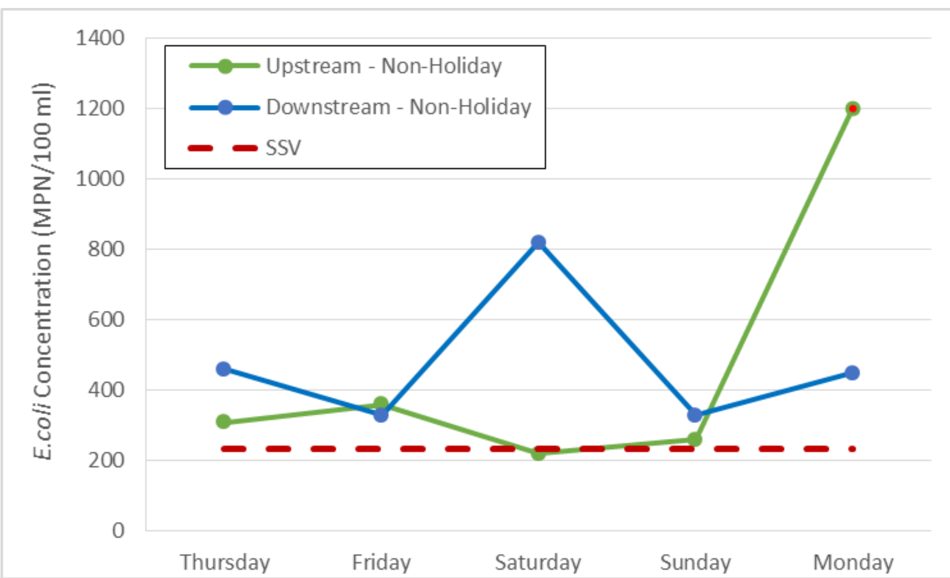

Figure 3-25 *E. coli* Concentrations Observed During a Non-Holiday Weekend in the Swim Study
(Circles with red fill indicate dogs detected in molecular analyses; The applicable SSV to evaluate the bacteria concentration is 235 MPN/ml. This is used where there is insufficient data to calculate a 30-day 5-sample geomean.)

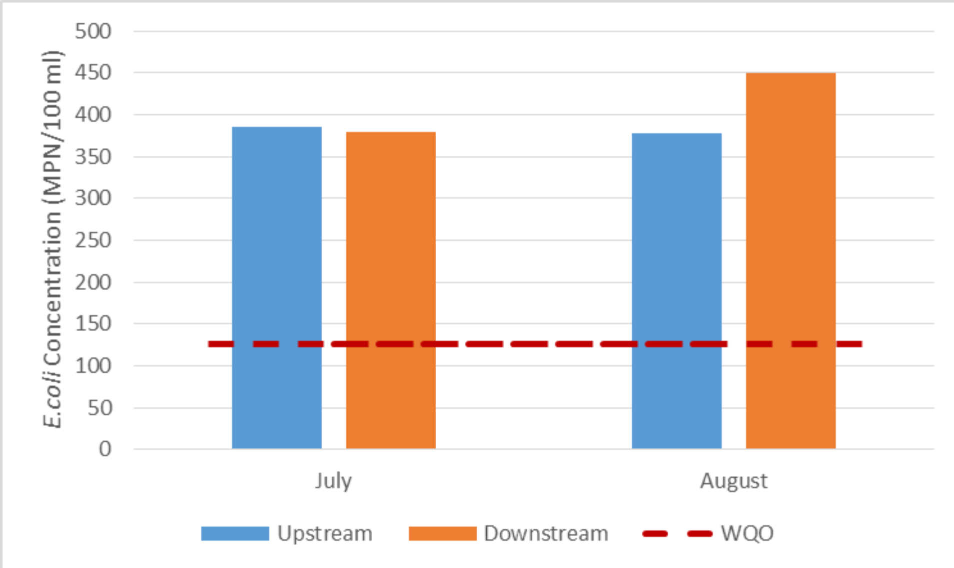

Figure 3-26 Geomean of *E. coli* Concentrations Observed During Holiday (July) and Non-Holiday (August) Weekends in the Swim Study

3.5.3 Transient Encampment Management

RCFC&WCD also conducted a transient encampment mitigation at (SAR at Market Street), where individuals have been observed using the SAR to bathe. RCFC&WCD analyzed bacteria concentrations in water samples collected prior to and after the cleanup (Table 3-6). While *E. coli* concentrations are generally low (less than 100 MPN/100 ml) at the site upstream of the homeless encampment, concentrations are higher downstream of the homeless encampment during two of the three sampling events. This finding suggests that the presence of the encampments cause an increase bacteria in concentrations in the SAR possibly due to bathing and other activities in the river. Average downstream bacteria levels were higher after the cleanup than before the cleanup (after: 900 MPN/100 ml, before: 254 MPN/100 ml). However, a detailed source investigation post cleanup was not conducted. Although humans were not detected in any sample collected, dogs were detected in both upstream and downstream samples from July 9, 2015.

Table 3-6 Data from Homeless Encampment Cleanup Conducted by RCFC&WCD

Analysis	Before Cleanup				After Cleanup	
	July 9, 2015		July 22, 2015		August 26, 2015	
	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream
E. coli **(MPN/100 ml)**	8	7	80	500	80	900
Human	BDL	BDL	BDL	BDL	BDL	BDL
Canine	+	+	BDL	BDL	BDL	BDL

Note: BDL = Below detection limit; + = positive detection
Source: RCFC&WCD

3.6 Horse Recreation Study

In the horse recreation study, water quality samples were collected on a single day during two weekends from both the water column and sediment. The two Saturdays, during which samples were collected, target a holiday and a non-holiday weekend to compare effects of more recreation during a holiday with that from less recreation during a typical weekend.

3.6.1 Visual Observations

Visual observations during the holiday weekend showed recreational activity at SAR at Etiwanda Avenue and SAR at Downey Street only. At SAR at Etiwanda Avenue, approximately 5 people as well as a horse and rider were in the river downstream of the study location (Figure 3-27). Considerably more people (more than 300) were found to be recreating in the river around SAR at Downey Street (Figure 3-28), however, no horses were observed. Although recreation by humans or horses were not observed at SAR at Mary Tyo Equestrian Area at the time of sample collection, horses were seen approaching the river as the field staff was leaving this study location. During the non-holiday weekend, recreational activity was not observed at SAR at Etiwanda Avenue and SAR at Mary Tyo Equestrian Area. However, people (>10) were seen recreating both upstream and downstream of the SAR at Downey Street study location (Figure 3-29).

Figure 3-27 A rider and horse at SAR at Etiwanda Avenue & 66th Street on July 4, 2015

Figure 3-28 People recreating at SAR at Downey Street & 64th Street on July 4, 2015

Figure 3-29 People recreating at SAR at Downey Street & 64th Street on August 15, 2015

3.6.2 Water Quality

E. coli concentrations were analyzed in water samples collected from three study locations along SAR on a holiday Saturday, July 4, and a non-holiday Saturday, August 15 (Table 3-7, Figures 3-30 and 3-31). Although observed ranges of *E. coli* levels in water samples for this study were similar during holiday and non-holiday samples (150 to 350 MPN/100ml and 120 to 320 MPN/100 ml, respectively), *E. coli* levels in water samples from all but one site were higher during the holiday weekend than the non-holiday weekend by as much as two-fold. Average concentrations in water samples from SAR at Downey Street during both holiday and non-holiday weekends (195 and 120 MPN/100 ml, respectively) were slightly lower than average concentrations at the other two sites (SAR at Etiwanda Avenue: 325 and 165 MPN/100 ml; SAR at Mary Tyo: 300 and 250 MPN/100 ml). *E. coli* levels are similar across transects at all sites with the exception of the non-holiday sediment samples.

Molecular analyses did not detect the presence of horse in any water sample, despite 83 percent and 17 percent of July and August samples exceeding the single sample numerical target, respectively.

Although horses were not detected in this study, it is possible that horses are contributing to fecal contamination based on visual observations of horse presence in and around SAR but that horse DNA signals may be low at the time of sampling. Studies have shown a host-specific genetic markers decay rapidly (within one day) and that detections may reflect only very recent fecal deposition (Bae and Wuertz, 2015; Balleste and Blanch, 2010). These study locations are also inhabited by other wildlife as well as humans, however, these sources were not tested as part of this study. It is also possible that the bacteria concentrations are influenced by upstream activity, including human recreation observed at the time of sample collection. Human recreation at the study locations could lead to sediment resuspension that releases bacteria into the water column. Resuspended bacteria may be transported downstream over time as well, which could contribute to elevated levels at downstream study locations (Walters et al, 2014; Curtis and Trapp, 2014).

During the holiday weekend, *E. coli* concentrations in sediment were over three orders of magnitude higher than concentrations in corresponding water samples. Levels in sediment samples from the non-holiday weekend were generally one order of magnitude greater than corresponding water samples. Concentrations from the holiday weekend were also over three orders of magnitude higher than non-holiday concentrations (holiday: 8.5×10^5 to 1.9×10^6 MPN/100 g; non-holiday: non-detects to 3.1×10^3 MPN/100 g). *E. coli* concentrations in August sediment samples are generally lower than concentrations reported in other studies. Molecular analyses did not detect the presence of horse in any sediment sample, however the MST analyses for this study was limited to only horse. It is possible other uncontrollable sources that were not analyzed for may have contributed to bacteria levels at these study locations. It is interesting to note that sediment *E. coli* concentrations were approximately three orders of magnitude higher during the holiday weekend than the non-holiday weekend, although it is unclear what the source is.

Table 3-7 *E. coli* Concentrations Observed in the Horse Study (MPN/100 ml & MPN/100 g)

Study Location	Study Site	Water		Sediment	
		Holiday (July 4)	Non-Holiday (Aug 15)	Holiday (July 4)	Non-Holiday (Aug 15)
SAR @ Etiwanda Ave	North Bank	350	190	1,900,000	1000
	South Bank	300	140	1,500,000	ND
	Average	**325**	**165**	**1,700,000**	**n/a**
SAR @ Mary Tyo Equestrian Area	East Bank	320	180	1,500,000	1000
	West Bank	280	320	850,000	ND
	Average	**300**	**250**	**1,175,000**	**n/a**
SAR @ Downey Street & 64th Street	North Bank	150	120	n/a [A]	1000
	South Bank	240	120	n/a [A]	3100
	Average	**195**	**120**	**n/a**	**n/a**

[A] Sediment samples were not collected from this study location on July 4, 2015

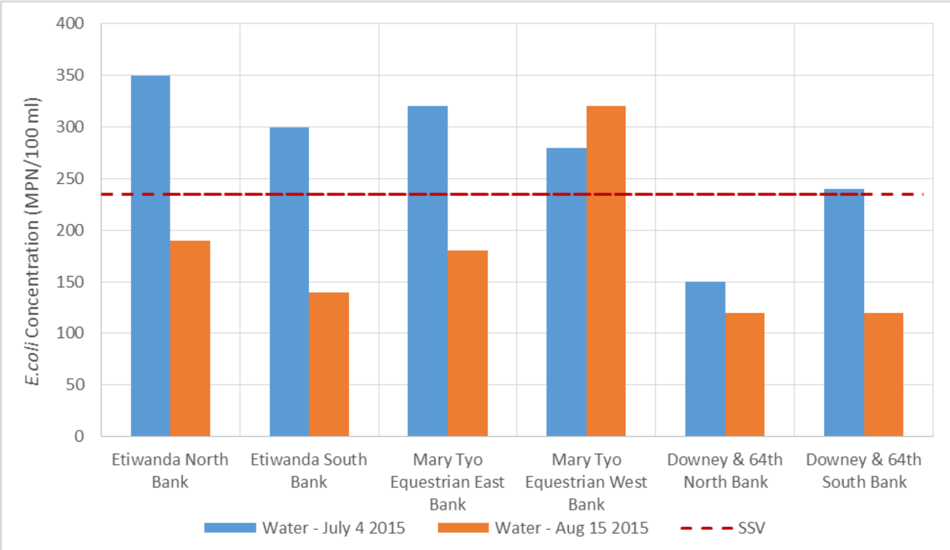

Figure 3-30 *E. coli* Concentrations Observed in the Horse Study Water Samples

(The applicable SSV to evaluate the bacteria concentration is 235 MPN/ml. This is used where there is insufficient data to calculate a 30-day 5-sample geomean.)

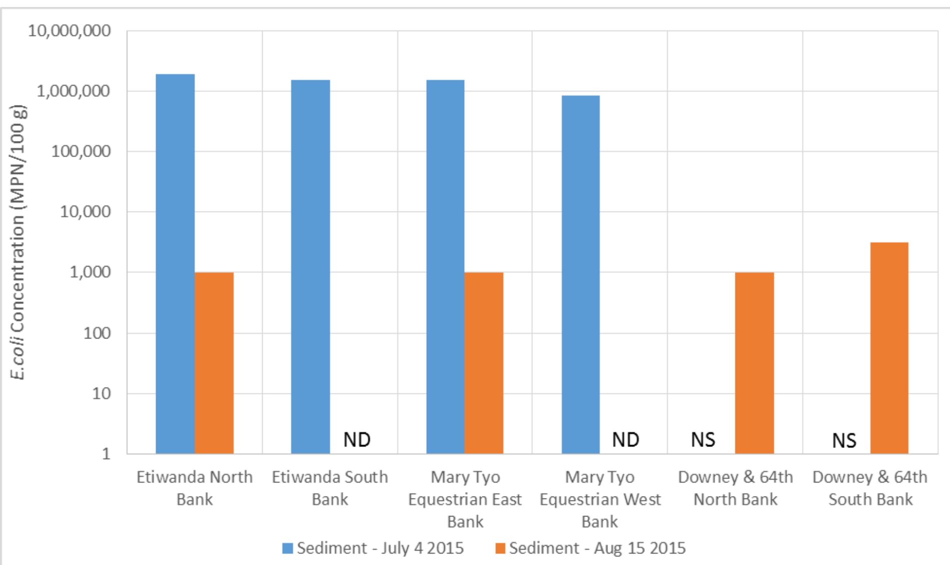

Figure 3-31 *E. coli* Concentrations Observed in the Horse Study Sediment Samples

(Note: July 4 sediment samples from SAR at Downey & 64th were not collected (NS – no sample). August 15 sediment samples were below detection limits (ND – non-detect).)

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Section 4

Discussion and Conclusion

Previous monitoring data has suggested that bacteria levels in the MSAR watershed cannot be explained solely by urban runoff. Bacteria concentrations within the SAR during the 2015 dry season showed significant fluctuation, with weekly samples varying by 1 to 2 orders of magnitude, despite the conditions of similar POTW effluent rates. The methods used to develop the source contribution analysis in the 2016 Triennial Review Report[11] were applied to weekly downstream concentrations during the 2015 dry season to determine the deviation from flow-weighted averages of *E. coli* in DWF from MS4s that would explain weekly variability measured downstream. This analysis found that deviations would be outside of the typical range for *E. coli* in DWF from all MS4s outfalls to the SAR. Most notable were two of the twenty weekly samples during 2015 dry season (July 5 and September 20). For example, the source contribution analysis for the 2016 Triennial Review estimated that downstream concentrations should be approximately 100 cfu/100 ml in the SAR at Pedley Avenue, based on historical MS4 outfall monitoring and 2015 POTW effluent rates. On July 5, 2015, the concentration of *E. coli* in the SAR at Pedley Avenue was approximately 2,200 cfu/100 ml. To be attributable to increased loads from MS4 discharges, *E. coli* in urban DWF would have to nearly simultaneously exceed 10,000 cfu/100mL for all eight major drainage areas with consistent DWF discharges to the SAR. Thus, it is completely plausible to consider that other uncontrollable sources of FIB are responsible for a significant fraction of downstream *E. coli*.

The UBSS was implemented to help identify to the extent possible whether specific uncontrollable sources of bacteria were contributing to the elevated levels observed in the watershed. Results for each of the six pilot studies are presented in Section 3 above. The UBSS investigated sources of fecal bacteria that can be categorized as host-specific (human, bird, dog, rumen, and horse) or naturalized (born in the environments such as in channel bottoms). The following sections synthesize the key findings from these two categories of fecal bacteria origin.

4.1 Host-Specific Bacteria Sources

Four of the pilot studies were conducted to assess whether there is a specific source (human, bird, dog, rumen, and horse) of fecal bacteria to the Santa Ana River and Cucamonga Creek. Multiple lines of evidence were developed to support the investigations, including FIB concentrations, biological surveys, microbial source tracking, and isolation of a Santa Ana River segment with no urban DWF discharges. Host specific sources were not consistently detected by MST analyses in samples that were hypothesized to be impacted, from sites downstream of 1) active riparian bird habitat areas, 2) bridges with nesting bird activity, 3) swimming recreation by humans and dogs, 4) equestrian use. Moreover, detections of a specific host were not well correlated with *E. coli* concentrations, suggesting that while these sources could contribute to elevated bacteria levels, they may not be the predominant source of fecal indicator bacteria at those monitoring sites. It is also possible that specific hosts were

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[11] Middle Santa Ana River Bacterial Indicator TMDL Implementation Final Report; submitted to the Santa Ana Watershed Project Authority on behalf of the MSAR TMDL Task Force, February 2016

not detected by MST analyses due to low levels in the samples, rapid decay of markers, or detections reflecting recent fecal deposition (Bae and Wuertz, 2015; Ballaste and Blanch, 2010).

Separate from the UBSS, RCFC&WCD conducted a transient encampment water quality assessment during the summer of 2015. This assessment found that *E. coli* concentrations were substantially higher downstream of transient encampments but does not identify the encampments as the only contributor to high *E. coli* concentrations at that site. Further study of the impact of such encampments may provide additional insight to this potential source.

4.2 Naturalized Bacteria

Although fecal bacteria directly from human and wildlife sources were not well correlated with *E. coli* concentrations, these sources may indirectly influence fecal indicator bacteria levels in the environment. Fecal bacteria from a specific host released to the environment can settle to the channel bottom and survive within sediments or biofilms for weeks or months over a wide range of temperature and moisture conditions (Balzer et al, 2010). Growth of these initially deposited fecal bacteria within channel bottom sediments and biofilms results in colonies, where the majority of the population may be considered naturalized, reproducing outside of a specific organism (Ishii et al, 2007; Byappanahalli et al, 2012; Ran et al 2013). Regrowth in biofilms has been recognized as a process influencing in-stream dynamics of fecal indicator bacteria levels. Balzer et al (2010) conducted a study on biofilms in several German Rivers. They found that fecal indicator bacteria were two-orders of magnitude higher in biofilms than overlying water, demonstrating that they may be able to integrate into existing biofilms and multiply – and thus, be a reservoir for indicator bacteria in the environment.

Although growth varies based on a number of factors including environmental conditions, using typical growth rates between 0.1 to 0.3 hr^{-1} (Jiang et al, 2007), the portion of the fecal bacteria population attributed to the initial host may be less than 5 percent within the first 12-24 hours of deposition (Figure 4-1). Even higher exponential growth rates up to 2 hr^{-1} may be expected shortly after colonization when food is abundant (Chapra, 1997). Thus, bacteria source tracking methods used in this study and by others are often unable to determine the ancestral host organism(s) in samples comprised of mostly naturalized fecal bacteria as methods were developed and tested using more laboratory spikes of fecal sources or recent fecal deposition (personal communication with Menu Leddy, October 20, 2015; Bae and Wuertz, 2015).

The physical processes that releases bacteria from sediment and biofilms to the water column may be just as important as factors that control colonization and growth (Grant, 2011). Fecal bacteria are not like chemical pollutants that have interactions between solid and dissolved phases by adsorption and desorption. Instead, bacteria may shed from a colony with weakened attachment from aging and when exposed to increased shear stresses.

By process of elimination, the UBSS results suggest that host specific sources do not represent the majority of *E. coli* fecal indicator bacteria in downstream waters. Therefore, the processes of colonization within channel bottoms and subsequent resuspension may be the most important controls on the concentration of *E. coli* in surface water. Two potential transport mechanisms by which naturalized bacteria are shed from channel bottoms were described by Grant (2011), including:

- Resuspension of sediment and attached *E. coli*, where flows exceeding critical shear stress releases loosely attached *E. coli* into overlying water column.

- Advection of *E. coli* in porewater, where flow drives exchanges between the porewater of channel bottom sediments and biofilms and overlying water column.

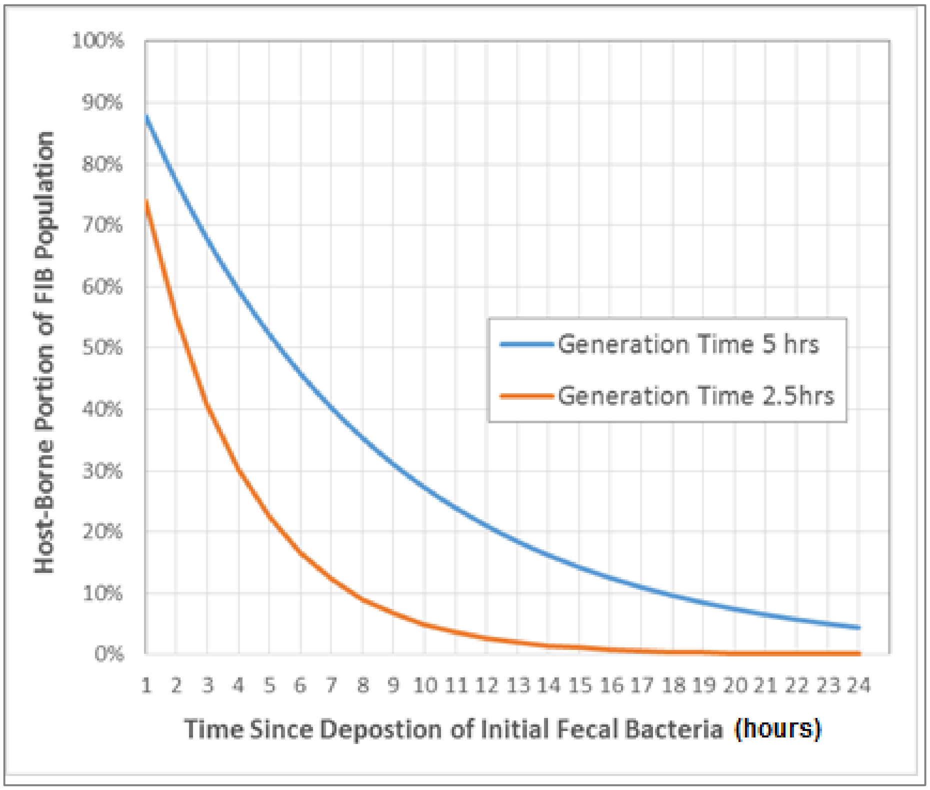

Figure 4-1 Ratio of Host-Borne to Naturalized FIB with a Range of Exponential Growth Rate Constants

Sediment is resuspended in streams when shear stress on the streambed exceeds the critical shear stress on the streambed. Resuspension of sediment depends on the type of sediment on the streambed and may be influenced by factors such as density, particle size, and the consolidation of the streambed. Resuspension of sediment particles has been identified to be a source of bacteria to overlying water, generally in wet weather conditions when high flows resuspend the sediment or in coastal areas where wave action can act to resuspend the sediment (Byappanahalli et al. 2003; Jamieson et al. 2005; Reeves et al. 2003; Solo-Gabriele and Perkins 1997; Whitman and Nevers 2003). In addition, work by Fries et al., 2006 and Fries et al, 2008 provided further evidence that resuspension of sediment can play an important role in the elevated concentrations of *E. coli* during and following rain events.

During dry weather conditions in the SAR and Cucamonga Creek, sharp increases in flow rate do occur in the form of increased POTW effluent, pulses of runoff from summer thunderstorms in far upstream mountains, and *de minimus* discharges. The final pilot study was intended to investigate whether flows that are not a source of bacteria could result in elevated downstream levels of *E. coli* through shearing effects. Given the unpredictability of these discharges, previous efforts were unable to provide new data, however, there is ongoing coordination occurring to utilize upcoming opportunities that will allow for further investigation.

4.3 Growth Factors for *E. coli*

Although the pilot studies did not investigate specifically what environmental factors may influence instream *E. coli* concentrations, it is important to consider other variables that may have impacted results observed in the six pilot studies. Resuspension may be an important mechanism for *E. coli* release into overlying water, however, variables influencing *E. coli* growth in the environment may be important as well. As bacterial growth, decay, and survival in environmental conditions may be related to a number of factors, it is likely that bacteria colonies persist and thrive in the environment under limited conditions. Not only could flow and resuspension result in dispersed colonies, areas or "hotspots" where conditions promote growth or decay may contribute to bacteria levels varying spatially. A study by Surbeck et al in 2010 suggested that nutrients, specifically dissolved organic carbon (DOC) and phosphorus, are correlated with *E. coli* growth trends observed in Cucamonga Creek. Microcosm experiments indicated that runoff is a significant source of bacteria, however, the experiments also suggested that nutrient levels may have a more important impact on how bacteria persists. *E. coli* growth was only observed when phosphorus and DOC were present at threshold levels and growth rates doubled with increasing DOC content. While Surbeck et al, 2010 suggested that treated wastewater effluent was not a source of bacteria, the study indicated that nutrient content (DOC, phosphorus, nitrate, and ammonium) in Cucamonga Creek is strongly correlated with the treated wastewater effluent.

4.4 Conclusion

Source contribution analysis conducted for the CBRP compliance analysis and in subsequent Triennial Reports have demonstrated that a significant portion of bacteria in the MSAR TMDL waterbodies during dry weather is not attributable to discharges from MS4s. The UBSS aimed to better understand and quantify the influence of other uncontrollable sources on bacterial indicator concentrations in these waterbodies. Findings from the six pilot studies include:

- Microbial source tracking analyses detected only birds and dog, mostly birds. However, these detections were not found consistently with higher *E. coli* concentrations in corresponding water and sediment or biofilm samples or consistently downstream of suspected sources.
- Extrapolation based on the gradual rise of *E. coli* concentrations observed in the natural sources study suggests there is the potential for bacteria levels to exceed WQO at further downstream locations and that natural sources may account for a majority of downstream bacteria.
- In the study targeting human recreation (swimming) as a source, *E. coli* concentrations were slightly elevated during the holiday weekend after presence of humans were observed to be high, however, humans were not detected in molecular analyses. Additionally, the highest *E. coli* concentration was observed when canine was also detected.
- Data collected by RCFC&WCD as part of a transient encampment water quality assessment showed higher *E. coli* levels downstream of the encampment before and after cleanup activities.
- In studies involving sediment or biofilm samples, *E. coli* concentrations were substantially higher in the sediment and biofilms than in the overlying water. However, sediment / biofilm concentrations were still lower than that observed in other studies.

Although the pilot studies did not suggest any human or wildlife source as a consistent significant contributor to elevated bacteria levels, the fact remains that unaccountable sources of *E. coli* are present. By process of elimination then, the UBSS results infer that the majority of uncontrollable *E. coli* in the impaired waters may be from releases from naturalized colonies in channel bottom sediment and biofilms. As noted above in Section 4.2, fecal bacteria from a specific host released to the environment can settle to channel bottom and survive within sediments or biofilms for weeks or months over a wide range of temperature and moisture conditions. Growth of these initially deposited fecal bacteria within channel bottom sediments and biofilms results in colonies, where the majority of the population may be considered naturalized, reproducing outside of a specific organism.

Resuspension of bacteria from channel bottoms may occur because of increased DWF (e.g., from *de minimus*, POTW effluent or dry weather MS4 discharges). As noted above, through the work of others, nutrients and DOC are examples of constituents that can influence bacteria growth rates in stream. If *in situ* growth is found to be a key source, then alternatives to reduce this growth could be evaluated. This evaluation could include additional studies to determine instream threshold levels for constituents that affect bacterial growth.

Finally, it is important to note that the UBSS represented pilot studies, which were developed and implemented as a preliminary effort to better understand uncontrollable sources of bacteria in the MSAR watershed to the extent possible. Though the study locations and monitoring sites did not identify specific uncontrollable sources as significant contributors of bacteria, it is important to note that it can be challenging to capture samples with detectable sources due to the high spatial variability of bacteria in the environment. Lack of detections may reflect absence of the source, however, it may also reflect a low, undetectable signal. While host-specific qPCR methods are often used in source tracking studies, it is also possible that alternative MST methods (e.g., library-dependent) could be more effective. It is likely that further investigation with additional and/or alternative study locations, MST methods, and additional analyses of uncontrollable sources would provide helpful and potentially more conclusive information to help better understand causes of elevated bacteria concentrations in the MSAR watershed.

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Section 5

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Appendix A

Literature Review Technical Memorandum

Memorandum

To: *Riverside County Flood Control and Water Conservation District*

From: *CDM Smith*

Date: *July 31, 2015*

Subject: *Literature Review for Uncontrollable Bacteria Sources*

The MSAR Bacterial Indicator TMDL includes a concentration based wasteload allocations (WLA) for MS4 Permittees for *E. coli* of 113 cfu/100mL, which is equal to the numeric water quality objective (126 cfu/100mL) minus a ten percent margin of safety (MOS). There is no data currently available to assess the portion of fecal indicator bacteria (FIB) measured at the TMDL compliance sites that may be attributed to uncontrollable sources, as defined in the recently adopted Basin Plan Amendment (BPA). Consequently, six technical studies are underway and comprise the Uncontrollable Source Monitoring Program (Program) for the MSAR watershed. The purpose of the Program is to better understand and quantify the influence of uncontrollable sources of FIB in waterbodies in the MSAR watershed.

A key task for the six uncontrollable sources studies is to conduct a literature review to determine the current scientific understanding for each of the fecal bacteria sources under investigation. This technical memorandum provides the literature review in the format of an annotated bibliography. For each reference, a brief summary of the study methods and pertinent findings is provided. The reference material is organized into four sections that represent the six sources of fecal indicator bacteria (FIB) under investigation as follows;

- **Direct inputs from wildlife** – Two studies are underway to evaluate the importance of wildlife in the Santa Ana River (SAR) and Cucamonga Creek. First, is a 'Natural' source study that collects samples from a segment of the SAR that has zero inputs from MS4s during dry weather and second is a 'Bird' source study that evaluates FIB upstream and downstream of two bridges; Mission Avenue over the SAR and Schleismann Road over Cucamonga Creek.

- **Resuspension from sediment/biofilm** - Two studies are underway to evaluate the importance of resuspension of FIB from sediment and or biofilm within conveyance facilities. The first study is of 'Sediment/Biofilm', and involves sampling of channel bottom sediment and biofilm from four tributaries of the SAR to determine the magnitude of FIB available for resuspension. The second study is of the impact of 'Non-MS4 flows' that cause a flow condition that may cause resuspension of FIB from sediment and biofilm on channel bottoms, such as from well blow-offs.

- **Shedding during swimming** – While the TMDL is intended to protect swimmers from potentially harmful pathogens, it is possible that the act of swimming could release FIB to the receiving water. A study is underway to evaluate FIB upstream and downstream of one popular swimming holes in the SAR.

- **Equestrian recreational use** – Equestrian uses exist within the SAR riparian area. A study is underway to evaluate whether feces from horses deposited along trails or directly to the river is a key contributor to downstream FIB concentration.

Direct Inputs from Wildlife

Byappanahalli, Muruleedhara N., Meredith B. Nevers, Richard L. Whitman, Zhongfu Ge, Dawn Shively, Ashley Spoljaric, Katarzyna Przybyla-Kelly (2015). Wildlife, urban inputs, and landscape configuration are responsible for degraded swimming water quality at an embayed beach, *Journal of Great Lakes Research*, v41: 1456-163.

Water samples were collected weekly between June-August 2010 from three sites at knee depth from Jeorse Park Beach in southern Lake Michigan. A total of 54 water samples were analyzed using culture based methods, Colilert-18 for *E. coli* and membrane filtration for *Enterococci*, and molecular methods, quantitative polymerase chain reaction (qPCR) for *Enterococci*, *Bacteroides* marker (HF183) for human and *Catellicoccus* for gull. Genomic DNA extraction was performed for fecal samples collected from gull, goose, and cormorants. *Enterococci* concentrations measured using qPCR (CCE) were significantly higher than by culture based membrane filtration (CFU); however the resulted were positively correlated. Host-specific makers for human and gull were detected in 15 and 37 percent of the water samples respectively. No relationship was found between the detection of the gull marker and indicator bacteria concentration. Lastly, a hydrodynamic model showed that the sampled beach exists within an embayment that has highly stagnant water and a circulation patterns that tends to entrain up-current contamination sources. A wide range of potential control strategies are discussed as well as planned activities for Jeorse Park. The investigators point to a gap in current microbial source tracking approaches that does not allow for quantification of the relative contribution from host-specific sources to total FIB levels.

Edge, Thomas A. and Stephen Hill (2007). Multiple lines of evidence to identify the sources of fecal pollution at a freshwater beach in Hamilton Harbour, Lake Ontario, *Water Research*, v41: 3585-3594.

Weekly samples were collected during the 2004 bathing season from beach sand, water at ankle and knee depth and two offshore sites. Concentrations of *E. coli* measured by membrane filtration methods were similar to other studies for water (10^2 to 10^5 cfu/100mL) and sand (10^4 to 10^7 cfu/100g[12]). Two library dependent methods, antimicrobial resistance and Rep PCR DNA fingerprinting analyses, were used to develop a library from approximately 2,000 isolates collected from numerous fecal samples from gulls, geese, ducks, dogs, cats, WWTP effluent, CSOs, and beach sand. Several accuracy measures documented the level of correctness of the applied methods. These methods were used to enumerate the relative contribution to *E. coli* in a sample from specific

[12] Roughly, one milliliter of water weighs one gram, thus the concentrations in sediment and water are equivalent

sources. The investigators found that birds and beach sands were the prominent sources of *E. coli* in samples collected from near shore sites.

Jiang, Sunny C., W. Chu, B. H. Olson, J.-W. He, S. Choi, J. Zhang, J. Y. Le, and P. B. Gedalanga (2007), Microbial source tracking in a small southern California urban watershed indicates wild animals and growth as the source of fecal bacteria. *Applied Microbiology and Biotechnology,* 76(4):927-34.

This study involved collection of samples from a small Orange County subwatershed for microbial source tracking (MST) using three methods; antibody resistance analysis (ARA), polymerase chain reaction (PCR) for *E. coli* toxin genes, and PCR detection of human adenovirus and enterovirus. Agreement between the ARA and toxin gene biomarkers was achieved. There were no detection of human enterovirus or adenovirus. Results indicated that human sources were not a major contributor. The most significant sources included birds, cows, and rabbits (one sample). Investigators discussed the lack of any cows within this drainage area and suggest that persistent detection of cows may be from organic mulch used in local landscaping. The City of Laguna Niguel found high levels of fecal coliform in organic compost collected from the same neighborhood in an independent study. Lastly, samples of dry weather runoff from street gutters were collected and used in a laboratory microcosm assay to measure *E. coli* growth potential. Results showed an increase in *E. coli* of 4-5 logs within 6-7 days, and translate to an exponential rate constant of 5.4 hr^{-1}.

Sejkora, Patrick, Mary Jo Kirisits, and Michael Barrett (2011). Colonies of cliff swallows oh highway bridges: a source of *Escherichia coli* in surface waters, *Journal of the American Water Resources Association*, v47(6): 1275 – 1284.

A study was conducted in 2009-2010 to assess the impact on bacteriological water quality from nesting cliff swallows under a Bridge over Bull Creek in Austin, Texas. Approximately 100 nests were directly above Bull Creek and another 275 were over land in the vicinity of the bridge. Samples were collected upstream and downstream of the bridge when swallows were present during dry (n=23) and wet (n=4) weather conditions. Results for dry weather samples showed a statistically significant increase in *E. coli* geometric mean concentration as water passed under the bridge from the upstream site (43 MPN/100mL) to the downstream site (106 MPN/100mL). This difference was not significant when the data was constrained to samples collected only during foraging periods, before and after the ~45 day nesting period. The greatest differences occurred during the nesting period when birds were more likely to deposit feces directly into Bull Creek. For the small (not sufficient for statistical t-test) dataset of wet weather when swallows were present, downstream samples had a substantially higher geometric mean concentration of *E. coli* (688 MPN/100mL) than upstream (78 MPN/100mL), which was attributed to mobilization of feces from land in the vicinity of the bridge. A supplemental sampling for *E. coli* at six hour intervals over the course of a single day was conducted to assess temporal variability. Results showed a fairly similar concentration of *E. coli* over the course of a day in Bull Creek, and a persistent patterns of higher downstream concentrations. Lastly, a load analysis was developed and used to estimate the contribution of *E. coli* from a single over-water nest of $3.1E^8$ MPN/100mL.

CDM Smith

Tiefenthaler, Liesl, Eric D. Stein, and Greg S. Lyon (2008). Fecal indicator bacteria levels during dry weather from southern California reference streams, Southern California Coastal Water Research Project Technical Report 542, January 2008.

Fecal indicator bacteria, *E. coli*, Enterococcus, and total coliforms, were measured weekly over the course of a year (2007-2008) from 15 unimpaired 'reference' streams in Southern California[13]. Reference streams have no upstream development. Median concentration from all samples at all sites was 10 MPN/100mL for *E. coli* and 20 MPN/100mL for enterococcus. FIB were significantly positively correlated with stream temperature and exceedences of water quality objectives for *E. coli* only occurred in the summer and from less shaded and lower elevation sites within Orange and San Diego Counties. Of all the samples, there was no detection of *B. thetaiotaomicron,* indicating FIB in reference streams were likely of nonhuman origin.

Wither, A., M. Rehfisch, and G. Austin (2005). The impact of bird populations on the microbiological quality of bathing waters, *Water Science and Technology*, v 51(3-4): 199-207.

A study was conducted to relate bird densities with FIB on the Flyde coast of northwest England. Bird surveys underneath several piers along this coast supported roosts of Starlings with population of over 30,000. Fecal matter deposited underneath the roosts was collected over a site and then extrapolated over the total area under the piers to estimate the total fecal load from birds of 210 kg/night. The geometric mean of *E. coli* concentration in sampled fecal matter of $4.6E^{9}$ cfu/100g (range of $6.0E^{8}$ – $2.4E^{11}$ cfu/100g), results in an estimated number of *E. coli* of $9.6E^{12}$ cfu/night.

Resuspension from Sediment/Biofilm

Balzer, M., N. Witt, H.-C. Fleming and J. Wingender (2010). Faecal indicator bacteria in river biofilms, *Water Science and Technology*, v61(5): 1105-1111.

Samples of water and biofilms were collected from three German streams for ten events in 2004-05 and analyzed for total coliform population and culturable *E. coli* and Enterococci. Biofilm samples were categorized as being sourced from epilithic biofilm or sediment. Results showed greater geometric means for *E. coli* concentration within biofilms (20,000 MPN/100g) than the overlying water (25 MPN/100mL), with at least one order of magnitude difference in all samples. The same pattern occurred in results for Enterococci. The study also showed a lower fraction of culturable to total coliform bacteria in biofilms than water, which is supported by other studies of autochthonous faecal bacteria (originating from growth within biofilm).

Ksoll, Winfried B., Satoshi Ishii, Michael J. Sadowsky, and Randall E. Hicks (2007). Presence and source of fecal coliform bacteria in epilithic periphyton communities of Lake Superior. *Applied and Environmental Microbiology*, v73(12): 3771-3778.

This study evaluated fecal coliform and *E. coli* in periphyton communities from three sites on the Minnesota shoreline of Lake Superior. The study found an increase of fecal bacteria in periphyton of

[13] No sites were selected within the Middle Santa Ana River watershed in Riverside County

four orders of magnitude in the spring. Library based DNA fingerprinting using horizontal, fluorophore-enhanced repetitive (HPERP) methods were used and compared with the Duluth source library (including HPERP fingerprint for *E. coli* strains from numerous isolates of deer, geese, gulls, terns, beavers, and sewage). Results indicate that waterfowl were the predominant source of *E. coli* within periphyton communities of the identifiable fraction, a minority of the total *E. coli* from the three sites (specifically 2, 23, and 44 percent). Unidentified periphyton strains were added to the source library as two groups, those that were discovered to be unique to periphyton and those that were non-unique. Subsequent HPERP analysis of samples of the overlying water (collected at the time of periphyton sampling) provided a relative contribution from different sources of *E. coli*, with the major sources including waterfowl, sewage, and periphyton. The study also involved a microcosm experiment which found 99 percent of *E. coli* cells remained on periphyton-covered rocks unless agitated.

Moreira, Stefan, A. Brown, R. Ha, K. Iserhoff, M. Yim, J. Yang, B. Liao, E. Pszczolko, W. Qin and K.T. Leung (2012). Persistence of Escherichia coli in freshwater periphyton: biofilm-forming capacity as a selective advantage. *Federation of European Microbiological Societies Microbiology Ecology*, v79: 608-618.

This study involved characterization of biofilm forming capacity of *E. coli* from various sources, including naturalized periphytic *E. coli* isolates, from three temperate freshwater lakes in Canada. The experiment employed a crystal violet assay to differentiate the growth of bacteria associated with biofilm as opposed to planktonic (floating) in a series of microsm assays. Results showed the periphytic *E. coli* were significantly more competent at forming biofilms than isolates from any other source grouping, which included bovine, human, and known Shiga-like toxin producing serotypes from a mix of human and bovine hosts. They study also employed an assay for the curli expression (a surface protein key to attachment stage of biofilm formation), which has been hypothesized to be primary controlling factor for environmental biofilm formation and subsequent colonization and persistence of periphytic *E. coli*. The study results showed little correlation and suggest that other factors are important for periphytic *E. coli* in biofilms.

Skinner, J.F., Kappeler, J., and Guzman, J. (2010). Regrowth of enterococci and coliform in biofilm. *Stormwater*, Santa Barbara, California.

In a Newport, CA residential neighborhood, a study was conducted to assess the potential release of FIB from biofilms in a street gutter. Bacteria free hose water was discharged to the street gutter and samples were collected at 10, 45, and 100 meters downstream prior to the flow entering a street inlet. Results showed an increasing fecal coliform concentration as the flow moved downstream reaching 14,000 cfu/100mL at 100 meters. A second test was performed following street sweeping and found fecal coliform at the same 100 meter downstream site at 870 cfu/100mL. Biofilm samples were also collected from the street gutter and showed very high concentrations of FIB; ranging from $4.1E^4$ to $9.0E^6$ *Enterococci*/100g and $1.0E^4$ to $6.0E^6$ fecal coliform/100g. The lowest concentrations of FIB in biofilm samples were in samples with the shortest duration since rain or manual scraping had removed biofilm from the gutter surface.

Solo-Gabriel (2000). Ecological control of fecal indicator bacteria in an urban stream. *Environmental Science and Technology*, v44(2): 631-637.

Monitoring of the North Fork of the New River, a coastal river in an urbanized region of south Florida was conducted to assess sources of *E. coli* using a sampling design to characterize spatial and temporal variability of *E. coli*, including sampling of sediment. Samples collected from the river were greater than from storm sewers, and two hotspots were identified for more detailed investigation. To assess temporal variability, autosamplers were used to collect hourly samples for a one week period from the two sites. Results showed increasing *E. coli* during high tide. At the same sites, intensive grids (n=35 and n=21) were used to characterize the river segment. Results showed the greatest water column concentrations along the river banks. Sediment samples (n=40) were also collected from transects of five river bank segments, three of which were centered on the hotspots in the river. The highest concentrations in riverbank sediments occurred near the hotpsots in long, shallow, and shaded embankments. The study also included a laboratory experiment to determine if *E. coli* can grow in riverbank sediment samples under different conditions of wetting and drying representing the impact of tidal fluctuations. Results showed that the sediment that was allowed to dry for longer periods of time had higher *E. coli* concentrations than if kept wet throughout the experiment. *E. coli* are able to survive longer period of drying than predators, which could explain the greatest concentrations in sediment and water at the outer fringes of the river banks.

Surbeck, C. Q., S. C. Jiang, and S. B. Grant (2010). Ecological control of fecal indicator bacteria in an urban stream. *Environmental Science and Technology*, v44(2): 631-637.

This study attempted to characterize the changes in FIB, *E. coli* and Enterococcus, within Cucamonga Creek by collecting water samples upstream of the POTW effluent and at several sites downstream for seven events during 2005-06. In many instances downstream samples showed higher *E. coli* concentrations than would be expected with a loading analysis. Microcosm studies were conducted for sample water to assess the potential growth or decay of FIB in a controlled environment. Results showed that dissolved organic carbon (DOC) concentration is a controlling factor in FIB survival within Cucamonga Creek. A threshold of 7 mg/L DOC was identified as indicating increased potential for growth (>7 mg/L) or decay (<7 mg/L).

Ferguson, Donna (2006). Growth of *E. coli* and Enterococci in Strom Drain Biofilm, presentation at the National Beaches Conference, October 13, 2006, Niagara Falls, New York.

Biofilm and overlying water was sampled from Costa Mesa Channel and analyzed for FIB; *E. coli* and *Enterococci*. Results showed very high concentration of *E. coli* ($1.8E^6$ cfu/100g) and *Enterococci* ($4.6E^6$ cfu/100g) in biofilm. The slideshow also described a laboratory method to assess biofilm and bacteria growth on a glass slide by placing it into a stormwater sample and inoculating with *E. coli* and *Enterocci faecium*. Results are shown visually but no quantification is provided.

Shedding during Swimming

City of Newport Beach and Santa Ana Regional Water Quality Control Board (2002). Swimmer Shedding Study in Newport Dunes, California. Report prepared by Sunny Jiang, Charlie McGee, Linda Candelaria, Garry Brown, and Dani Gold, http://www.waterboards.ca.gov/rwqcb8/water_issues/programs/tmdl/docs/swimmerreport.pdf

A study was conducted by the City of Newport Beach to investigate whether swimming uses increases FIB in waters. The study site was the Newport Dunes Resort, one of southern California's most popular family vacation spots. Results showed that water quality objectives were met in most samples and did not indicate any difference in FIB concentration at sites or sampling times with more swimmers.

Elmir, Samir M., Mary E. Wright, Amir Abdelzaher, Helena M. Solo-Gabriele, Lora E. Fleming, Gary Miller, Michael Rybolowik, Meng-Ta Peter Shih, Segaran P. Pillai, Jennifer A. Cooper, and Elesi A. Quaye (2007). Quantitative evaluation of bacteria released by bathers in a marine water, Water Research, v41: 3-10.

This paper summarized findings from two experiments of fecal bacteria shedding from bathers; referred to as the 'large pool' and 'small pool' studies. The large pool study evaluated the shedding of fecal indicator bacteria (*Staphyloccocus aureus* and *Enterococci*) from 10 test subjects into a sterilized inflatable pool filled with off-shore water from a marine beach in Miami-Dade County, Florida. The greatest concentrations of fecal bacteria were recorded following the first of four immersions. Shedding from the first immersion amounted to $6.1E^6$ cfu/100mL of *S. aureus* and $5.5E^5$ cfu/100mL of *Enterococci*. These shedding rates are comparable to other studies of bather shedding that involved immersion in freshwater and in supplemental studies by the investigators in 2009[14]. The small pool study was designed to measure the amount of sand and associated fecal bacteria transported from single subjects after recreating on beach sand for 15-30 minutes. Results show that *Enterococci* from shedding of sand was small relative to the total shedding from bathers.

Gerba, Charles P. (2000). Assessment of enteric pathogen shedding by bathers during recreational activity and its impact on water quality, *Quantitative Microbiology*, v2:55-68.

A literature review of pathogen shedding by swimmers is presented in this paper. Only one study was found that has evaluated the release of enteric pathogens to recreational waters from swimming just downstream of a groundwater spring at the headwaters of Oak Creek, Arizona (Rose et al, 1987[15]).

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[14] Elmir, Samir M., Tomoyuki Shibata, Helena M. Solo-Gabriele, Christopher D. Sinigalliano, Maribeth L. Gidley, Gary Miller, Lisa R.W. Plano, Jonathan Kish, Kelly Withum, and Lora E. Fleming (2009). Quantitative evaluation of *Enterococci* and *Bacteroides* released by adults and toddlers in marine water, *Water Research*, v43: 4610-4616.

[15] Rose, J.B., R.L. Mullinax, S.N. Singh, M.V. Yates, C.P. Gerba (1987). Occurrence of rotaviruses and enteroviruses in recreational waters of Oak Creek, Arizona, *Water Research*, v21: 1375-1381.

Based on the concentration and flow rate, it was estimated that bathers shed over 16,000 viruses during the 30 minute period of the monitoring. The paper uses this shedding rate to extrapolate the potential shedding into a southern California reservoir with frequent water contact recreational use. Other literature was presented that analyzed fecal indicator bacteria shed from swimmers or bathers, which showed a common finding of 10^5 to 10^6 fecal coliforms per bather are shed, mostly within the first 15 minutes of water contact. One study by Rose et al. (1991[16]) found substantially higher fecal coliform concentration in bathwater from young children (10^5 MPN/100mL) compared with adults (10^1 to 10^2 MPN/100mL).

Zhu, Xiaofang, John D. Wang, Helena M. Solo-Gabriele, and Lora E. Fleming (2011). A water quality modeling study of non-point sources at recreational marine beaches, *Water Research*, v45: 2985-2995.

This study involved the use of a hydrodynamic model of a coastal recreational marine beach in Biscayne Bay near Miami. Bacteria inputs from three non-point sources were simulated, including a single fecal event by a large dog, a holiday day of recreational swimming, and release from beach sand during high tide for one hour. Literature values were used for inputs of per bather shedding of FIB of ~10^6 cfu/event. These rates were then extrapolated to the number of swimmers observed in images collected by an on-site surveillance camera. When the load from bathers was added to the hydrodynamic model, increases in *Enterococci* concentration accounted for less than 1 cfu/100mL. Thus, for this receiving water, recreational swimming is most likely not a source of FIB contamination.

Equestrian Use

Airaksinan, S., M.-L. Heiskanen, and H. Heinonen-Tanski (2007). Contamination of surface runoff water and soil in two horse paddocks, *Bioresource Technology*, v98: 1762-1766.

Wet weather surface runoff samples were collected from three sites at two horse paddocks in Eastern Finland during three storm events in 2002. Analyses included nutrients as well as indicators of microbial water quality. Soil samples were also collected from the sites but only evaluated for nutrients. Three horses resided in each paddock over the course of the study. One of the paddocks was cleaned daily and the other was left uncleansed. The quantities and concentration in the cleaned and uncleansed paddocks were similar over the three sampling events.

Tiefenthaler, L., E. D. Stein and K. C. Schiff (2011). Levels and patterns of fecal indicator bacteria in stormwater runoff from homogenous land use sites and urban watersheds, *Journal of Water and Health*, v9(2): 279-290.

Regional monitoring was conducted to estimate land use based EMCs for southern California. Monitoring spanned over 13 storm events in 5 southern California watersheds during the 2000–2005 storm seasons, and the selected stations were representative of 8 different LU types. The highest mean FIB concentrations were measured at the station downstream of mostly recreational land use;

[16] Rose, J.B., G.-S. Sun, C.P. Gerba, N.A. Sinclair (1991). Microbial quality and persistence of enteric pathogens in graywater from various household sources, *Water Research*, v25: 37-42.

with a confidence interval for *E. coli* of 5.3 +/- 1.7E^{5} MPN/100mL and statistically significant difference relative to other land use types, including commercial, high density residential, industrial, and transportation. The investigators suggest that the high bacteria from recreational land use could be due to the site being an equestrian facility.

Long, Sharon C. and Jeanine D. Plummer (2004). Assessing land use impacts on water quality using microbial source tracking, *Journal of American Water Resources Association*, v40(6): 1433-1448.

Samples were collected from 13 sites within the watershed to the Wachusett Reservoir that were determined to have drainage areas characterized by a single predominant land use type. Land uses types characterized by the study included residential, horse and dairy (grazing animal) operations, and forested. One site was downstream of a large pasture land and horse farm with a resident population of 7-15 horses. Fecal coliform samples collected during summer dry weather conditions from this site were the highest (1,200 cfu/100mL) of the 13 sampled sites (average of 233 cfu/100mL). *R. coprophilus* is a microbial source tracking indicator that is found at high levels in manure of grazing animals (although it does not originate in the gastrointestinal tract of these animals). Only the horse farm site detected this indicator above a threshold that indicates the presence of manure from grazing animals. The investigators used this MST tool to suggest that the source of bacteria at this site was from horse manure. Samples were also collected from the 13 sites during wet weather and winter dry weather. Statistically significant differences for the pooled data were detected based on season and weather condition.

Appendix B

Biological Assessment Technical Memorandum

Memorandum

To: *Mr. Steven Wolosoff, CDM Smith*

From: *Jennifer Jones, CDM Smith*

Date: *May 28, 2015*

Subject: *Findings of the Biological Survey*

Introduction

This memorandum presents the findings of a biological survey conducted by Jennifer Jones, CDM Smith biologist, on May 21, 2015. A nesting bird survey was conducted at the Cucamonga Creek and Mission Boulevard bridge sites in conjunction with water quality monitoring performed by the Riverside County Flood Control and Water Conservation District. In addition, a wildlife habitat assessment was conducted along the "Natural Study" reach.

Cucamonga Creek- Schleisman Road Bridge Site

Cucamonga Creek at this location is a concrete-lined, trapezoidal box channel (Photo 1). Water was flowing over approximately 40 feet of the channel width, and was approximately six inches deep in the deepest spot in the center of the channel. Wire netting was observed to cover over half of the underside of the bridge, presumably to keep birds from nesting in that area. The downstream one third of the bridge underside was not covered in netting.

Cliff swallows were observed flying over the channel and under the bridge. Approximately 60 birds were observed flying over the channel and visiting nests. The swallows were visibly disturbed by the biologist's presence and would not approach the nests if the biologist was standing under the bridge.

Adult birds were observed sitting in nests; nestlings were also observed in some nests (Photo 2). Adults visited nests on average every 2-3 minutes. A nest was considered active if it was observed to have a bird in it (either adult or nestling), or an adult visited the nest (but no other bird could be seen in the nest). Twenty-six active nests were observed: 17 located over the water and nine located over the dry part of the channel. Fecal waste was observed to be accumulating in the dry parts of the channel under the nests. The total number of cliff swallow nests (active and inactive) was 293 nests. Of these, approximately 170 were not located over the water.

Other birds observed near the bridge included barn swallow (1 individual), black phoebe (2), Brewer's blackbird (12), American crow (2), turkey vulture (1), and merlin (1). One barn swallow was observed to be visiting a nest in a storm drain channel upstream of the bridge.

Photo 1. Cucamonga Creek at the Schleisman Road Bridge

Photo 2. Active cliff swallow nests at the Schleisman Road Bridge. Adult birds can be seen in the lower two nests.

Santa Ana River- Mission Boulevard Bridge Site

The Mission Boulevard Bridge is very wide (over 1,000 feet), with water flowing only along the far western end of the channel. The biologist observed one half (the western half) of the underside of the bridge. The water was approximately one foot deep in the deepest part of the channel under the bridge, which appeared to be a depositional area where sand accumulates (Photo 3). Upstream and downstream of the bridge, the water appeared to be deeper (Photo 4). Several tents, homeless/vagrant persons and trash (shopping carts, spray paint cans, etc.) were observed under the bridge.

Approximately 45 cliff swallows were observed flying over the channel and visiting nests. Adult birds and nestlings (Photo 5) were observed sitting in nests. A total of 30 active nests were identified. Of the active nests, 18 were located over the water. The total number of cliff swallow nests observed under the western half of the bridge was 128. Nests were more dispersed than at the Cucamonga Creek bridge site, and there were more old nests that had been used in previous years.

Approximately 15 rock doves (pigeons) were also present and appeared to be nesting and/or roosting under the bridge. Courtship and territorial behavior was observed among rock doves, although nests were not visible.

Photo 3. Water flow was confined to one section of the Mission Boulevard Bridge.

Other birds observed included black phoebe (2), house wren (2), yellow warbler (1), common yellowthroat (1), Wilson's warbler (1), bushtit (10), Anna's hummingbird (1), and house finch (2). Several ground squirrels were observed. Habitat within the Santa Ana River channel in this area likely supports many songbirds, waterbirds, reptiles and amphibians, and mammals such as raccoon, possum, and coyote.

Photo 4. The Santa Ana River upstream of the Mission Boulevard Bridge.

Photo 5. Cliff swallow nestings in a nest under the Mission Boulevard Bridge.

Natural Study Reach

The "Natural Study" reach of the Santa Ana River extends from the wastewater treatment plant downstream to the Riverside Avenue Bridge. The biologist performed a general reconnaissance of portions of this reach to assess habitat and the potential for wildlife use.

The Santa Ana River in this location consists of a wide sandy wash with a narrow but well-vegetated riparian community of cottonwoods and willows along the main channel (Photo 6). Dominant shrub species include mulefat and coyote bush. Invasive plant species are prevalent throughout the reach and include castor bean, fennel, and Arundo (giant reed). At the time of the site visit, water was flowing approximately 25 feet wide in the main channel which is located along the western bank portion of the reach. In some areas, the channel has split into two channels with a vegetated sand bar in the middle. Aerial photos (from Google Earth) indicate that the wetted channel moves to the eastern portion of the reach further downstream toward Riverside Avenue. This portion of the channel was not observed during the site visit.

The reach is used by horse riders, as evidenced by prevalent horse tracks. Domestic dogs are likely to use the reach in association with horse riders, and a feral dog was observed in the vicinity. Other tracks observed included raccoon and rabbit. In addition, off-road vehicle tracks were observed along with some evidence of use by homeless and/or vagrants.

Small numbers of several bird species were observed during the site visit, including mallard, common raven, bushtit mourning dove, olive-sided flycatcher, blue grosbeak, Wilson's warbler, yellow warbler, common yellowthroat, song sparrow, house finch, and American goldfinch. Other animals observed included a group of several juvenile Western toads, and a side-blotched lizard.

Riparian and in-channel habitat within the Natural Study reach likely support many songbirds, waterbirds, reptiles and amphibians, and mammals such as raccoon, possum, and coyote. The adjacent La Loma Hills located to the east of the Santa Ana River in this area provide open space with sparse scrub vegetation. However, the general lack of cover provided by the scrub vegetation likely precludes use of the area by large mammals such as mule deer or mountain lion. The area is surrounded by freeways on the north, east, and south sides, with the Santa Ana River to the west.

Limitations

This memo provides information gathered during brief bridge nest surveys and site reconnaissance conducted on May 21, 2015. While this is an active time for migratory songbirds, including nesting cliff swallows, other wildlife may be more prevalent and active during other times of the year. For instance, gulls have been observed using the Santa Ana River in large numbers during the winter. Other potentially important sources of bacterial contamination in the River, such as number and extent of homeless encampments, were not assessed during the biological site visit.

Photo 6. Riparian vegetation along the Natural Study reach of the Santa Ana River.

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