

Lower San Luis Rey River
Bacteria Source Identification Study

SWRCB Grant Agreement No. 07-577-550-2

Final Project Report

September 2011

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Submitted by:

City of Oceanside
Water Utilities Department
300 North Coast Highway
Oceanside, California 92054

ACKNOWLEDGEMENTS

This Draft Project Report was prepared by the Project team in close collaboration with the City of Oceanside and the Technical Advisory Committee. The following entities made major contributions to this report.

Project Team:

MACTEC

UNC INSTITUTE of MARINE SCIENCES

USC UNIVERSITY OF SOUTHERN CALIFORNIA

Technical Advisory Committee:

City of Vista, Stormwater Department

County of San Diego, Department of Environmental Health

County of San Diego, Department of Public Works/Watershed Protection Program

San Diego Coastkeeper

San Diego Regional Water Quality Control Board

Funding for this project has been provided in full or in part through an agreement with the State Water Resources Control Board. The contents of this document do not necessarily reflect the views and policies of the State Water Resources Control Board, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. (Gov. Code, § 7550, 40 CFR 31.20).

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ACRONYMS AND ABBREVIATIONS

AB411 – California State Assembly Bill Number 411 (1999)
ADCP – Acoustic Doppler Current Profiler
AVB – Area Velocity Bubbler
Bacteria I TMDL – Total Maximum Daily Load for Indicator Bacteria, Project I for Beaches and Creeks in the San Diego Region
Basin Plan – Water Quality Control Plan for San Diego Basin Region 9
BLRP – Bacterial Load Reduction Plan
BMP – Best Management Practices
CASQA – California Stormwater Quality Association
CEQA – California Environmental Quality Act
Cfs – Cubic feet per second
CFU – Colony Forming Units
CWA – Clean Water Act
DCA – Detrended Correspondence Axis
DEH – Department of Environmental Health
DQO – Data Quality Objective
DST – Defined Substrate Technology
ESP – *Enterococcal* Surface Protein
FIB – Fecal Indicator Bacteria
GPS – Global Positioning System
HA – Hydrologic Area
HF – Human Fecal
MP – Monitoring Plan
MPN – Most Probable Number
MSL – Mean Sea Level
MST – Microbial Source Tracking Program
NEPA – National Environmental Policy Act
NRPI – National Resource Projects Inventory
NWS – National Weather Service
PAEP – Project Assessment and Evaluation Plan
PCR – Polymerase Chain Reaction
Prop 50 Grant – Proposition 50 Clean Beaches Grant Program
QAPP – Quality Assurance Project Plan
QA/QC – Quality Assurance/Quality Control
QPCR – Quantitative Polymerase Chain Reaction
Q-RTPCR – Quantitative Real Time Polymerase Chain Reaction
RPD – Relative Percent Difference
SCCWRP – Southern California Coastal Water Research Project
SDRWQB – San Diego Regional Water Quality Board
SLR – San Luis Rey
SWAMP – Surface Water Ambient Monitoring Program
SWRCB – State Water Resources Control Board
TAC – Technical Advisory Committee
TMDL – Total Maximum Daily Load
TRFLP – Terminal Restriction Fragment Length Polymorphism
UNC – University of North Carolina, Chapel Hill
USC – University of Southern California
USEPA – United State Environmental Protection Agency

USGS – United States Geological Survey
WLA – Waste Load Allocation
WQO – Water Quality Objective
WURMP – Watershed Urban Runoff Management Program

EXECUTIVE SUMMARY

Background

The Pacific Ocean shoreline at the outlet of the San Luis Rey (SLR) River mouth, located in the City of Oceanside (City), California, is a stretch of coastline with a large sandy beach and an easily accessible surf break, making it one of the City's most popular places for recreation. A portion of the beach (0.03 miles of shoreline) near the outlet of the San Luis Rey (SLR) River mouth is an impaired water body under Section 303(d) of the Clean Water Act (CWA) for indicator bacterial standards and is included in the recently adopted Total Maximum Daily Load (TMDL) for Indicator Bacteria, Project I for Beaches and Creeks in the San Diego Region (Bacteria I TMDL). To address the elevated levels of fecal indicator bacteria (FIB), and in anticipation of the TMDL, the City applied for and was awarded a State Water Resources Control Board (State Water Board) Proposition 50 Clean Beaches Grant Program (Prop 50 Grant) to implement the Lower SLR River Bacterial Source Identification Project (the Project) in the Lower SLR River and river mouth (Grant Agreement No. 07-577-550-0 (Grant Agreement)). The State Water Board, under the Prop 50 Grant, allocated a total of $554,375 for the Project. The City, County of San Diego, and other TAC members anticipated a contribution of an additional $141,750 in in-kind services to the Project.

Project Approach

The Lower SLR River Bacterial Source Identification Project was designed as the initial phase of a source investigation to provide a broad characterization of bacterial concentrations throughout the SLR River and river mouth. The Project was designed to attain the following goals:

- Assess what sources and activities have contributed most to the bacterial impairment of the SLR River mouth and from where those sources and activities may have originated.
- Analyze potential bacterial source elimination or reduction practices targeted at the identified sources and activities.
- Contribute to future achievement of bacterial TMDL objectives by identifying potential management measures (MMs) and follow-up studies to target sources and activities more effectively.

The Project goals were accomplished by integrating data from the following independent monitoring programs:

- The Microbial Source Tracking (MST) Program was implemented by the Prop 50 Grant Project team. The MST Program included monitoring 11 sites within the lower 7.5 miles of the SLR River during wet and dry conditions, a tiered analytical approach for microbial source tracking,

and a visual observation program to identify upstream activities in the watershed that may be potential sources of bacteria.

- The Joint Monitoring (JM) Program was completed by the City of Oceanside and the County of San Diego, Department of Public Works as in-kind services to the Grant. Monthly dry weather monitoring was conducted at 18 locations within the lower 19.2 miles of the SLR Watershed.
- The Department of Environmental Health (DEH) AB411 Program was conducted by the County of San Diego, Department of Environmental Health. Weekly dry weather monitoring of the Pacific Shoreline 75 feet south of the SLR River outlet.

MACTEC Engineering and Consulting, Inc. (MACTEC) was awarded the contract by the City to design, implement, and report findings of the Project. The MACTEC project team included MACTEC Engineering and Consulting, Inc. (MACTEC), Dr. Rachel Noble from the University of North Carolina, Chapel Hill (UNC), Dr. John Griffith from the Southern California Coastal Water Research Project (SCCWRP), and Dr. Jed Fuhrman from the University of Southern California (USC). In addition, a Technical Advisory Committee (TAC) was established to oversee and guide the development, implementation, and reporting of the study. The TAC was comprised of representatives from the following agencies: the City of Oceanside; the City of Vista; the County of San Diego, Department of Public Works/Watershed Protection Program; the County of San Diego, Department of Environmental Health; San Diego Coastkeeper; and the San Diego Regional Water Quality Control Board (SDRWQCB).

Monitoring Programs

For the MST Program, water samples were collected during dry and wet weather for FIB and microbial source tracking analysis in the SLR River mouth, the main stem of the river, and tributary locations. Visual observations were conducted in conjunction with sampling activities, when possible, within 14 zones of residential, commercial, and/or park areas. The MST dry season monitoring was implemented in 2008 and 2010 between the months of May to August. There were a total of four dry season sampling events. Due to the Stop Work Order from the State, there was no activity on the Project during 2009. Wet season monitoring was conducted in 2010 and 2011 during the months of December to February. Wet season monitoring events consisted of a dry weather baseline event, two wet weather (storm) events, and two (dry weather) base flow events.

All FIB results from the MST Program were compared to selection criteria based on the Basin Plan Rec-1 WQOs in order to identify samples for further microbial source analyses. This multi-tiered approach utilizes a tool box of traditional fecal indicator bacterial analyses and quantitative molecular assays that are optimal for microbial source tracking in urban environments. Three quantitative polymerase chain reaction (QPCR) fecal *Bacteroides* spp. assays were conducted to confirm the presence of human fecal

bacteria, taking into consideration a spectrum of specificity and sensitivity. Enterovirus assays quantified the enteric viral concentrations present in water samples and were used as a tool to characterize the threat to human health. A gull specific marker, *Catellicoccus marimammalium*, was used to provide important information about the impact of seagulls in the SLR River. A bacterial community analysis was used to characterize the bacterial communities in the water column and sediment in the river mouth during dry weather.

The City of Oceanside and the County of San Diego conducted the JM Program over the entire project duration. Water samples were collected monthly at main stem, tributary, river mouth, and AB411 locations during dry weather for the JM Program.

The FIB results from both the Joint Monitoring Program and AB411 Program were used to support the seasonal patterns observed in the MST FIB data. A summary of FIB samples collected for each program is provided below:

- MST Program: 111 water samples were collected during discrete wet and dry season events. 48 samples were collected in the main stem of the river, 19 samples were collected at the two tributary sites, and 44 samples were collected in the river mouth. An average of 10 samples was collected at each monitoring location.
- Joint Monitoring Program:
  - 343 water samples were collected within the City of Oceanside from January 2007 to March 2011: 177 samples were collected in the main stem of the river, 69 samples were collected at the two tributary sites, 49 samples were collected in the river mouth, and 48 samples were collected at the AB411 site.
  - 217 water samples were collected upstream within the County of San Diego from January 2007 to June 2010.
- DEH AB411 Program: 74 samples were collected from April 2010 to February 2011 at the SLR AB411 monitoring location.

The MST program samples were selected for further molecular marker analyses based on the FIB concentrations. An overall breakdown of the microbial, viral, and community fingerprinting analyses conducted is as follows:

- 72 samples were analyzed for fecal *Bacteroides* spp., BacHum-Human *Bacteroides*, and Human-specific HF183 marker. In 2008, six dry season samples from main stem river and tributary locations were selected for further molecular analysis, but were not analyzed due to holding time exceedances as a result of the Stop Work Order in December 2008.
- 68 samples were analyzed for enteroviruses.
- 72 samples were analyzed for the gull specific marker.

- 13 water samples and 13 paired sediment samples from the river mouth were selected for Community Fingerprinting Analyses.

Conclusions

The Prop 50 Grant Project was effective in characterizing bacterial concentrations throughout the Lower SLR River and river mouth during wet and dry seasons. A summary of conclusions is provided below.

River Mouth:

- FIB concentrations were generally below WQOs in the river mouth during the summer months when human exposure and health risks are highest at the Pacific Ocean.
- Although FIB concentrations were below WQOs, multiple MST molecular markers for human-related bacterial sources were present in August when the river mouth was closed to ocean inputs and SLR River flow was significantly reduced. Human sources of contamination have been identified subsequently in this area, including an active sewer pipe. Other factors could be important to the retention of fecal contamination in this area, including low residence time and hydrologic conditions in the river mouth during low flow.
- The bacterial community analysis showed a lack of relationship between the sediment and water samples in May when the river mouth is open to tidal exchange. Conversely, there was a strong relationship between the sediment and water samples in August when the river mouth is closed. The sediment bacterial community is stable during the dry season and was not analyzed during the wet season. It should be noted that the bacterial community analysis was only conducted on river mouth samples and no analysis was conducted to evaluate the relationship between the SLR River's main stem and tributaries and the river mouth monitoring locations.
- No enteroviruses were recorded during the dry season.
- A strong signal of gull feces was found in the river mouth during the wet and dry seasons.
- FIB concentrations significantly increased in the wet season resulting in a higher frequency of WQO exceedances.
- There was evidence of potential human-related bacterial sources (a minimum of two out of three markers) at River Mouth 1 and River Mouth 3 throughout the wet season, while at Point Zero there was evidence of potential human-related bacterial sources (a minimum of two out of three markers) at a lower frequency.
- One sample collected at River Mouth 1 during Wet Event 1 was positive for enteroviruses.
- Of the 74 samples from the DEH AB411 program, 12 results for *Enterococcus* sp, and 4 results for fecal coliform were above the respective WQOs, whereas no total coliform results exceeded the WQO.

SLR River Main Stem:

- The MST Program study data at the SLR main stem reinforced trends in elevated FIB concentrations seen in the data collected by the Joint Monitoring Program.
- In May of 2010, there were potential (two of three markers) human-related bacterial sources present at Bonsall Bridge, Douglas Bridge, and the Critical Point.

- No enteroviruses were detected in samples at the main stem monitoring locations during dry or wet seasons.
- During the wet season, the Douglas Bridge and Critical Point locations exhibited strong positive signals for all three MST molecular markers of human fecal *Bacteroides* spp.
- FIB concentrations and loadings did not change significantly in the river during base flow conditions.
- A trend toward delivery of bacterial loadings in the first half of the storm event was demonstrated by a simple first flush analysis. This indicates a possible first flush effect and may aid in the future selection and design of appropriate BMPs.

Tributaries:

- The MST Program study data at the SLR tributaries reinforced trends in elevated FIB concentrations seen in the data collected by the Joint Monitoring Program.
- In May of 2010, there were potential human-related bacterial sources present at Sleeping Indian and Pilgrim Creek.
- No enteroviruses were detected in samples at the tributaries during dry or wet seasons.
- During the wet season, Sleeping Indian and Pilgrim Creek exhibited strong positive signals for all three MST molecular markers of human fecal *Bacteroides* spp.
- FIB concentrations and loadings did not change significantly in the tributaries after storms passed during base flow conditions.
- The assessment of flow relative to concentrations of FIB and human-specific markers indicates the importance of bacterial sources stemming from low flow areas or low dilution (i.e., tributaries).
- A trend toward delivery of bacterial loadings in the first half of the storm event was demonstrated by a simple first flush analysis. This indicates a possible first flush effect and may aid in the future selection and design of appropriate BMPs.

Recommendations

The Project was designed as the first phase in source identification to assess the magnitude and extent of elevated FIB and to characterize sources on a broad scale. Based on results from this study, recommendations were developed to provide further information to help select the most feasible and effective management strategies necessary to reduce bacterial concentrations. Recommendations included follow-up source investigations, an assessment of the relationship between the river mouth and ocean, and focused management measures including source controls, decentralized treatment controls and centralized treatment controls.

Some general recommendations include:

- To identify possible sources of overflow or leaks along the main stem river and tributaries, a desktop GIS analysis of the City's sewer infrastructure and septic systems may be conducted. Field surveys, such as dye testing or the use of closed circuit television (CCTV), may be considered to identify leaks or illicit connections.
- Focus education and outreach based on activities documented in visual surveys and known land use types.
- Higher resolution data is required for an evaluation of loading and possible implementation of structural treatment control BMPs. This may be accomplished through continuous flow and higher resolution FIB sampling at the outlets during the wet season to characterize conditions over the course of the hydrograph and continuous flow and higher resolution FIB sampling at the outlet over a 24 hour period during dry weather (including base flow conditions and dry season, when flow is present).
- Characterize the flow patterns within the river mouth system to understand the general transport of fecal bacteria sources and their ultimate fate.
- Design a groundwater study to gain a better understanding of groundwater dynamics and their relationship to surface water in the SLR River and river mouth during base flow conditions.
- To contribute to future achievement of the Bacteria TMDL, it will be important to assess the interaction between the river mouth and the Pacific Ocean. To characterize the dispersal of the bacterial and viral material from the SLR River outlet along the shoreline, multiple sampling locations between the river mouth and the AB411 monitoring location may be monitored.
- To allow for accurate loading calculations and an assessment of bacterial loads and flux over the course of the monitoring period at the river mouth, pollutograph monitoring may be employed at a river mouth location to characterize bacteria on a higher resolution time frame. Acoustic Doppler current profilers (ADCPs) may be utilized to collect continuous flow measurements during sampling events.
- To identify potentially failing septic (onsite) wastewater systems viral tracking, dye testing, inspections and/or other methods may be utilized. Once identified, an inspection and maintenance schedule may be developed and revised to prioritize the septic systems in the SLR watershed, based on threats to groundwater and receiving water quality. Based on available resources and feasibility, a capital improvement plan for conversion of septic systems to sanitary sewers may be developed in appropriate areas.
- To address the human-related bacterial sources present in the river mouth during the dry season, when the river mouth is closed to ocean inputs and the SLR River flow is significantly reduced, the feasibility of hydraulic modifications to the river mouth and/or the SLR River outlet may be evaluated to reduce residence time and increase flushing during the dry season. A feasibility study may be implemented after additional monitoring has been conducted to better understand the hydrology of the river mouth and retention of FIB in the river mouth. Based on available resources, the feasibility study could identify benefits and constraints for current and alternative hydraulic configurations of the river mouth.

Next Steps

The SLR Watershed TMDL Responsible Parties are in the process of developing a Comprehensive Load Reduction Plan (CLRP) for the SLR Watershed to address the Bacteria I TMDL and other pending TMDLs. The CLRP will provide a watershed-based approach to assess impairments and sources and implement a phased approach to pollutant reduction strategies. The results of this project will be used immediately by the SLR TMDL Responsible Parties in the development of the CLRP addressing bacteria. Project data will be used to focus the initial phase of CLRP implementation by assisting in the prioritization of source and sub-watershed activities.

With preliminary results indicating human sources of bacteria, the City of Oceanside has taken a proactive approach and already has begun to implement a key recommendation from this project, initiating the desktop GIS analysis of the City's sewage infrastructure. The City's Clean Water Program is working closely with the City's Sewer Division to develop an action plan to investigate the City's sewage infrastructure.

1.0 PROJECT SUMMARY

The Pacific Ocean shoreline at the outlet of the San Luis Rey (SLR) River mouth, located in the City of Oceanside (City), California, is a stretch of coastline with a large sandy beach and an easily accessible surf break, making it one of the City's most popular places for recreation. Due to historical fecal indicator bacteria (FIB) exceedances at the beach, 0.03 miles of shoreline are included as an impaired water body under Section 303(d) of the Clean Water Act (CWA) for FIB standards and included in the recently adopted Total Maximum Daily Load (TMDL) for Fecal Indicator Bacteria, Project I for Beaches and Creeks in the San Diego Region (Bacteria I TMDL).

To address the elevated levels of FIB and in anticipation of the TMDL, the City applied for and was awarded the State Water Resources Control Board (State Water Board) Proposition 50 Clean Beaches Grant Program (Prop 50 Grant) to implement the Lower SLR River Bacterial Source Identification Project (the Project) in the Lower SLR River and river mouth (Grant Agreement No. 07-577-550-0 (Grant Agreement)). The overall goals of the Project were to identify hotspots of FIB within the Lower SLR River, identify potential sources, and prioritize those sources and/or locations for future management measures to reduce bacteria concentrations.

The MACTEC Engineering and Consulting, Inc. (MACTEC) project team was awarded the contract by the City to design, implement, and report the Project findings. The MACTEC project team included MACTEC Engineering and Consulting, Inc. (MACTEC), Dr. Rachel Noble from the University of North Carolina, Chapel Hill (UNC), Dr. John Griffith from the Southern California Coastal Water Research Project (SCCWRP), and Dr. Jed Fuhrman from University of Southern California (USC). In addition, a Technical Advisory Committee (TAC) was established to oversee and guide the development, implementation, and reporting of the study. The TAC was comprised of representatives from the following agencies:

- City of Oceanside
- City of Vista
- County of San Diego, Department of Public Works/Watershed Protection Program
- County of San Diego, Department of Environmental Health
- San Diego Coastkeeper
- San Diego Regional Water Quality Control Board

The Project goals were accomplished by integrating data from the following independent monitoring programs:

- The Microbial Source Tracking (MST) Program was implemented by the MACTEC project team. The MST Program included monitoring 11 sites along the SLR River during wet and dry conditions, a tiered analytical approach for microbial source tracking, and a visual observation program to identify activities upstream in the watershed that may be potential sources of bacteria.
- The Joint Monitoring (JM) Program was completed by the City of Oceanside and County of San Diego, Department of Public Works as in-kind services to the Grant. Monthly dry weather monitoring was conducted at 18 locations within the lower 19.2 miles of the SLR Watershed.
- The Department of Environmental Health (DEH) AB411 Program was conducted by the County of San Diego, Department of Environmental Health. Weekly dry weather monitoring of the Pacific Shoreline 75 feet south of the SLR River outlet.

This project report summarizes all activities conducted under the Project from November 2007 through July 2011.

1.1 BACKGROUND

In 1999, the California State Assembly Bill No. 411 of 1999 (AB411) added new bacteriological ocean water quality standards and monitoring requirements to the California Health and Safety Code. The City and the County of San Diego, Department of Environmental Health conducted weekly AB411 monitoring at the SLR River Outlet/Harbor Beach shoreline station. Based on monitoring results from January 2002 to May 2008, the SLR River outlet and shoreline had more beach postings and closures than any other beaches in the City during that time period.

An objective of the SLR Watershed Urban Runoff Management Program (WURMP) is to identify sources of the FIB at the river mouth and in the river. In March 2004, the City and County of San Diego began a voluntary WURMP activity to jointly monitor main stem and tributary sites throughout the Lower San Luis Rey Hydrologic Area. The results were inconclusive and indicated the need for focused sampling in the lower river and at the river mouth to determine if the river is the source of elevated FIB concentrations at the river mouth and along the shoreline. To address this issue, the City applied for and was awarded a State Water Resources Control Board Proposition 50 Clean Beaches Grant program to implement this MST Program. As a component of the grant match funding, the City and County continued the monthly joint monitoring program to supplement the MST Program.

The Bacteria I TMDL was developed by the San Diego Regional Water Quality Control Board (San Diego Regional Board) to establish TMDLs for impaired beaches and creeks listed on the 2002 303(d) list in order to attain and maintain water quality standards. Separate dry weather and wet weather TMDLs were calculated for each FIB (total coliform, fecal coliform, and *Enterococcus* sp.). The San Diego Regional Board recognized that exceedances of the Rec-1 Water Quality Objectives (WQOs) may be partially due to natural sources and developed the wet weather TMDLs to include an allowable exceedance frequency. The Bacteria I TMDL incorporates a reference system approach to account for bacterial loads contributed from natural sources that is based on the Leo Carrillo Beach in Los Angeles County, California. The Municipal MS4 dischargers have been assigned a waste load allocation (WLA) and will be responsible for reducing their bacterial load and demonstrate that their discharge is not causing exceedances of the numeric WQOs and allowable exceedance frequencies in the receiving water. The SLR Watershed TMDL Responsible Parties are in the process of developing Comprehensive Load Reduction Plan (CLRP) for the SLR Watershed to address the Comprehensive TMDL. The CLRPs will provide a watershed-based approach to assess impairments and sources and implement a phased approach to pollutant reduction strategies. The MST Program data will be used to focus the initial phase of implementation of the CLRP by assisting in the prioritization of source and sub-watershed activities.

1.2 SCOPE AND GOALS

Per the Grant Agreement, a Project Assessment and Evaluation Plan (PAEP) was developed to detail the methods and tasks for program implementation and evaluation. The Project scope contains four tasks per the PAEP, as described in Section 1.3: Project Management, Administration, and Reporting; establishment of a TAC; Development of a Quality Assurance Project Plan (QAPP) and Monitoring Plan (MP); and creation of a Project Design. These four tasks encompass the work to be performed required by the Grant Agreement. The Project was designed to attain the following goals as defined in the PAEP:

- Assess where and what sources and activities have contributed most to bacterial impairment of SLR River mouth.
- Analyze potential bacterial source elimination or reduction practices that are targeted at identified sources.
- Contribute to future achievement of bacterial TMDL objectives by identifying potential management measures (MMs) and follow-up studies to effectively target sources.

1.3 ACTIVITIES COMPLETED, TECHNIQUES USED, AND PARTNERS INVOLVED

Project activities completed are provided in Sections 1.3.1 to 1.3.4 below. Additional details regarding Project methodology are discussed in Section 2.0. Table 1-1 provides a list and dates for completed Project work items.

The Grant Agreement for the Project was approved in December 2007. The QAPP and MP were submitted in April 2008 and presented a monitoring approach for the Project comprised of a dry season monitoring in the Lower SLR River at main stem and tributary locations, wet season monitoring at main stem, tributary, and river mouth locations, followed by dry season monitoring focused in the river mouth. The Lower SLR River monitoring was completed during the summer of 2008. Before any wet weather monitoring was conducted, all Project activities were suspended per the California Department of Finance Budget Letter 08-33 on December 19, 2008.

On December 17, 2009, the State Water Resources Control Board gave the City notice that the Lower San Luis Rey Source Identification Project grant had been selected to restart. With an original grant end date of March 31, 2010, the City, on January 4, 2010, submitted a Request for Time Extension in order to complete the remaining two-thirds of the field and laboratory work required. On April 14, 2010, the City of Oceanside received the executed amendment to the Grant Agreement (07-577-550-1) and work began immediately to restart the Project. Samples selected to be analyzed for human-specific markers in 2008 were not analyzed due to holding time exceedances as a result of the stop work order issued in December 2008. Therefore, the majority of human-specific markers were analyzed in the 2010-2011 monitoring period. The monitoring approach was re-organized based on the new Project schedule and the dry season monitoring in the river mouth was conducted in May and August 2010. The City collected extra water samples at Lower SLR River main stem and tributary monitoring locations during the May event for possible follow up molecular analyses. Wet Season monitoring was conducted between December 2010 and February 2011.

On February 11, 2011, the City of Oceanside requested a time extension for the grant in order to capture the last storm event. On June 16, 2011, the City received the revised Grant Agreement (07-577-550-2).

Table 1-1: Completed Work Items

Work Item	Items for Review #	Due Date	Work Completed	Date(s) Submitted
EXHIBIT A Scope of Work	**A – Plans and Compliance Requirements**			
	A1 Global Positioning System (GPS) Information	October 2007	X	1/18/2008
	A2 Project Assessment and Evaluation Plan (PAEP)	October 2007	X	7/20/2007
	A3 Monitoring Plan	April 2008	X	1.0 - 4/28/08 2.0 - 6/11/08 3.0 - 6/18/08
	A4 QAPP	April 2008	X	1.0 - 4/28/08 2.0 - 6/11/08 3.0 - 6/18/08 3.1 - 5/21/10 3.2 - 2/11/11
	A5 California Environmental Quality Act (CEQA)/National Environmental Policy Act (NEPA) Documents	April 2008	X	4/20/2007
	B – Work to be Performed by Grantee			
	B1.1 List of TAC Members	November 2007	X	1/10/2008
	B1.2 TAC Meeting Documents	Quarterly, as needed	X	1/18/2008 4/17/2008 6/19/2010 10/11/2010
EXHIBIT B Invoicing, Budget, and Reporting	A – Invoicing	Quarterly	86%	1/18/2008 4/17/2008 7/17/2008 10/16/2008 1/16/2009 4/9/2009 7/17/2009 4/19/2010 7/19/2010 10/19/2010 1/19/2011 4/19/2011
	F – Reports			
	F1 Grant Summary Form	3/11/2008	X	4/11/2008
	F2 Progress Reports	Quarterly	86%	1/18/2008 4/17/2008 7/17/2008 10/16/2008 1/16/2009 4/9/2009 7/17/2009 4/19/2010 7/19/2010 10/19/2010 1/19/2011 4/19/2011
	F3 National Resource Projects Inventory (NRPI) Project Survey Form	Before Final Invoice	0%	To Be Submitted
	F4 Draft Project Report	8/1/2011	100%	8/1/2011
	F5 Final Project Report	9/1/2011	0%	9/1/2011

1.3.1 Task 1: Project Management, Administration, and Reporting

The City provided Grant invoices and developed quarterly progress reports for the State Water Board indicating the percentage of completed program activities. The City coordinated the TAC and worked closely with the MACTEC project team. The MACTEC project team implemented the monitoring program, managed analytical data, scheduled, and developed the project report.

1.3.2 Task 2: Establish Technical Advisory Committee

A TAC was comprised of stakeholders, regulators, and non-profit organizations based on their experience in genetic microbial source tracking methodologies, hydrology, watershed studies, and load calculations. The TAC consisted of staff from the City, the City of Vista, the County of San Diego – Department of Public Works/Watershed Protection Program and the Department of Environmental Health, the San Diego Coastkeeper, and the San Diego Regional Board. TAC meetings are summarized below:

- In November 2007, the TAC met prior to consultant selection to review the Scope of Work and timeline for the Project.
- In February 2008, the TAC convened to assist in selection of the Project team.
- In May 2010, the TAC met to review the MP objectives and activities completed in 2008, and re-examine the program approach and sample design.
- In October 2010, the TAC met to review the dry weather results, define the wet season events, and verify the logistical constraints.
- In April 2011, the TAC met to discuss the results of the Project to date, review the project report outline, and discuss Project outcomes.
- In July 2011, the TAC independently reviewed and provided comments on the Draft Project Report prior to submittal to the State.

1.3.3 Task 3: Develop Quality Assurance Project Plan and Monitoring Plan

A QAPP and MP were developed by the Project team in June 2008. The MP described the program design, the rationale for site selection and program structure, the sampling procedures, and the tiered analytical approach. The QAPP established the sampling and analytical methodologies, logistical constraints and approach, and data management protocols in order to produce a representative and scientifically-defensible data set. Per the Grant Agreement, the QAPP was developed in accordance with the Surface Water Ambient Monitoring Program (SWAMP) guidelines. After the Project was reinstated in 2010, the QAPP was amended to update Project and TAC members, incorporate additional microbial molecular marker analyses, and update the Project schedule and timeline. Table 1-2 describes the revisions made to the QAPP and MP.

Table 1-2: Revision History of MP and QAPP

Revision Number	Date	Revision Statement
1.0	04/28/08	• Original Draft Submittal.
2.0	06/11/08	• The document was revised to include comments provided by the SWRCB on the Monitoring Plan and by Moss Landing QA Research Group on the Draft QAPP.
3.0	06/18/08	• All references to CRG Marine Laboratories were replaced by EnviroMatrix Analytical, Inc. in the Final QAPP submitted to the State Water Resources Control Board (SWRCB) on 6/11/08. For the success of the Project, the City of Oceanside and the Project team have decided to change back to CRG. CRG is more experienced conducting the necessary filtration procedures required for the molecular marker analyses.
3.0	6/19/08	• Approved Amended Plan.
3.1	5/21/10	• The MACTEC Project Manager was changed from Hawkeye Sheene to Roshan Sirimanne. • The document was revised to incorporate new deadlines from the Grant Agreement amended on 4/12/2010 after the suspension of SWRCB bond grants. • The document was revised to incorporate a revised river mouth monitoring strategy to accommodate the decisions made by the stakeholders for the first monitoring event of 2010 and for the revival of the Project and shift in resources after the suspension and subsequent restart of the Project. An additional QAPP Amendment is planned to address all program components.
3.2	2/11/11	• The document was revised with the updated Project team members and list of TAC members (see attached list of TAC members). • The document was updated to include two additional laboratories that are being utilized to conduct the analysis of FIB replacing CRG Marine Laboratories in December 2010. • The document was revised to incorporate three additional types of molecular marker analyses: BacHum-Human *Bacteroides* marker, HF183 human-specific marker, Gull (*Catellicoccus marimammalium*) Bacterial Marker. • The *Enterococcal* Surface Protein (ESP) gene assay has been removed, since studies have shown that the ESP gene protocol is very time consuming, suffers from low sensitivity, and that the ESP gene is not specific only to humans (lots of cross reactivity). So effectively, the ESP gene protocol was replaced with the BacHum marker assay. • Updated flow monitoring techniques and equipment.

1.3.4 Task 4: Project Design

The Project included three components: (1) a Microbial Source Tracking (MST) Program, (2) the Joint Monitoring Program implemented by the City and County of San Diego, and (3) a visual observation program. These components are described in detail in Section 2.0.

The microbial source tracking design was developed by a project team comprised of MACTEC, Dr. Rachel Noble from UNC, Dr. John Griffith from SCCWRP, and Dr. Jed Fuhrman from USC. The monitoring approach established the following objectives in the QAPP to meet the overall Project goals:

- Identify point and non-point sources of bacterial contamination in the Lower SLR River and at the river mouth during dry and wet seasons.
- Estimate the bacterial loading from tributaries and along the main stem of the SLR River during dry and wet seasons.
- Recommend Best Management Practices (BMPs) to reduce and/or eliminate bacterial sources.

1.4 FUNDING

Funding for the Project was obtained from the State Water Board under a Prop 50 Grant, and was matched by the City, County, and TAC members, per the Grant Agreement. The original end date for the grant was April 2010. Some of the tasks were delayed due to a stop work order issued by the State on December 18, 2008. Funding was restored in late 2009 and the delayed tasks were resumed in May 2010. The grant contract was amended with a final work completed date of October 2, 2011. Table 1-3 provides a summary of funding received and the projected and actual costs for the Project, as anticipated and implemented under the Grant Agreement. Table 1-4 provides a summary of the remaining grant budget and matching funds to date. A final quarterly progress report will be submitted October 20, 2011, and will provide the final budget breakdown.

Table 1-3: Projected and Actual Costs

Expenditure Type/ Line Item	Projected Prop 50 Grant Amount Received	Projected Match Amount	Total Projected Costs	Actual Prop 50 Grant Amount Received (as of 6/30/2011)	Actual Project Match Amount Provided (as of 6/30/2011)	Total Actual Costs (as of 6/30/11)
Personnel Services	$10,000	$34,250	$44,250	$2,567.74	$21,408.63	$23,976
Operating Expenses	$0	$6,250	$6,250	$0.00	$3,357.31	$3,357
Professional/ Consultant Services	$544,375	$101,250	$645,625	$464,451.08	$98,370.34	$562,821.42
TOTAL	$554,375	$141,750	$696,125	$467,018.82	$123,136.28	$590,155.10

Table 1-4: Remaining Budget

Expenditure Type/ Line Item	Grant Allotment Remaining (as of 6/30/11)	Match Remaining (as of 6/30/2011)
Personnel Services	$7,432.26	$12,841.37
Operating Expenses	$0	$2,892.69
Professional/ Consultant Services	$79,923.92	$2,879.66
TOTAL	$87,356.18	$18,614.72

1.4.1 Alternate Project Funding Sources

The State Water Board, under the Prop 50 Grant, allocated a total of $554,375 for the Project. The City, County of San Diego, and other TAC members anticipated a contribution of an additional $141,750 in in-kind services to the Project. No additional funding sources were allocated to the Project.

1.4.2 Leveraged Funding Sources and Plans for Funding Future Activities

Additional grant funding may be sought in the future for source tracking and BMP implementation. No additional funding has been leveraged by the Project.

2.0 PROJECT METHODOLOGY

This section describes the methodologies implemented to attain the Project goals and objectives stated in Section 1.0.

2.1 PROJECT LOCATION

The SLR Watershed is located in the northern portion of San Diego County and is approximately 360,000 acres, or 562 square miles (WURMP Annual Report, 2011). The SLR River originates in the Palomar and Hot Springs Mountains, both of which are over 6,000 feet above mean sea level (MSL), and flows west over 55 miles to discharge into the Pacific Ocean at the western boundary of the City of Oceanside. The City of Oceanside, City of Vista, County of San Diego, and Caltrans have jurisdiction within the SLR watershed. Over 54 percent of the land in the watershed is vacant or undeveloped. The next largest land uses in the watershed are residential (15%) and agricultural (14%). The highest population concentration is located in the Lower San Luis Hydrologic Area (HA 903.1) which is the furthest downstream HA, as well as the largest. To assess the sources of bacteria reaching the beach, the geographical scope of the Project concentrated on the most populated lower reaches of the Lower SLR River and river mouth. The monitoring component of the grant funded MST Program was conducted within approximately the lower 7.5 river miles, from the river mouth to just east of the City's jurisdictional boundary. The Joint Monitoring Program was conducted within approximately the lower 19.2 river miles from the shoreline, ocean monitoring site to just east of Interstate 15.

2.2 MICROBIAL SOURCE TRACKING PROGRAM

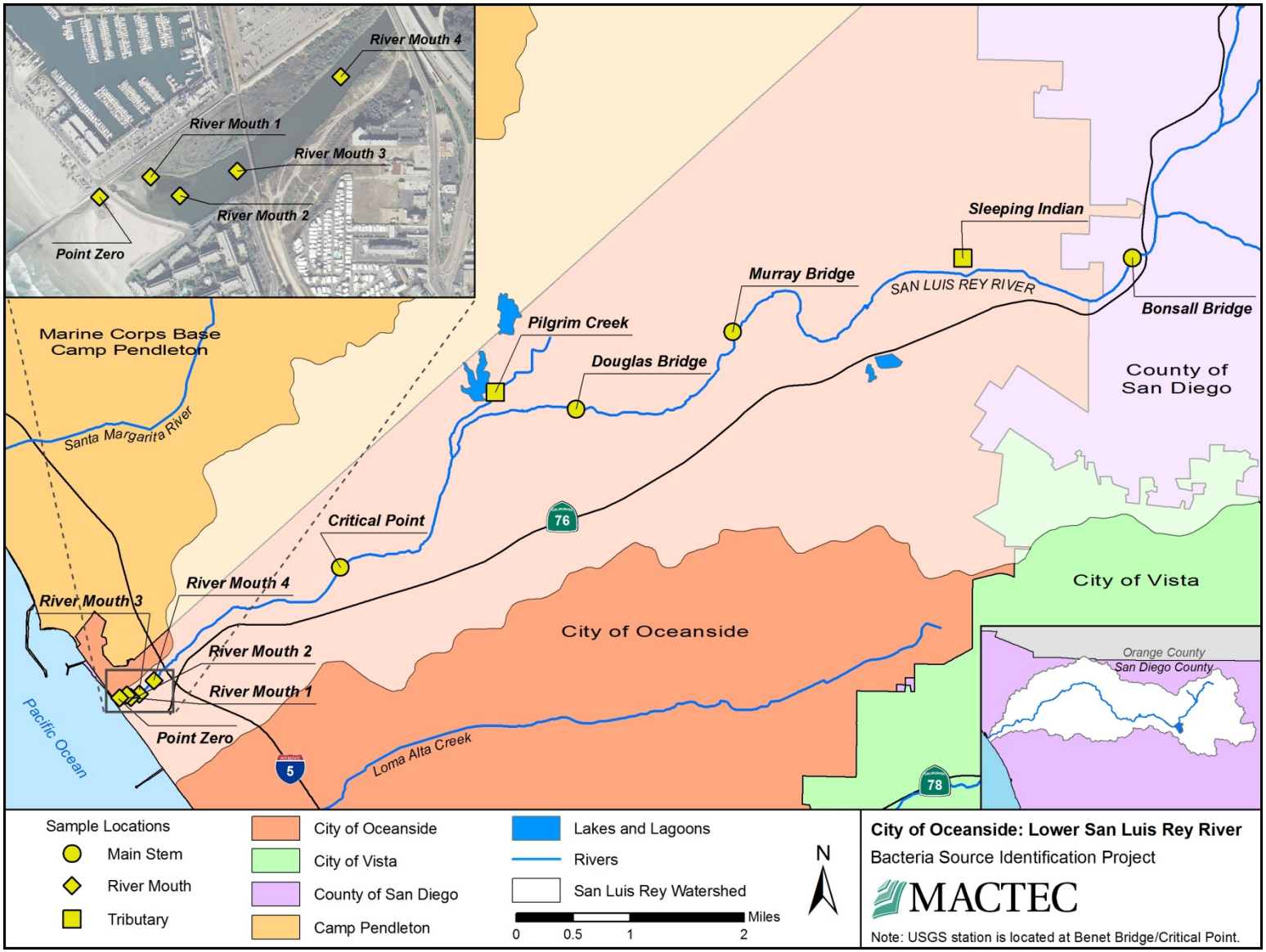
This section describes the sample locations, events, sample and flow collection methods, and visual observation protocols. Monitoring occurred during both the dry season (May to September) and the wet season (October to April). The visual observation program was implemented to correspond with monitoring activities, when feasible.

2.2.1 Monitoring Locations and Events

Samples for the MST Program were collected in the lower 7.5 miles of the river wholly within the Lower SLR River (HA 903.1). Sampling locations included various points along the main stem of the SLR River, its tributaries, and the river mouth down to the mean high tide line where the SLR River discharges into the Pacific Ocean. Four main stem river locations were monitored to characterize a transect from east of the upper boundary of the City to the Benet Bridge, or the Critical Point, where the SLR River

transitions from freshwater to brackish conditions in the river mouth. Two tributaries were monitored as the main inputs to the SLR River. Three to five river mouth locations were monitored to characterize the spatial variability within the river mouth, including the "Point Zero" location, at the outlet to the ocean, to assess human health risk exposure. MST Program monitoring locations are shown in Figure 2-1. Appendix A provides a detailed description of monitoring sites, including Global Positioning System (GPS) coordinates.

Figure 2-1: Grant Monitoring Locations



2.2.1.1 Dry Season

A total of four dry season events over two years were planned. The first year's dry events included main stem and tributary sampling only due to construction of the Pacific Street Bridge at the river mouth. The second year's dry events included river mouth, main stem, and tributary sites. Dry Season monitoring occurred during the months of May to September, 2008 and 2010. There were a total of four dry season sampling events, each preceded by a minimum of 72 hours of dry weather which was defined as less than 0.1 inch of rainfall. Each sampling event consisted of two to three consecutive days of sampling to identify intermittent and chronic sources. Samples and flow measurements were collected when flow was present. In order to minimize sun exposure which can cause die-off of bacteria, samples were collected prior to 11am when feasible. To collect a sample representative of the SLR River with minimal influence from tidal exchange, sampling was coordinated with ebb low tide when feasible.

2.2.1.2 Wet Season

Wet season monitoring occurred during the months of October to April, 2010-2011, in the river mouth, main stem, and tributaries. Wet season monitoring events consisted of a dry weather baseline event, two wet weather events, and two dry weather base flow events. For the purposes of this study, base flow is defined as the groundwater flow or dry/background flow in the stream. The dry weather baseline event was defined to characterize wet season dry conditions not influenced by a specific rain event. Two wet weather events were monitored to capture bacteria levels during the rising and falling limbs of the hydrograph. The storm events met mobilization criteria, including no rain exceeding 0.1 inches in precipitation for at least 72 hours, a minimum of a 60-percent chance of precipitation, and a minimum of 0.25 inches of predicted rain. Monitoring activities were conducted during daylight hours due to safety concerns and site conditions. Two base flow events were conducted to examine the post-storm exceedances during dry conditions within the river, its tributaries, and the river mouth. Base flow events were conducted with a minimum of 72 hours of antecedent dry conditions after the monitored storm event.

2.2.2 Water Sampling

Water samples collected during the dry and wet seasons were analyzed for bacterial indicators. Five one-liter aliquots were manually collected and were composited for dry season monitoring, with a 30-minute interval between aliquots, for a total of five-liters per sample. Six one-liter aliquots were manually collected and were composited for wet season monitoring, with a 30-minute interval between aliquots, for a total of six-liters per sample. Two samples were collected during each storm event to capture the rising

and falling limb of the hydrographs. All materials and containers were field rinsed. In between sample aliquot collection, site information and conditions were recorded on the Field Data Sheet.

Sampling for the third dry season event was coordinated with the Joint Monitoring Program to provide a cost-effective solution to collect additional data at the main stem and the tributary locations of the River. Sampling on the first day of the third event was conducted by the City at the main stem and tributary locations presented in Figure 2-1. Samples were collected according to the Joint Monitoring Program protocols provided in the QAPP and consisted of a composite collected at multiple points along the stream cross-section rather than the time-weighted composite sample, as described above.

2.2.3 Sediment Sampling

Sediment samples were only collected during dry season monitoring in the river mouth for use in the bacteria-based community analysis. The sediment samples were paired with water samples to assess the relationship between stored reservoirs of bacteria communities within the floor of the lagoon with those in the water column. Sediment samples included a spatial composite of six to ten samples from a three square-foot area. The samples were collected after water sample collection to prevent cross-contamination of the water column samples, and were collected using pre-cleaned syringes from the top two centimeters of sediment.

2.2.4 Stream Flow Estimations

Stream flow estimates were calculated for the locations listed in Table 2-1, using methodologies selected based on the site conditions during the sampling event and other available resources. For dry season monitoring, the stream flow estimates were made in accordance with United States Geological Survey (USGS) methodology (Rantz et al., 1982) for cross-sectional profiles measuring velocity at equal increments throughout a cross-section of the stream. Stream flow estimations were calculated using the flow continuity equation provided in the QAPP. During wet season monitoring, flow measurements and estimations were calculated using a combination of USGS data, installed flow meters, and installed water level data loggers as summarized in Table 2-1.

Table 2-1: Stream Flow Estimation Methodologies

Sampling Location	Dry Season Monitoring	Wet Season Monitoring		
	USGS Equation	USGS Site 11042000	Flow Meter with AVB Probe[(a)]	Onset HOBO U-20 Water Level Data Loggers[(b)]
Bonsall Bridge	X			X
Sleeping Indian[(c)]	X		X	X
Murray Bridge	X		X	
Douglas Bridge	X			X
Pilgrim Creek	X		X	
Critical Point	X	X		

(a) Area velocity bubbler (AVB) probes.
(b) Discharge estimations from locations that only had water level data loggers were calculated using water level data and the Manning's equation in conjunction with interpolated data from nearby sites containing flow meter installations.
(c) At the Sleeping Indian location, total discharge estimations were made by summing the discharge from all four pipes at the site. Discharge for the three pipes with water level loggers was estimated by applying the velocities measured in the pipe with a flow meter to the water level data from the other three pipes.

2.2.5 Visual Observations Program

The visual observations program was conducted in conjunction with sampling activities, when possible, to survey the upstream drainage areas and document bacterial sources and/or pollutant generating activities. A zone map was developed to identify zones of residential, commercial, and park areas within drainage areas adjacent to monitoring locations that could be surveyed in one-hour increments as shown in Table 2-2. A zone may represent a mixed land use types as described in Table 2.2. Field crews conducting visual observations included City staff, MACTEC personnel, and other interested parties. Visual observations were recorded by field crews on the SLR River Source Identification Project Visual Observation Data Sheet (Observation Sheet) and site maps. Observation Sheets and maps are provided in Appendix D. The Observation Sheet contained the following information:

- General Site Descriptions: Zone ID, date, time, observers, and address/intersection.
- Environmental Conditions: Weather, water flow, and whether flow was reaching the nearby storm drain inlets.
- Runoff Characteristics: Odor, clarity, floatables and deposits, and presence of biological indicators.
- A Flow Estimation Worksheet for use at flowing creeks or box culverts, flowing pipes, and/or areas with ponded water.
- Activities and Behaviors in or near runoff, including: Human behavior, maintenance procedures, and wildlife distribution.

Table 2-2: Description of Visual Observation Zones

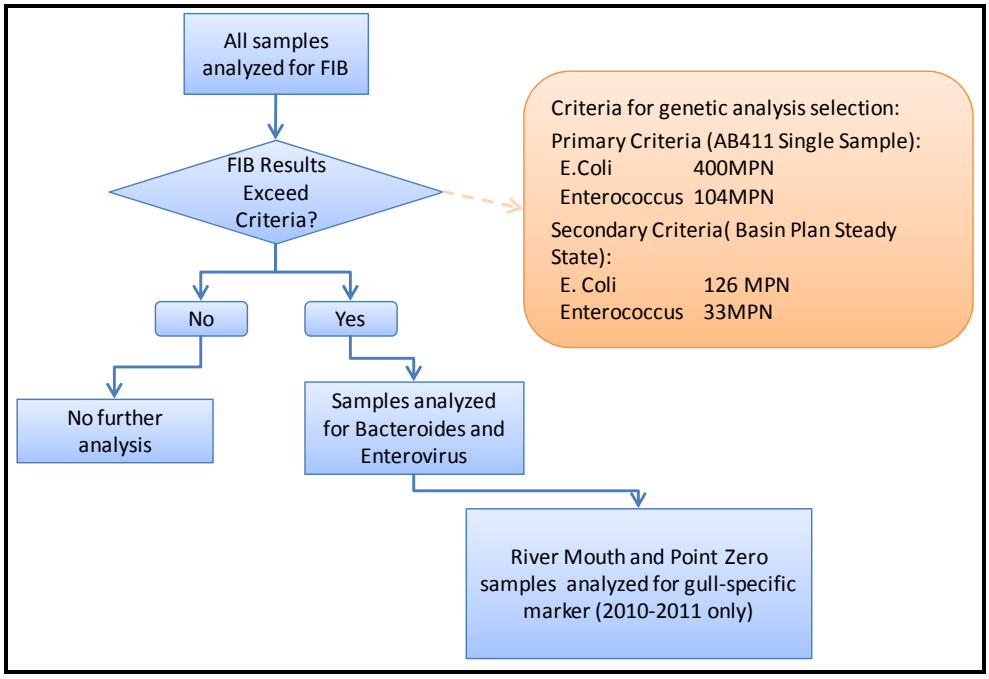
Zone	Land Use Type	Nearest Sample Location
1	Recreational/Water	River Mouth
2	Recreational/Industrial	River Mouth
3	Commercial	River Mouth
4	Residential	River Mouth
5	Business	Critical Point
6	Residential/Recreational	Critical Point
7	Commercial	Critical Point
8 and 8a	Residential	Pilgrim Creek
9	Residential	Douglas Bridge
9a	Residential	Pilgrim Creek
10	Residential	Douglas Bridge
11	Residential	Murray Bridge
12	Commercial	Murray Bridge
13	Rural Residential/Agricultural	Sleeping Indian
16	Residential	Murray Bridge

2.3 MULTI-TIERED ANALYTICAL APPROACH

The MST Program was conducted primarily to assess the presence, and quantify markers of human fecal bacteria sources, and evaluate whether the bacteria sources at specific sites were chronic or intermittent. In addition, a gull specific marker was utilized to verify the presence and impact of the large population of seagulls at the river mouth.

The multi-tiered analytical approach utilized a tool box of traditional FIB analyses and quantitative molecular assays for microbial source tracking in urban environments. Figure 2-2 shows the multi-tier analytical approach followed. Water samples collected during the dry and wet season sampling events were initially analyzed for the FIB *E. coli* and *Enterococcus* sp. For each water sample, 200-ml was sub-sampled for FIB analysis and the remaining volume was filtered and frozen for follow-up molecular, viral, and/or community fingerprinting analyses. Based on results of the initial FIB analyses, samples were selected for further molecular marker analysis consisting of fecal *Bacteroides* spp., HF183 marker, BacHum marker, and Enterovirus. Analysis for the gull specific marker, *Catellicoccus marimammalium*, was performed on river mouth samples to assess the bacterial inputs from the resident gull community. Details on the various analyses and methods are provided in the following sections.

Figure 2-2: Multi-Tiered Analytical Approach



All FIB results for all samples, with the exception of Dry Events 3 and 4, were compared to the following primary selection criteria threshold to identify samples for further molecular marker analysis: 104 most probable number (MPN) or colony forming units (CFU) per 100 ml for *Enterococcus* sp., or 400 MPN or CFU per 100 ml for fecal coliform. The selection criteria was based on the Contact Water Recreational (Rec-1) single-sample WQOs assigned to beaches and creeks that discharge to those beaches as required by Water Quality Control Plan for San Diego Basin Region 9 (Basin Plan) and now adopted by the Bacteria I TMDL.

During Dry Events 3 and 4, the FIB results for all river mouth samples were below the primary selection criteria threshold used to identify samples for further molecular marker analysis. The secondary criteria threshold was taken from the steady state criteria (30-day geomean) listed in the Basin Plan: 33 MPN per 100 ml for *Enterococcus* sp., and 126 MPN per 100 ml for *E. coli*, to select samples for further molecular marker analysis. Sediment samples were selected for community fingerprinting analyses based on the FIB results of the paired water samples.

2.4.1 Fecal Indicator Bacteria

Fecal indicator bacteria (FIB) are surrogates used to measure the potential presence of bacteria, fecal material, and associated fecal pathogens. Fecal indicator bacteria such as fecal coliform and *Enterococcus* sp. are part of the intestinal flora of warm-blooded animals. Fecal indicator organisms have long been used to protect swimmers from illnesses that may be contracted from recreational activities in surface waters contaminated by fecal pollution. These organisms often do not cause illness directly, but have demonstrated characteristics that make them good indicators of harmful pathogens that may be present in water bodies (RWQCB, 2007). The United States Environmental Protection Agency (USEPA) recommends FIB quantification for monitoring of ambient waters because studies have demonstrated that *E. coli* and *Enteroccocci* are better predictors of the presence of gastrointestinal illness-causing pathogens than fecal and total coliforms, and, therefore, provide a better means of protecting human health. Some states, such as the State of California, still utilize all three FIB groups for monitoring.

E. coli was measured using the Colilert Method, which is an IDEXX patented defined substrate technology (DST). *Enterococcus* sp. was measured using the Enterolert Method, also an IDEXX patented DST. During the first day of the third dry season event, sampling was coordinated with the Joint Monitoring Program. Samples collected under this program were analyzed for FIB analyses that included *Enterococcus* sp., fecal coliform, and total coliform. Total coliform was measured using Method SM 9221B, fecal coliform using Method SM 9221E, and *Enterococcus* sp. using the Enterolert Method for the Joint Monitoring Program.

Although, *E. coli* is a fraction of the fecal coliform bacteria, a standard conversion factor or ratio of *E. coli* to fecal coliform has not been determined for California. Therefore, it is unknown what fraction of a fecal coliform result is comprised of *E. coli* and it was deemed inappropriate to apply an estimated value to transform the data or WQOs. It is standard practice in southern California to compare *E. coli* concentrations directly to fecal coliform concentrations and to the fecal coliform Rec-1 WQOs for discussion purposes.

2.4.2 Filtration of Water Samples

Water samples were sub-sampled for FIB analysis and follow-up molecular, viral, and/or community fingerprinting analyses. Prior to splitting the sample, the field crew ensured that the sample was homogeneous and the water sample was placed in the appropriate sample container. The laboratory vacuum filtered the remaining sample volume twice through 47-mm diameter, 0.4-um pore-size

polycarbonate filters. The resulting polycarbonate filters were DNA extracted using crude bead beating along with the UltraClean™ Fecal DNA Isolation Kit (MoBio Laboratories, Inc.,). For enterovirus analysis 47-mm, 0.4-µm type HA mixed ester cellulose filters (Millipore) were used with replicate filtrations of 100 ml, and filters were RNA extracted immediately using the RNeasy kit by QIAGEN (Gregory et al. 2006, Fuhrman et al. 2005, Noble et al. 2006).

2.4.3 Microbial Source Tracking

Microbial source tracking methods have historically been categorized into two types of approaches – library dependent approaches and library independent approaches. Library dependent methods rely on matching known source "fingerprints" or isolate patterns generated by molecular or phenotypic methods to unknowns collected from the environment. Library independent approaches are based upon detection (often presence/absence) or quantification of specific markers of fecal bacteria sources and these methods often include polymerase chain reaction (PCR) or quantitative PCR (QPCR) analysis for host-specific markers, or specific groups of bacteria, viruses, or protozoans that are specific to a certain type of gut flora. Conventional PCR requires the use of specific primers that are complementary to the target sequence of interest combined with the heating and cooling of a PCR machine. The results are often reported as presence/absence, or at best by using a serial dilution technique yielding semi-quantitative information (e.g., Noble et al. 2001). QPCR is a novel primer-based molecular technique that combines the specificity of conventional PCR with the quantitative measurement of fluorescence for quantification of target nucleic acid sequences. As opposed to conventional PCR, which is limited to a presence/absence result, QPCR provides for quantification over a wide dynamic range, from one to 10,000,000 cells, viruses, or spores/cysts. The platforms currently being used for QPCR include hardware with proven rapid cycling, sensitive optics, and multiplex capabilities, making possible to rapidly analyze many samples in a single run.

In 2004, a comparative study was published by Stoeckel et al. (2004) to assess the performance of microbial source tracking methods based upon typing *E. coli*, including two approaches for assigning *E. coli* isolates to specific hosts. None of the protocols tested correctly assigned the majority of the *E. coli* isolates to the correct fecal source host. Since then an array of scientists have published on some of the merits of library-dependent studies, but the variability and incorrect classification of source types in library-dependent studies has been too great to ignore, and has led most to begin to rely more heavily on library-independent methods, such as QPCR and others. One of the main reasons for this is that the resulting information from the library dependent methods is often reported in a percentage scheme of classification. However, a major drawback is the bias associated with the enrichment, selection, and

classification steps of isolates for analysis. There are statistical issues associated with trying to link the distribution of selected isolates back to the original colony counts from membrane filtration, making the library dependent results at best, semi quantitative. A few researchers that have access to particularly large scat and "known" libraries still use the library dependent approaches successfully, but many scientists have abandoned these more cumbersome methods for the library independent methods.

To verify the absence or presence of a human source of bacteria within the SLR River, several human-specific markers were chosen for the MST Program to provide a weight-of-evidence approach. Fecal *Bacteroides* spp. make up approximately one-third of the human fecal micro flora, considerably outnumbering *Enterococcus* sp. and *E. coli*. The *Bacteroides* group belongs to a group of non-spore-forming, gram-negative, obligate anaerobes, so there is little concern over re-growth in the environment. The three *Bacteroides* assays chosen for this project have been specifically developed to be conducted together to confirm the presence of human fecal contamination, taking into consideration a spectrum of specificity (i.e., high specificity=low likelihood for cross reactivity of the marker with other types of non-human fecal contamination), and sensitivity (i.e., a *Bacteroides* marker assay with good sensitivity means that when human fecal contamination is present the marker is found in high concentrations in water, and can be quantified). Of the three assays, the fecal *Bacteroides* spp. assay is the least specific, but has the best sensitivity because fecal *Bacteroides* spp. are a broad subgroup of *Bacteroides* found in the human gut. The human-specific marker assay (HF183) is the most specific (has the lowest incidence of cross reactivity with other non-human types of fecal contamination), but is the least sensitive, because the marker is not found in all humans, and is found in relatively low concentrations in human feces (as compared to the fecal *Bacteroides* spp.). Therefore, in most cases throughout the study, the interpretation of the fecal *Bacteroides* spp. markers was first conducted by examining when all three markers, or at least two of the markers, were present. Secondary examination was conducted of the trends in concentrations across markers throughout the system. The following *Bacteroides* assays were conducted for this study using QPCR methods:

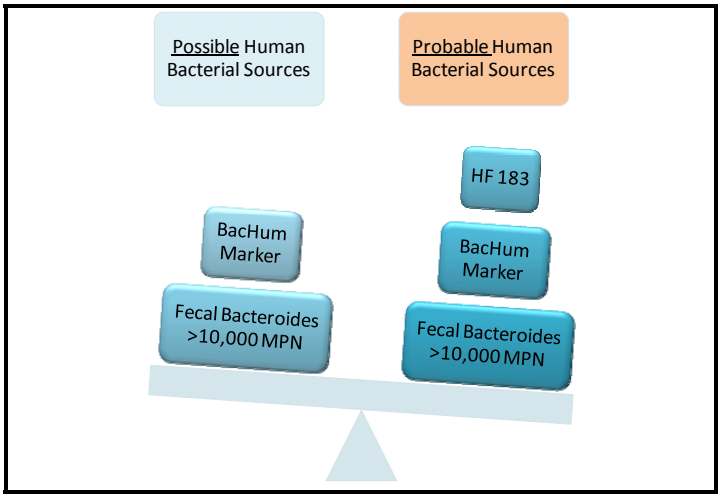
- Fecal *Bacteroides* spp. QPCR assay (Converse et al. 2009) relies on Taqman chemistry and all the reagents are in a liquid formulation, except the OmniMix. The assay quantifies a cohort of bacteria found in high concentrations in the human gut, including *Bacteroides thetaiotaomicron, Bacteroides distastonis*, and *Bacteroides fragilis*. However, the method is not human specific. The assay has been tested against a range of different fecal samples types, and has been shown to be capable of quantifying over a wide range of concentrations, and to be sensitive at concentrations relevant to water quality source tracking studies. When using the QPCR approach for fecal *Bacteroides* spp., strong relationships have been observed within a wide array of human sewage collected from areas on both the eastern and western US coastlines. The assay is highly sensitive and the target bacteria that are enumerated have been shown to be a predictor of human

health in both sand and recreational waters (Wader et. al. 2011, Heaney et al. 2011) during large-scale EPA-run epidemiology studies. This is a fully QPCR-based assay that is being used in an array of studies in stormwater contaminated areas and that, with the use of other additional confirmatory methods, can be used to both identify potential hot spots of human fecal contamination (Converse et al, 2009).

- BacHum Human Marker: A separate QPCR assay was utilized to quantify the BacHum molecular markers reported by Kildare et al., 2007. The assay has been widely tested for specificity against a range of fecal sample types and has shown high capacity for discrimination against human and animal fecal types (Ahmed et al., 2009). The assay is conducted in conjunction with a specimen processing control for full quantification, even samples that exhibit inhibition of the QPCR amplification can be diluted and reanalyzed to provide quantitative information.
- HF183 (human fecal): Human-specific marker by QPCR has been conducted previously by Bernhard and Field (2000) and updated by Seurinck et al., 2006. This assay is specific to a region of ribosomal rDNA within the fecal *Bacteroides* spp. that is found almost exclusively in human feces. The assay has been tested repeatedly in a range of different environments for cross reactivity with other types of fecal material, and researchers have found either a 99-percent or a 100-percent ability to discriminate between human and animal feces when using this assay. The assay, however, can be problematic when used alone, because the target copy concentration in fecal material contributed to receiving water environments can be quite low due to dilution and the assay has a relatively low sensitivity.

A conservative, weight-of-evidence approach was used to identify samples with human bacterial sources by 1) building on the quantitative results of fecal *Bacteroides* spp. 2) supported by the quantitative BacHum marker results provides evidence of possible human bacterial sources and 3) the presence of the HF183 marker provides confidence in probable human bacterial sources and 4) evaluating the trend of concentration across the three markers and throughout the river system (Figure 2-3). A strong positive signal is one that is significantly above the detection limit for the selected molecular methods, estimated at 80-200 cells or gene copies per 100 ml. Each site had mean values greater than 500 cells or gene copies per 100 ml for at least two markers on more than two sampling days during the wet season.

Figure 2-3: Weight-of-Evidence Approach for Fecal *Bacteroides* spp. Assay



Note: This is a simplified schematic of the weight-of-evidence approach. The trend of concentrations across markers and throughout the river system was taken into consideration, although, not depicted in this schematic.

Another host specific marker QPCR assay that was utilized in this study is specific for fecal bacteria from seagulls. The assay targets the bacteria *Catellicoccus marimammalium* (Lu et al. 2007). *Catellicoccus marimammalium* is a gram-positive, catalase-negative bacterium. The assay has now been widely tested throughout the USA and Canada. This assay has provided important additional information about the impact of seagulls in the SLR River. The gull specific marker was used to identify the gull input at the river mouth. Additionally, analysis for the gull specific marker was performed on river samples to assess the specificity to gull versus other species of birds. The results of this project do demonstrate that the gull assay is highly specific. This assay was designed by Santo Domingo and Sinigalliano, 2010, and has been used as a marker to quantify the presence of gull fecal material (not specific to other water fowl species). The assay is highly specific and sensitive, and is a strong complement to the other human-specific assays listed here.

2.4.4 Viral Source Tracking (Detection and Quantification)

In addition to fecal source tracking using markers that are bacterial in nature, quantification was also conducted for enterovirus on a subset of samples collected from the SLR River. This quantification followed the previously developed protocols of Gregory et al. (2006) and Fuhrman et al. (2005) for real time polymerase chain reaction quantification (Q-RTPCR) of enteroviruses. This work was conducted by Dr. Jed Fuhrman at the University of Southern California's Department of Biological Sciences. Viruses are known to cause a significant portion of waterborne illnesses. Enterovirus assays quantify the enteric

viral concentrations present in a water samples and were used as a tool to characterize the threat to human health.

The assays utilized in this study are novel with respect to the attention given to quality assurance controls, attributes that are not routinely found with other viral QRTPCR assays. After the first set of analyses, those samples that appeared to have any inhibition of the QRTPCR were diluted and reanalyzed. Inhibition is the "blocking" or "interference" of the result and can occur due to the nature of the sample matrix. In some studies, a correction factor can be used to adjust the data set and estimate the result. No data was adjusted or estimated in this data set and is shown on figures as not detected. Standard curves were generated using a synthetic enterovirus transcript that was quantified using fluorometric analysis, and sample genome concentrations were interpolated from the standard curve using the manufacturer's curve-fitting software. The filter volumes utilized for the enterovirus analysis within this project (100 ml) were selected for consistency and to save time. In retrospect, it appears that the filtration volumes might have benefited from being increased (i.e., greater chance for quantifying enterovirus if a greater volume was filtered). Increased sample volume would be recommended for any future enterovirus quantification work conducted in the SLR River.

2.4.5 Community Fingerprinting Analyses

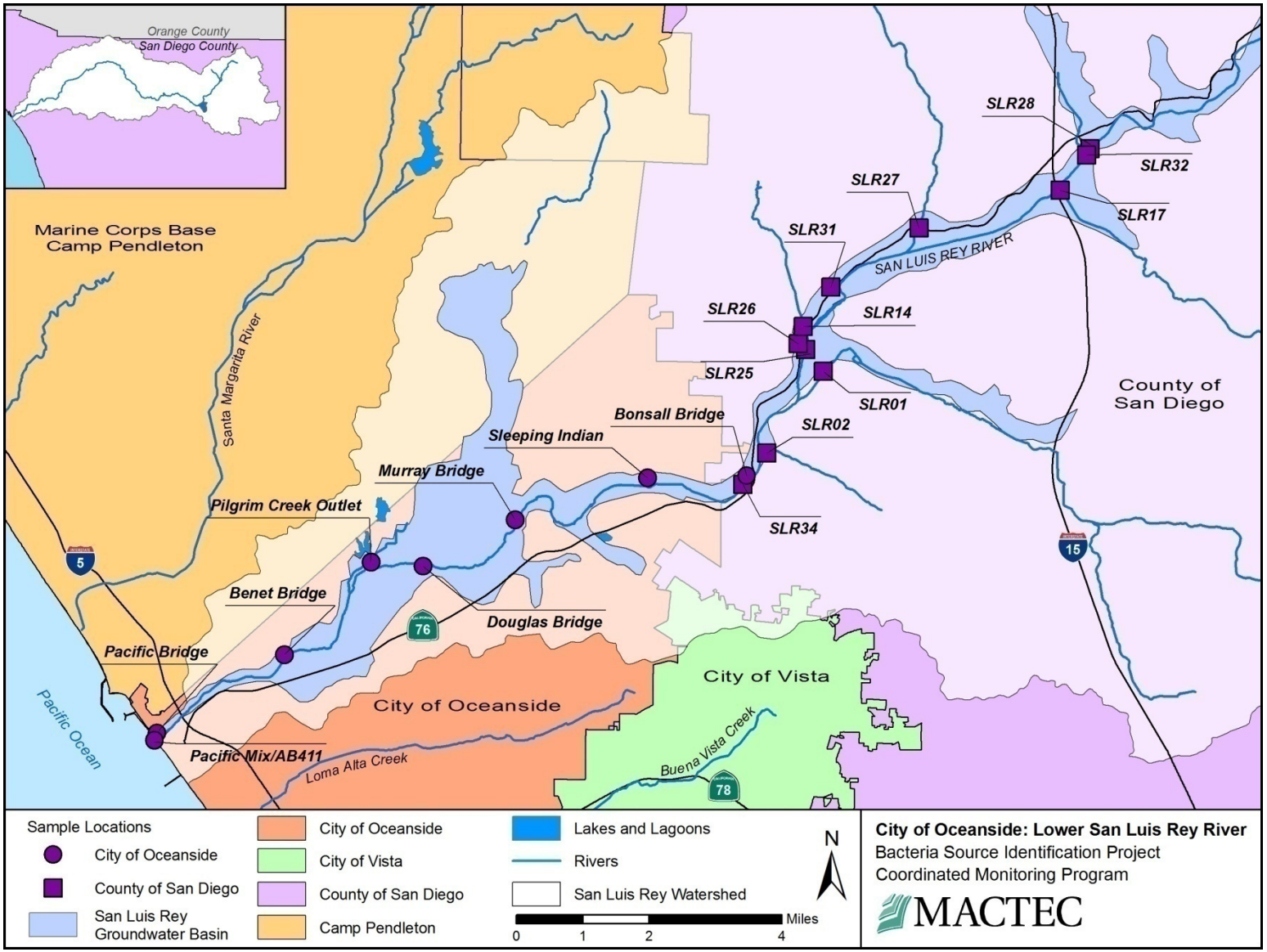
The community fingerprinting analysis is a comparison of bacterial populations from varied sources. This analysis was used to characterize the bacterial communities in the water column and sediment of the river mouth. The analysis was conducted using terminal restriction fragment length polymorphism (TRFLP). DNA was extracted from two-gram sediment samples using the UltraClean Soil DNA kit (MoBio Laboratory, Inc., Solana Beach, CA) following the manufacturer's protocol. Extracted DNA was then purified by a size-exclusion-column, checked for quality by assessing the A260/230 ratio, and quantified by SYBR Green fluorometry. Genes encoding 16S rRNA were PCR-amplified from purified community DNA using universal eubacterial primers 8F hex (fluorescently labeled forward primer; 5'AGAGTTTGATCCTGGCTCAG) and 1389R (5'ACGGGCGGTGTGTACAAG). The PCR products were purified and digested with *Hha*I. The restriction enzyme was inactivated by heating (at 65 degrees Celsius for 15 minutes), and the lengths of fluorescently labeled terminal restriction fragments (TRFs, corresponding to peaks in the TRFLP electropherogram) were determined with an Applied Biosystems Instruments (ABI; Foster City, CA) Model 373A automated sequencer.

2.4 JOINT MONITORING PROGRAM

As part of the SLR WURMP, the City and County of San Diego conducted joint monitoring to characterize baseline conditions and trends of bacteria levels within the lower 19.2 river miles of the SLR Watershed encompassing the entire length of the Lower San Luis HA. Between March 2004 and March 2011, 18 locations were sampled, as presented in Figure 2-4. A total of eight locations were monitored by the City and an additional ten were monitored by the County of San Diego. Appendix A provides a detailed description of monitoring sites, including Global Positioning System (GPS) coordinates. Both agencies collected samples on the same date, when possible. If collection on the same date was not possible, samples were collected within one to two days of the initial sampling event.

This data helped assess the magnitude of the bacterial contamination in the Lower SLR River over the entire Project duration, and to allow the Project team to put the results into context within the watershed (i.e., to conduct comparisons of Project observations with historical data). The City's sample locations included one ocean, one river mouth, four main stem, and two tributary locations. The County's sample locations include three main stem and seven tributary locations. One site along the River's main stem, Bonsall Bridge, was sampled by both agencies for quality control. All samples were collected during dry weather (at least 72 hours following any rain event with precipitation greater than or equal to 0.10 inches). The composite samples were taken from the three points across the river using a 300-milliliter (ml) IDEXX bottle supplied with sodium thiosulfate to neutralize potential impacts from chlorine in the water. Samples were stored at four degrees Celsius and were transported to the laboratory to be analyzed for total coliform, fecal coliform, and *Enterococcus* sp. employing multiple tube fermentation and IDEXX methods to quantify bacteria. City data collected from January 2007 through March 2011 and County data collected from January 2007 through June 2010 was used in the data analysis.

Figure 2-4: City and County Coordinated Joint Monitoring Program Locations



3.0 MONITORING RESULTS AND DISCUSSION

This section includes a summary and discussion of the hydrological, analytical, and visual observation results from the MST Program. The FIB analytical results from both the Joint Monitoring Program and AB411 Monitoring Program were used to support the seasonal patterns observed in the MST FIB data.

3.1 MONITORING EVENTS AND HYDROLOGY

There are three general water sources into the SLR River system: wet weather runoff, dry weather runoff, and ground water inputs. This program focused on assessing wet weather and dry season discharges and did not evaluate ground water contributions. The groundwater and surface water in the SLR watershed are an integrated system (WURMP, 2008). There are three shallow alluvial groundwater basins within the lower San Luis Rey Watershed that provide base flow to the river: Bonsall, Mission and Moosa Canyon. Historically, the SLR River was considered ephemeral and only flowed when there was above normal precipitation. Water in the upper extent of the SLR watershed was impounded by Henshaw Dam and diverted downstream to Lake Wohlford (WURMP, 2010). By the late 1960s, the SLR River was becoming perennial through Oceanside. Although, the SLR River has increased in annual flow rate there are segments of the river that typically remain dry for a period of time throughout the dry season.

3.1.1 Dry Season Events

Monitoring was conducted during four events at various sites in the summers of 2008 and 2010, as outlined in Table 3-1. The dry season monitoring program was conducted in early summer (June 2008) and again in mid-summer (July 2008) at all sampling locations upstream of the river mouth. The river mouth dry season monitoring was conducted in spring (May 2010) and late-summer (August 2010) to characterize the unique sources and hydrology of the river mouth.

Table 3-1: Dry Season Sampling Summary

Sampling Event	Dates	Locations Sampled		Event Notes
Dry Event 1	6/18/2008 – 6/19/2008	Bonsall Bridge Sleeping Indian Murray Bridge	Douglas Bridge Pilgrim Creek Critical Point	The river mouth was not sampled due to construction of the Pacific Street Bridge.
Dry Event 2	7/23/2008 – 7/24/2008	Bonsall Bridge Sleeping Indian Murray Bridge	Douglas Bridge Critical Point	The river mouth was not sampled due to construction of the Pacific Street Bridge. Pilgrim Creek was dry, so no sample was collected.
Dry Event 3	5/18/2010 – 5/20/2010	Bonsall Bridge Sleeping Indian Murray Bridge Douglas Bridge Pilgrim Creek Critical Point	River Mouth 4 River Mouth 3 River Mouth 2 River Mouth 1 Point Zero	City of Oceanside participated in the sampling event on 5/18/2010 with the grant Project team completing sampling on the following two days. Sleeping Indian, Murray Bridge, Douglas Bridge, and Pilgrim Creek were only sampled on 5/18/2010.
Dry Event 4	8/03/2010 – 8/04/2010	River Mouth 4 River Mouth 3	River Mouth 1 Point Zero	Critical Point was dry, so no sample was collected. The river mouth had closed. A sampling point nearest to the Point Zero site was included to examine spatial variability.

During dry season events, instantaneous flow estimates were made at the time and point of sample collection. Flow estimates were made at main stem river and tributary monitoring locations using USGS protocols as described in Section 2.2.4. The flow estimate values are shown in Table 3-2. A summary of dry season flow conditions at main stem and tributary locations are provided below:

- Dry Event 2 flows were lower than Dry Event 1 flows, with the exception of Sleeping Indian, where flow stayed level.
- Dry Event 3 flows were the highest of the four dry events at all monitoring locations, except at Sleeping Indian.
- Cross-sectional flow measurements were made at river monitoring locations once during Dry Event 3 and used for all three days of the event since no significant change in stage was observed.
- The Critical Point site signifies the main input from the SLR River to the river mouth and was the only river site scheduled to be sampled during Dry Event 4. By August, the Critical Point (Benet Bridge) site was dry (without flow) and no sample was collected.

Table 3-2: Dry Season Instantaneous Flow Estimates

Site	Dry Event 1 Flow (cfs)		Dry Event 2 Flow (cfs)		Dry Event 3 Flow (cfs)		
	6/18/2008	6/19/2008	7/23/2008	7/24/2008	5/18/2010	5/19/2010	5/20/2010
Bonsall Bridge	6.49	5.52	4.06	3.26	14.56	14.56	14.56
Sleeping Indian	0.05	0.06	0.06	0.06	0.01	NS	NS
Murray Bridge	6.51	6.11	0.95	0.91	35.60	NS	NS
Douglas Bridge	4.73	4.63	0.21	0.05	17.00	NS	NS
Pilgrim Creek	NM	0.04	DRY	DRY	0.24	NS	NS
Critical Point	7.45	6.55	3.00	2.80	10.82	10.82	10.82

Cfs = Cubic feet per second.
DRY = Site dry; sample not collected.
NS = Site not selected to be monitored during this event.
NM – Trickle flow was present but not measureable.

Dry season monitoring in the river mouth was scheduled to account for tidal cycles and solar radiation exposure. The majority of river mouth samples were collected prior to 9 am. A summary of dry season flow conditions at river mouth locations are provided below:

- During Dry Event 3, the river mouth was open to tidal exchange with the Pacific Ocean (Figure 3-1) and river mouth samples were collected during an ebb tide.
- During Dry Event 4, the river mouth was closed, a common occurrence during late summer, as shown in Figure 3-1. The flow rate was reduced within the river mouth due to reduced input from the river and lack of tidal exchange. A sample was not collected at the 'true' Point Zero site, which represents the exchange between the river mouth and Pacific Ocean. A sampling point nearest to the Point Zero site was selected as one of the four sampling locations in order to examine spatial variability.

Figure 3-1: Point Zero in May 2010 (Left) and August 2010 (Right)

Key visual observations recorded during the dry season monitoring activities from 2008 and 2010 included:

- Most water samples were colorless and clear at all main stem, tributaries, and river mouth locations.
- In May 2010, the number of sea gulls was observed on the beach shoreline during sampling activities, sea weed wrack line was noted throughout the river mouth, and an algal sheen and organic/algal debris was observed in all river mouth samples.
- In August 2010, 30-40 sea gulls were observed at the River Mouth 1 site.

3.1.2 Wet Season Events

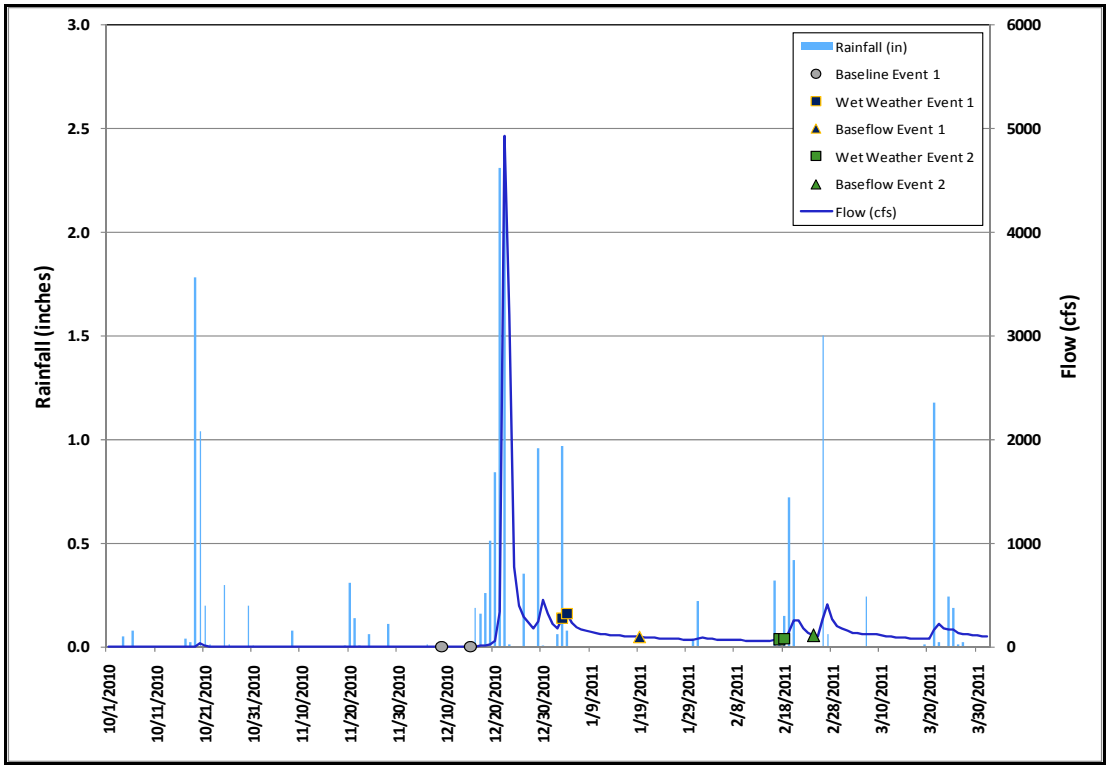
Wet season monitoring was designed to quantify fecal concentrations and loads during storm events and dry weather conditions, to identify chronic and intermittent fecal concentration and loads and potential sources. Wet season monitoring took place on the following five occasions, as outlined in Table 3-3: one Baseline event, two wet weather events, and two base flow events. Wet season monitoring took place during the 2010-2011 portion of this project.

Table 3-3: Wet Season Sampling Summary

Sampling Event	Dates	Locations Sampled		Event Notes
Baseline 1	12/9/2010 & 12/15/2010	Bonsall Bridge Murray Bridge Douglas Bridge Pilgrim Creek	Critical Point River Mouth 3 River Mouth 1 Point Zero	The event was preceded by 11 days without rain and a total cumulative rainfall of 5.04 inches since 10/1/2010. Sleeping Indian site was dry. The Critical Point was sampled 12/15/2010 since it was dry on the morning of 12/9/2010 but flow began later that day. There was no rainfall between 12/9/2010 and 12/15/2010. River mouth samples were collected during flood tide conditions.
Wet Event 1	1/3/2011 – 1/4/2011	Bonsall Bridge Sleeping Indian Murray Bridge Douglas Bridge Pilgrim Creek	Critical Point River Mouth 3 River Mouth 1 Point Zero	Proceeded by 4 day antecedent dry period and a total rainfall of 1.11 inches. No flow data was recorded at Sleeping Indian due to equipment malfunction. River mouth samples were collected during an ebbing tide.
Base Flow Event 1	1/20/2011	Bonsall Bridge Sleeping Indian Murray Bridge Douglas Bridge Pilgrim Creek	Critical Point River Mouth 3 River Mouth 1 Point Zero	Proceeded by 16 day antecedent dry period. There was a sewage collection system spill less than 0.5 miles upstream of the Pilgrim Creek site 6 days prior to the event. River mouth samples were collected bracketing the morning high tide.
Wet Event 2	2/17/2011 – 2/18/2011	Bonsall Bridge Sleeping Indian Murray Bridge Douglas Bridge Pilgrim Creek	Critical Point River Mouth 3 River Mouth 1 Point Zero	Proceeded by 15 day antecedent dry period and a total rainfall of 0.35 inches. River mouth samples were collected between 10:30 and 11:30 during a peak ebb tide on both days of the event.
Base Flow Event 2	2/24/2011	Bonsall Bridge Sleeping Indian Murray Bridge Douglas Bridge Pilgrim Creek	Critical Point River Mouth 3 River Mouth 1 Point Zero	Proceeded by 3day antecedent dry period. A rainfall event of 1.29 inches occurred between Wet Event 2 and the Base Flow Event 2.

Daily and monthly rainfall data for the 2010-2011 wet weather season (October through March) were obtained from the National Weather Service (NWS) Oceanside Municipal Airport Station, located approximately 0.5 miles east of the Critical Point monitoring location. Oceanside Municipal Airport recorded 18.99 inches of rainfall between October 1, 2010, and March 1, 2011 (NWS, 2010). Figure 3-2 depicts the rainfall for the 2010-2011 wet season and days of sample collection for wet season events.

Figure 3-2: Wet Season Rainfall and Monitoring Events



Note: Flow data is from the USGS Site 11042000. Rainfall date is from the Oceanside Municipal Airport (OKB).

During the wet season, the ground typically becomes more saturated as more rain falls, resulting in increased base flows. Between July and December 2010, the Critical Point was dry (Figure3-2). After several rainfall events in October and November, base flow conditions in the SLR River were recharged and flow returned. The Baseline Event was preceded by an 11 day antecedent dry period and a total of 5.04 inches of cumulative rainfall was recorded from October 1, 2010. The Critical Point sustained an average flow rate of 4.4 cfs between December 9, 2010, when flow began, and December 15, 2010, when the baseline sample was collected.

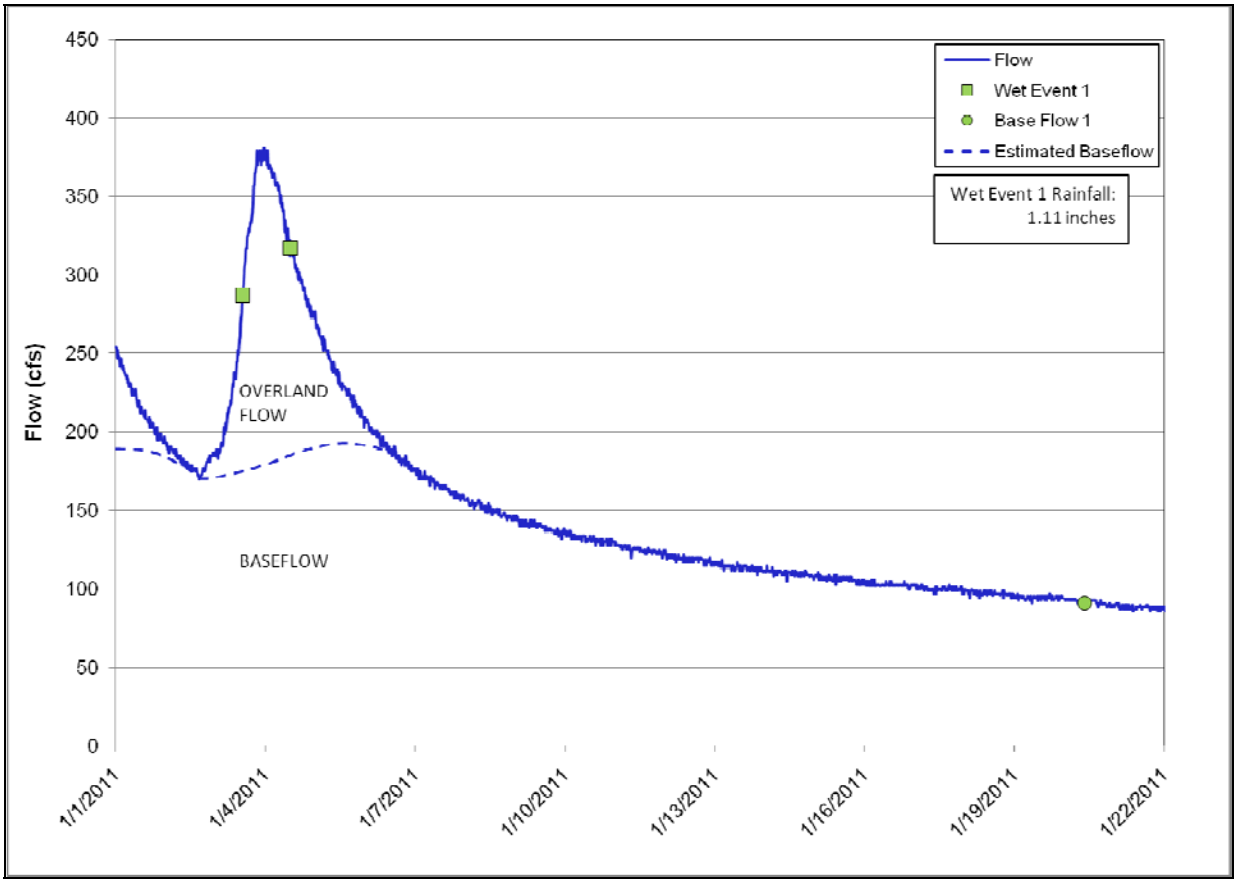
A series of storm events occurred between December 16, 2010 and December 30, 2010 without a 72 hour window to meet mobilization criteria. The SLR River reached a peak flow of 11,500 cubic feet per second (cfs) during these events and returned to a base flow of 110 cfs prior to Wet Event 1. A total of 12.51 inches of cumulative rainfall was recorded from October 1, 2010, through the start of Wet Event 1.

Wet Event 1 was preceded by a four-day antecedent dry period and had a total of 1.11 inches of rainfall during the event. At the Critical Point, a peak flow of 381 cfs occurred at 23:30 on January 3, 2011, as shown on the hydrograph for Wet Event 1 (Figure 3-3).

Base Flow Event 1 was conducted 16 days subsequent to Wet Event 1. At the time of sampling, the Critical Point was flowing at a rate of 91 cfs. A sewage spill was recorded less than half a mile upstream of the Pilgrim Creek sampling location six days prior to the sampling event. Figure 3-3 depicts a hydrograph for the Critical Point during Wet Event 1 and sample times for Wet Event 1 and Base Flow Event 1.

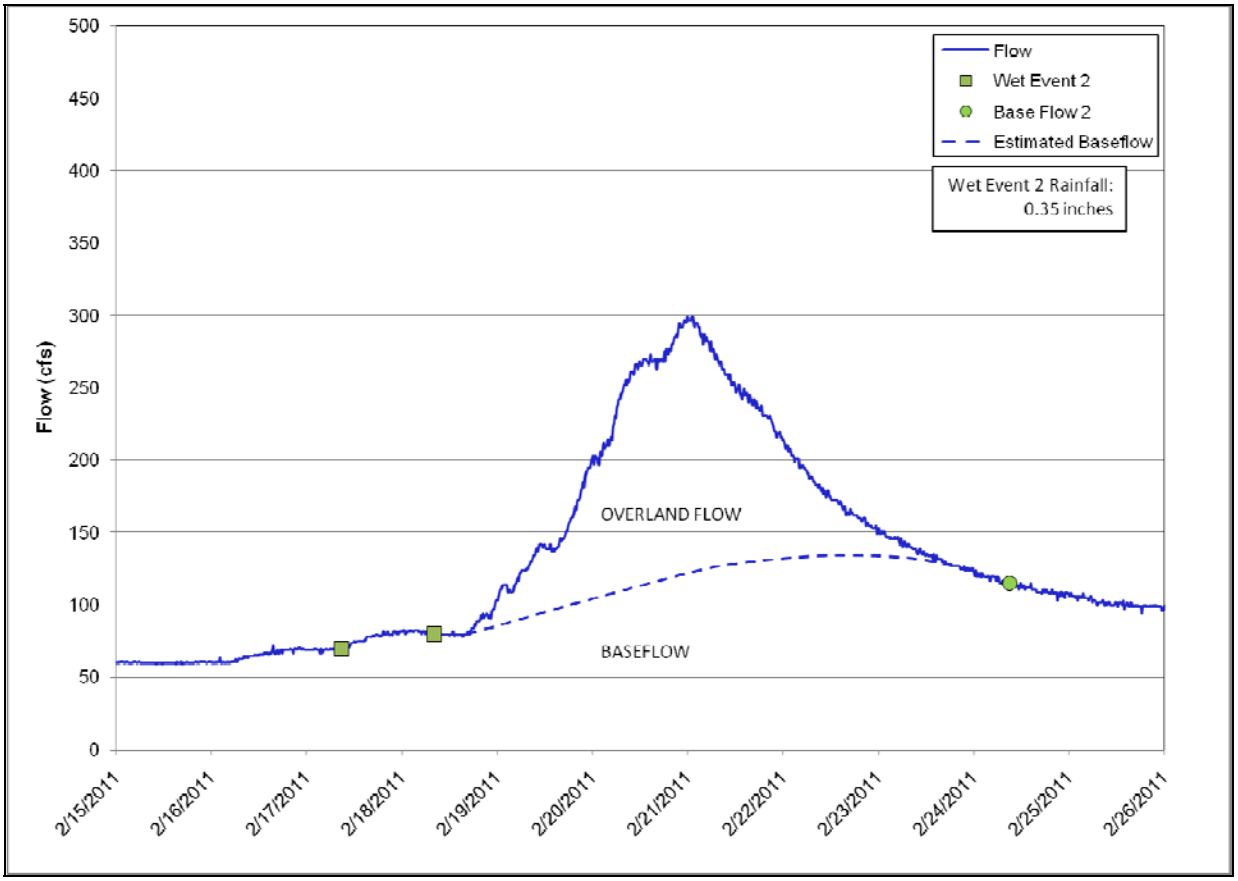
Wet Event 2 was preceded by a 15-day antecedent dry period, and a total of 13.88 inches of cumulative rainfall was recorded from October 1, 2010, through the start of Wet Event 2. A total of 0.35 inches of rainfall fell during the event. A rainfall event of 1.29 inches occurred between Wet Event 2 and Base Flow Event 2. Base Flow Event 2 was conducted after a three-day antecedent dry period. A hydrograph for the Critical Point during Wet Event 2 and the times of sample collection for Wet Event 2 and Base Flow Event 2 are provided in Figure 3-4.

Figure 3-3: Wet Event 1/Base Flow Event 1 Critical Point Hydrograph



Note: Flow data is from the USGS Site 11042000.

Figure 3-4: Wet Event 2/Base Flow Event 2 Critical Point Hydrograph



Note: Flow data is from the USGS Site 11042000.

Key visual observations recorded during wet season monitoring activities included:

- During the Wet Season, the majority of sea gulls were observed at the River Mouth 1 location with estimates ranging from 20 to 150 birds. Sea gulls were also present at Point Zero, with estimates ranging from 10 to 60 birds.
- During the Baseline Event, there was evidence of an encampment at the Critical Point, including trash, clothing, and a backpack (Figure 3-5).
- During Wet Event 2, a large accumulation of organic debris, algae, and trash was observed upstream of the Pilgrim Creek monitoring location (Figure 3-5).
- At main stem river sites, trees and vegetation were knocked over as a result of high storm flows, possibly from storm events preceding Wet Event 1.
- Wet Event 1 produced a highly turbid storm flow that was noted at all main stem river and river mouth monitoring locations. During this event, main stem river sites and tributaries were described as brown and cloudy to opaque on the first day of the event and then brown and clear to slightly-cloudy on the second day (Figure 3-6). On the contrary, river mouth sites were described

as brown and clear on the first day of the event and brown and cloudy on the second day of the event.

- The storm drain on the south side of the Pacific Street Bridge was discharging at the time of sampling at River Mouth 3 during Wet Event 1. However, the storm drain was not discharging during Wet Event 2 sampling.
- During Base Flow Events 1 and 2, an encampment was observed on the north bank under the Old Bonsall Bridge.

Figure 3-5: Encampment at Critical Point (Left) and Debris at Pilgrim Creek (Right)

Figure 3-6: Highly Turbid Flow at Douglas Bridge (Left) and Point Zero (Right)

Figure 3-7: Pilgrim Creek Flow Increase between Baseline (Left) and Base Flow Event 1 (Right)

3.1.3 Tributary Input Characterization

Flow input from the two monitored tributaries varied between wet season and dry season conditions. During the dry season, flows were more consistent at Sleeping Indian than Pilgrim Creek. Pilgrim Creek was generally dry between July and October/November based on the City data. Flows at Sleeping Indian accounted for up to three percent of the Critical Point daily flows. Pilgrim Creek accounted for approximately one to four percent of the Critical Point daily flows during both wet and dry season conditions, with the exception of Wet Event 1. During that event, Pilgrim Creek accounted for approximately 15 percent of the Critical Point daily flows. Sleeping Indian accounted for less than one percent of the total daily flow measured at the Critical Point during wet season conditions.

The differences between the two monitored tributary sites may be attributable to the land uses within their respective drainage areas. The Sleeping Indian drainage area is mostly comprised of rural residential and agricultural land uses with pervious surfaces. This may explain the decreased proportion of discharge during wet weather events due to a higher level of infiltration compared to impervious surfaces. The increased proportion of discharge during the dry season may also be attributable to agricultural land uses and the associated irrigation that occurs on these lands. During dry season conditions surface runoff is much lower throughout the Sleeping Indian system. Irrigation, therefore may account for a greater proportion of the overall discharge.

The Pilgrim Creek drainage area, conversely, has more development, including residential areas, resulting in a lower amount of pervious surface area. This may account for the more inconsistent flows observed throughout wet and dry seasons, as well as, for the higher proportion of discharge to the overall system

observed during the first wet weather event. This event had a high amount of rainfall and produced a large runoff volume.

3.1.4 Seasonal Comparison

Flow data from the USGS Station at the Critical Point over the past five years (2006 through 2010) was reviewed to assess seasonal patterns throughout the system. Averages of the monthly total flows were compared to the sum of the monthly average flows to determine the periods of the year that account for the various proportions of flow. For this assessment, the months of the years were categorized by season according to the following groups: winter (December, January, February), spring (March, April, May), summer (June, July, August), and fall (September, October, November). Based on the past five years of data, the winter months accounted for 71 percent of the average total discharge, the spring months accounted for 25 percent of the total discharge, and the summer and fall months accounted for three and one percent of the total discharge, respectively (Table 3-4). Discharge decreases to nearly zero or zero during summer and remains at that level throughout summer and fall, which is indicative of an ephemeral stream. The river mouth generally closes between July and October until a large storm or series of storm flows push through the sand berm.

Table 3-4: Assessment of Seasonal Flow

Season	Months	Total Average Discharge (cfs)	Discharge Proportion
Winter	December - February	8,313	71%
Spring	March - May	3,007	25%
Summer	June - August	312	3%
Fall	September - November	129	1%
Total Average Annual Discharge		11,760	100%

Based on USGS historical data from 2006 to 2011.

3.1.5 Visual Observation Results

Visual observations recorded potential sources of bacteria and/or pollutant generating activities throughout the SLR watershed during sampling activities. Visual observations were conducted during Dry Event 2, Dry Event 3, Dry Event 4, the Baseline Event, prior to Wet Event 2, and during Base Flow Event 2. Table 3-5 presents a summary of the visual observation results by event. Visual observations were conducted in each zone at least once over the course of the study. Figure 3-8 depicts the Lower SLR River visual observation zones. For each event, zones were selected based on monitoring activities. For

example, during Dry Events 3 and 4, visual observations were conducted in Zones 1 through 4, which were within the drainage area to the river mouth. Wet season observations were not comparable to the dry season observations since different zones and land use types were monitored. During Dry Event 2, the format of visual observations was more qualitative and after this event, the Visual Observation Sheet was restructured to be more quantitative with increased detail to facilitate a comparison of overall sources between zones and events. A summary of Observations is outlined below:

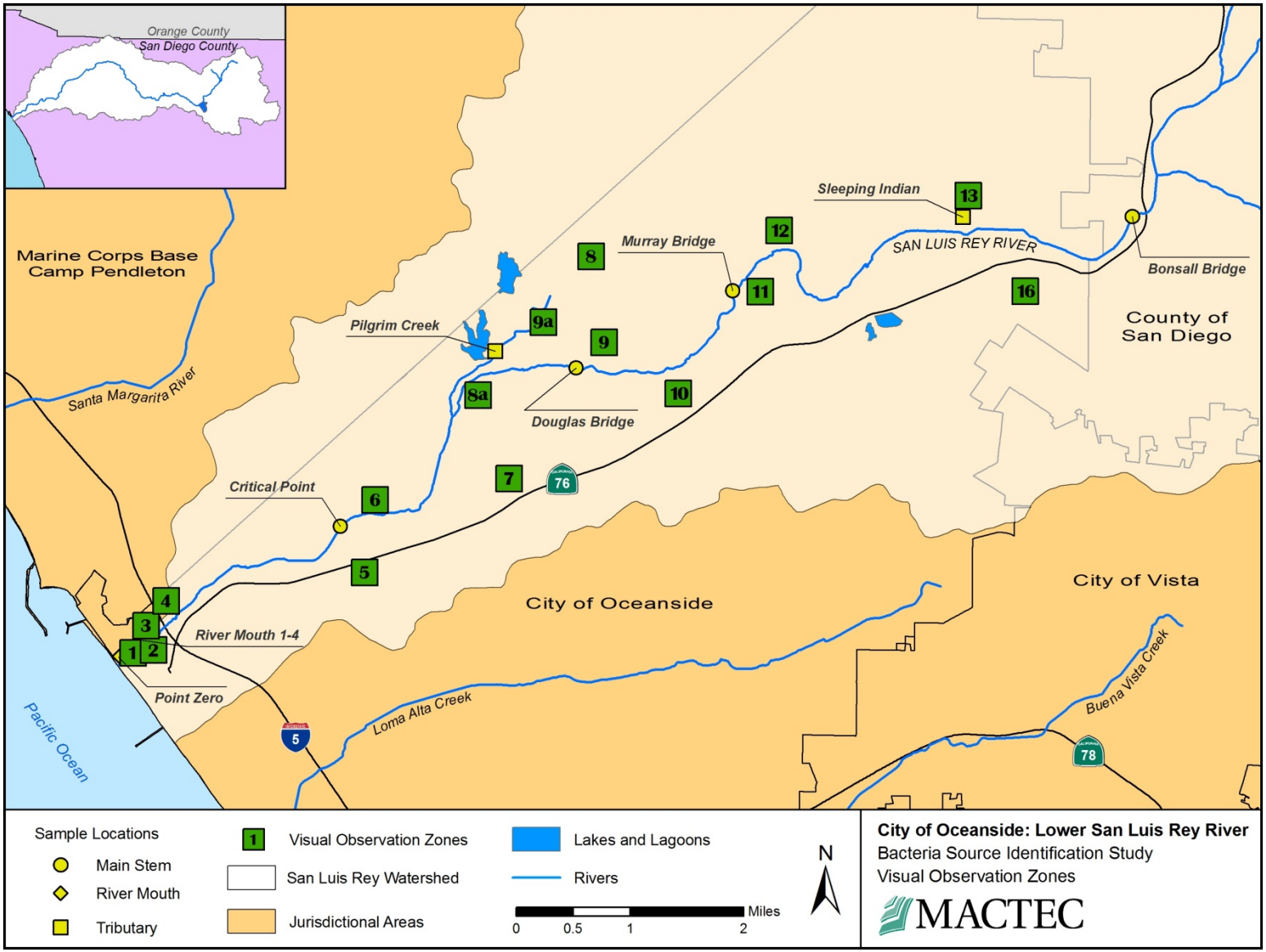
- Flowing and ponded water was observed more frequently during the Baseline event and prior to Wet Event 2 when observations were conducted in predominantly residential areas.
- The highest number of observations of flowing water, ponded water and irrigation runoff was recorded prior to Wet Event 2 in Zones 6, 8, 9, 11, and 12.
- Trash was observed at relatively the same frequency for all events except Base Flow Event 2, where it was less, potentially due to preceding storm events.
- Overall, Base Flow Event 2 had very few sources present in the drainage areas since it was conducted subsequent to a storm event when trash would have washed downstream.
- Birds and kelp were present at the river mouth in Zone 1 during Dry Event 3 and Base Flow Event 2.
- Additionally, a dog was observed in the river mouth during Base Flow Event 2.

Table 3-5: Summary of Visual Observations by Event

Season	Event	Zones	Flowing	Ponded Water	Flow Reached Inlet	Trash	Bubbles/Foam	Fecal Matter	Sediment/Gravel	Organic Debris	Food	Green Waste	Hosing Hard/Paved Surfaces	Washing Equipment/Supplies	Dumpster Overflow	Leaking Trashcan	Broken/Sprinkler/Irrigation Overflow	Washing Vehicles On Hard/Paved Surfaces	Pet Excrement	Domestic Animal In River/At Beach	Birds Present at River Mouth And Beach Shoreline	Algae	Pet Waste	Kelp
Dry	Dry Event 2[(a)]	8a, 9a, 11, 16	8	0	0	2	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0
	Dry Event 3	1, 2, 3, 4	3	5	2	5	0	0	2	4	0	2	2	1	0	0	3	0	0	1	100	1	0	1
	Dry Event 4	1, 2, 3, 4	11	9	1	7	0	0	4	6	1	8	1	0	0	0	11	0	0	0	0	5	0	0
Wet	Baseline	5, 7, 8, 9, 10	21	20	5	7	1	0	11	15	0	1	1	0	0	0	10	2	1	0	0	3	1	0
	Wet Event 2	5, 6, 8, 9, 11, 12	33	30	8	9	2	0	11	13	2	7	2	0	2	0	17	2	4	0	5	11	1	0
	Base Flow 2	1, 5, 6, 11, 12, 13	2	2	2	2	0	0	1	0	0	0	0	0	0	0	0	0	0	1	94	0	0	1
Total Count			**68**	**66**	**17**	**35**	**3**	**0**	**28**	**38**	**3**	**18**	**6**	**1**	**2**	**0**	**41**	**4**	**5**	**2**	**199**	**20**	**2**	**2**

(a) Observations were recorded in a different format during Dry Event 2. After this initial event, the visual observations program was restructured to make the visual observations more quantitative.

Figure 3-8: Visual Observation Zones



3.2 MICROBIAL CONTAMINANT RESULTS AND DISCUSSION

This section presents the FIB results for the Microbial Source Tracking (MST) Program, the Joint Monitoring Program, the San Diego County/DEH AB411 Monitoring Program, and results from the genetic, viral, and community fingerprinting analyses.

3.2.1 Fecal Indicator Bacteria Results and Discussion

Fecal indicator bacteria results were used to assess seasonal and spatial patterns. The overall grant program integrates FIB results from the MST Program, the Joint Monitoring Program, and the AB411 Program. Each program was implemented independently with varying sampling frequencies and FIB analyses. To facilitate comparisons, the data have been categorized as dry season data (May to September) and wet season data (October to April) and will focus on *Enterococcus* sp. and fecal coliform/*E. coli*.

A summary of samples collected for each program is provided below:

- MST Program: 111 water samples were collected during discrete wet and dry season events. 48 samples were collected in the main stem of the river; 19 samples were collected at the two tributary sites; and 44 samples were collected in the river mouth. An average of 10 samples was collected at each monitoring location.
- Joint Monitoring Program:
  - 128 water samples were collected within the City of Oceanside from July 2009 to March 2011. 177 samples were collected in the main stem of the river; 69 samples were collected at the two tributary sites; 49 samples were collected in the river mouth; and 48 samples were collected at the AB411 site.
  - 217 water samples were collected upstream within the County of San Diego from January 2007 to June 2010.
- DEH AB411 Program: A total of 74 samples were collected from April 2010 to February 2011 at the SLR AB411 monitoring location.

<u>Dry Season</u>

The distribution of dry season results in comparison to Rec-1 WQOs for the MST Program, Joint Monitoring Program, and AB411 Program are presented in Figures 3-9 to 3-16. During the dry season, 24 percent of samples collected for the MST Program exceeded the WQO for *Enterococcus* sp., and 10 percent of samples exceeded the WQO for *E. coli.* For the MST Program, all WQO exceedances occurred at main stem and tributary sites, with Sleeping Indian having the highest exceedance frequency for both *Enterococcus* sp. (80 percent) and *E. coli* (50 percent). Main stem MST samples exceeded WQOs at a rate

of 35 percent for *Enterococcus* sp. and 15 percent for *E. coli*. For samples collected at the tributary sites, 50 percent exceeded the WQO for *Enterococcus* sp. and 33 percent exceeded the WQO for *E. coli*. The higher exceedance frequency at tributaries may be due to the lower flow rates. During low flow conditions, lower flows and stagnant conditions can provide an environment conducive to higher bacteria concentrations. Additionally, nuisance flows from urban land use activities, such as car washing, sidewalk washing, and over-irrigation, tend to occur more frequently during the summer and fall months. These nuisance flows can transport bacteria, leading to elevated bacterial concentrations in the main stem and the tributaries of the SLR River.

At the river mouth sites and the AB411 station, most FIB concentrations were below the WQOs during the dry season. During the late summer and fall months, a natural sand berm accumulates and closes the river mouth from its exchange with the Pacific Ocean. Summary tables of percent exceedances by site and season for each program are provided in Appendix E. Additional details of dry season patterns based on the distribution of percent exceedances is provided below:

- Fecal indicator bacteria data collected by the City through the Joint Monitoring Program shows a similar range and low exceedance frequency for Bonsall Bridge and Critical Point, which are the upstream and downstream extents of the main stem MST study area. For City' Joint Monitoring samples at the main stem sites, 51 percent exceeded the WQO for *Enterococcus* sp. and 26 percent exceeded the WQO for fecal coliform.
- Data from the tributary sites, Sleeping Indian and Pilgrim Creek, were similar in that the majority of samples collected were above the respective WQOs as well as being elevated above most main stem sites (Figures 3-11 and 3-12). For samples at the tributary sites, 95 percent exceeded the WQO for *Enterococcus* sp. and 32 percent exceeded the WQO for fecal coliform.
- Based on three years of monitoring data, samples collected upstream of Bonsall Bridge within the County of San Diego had elevated levels of *Enterococcus* sp. during the dry season (Figure 3-13).
- The City's Joint Monitoring data show exceedances did occur at the Pacific site, similar to the Point Zero location, during the dry season (May to September). No exceedances occurred at Point Zero during the MST Program in May or August events.

Figure 3-9: Microbial Source Tracking Program Dry Season *Enterococcus* Results

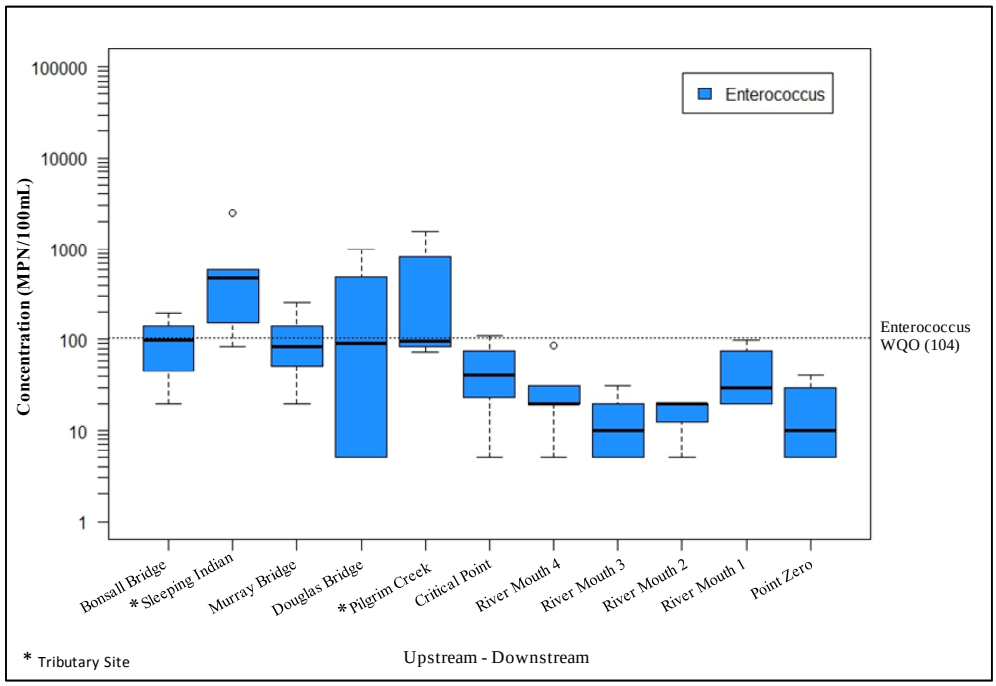
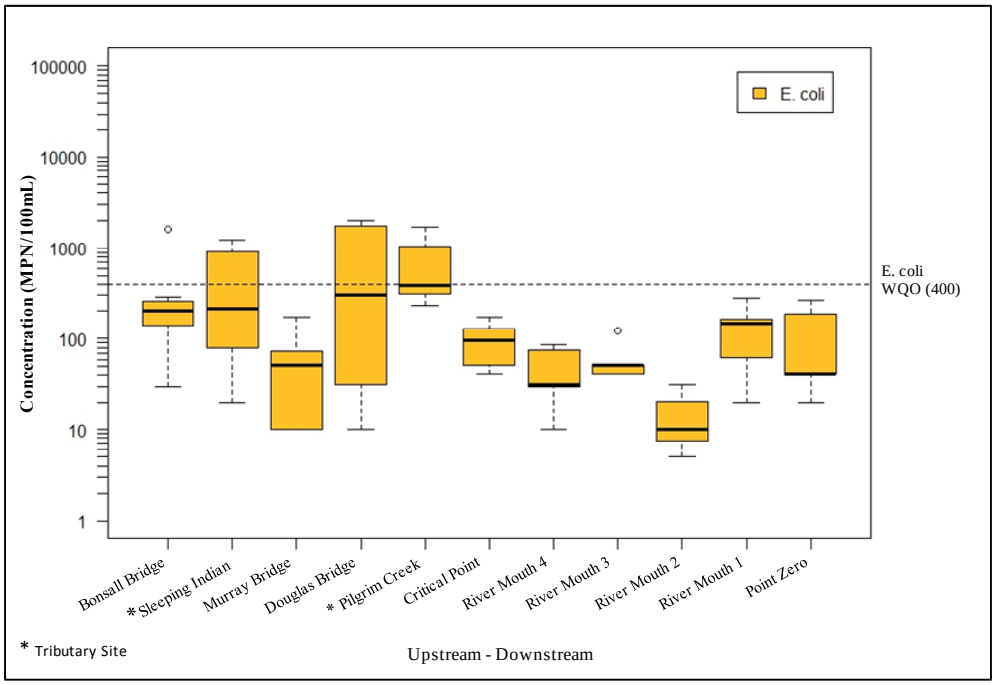


Figure 3-10: Microbial Source Tracking Program Dry Season *E. coli* Results



1. **Outlier (*)** - Observation that is beyond the upper or lower whisker.
2. **Upper whisker** - Extends to the maximum data point within 1.5 box heights from the top of the box.
3. **Interquartile range box** - Middle 50% of the data.
4. **Lower whisker** - Extends to the minimum data point within 1.5 box heights from the bottom of the box.

Figure 3-11: City of Oceanside – Joint Monitoring Program Dry Season *Enterococcus* Results

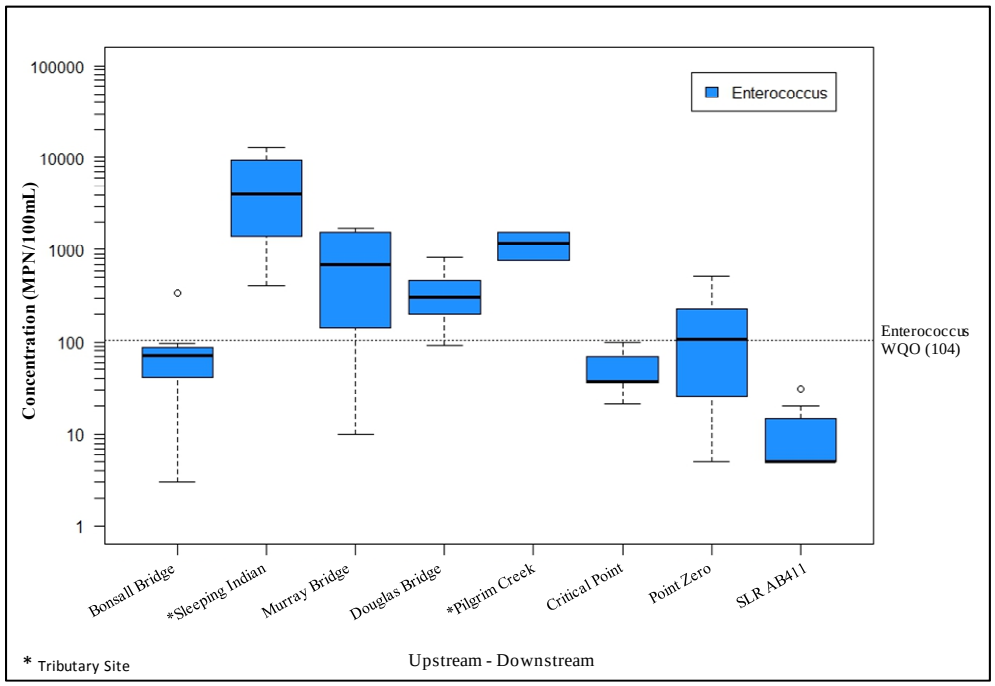
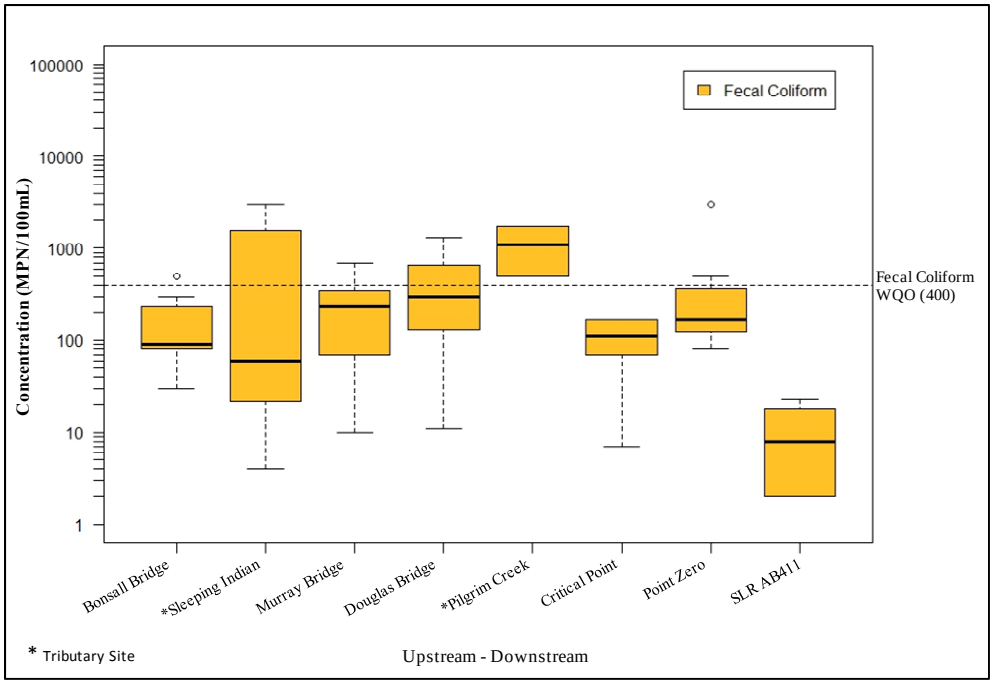


Figure 3-12: City of Oceanside – Joint Monitoring Program Dry Season Fecal Coliform Results



1. **Outlier (*)** - Observation that is beyond the upper or lower whisker.
2. **Upper whisker** - Extends to the maximum data point within 1.5 box heights from the top of the box.
3. **Interquartile range box** - Middle 50% of the data.
4. **Lower whisker** - Extends to the minimum data point within 1.5 box heights from the bottom of the box.

Figure 3-13: County of San Diego – Joint Monitoring Program Dry Season *Enterococcus* Results

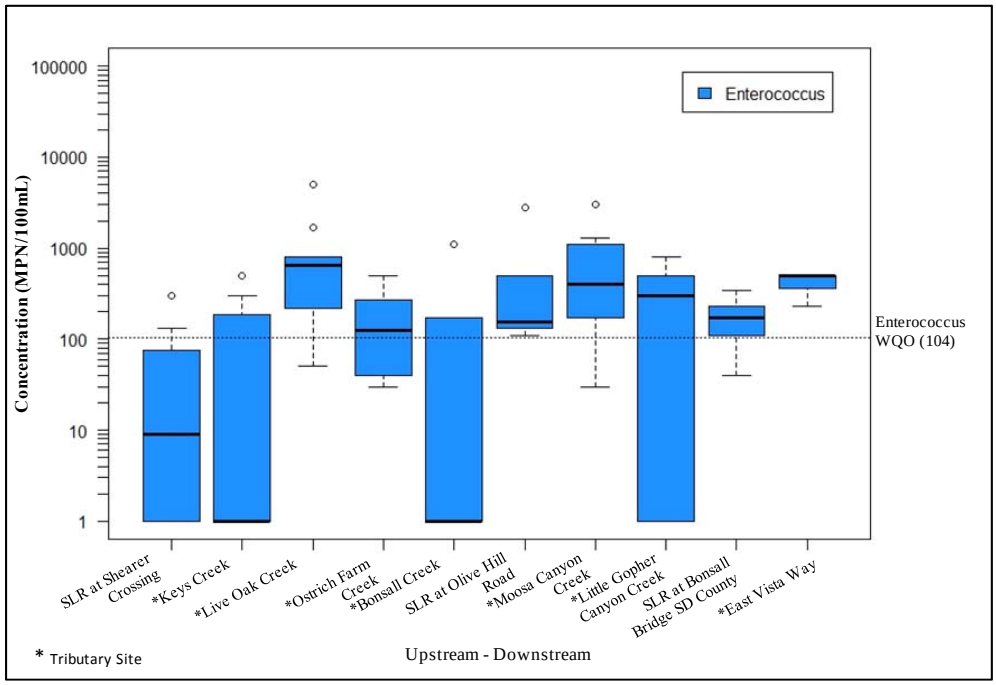
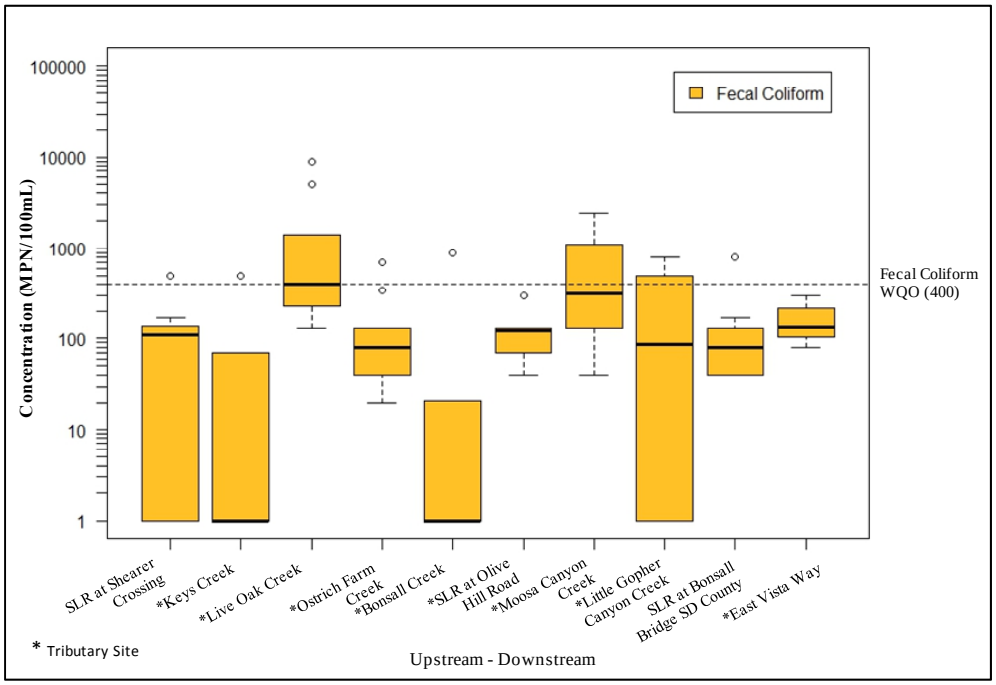


Figure 3-14: County of San Diego – Joint Monitoring Program Dry Season Fecal Coliform Results



1. **Outlier (*)** - Observation that is beyond the upper or lower whisker.
2. **Upper whisker** - Extends to the maximum data point within 1.5 box heights from the top of the box.
3. **Interquartile range box** - Middle 50% of the data.
4. **Lower whisker** - Extends to the minimum data point within 1.5 box heights from the bottom of the box.

Figure 3-15: DEH AB411 Monitoring Program Dry Season *Enterococcus* Results

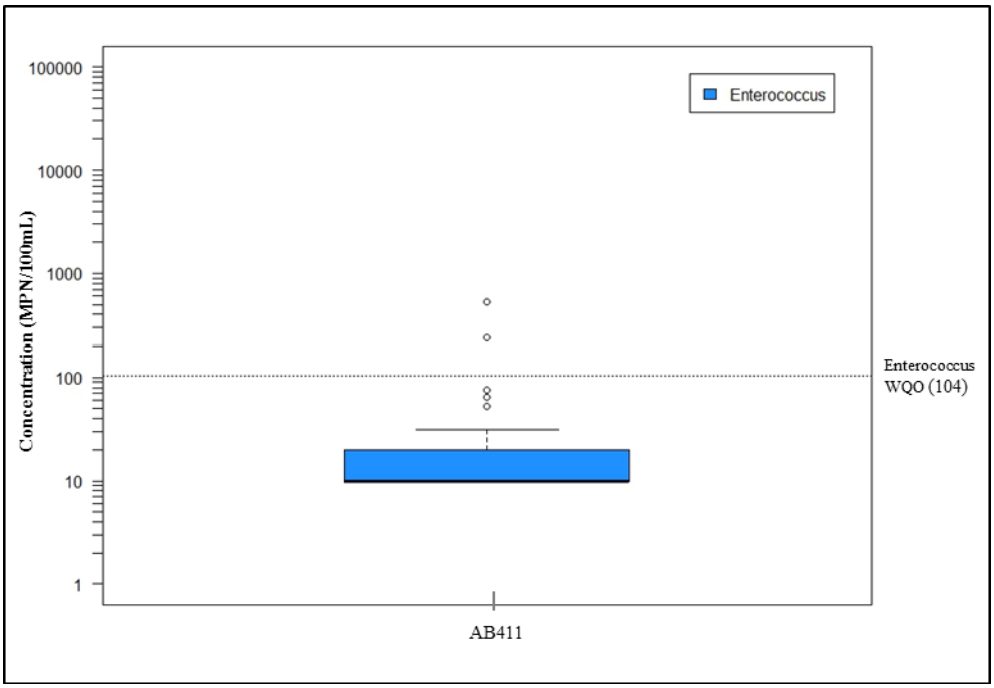
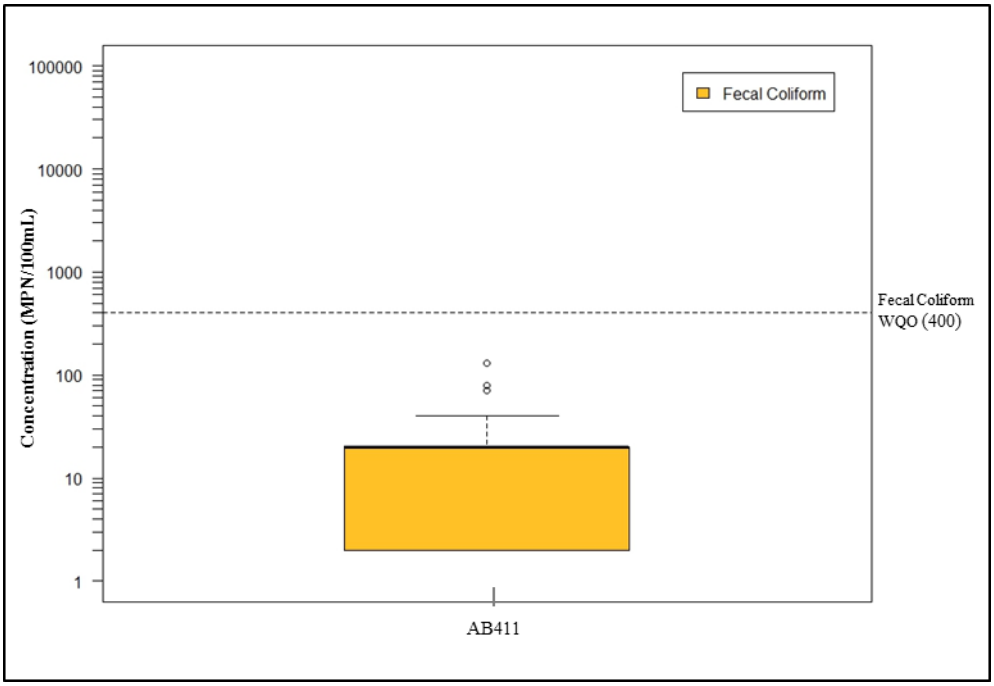


Figure 3-16: DEH AB411 Monitoring Program Dry Season Fecal Coliform Results



1. **Outlier (*)** - Observation that is beyond the upper or lower whisker.
2. **Upper whisker** - Extends to the maximum data point within 1.5 box heights from the top of the box.
3. **Interquartile range box** - Middle 50% of the data.
4. **Lower whisker** - Extends to the minimum data point within 1.5 box heights from the bottom of the box.

Wet Season

The MST Program wet season samples represent both storm events and dry weather conditions (baseline and base flow events). Fecal indicator bacteria concentrations at most sites show both a wider range and a higher frequency of exceedance during the wet season than the dry season. The distribution of wet season results in comparison to Rec-1 WQOs for the MST Program, Joint Monitoring Program, and AB411 Program are presented in Figures 3-17 to 3-24. During the wet season, 79 percent of samples collected for the MST Program exceeded the WQO for *Enterococcus* sp., and 52 percent of samples exceeded the WQO for *E. coli*. Main stem MST samples exceeded WQOs at a rate of 86 percent for *Enterococcus* sp. and 46 percent for *E. coli*. For samples collected at the tributary sites, 92 percent exceeded the WQO for *Enterococcus* sp. and 54 percent exceeded the WQO for *E. coli*. River mouth samples exceeded WQOs at a rate of 62 percent for *Enterococcus* sp. and 57 percent for *E. coli*. Fecal indicator bacteria concentrations at river mouth sites were significantly higher during the wet season as indicated by the exceedance percentages when compared to zero exceedances during the dry season. Additional details of wet season patterns based on the distribution of percent exceedances are provided below:

- Data from the City's Joint Monitoring Program, implemented monthly to measure FIB concentrations during dry conditions in the wet season, resulted in *Enterococcus* sp. concentrations above the WQO at most sites, while fecal coliform concentrations exceed the WQO at Pilgrim Creek and Critical Point, based on City data (Figures 3-19 and 3-20).
- For the City's Joint Monitoring Program at main stem sites, 61 percent exceeded the WQO for *Enterococcus* sp. and 19 percent exceeded the WQO for fecal coliform.
- Of the City's Joint Monitoring Program samples collected at the tributary sites, 94 percent exceeded the WQO for *Enterococcus* sp. and 43 percent exceeded the WQO for fecal coliform.
- Results from the County's Joint Monitoring Program, resulted in *Enterococcus* sp. concentrations that were above the WQO, whereas fecal coliform concentrations varied by site, as shown in Figures 3-21 and 3-22.
- AB411 samples exceeded WQOs at a rate of 14 percent for *Enterococcus* sp. and 5 percent for fecal coliform during the wet season (Figures 3-23 and 3-24).

Figure 3-17: Microbial Source Tracking Program Wet Season *Enterococcus* Results

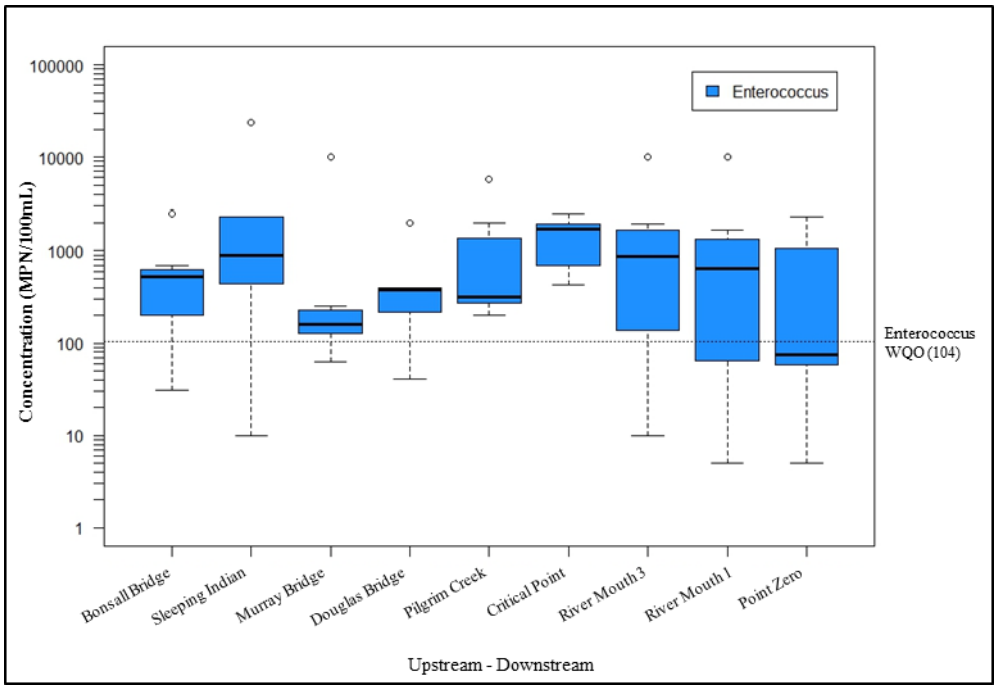
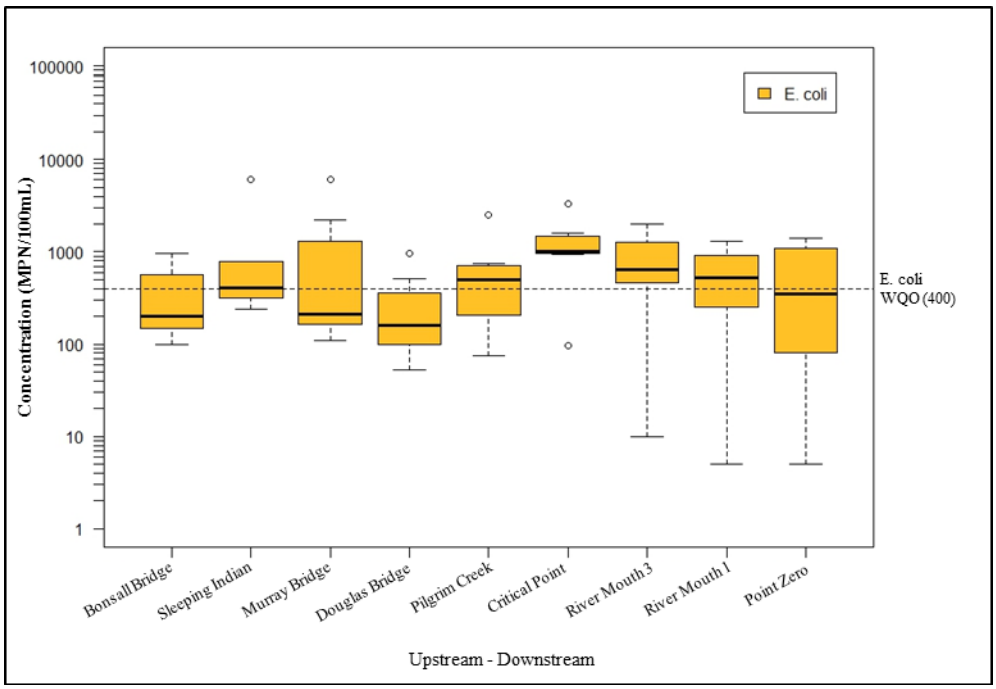


Figure 3-18: Microbial Source Tracking Program Wet Season *E. coli* Results



1. **Outlier (*)** - Observation that is beyond the upper or lower whisker.
2. **Upper whisker** - Extends to the maximum data point within 1.5 box heights from the top of the box.
3. **Interquartile range box** - Middle 50% of the data.
4. **Lower whisker** - Extends to the minimum data point within 1.5 box heights from the bottom of the box.

Figure 3-19: City of Oceanside – Joint Monitoring Program Wet Season *Enterococcus* Results

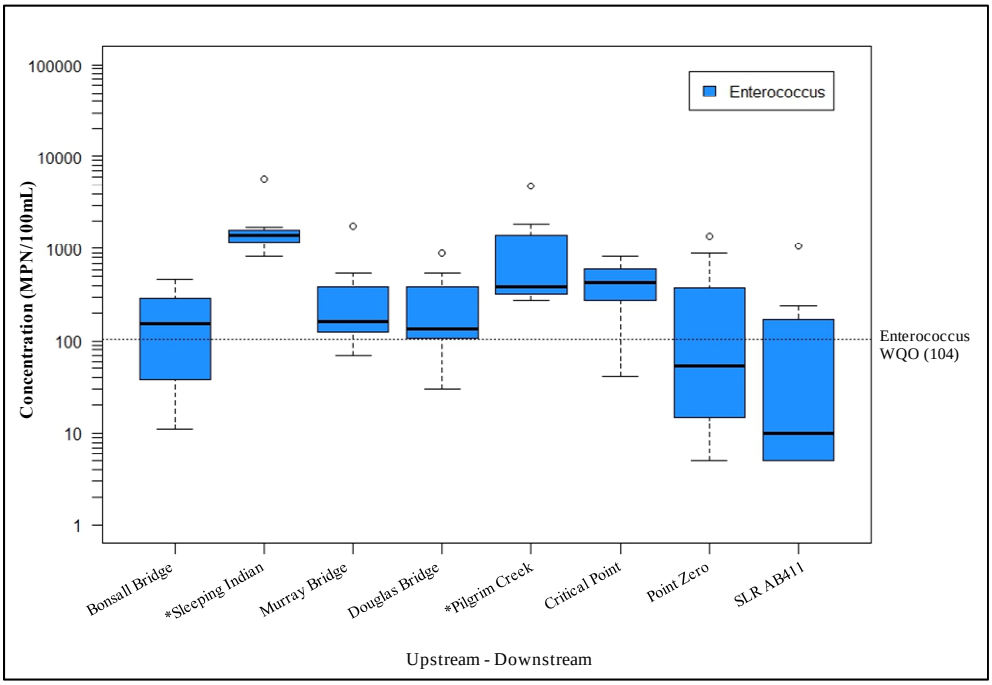
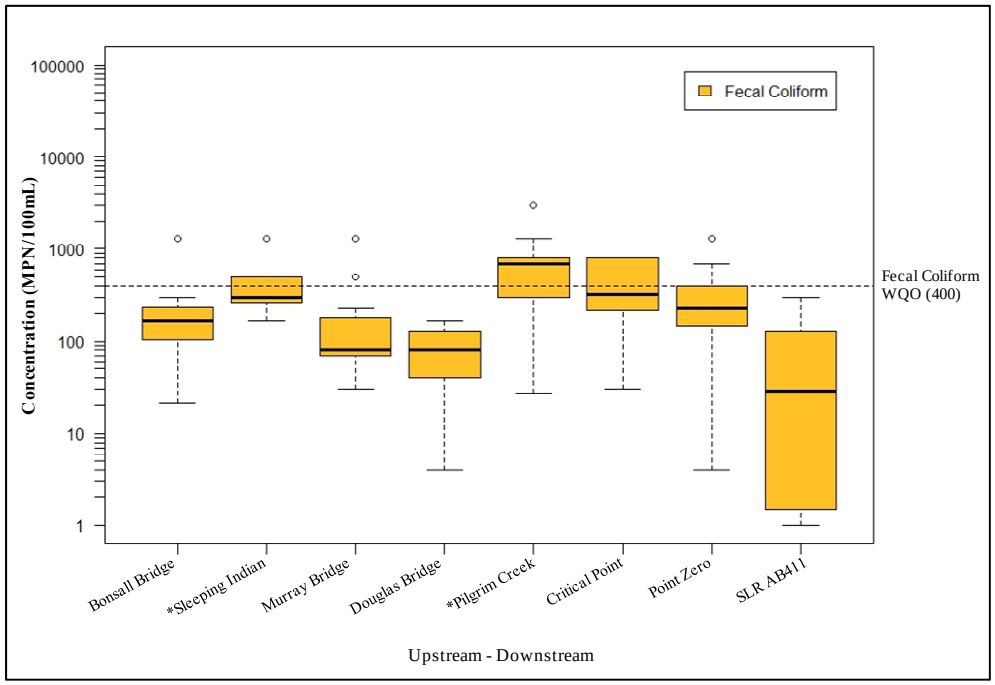


Figure 3-20: City of Oceanside – Joint Monitoring Program Wet Season Fecal Coliform Results



1. **Outlier (*)** - Observation that is beyond the upper or lower whisker.
2. **Upper whisker** - Extends to the maximum data point within 1.5 box heights from the top of the box.
3. **Interquartile range box** - Middle 50% of the data.
4. **Lower whisker** - Extends to the minimum data point within 1.5 box heights from the bottom of the box.

Figure 3-21: County of San Diego – Joint Monitoring Program Wet Season *Enterococcus* Results

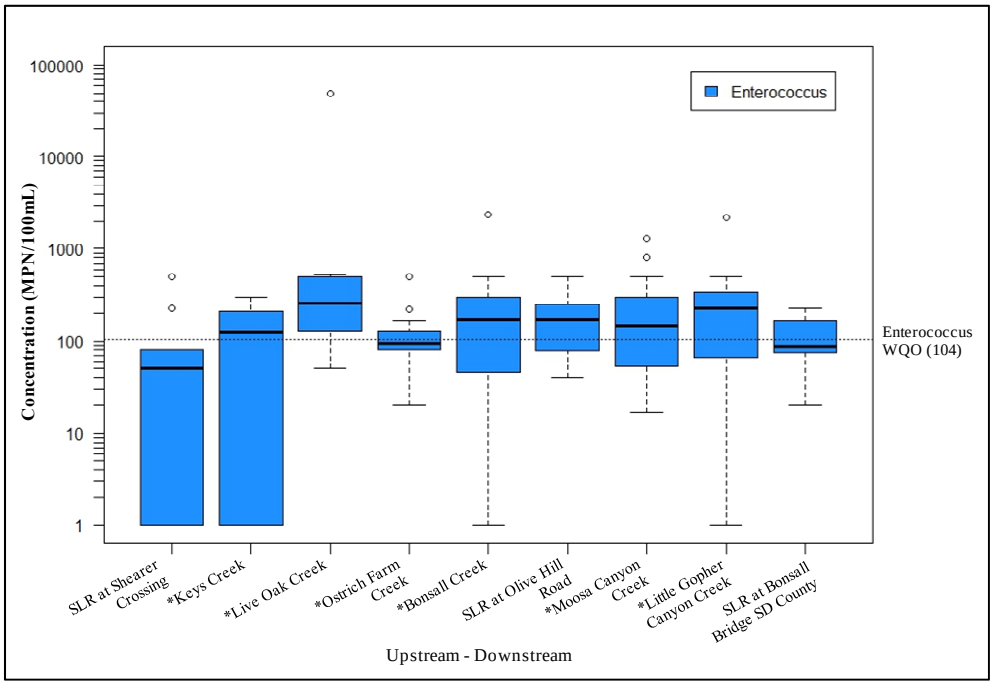
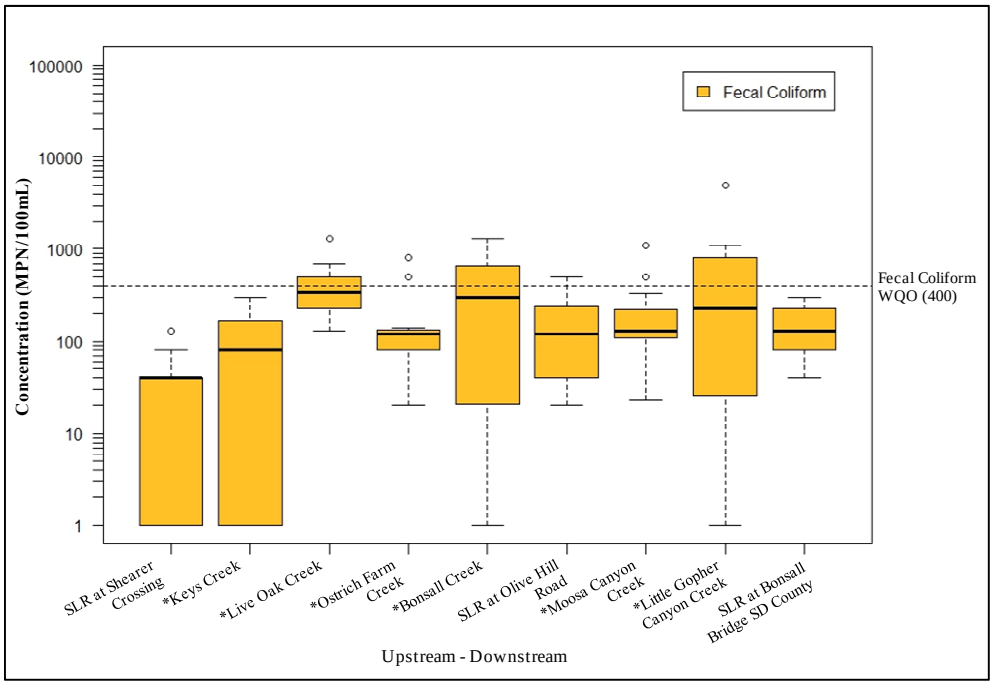


Figure 3-22: County of San Diego – Joint Monitoring Program Wet Season Fecal Coliform Results



1. **Outlier (*)** - Observation that is beyond the upper or lower whisker.
2. **Upper whisker** - Extends to the maximum data point within 1.5 box heights from the top of the box.
3. **Interquartile range box** - Middle 50% of the data.
4. **Lower whisker** - Extends to the minimum data point within 1.5 box heights from the bottom of the box.

Figure 3-23: DEH AB411 Monitoring Program Wet Season *Enterococcus* Results

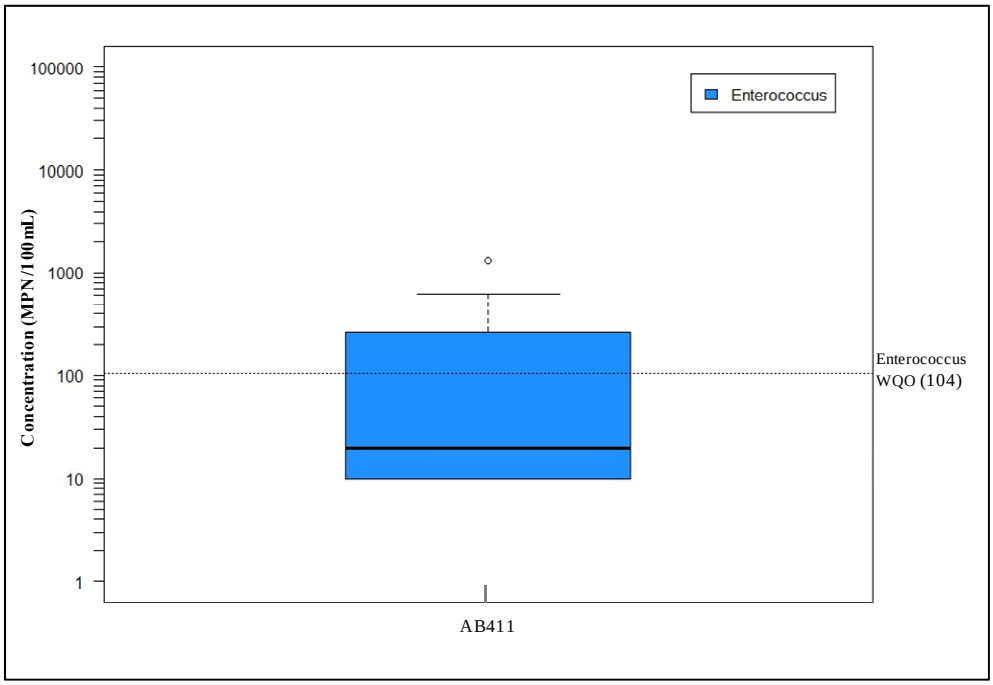
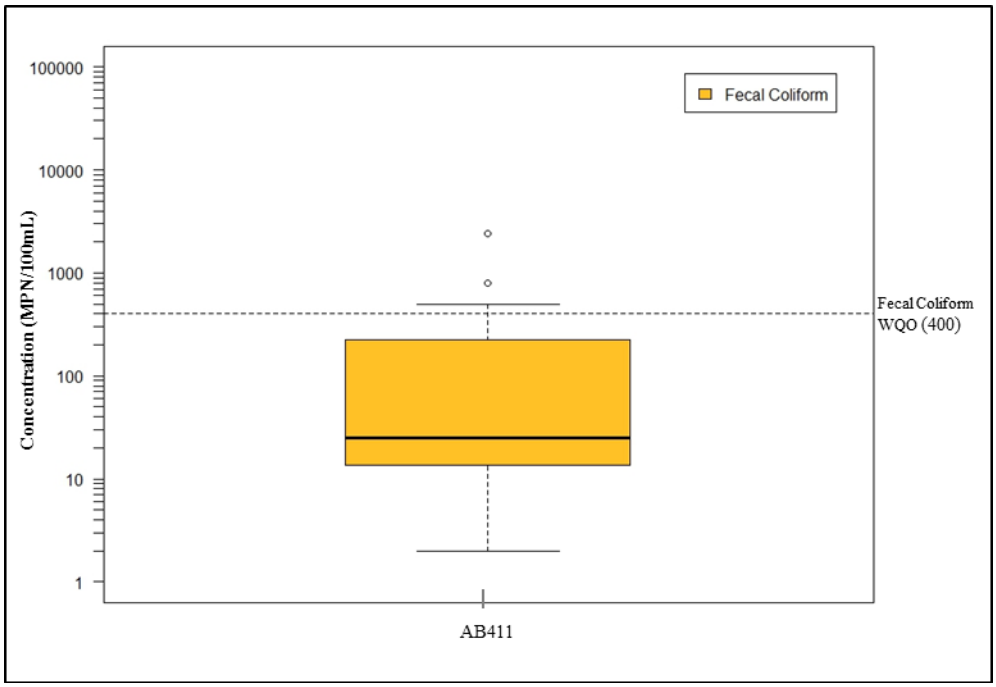


Figure 3-24: DEH AB411 Monitoring Program Wet Season Fecal Coliform Results



1. **Outlier (*)** - Observation that is beyond the upper or lower whisker.
2. **Upper whisker** - Extends to the maximum data point within 1.5 box heights from the top of the box.
3. **Interquartile range box** - Middle 50% of the data.
4. **Lower whisker** - Extends to the minimum data point within 1.5 box heights from the bottom of the box.

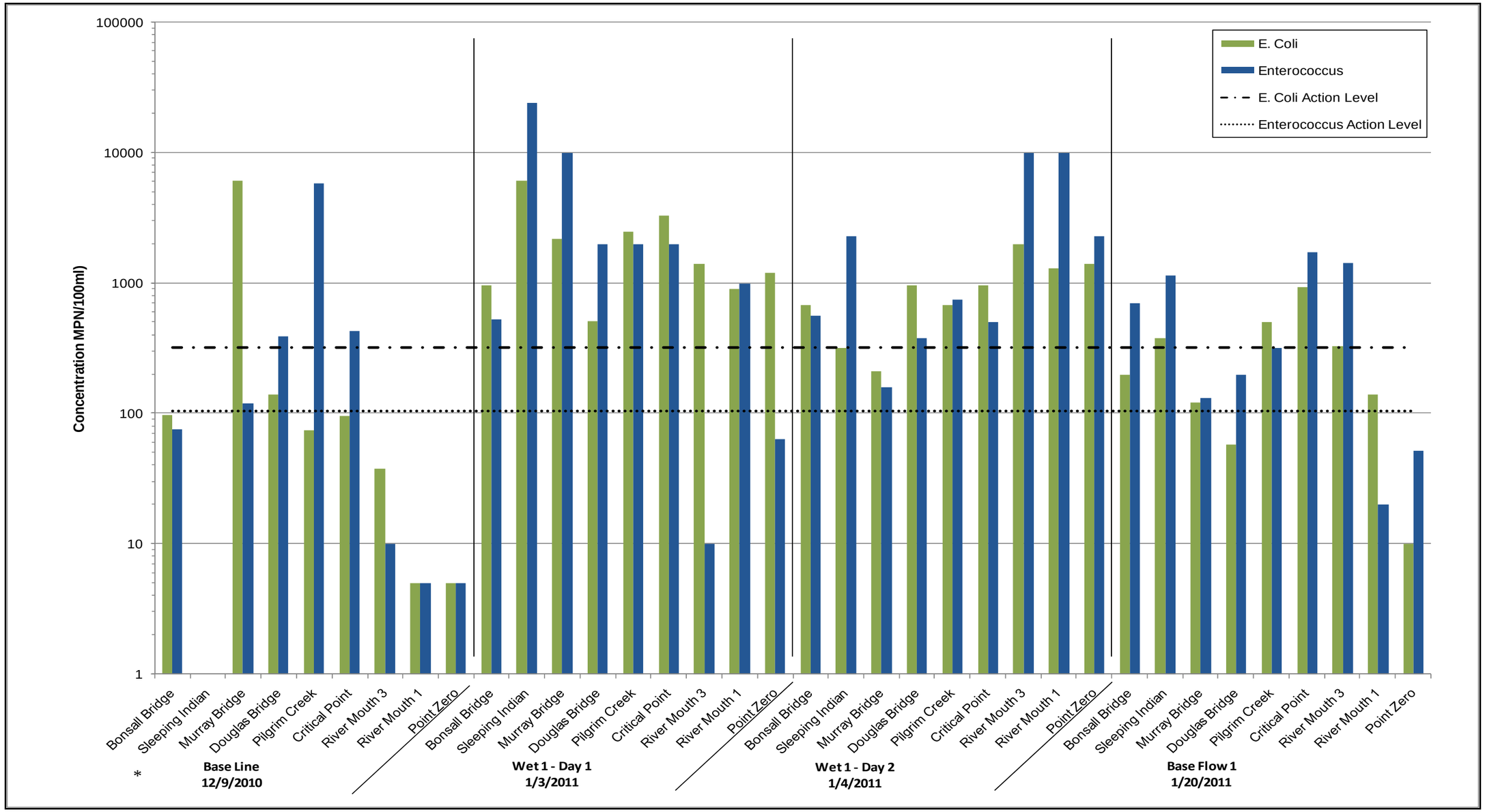
Figures 3-25 and 3-26 present the FIB concentrations by site for each sampling day during the wet season. The Baseline Event characterized FIB concentrations in December 2010 when flow was comprised only of surface water runoff and groundwater flows and was without the direct influence of a storm event. Main stem river sites (Bonsall Bridge, Murray Bridge, Critical Point) and one tributary (Pilgrim Creek) show exceedances of either *Enterococcus* sp. or *E. coli*, or both. Sleeping Indian was still dry during this time period. Fecal indicator bacteria concentrations were detected at River Mouth 3, but were not detected at River Mouth 1 and Point Zero. There was a substantial increase in concentrations of *E. coli* and *Enterococcus* sp. between the Baseline Event and Wet Event 1.

Fecal indicator bacteria concentrations were an order of magnitude higher during Wet Event 1 than Wet Event 2. The following factors may have influenced the higher concentrations during Wet Event 1: longer event duration, higher intensity of rainfall, and higher peak discharge. Samples were collected on two consecutive days of each storm event to evaluate the relative change in concentrations on the rising and falling limbs of the hydrograph. A noticeable shift of FIB concentrations at river and tributary locations from higher FIB concentrations on day 1 than day 2 occurs during both wet events (Figures 3-25 and 3-27). On the contrary, FIB concentrations at river mouth locations were higher on day 2 than day 1 of both monitored events. Pollutograph monitoring over the course of the hydrograph would provide more information regarding transport and trends throughout the river system.

Base flows increased throughout the wet season as the ground became more saturated. Base flows contribute to overall wet weather runoff, accounting for the majority of flows during wet season dry periods and spring flows after rain has ceased. Samples collected during the base flow events indicate elevated concentrations similar to those during the preceding storm events. During Base Flow Event 1, FIB concentrations persisted in the river 16 days after Wet Event 1. Main stem sites, Bonsall Bridge, Murray Bridge, Douglas Bridge, Critical Point, and River Mouth 3 exceeded *Enterococcus* sp. and/or *E. coli* WQOs. Both tributary sites exceeded *Enterococcus* sp. and/or *E. coli* WQOs. FIB concentrations at River Mouth 1 and Point Zero were below WQOs.

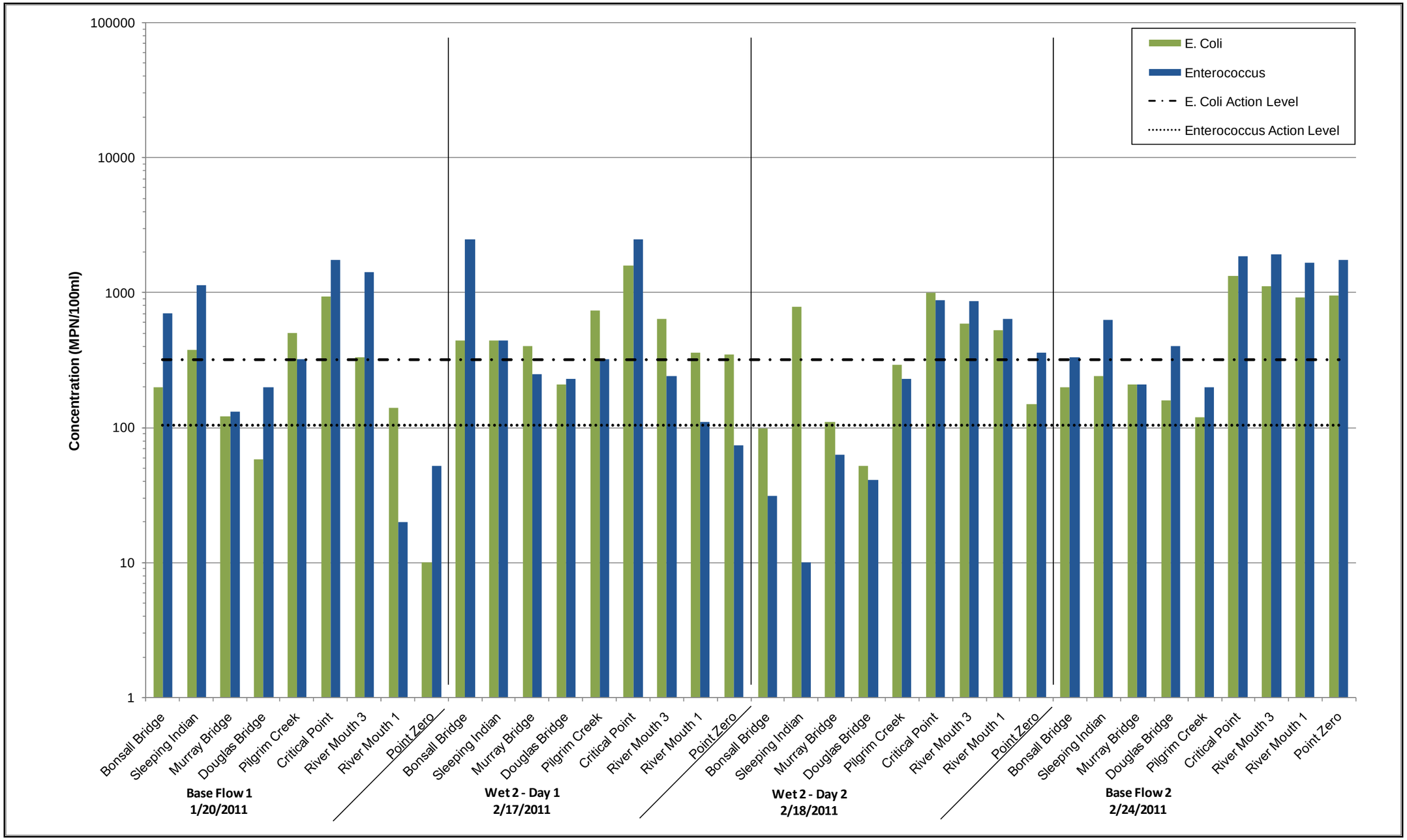
FIB concentrations were generally higher during Base Flow Event 2 in comparison to FIB concentration during Base Flow Event 1. The following factors may have influenced the higher concentrations: antecedent dry period, influence from Wet Event 2 and a subsequent storm event for a total rainfall of 1.64 inches. *Enterococcus* sp. results for all monitoring locations exceeded the WQO and *E. coli* results exceeded the WQO for all river mouth locations. Base flow conditions have proven to be critical periods within this river system as it relates to microbial sources.

Figure 3-25: FIB Concentrations by Site for Baseline Event, Wet Event 1, and Base Flow Event 1



* Sleeping Indian site was dry so no sample was collected.

Figure 3-26: FIB Concentrations by Site for Base Flow Event 1, Wet Event 2, and Base Flow Event 2



Discussion of FIB Concentration Patterns

In assessing all of the MST Program wet weather FIB concentration data, there was not a consistent decrease or increase from upstream to downstream locations; the concentrations of FIB in the upper reaches of the study area (Bonsall Bridge and Sleeping Indian) were generally higher than those in the mid-river locations, and then the concentrations increased again near the Critical Point and the river mouth. This general trend was not exceedingly strong, nor can it be statistically tested given the low number of storms samples. However, the results do permit speculation about the multiple possible reasons for this disconnect, including: (1) overland flow during storms in between sampling points, (2) additional non-storm driven FIB contributions (birds, humans, septic systems, leaking infrastructure), and (3) growth of reservoir populations of FIB at the river mouth locations where flow is generally slowed and the water is pooled.

Mean FIB concentrations from the MST Program during wet weather versus dry weather are summarized in Table 3-6. The average concentration of *E. coli* across all sampling events were more similar, while the average concentration for *Enterococcus* sp. was higher during wet weather (i.e., for *Enterococcus* sp., 168 MPN/100 ml dry, versus 1612 MPN/100 ml wet). *Enterococcus* sp. concentrations typically were high in the river mouth during wet weather conditions.

Table 3-6: Seasonal Summary Statistics for *E. coli* and *Enterococcus* sp.

Analyte and Season	Sample Count	Mean	Maximum	Minimum[(a)]	Standard Deviation
E. coli (MPN/100ml)	111	564	6,150	5	970
Dry	49	239	1,956	5	451
Wet[(b)]	62	821	6,150	5	1,177
Enterococcus sp. (MPN/100ml)	117	933	24,000	5	2,754
Dry	55	168	2,420	5	404
Wet[(b)]	62	1612	24,000	5	3,645

(a) For statistical analysis, non-detects were set to half the method detection limit of 10 MPN/100ml.
(b) Wet Season includes Baseline and Base Flow.

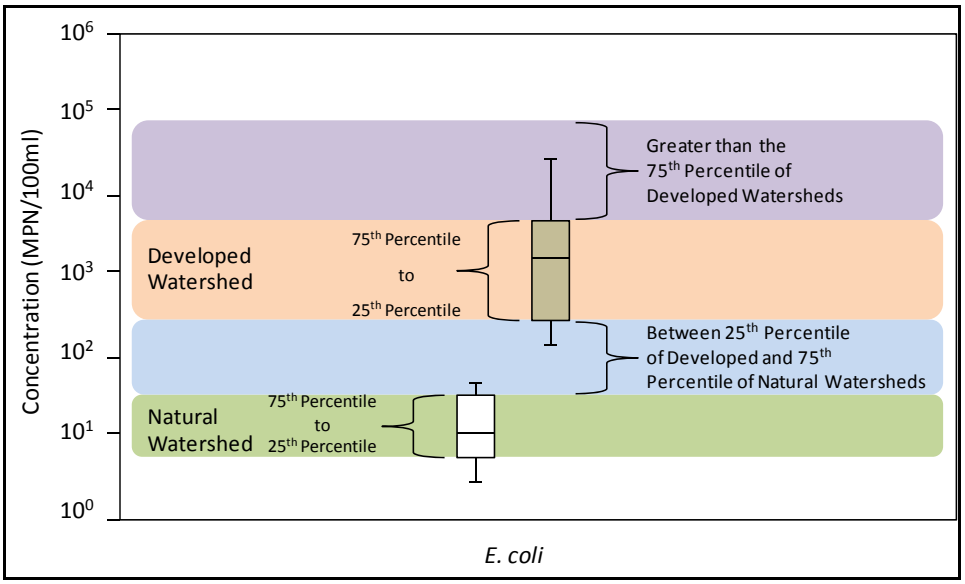
3.2.2 FIB Results Compared to Natural Landscapes

The SLR Watershed has an area of approximately 562 square miles, of which approximately 54 percent is undeveloped (Project Clean Water, 2011). It has been accepted that natural sources cause exceedances of indicator bacteria water quality objectives on their own, without contributions from anthropogenic sources (SDRWQB, 2008). Natural systems as a source of bacteria is further supported by the Assessment

of Water Quality Concentrations and Loads from Natural Landscapes (SCCWRP Natural Loading Study), where samples collected from natural landscapes exceeded WQOs 40 to 50 percent of the time (Stein & Yoon, 2007).

Results from samples collected during the MST Program were compared to the ranges of concentrations found for developed and natural watersheds in the SCCWRP Natural Loading Study. Comparisons were made using the average dry and wet season concentrations from each site monitored in this program. Main stem and tributary sites from the MST program were included in this comparison since a natural streams ranged in sizes comparable to the MST monitoring locations. The average *E. coli* and *Enterococcus* sp. concentrations from monitored sites were compared to the 25th to 75th percentile range for *E. coli* and *Enterococcus* sp. presented in the Natural Loading Study. For these comparison purposes four ranges were considered: the natural watershed, the developed watershed, the range between the natural and developed watersheds, and greater than the 75th percentile concentrations of the developed watershed. Figure 3-27 presents a schematic of ranges for comparison to the Natural Loading Study.

Figure 3-27: Natural Loading Study Comparison



Note: Plot is a diagram for informational purposes only. The plot does not depict real data.

Percentages of the sites that were within these ranges are presented in Table 3-7 for the dry season and Table 3-8 for the wet season.

Table 3-7: Dry Season Site Comparisons to SCCWRP Natural Loading Study

Constituent	Greater than 75th Percentile of Developed Watersheds	Within Range for Developed Watersheds (25th to 75th Percentile)	Between 25th Percentile of Developed and 75th Percentile of Natural Watersheds	Within Range for Natural Watersheds (25th to 75th Percentile)
E. coli	20%	10%	40%	30%
Enterococcus sp.	0%	10%	60%	30%
Overall	10%	10%	50%	30%

Note: Percentages represent the percent of sites monitored during this program that had average seasonal concentrations within these four groups based on watershed conditions as compared to SCCWRP Natural Loading Study.

Summary of Dry Season Comparison:

- During the dry season, average concentrations were generally less than the range of concentrations for developed watersheds.
- Douglas Bridge had an average *E. coli* concentration and Sleeping Indian had an average *Enterococcus* sp. concentration within the 25th to 75th percentile range for developed watersheds.
- The two tributary (Sleeping Indian and Pilgrim Creek) had average dry season *E. coli* concentrations greater than the 75th percentile concentration for developed watersheds.
- 30 percent of sites were within the ranges found in natural watersheds throughout southern California. These include average *Enterococcus* sp. concentration at Critical Point, average *E. coli* concentration at Murray Bridge, average concentrations of both indicators at River Mouth 2 and 4.

Table 3-8: Wet Season Site Comparisons to SCCWRP Natural Loading Study

Constituent	Greater than 75th Percentile of Developed Watersheds	Within Range for Developed Watersheds (25th to 75th Percentile)	Between 25th Percentile of Developed and 75th Percentile of Natural Watersheds	Within Range for Natural Watersheds (25th to 75th Percentile)
E. coli	0%	0%	33%	67%
Enterococcus sp.	0%	0%	67%	33%
Overall	0%	0%	50%	50%

Note: Percentages represent the percent of sites monitored during this program that had average seasonal concentrations within these four groups based on watershed conditions as compared to SCCWRP Natural Loading Study

Summary of Wet Season Comparison:

- During the wet season the average concentrations at all monitored sites were less than the 25th percentile concentration for developed watersheds.
- The majority (67 percent) of average *E. coli* concentrations were within the 25th to 75th percentile range found in natural watersheds in southern California. These include Bonsall Bridge, Douglas Bridge, Pilgrim Creek, River Mouth 1 and 3, and Point Zero.
- 33 percent of the monitored sites had an average *Enterococcus* sp. concentration within the natural watershed range. These include Bonsall Bridge, Douglas Bridge, and Point Zero.

3.2.3 Microbial Source Tracking Results

Samples were selected for further molecular marker analyses based on the FIB concentration thresholds described in Section 2.4. Table 3-9 presents a summary of the microbial source tracking tools utilized by event and site. An overall breakdown of the microbial, viral, and community fingerprinting analyses conducted is as follows:

- 72 samples were analyzed for fecal *Bacteroides* spp., BacHum-Human *Bacteroides*, and the human-specific HF183 marker. In 2008, six dry season samples from main stem river and tributary locations were selected for further molecular analysis, but were not analyzed due to holding time exceedances as a result of the stop work order in December 2008.
- 68 samples were analyzed for enteroviruses.
- 72 samples were analyzed for the gull specific marker.
- 13 water samples and 13 paired sediment samples from the river mouth were selected for Community Fingerprinting Analyses.

Table 3-9: Summary of Microbial, Viral, and Community Fingerprinting Analyses by Event

Sampling Event	Dates	Locations Sampled		Fecal *Bacteroides* spp.	BacHum Marker	HF183	Enteroviruses	Gull Specific Assay	Community Fingerprinting
Dry Event 1	6/18/2008 – 6/19/2008	Bonsall Bridge Sleeping Indian Murray Bridge	Douglas Bridge Pilgrim Creek Critical Point	NA	NA	NA	X	--	--
Dry Event 2	7/23/2008 – 7/24/2008	Bonsall Bridge Sleeping Indian Murray Bridge	Douglas Bridge Critical Point	--	--	--	--	--	--
Dry Event 3	5/18/2010 – 5/20/2010	Bonsall Bridge *Sleeping Indian *Murray Bridge *Douglas Bridge *Pilgrim Creek Critical Point	River Mouth 4 River Mouth 3 River Mouth 2 River Mouth 1 Point Zero	X	X	X	--	X	X
Dry Event 4	8/03/2010 – 8/04/2010	River Mouth 4 River Mouth 3	River Mouth 1 Point Zero	X	X	X	--	X	X
Baseline 1	12/9/2010 & 12/15/2010	Bonsall Bridge Murray Bridge Douglas Bridge	Pilgrim Creek Critical Point	X	X	X	X	X	--
Wet Event 1	1/3/2011 – 1/4/2011	Bonsall Bridge Sleeping Indian Murray Bridge Douglas Bridge Pilgrim Creek	Critical Point River Mouth 3 River Mouth 1 Point Zero	X	X	X	X	X	--
Base Flow Event 1	1/20/2011	Bonsall Bridge Sleeping Indian Murray Bridge Douglas Bridge Pilgrim Creek	Critical Point River Mouth 3 River Mouth 1 Point Zero	X	X	X	X	X	--
Wet Event 2	2/17/2011 –	Sleeping Indian Pilgrim Creek Critical Point	River Mouth 3 River Mouth 1 Point Zero	X	X	X	X	X	--
	2/18/2011	Sleeping Indian Pilgrim Creek	Critical Point River Mouth 3 Point Zero	X	X	X	X	X	--
Base Flow Event 2	2/24/2011	Bonsall Bridge Sleeping Indian Murray Bridge Douglas Bridge Pilgrim Creek	Critical Point River Mouth 3 River Mouth 1 Point Zero	X	X	X	X	X	--

Notes:
NA – Analysis requested but not analyzed as a result of holding time exceedances caused by the stop work order in December 2008.
X – Analysis was conducted on samples that met the selection criteria.
*Only sampled on 5/18/10.
Gull and community fingerprinting analyses were conducted on river mouth and Point Zero samples only.

Seasonal trends were observed for fecal *Bacteroides* spp., the BacHum marker, and the HF183 marker. To specify locations that are likely (probable) to be contaminated with human-related bacterial sources, locations where all three markers were positive were examined while taking the respective concentrations over the course of the events into consideration.

Bonsall Bridge, Douglas Bridge, Sleeping Indian, Pilgrim Creek, and the Critical Point exhibit potential signs (2 of 3 markers) of human-related bacterial sources during the dry season. Due to the stop work order in December 2008, main stem river and tributary samples (1 to 3 samples per location) were only analyzed for fecal *Bacteroides* spp., BacHum-Human *Bacteroides*, and HF183 marker during Dry Event 3. No enteroviruses were present in main stem river or tributary samples during the dry season.

The river mouth exhibits a more pronounced human-related bacterial source signal during the dry season when the river mouth is closed to tidal exchange (three of three markers were positive for the August 2010 sampling event) as shown in Figure 3-28. During dry season, there was only one sample (River Mouth 1, collected on August 3, 2010) that exhibited substantial inhibition, preventing accurate quantification of the fecal *Bacteroides* spp. Inhibition is the "blocking" or "interference" of the result and can occur due to the nature of the sample matrix. In some studies, a correction factor can be used to adjust the data set and estimate the result. No data was adjusted or estimate in this data set and is shown on figures as not detected. No enteroviruses were present in main stem river, tributary, or river mouth samples during the dry season. Additionally, the river mouth exhibits a very strong gull bacteria signal during both dry and wet weather.

The river mouth appears to exhibit potential human-related bacterial sources during the wet season; with two of the three bacteria markers strongly positive, and enterovirus being positive in River Mouth 1 during Wet Event 1. This potential for human-related bacterial sources is differentiated from a probable human-related bacterial source signal seen in the August sampling event. Given that only two of the three markers were positive throughout the wet season events further source investigation in the river mouth is warranted.

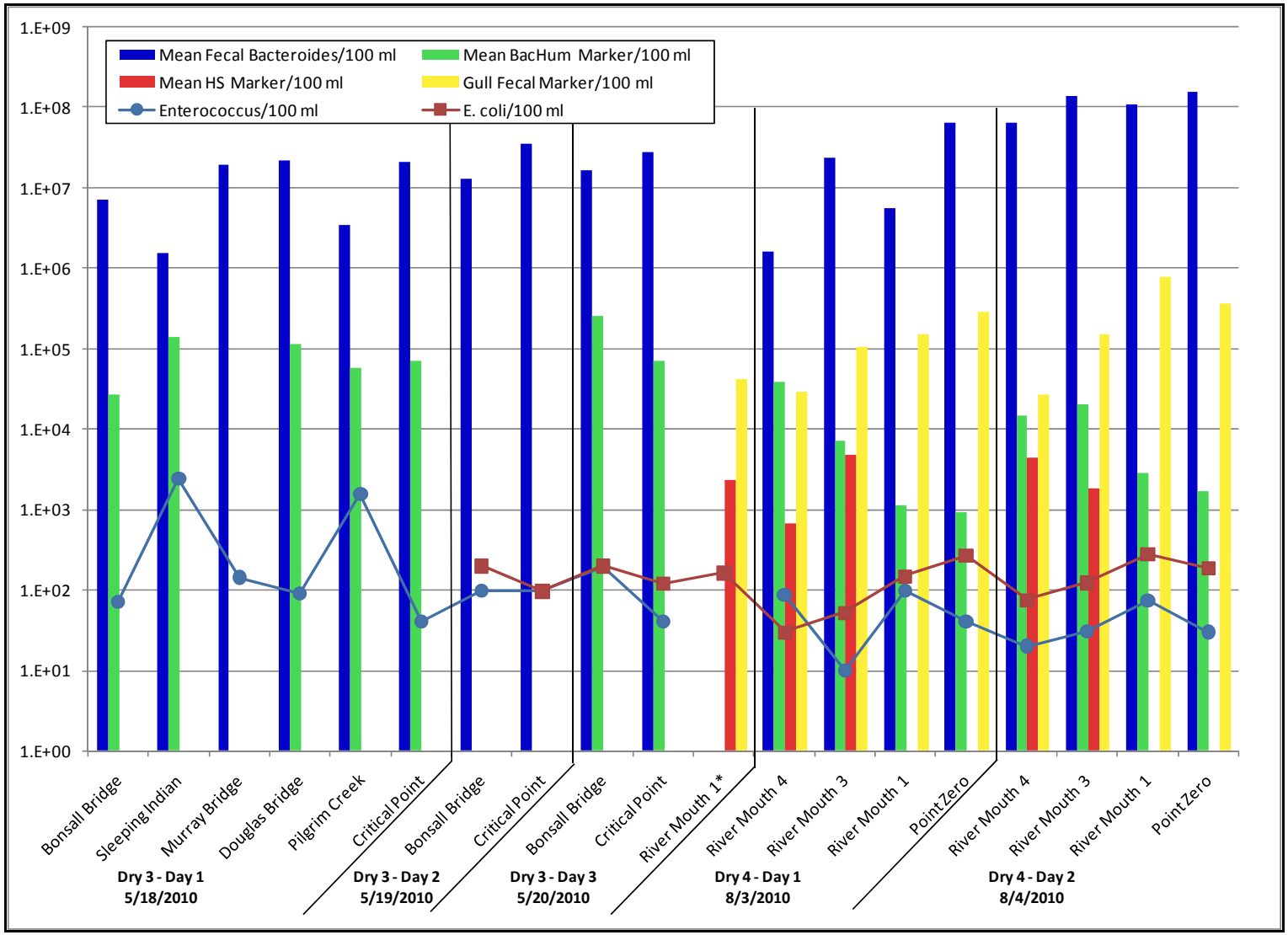
Generally, Wet Event 2 concentrations of fecal *Bacteroides* spp. and BacHum marker were an order of magnitude higher than Wet Event 1. This may have been influenced by the following factors: longer antecedent dry period, duration and intensity of rainfall, and a higher peak discharge. Figures 3-29 and 3-30 demonstrate that the main stem locations of Douglas Bridge and Critical Point exhibited strong positive signals for all three markers of human-related bacterial sources during wet weather. The two

tributaries, Sleeping Indian and Pilgrim Creek, also resulted in strong positive signals for three of three markers. In the future, it will be important to further quantify the human-related bacterial source signal at these locations over the duration of storm events, especially in consideration of the possible importance of groundwater and septic systems.

During the Baseline Event (December 15, 2010), a quantitative result from the Critical Point was not achieved for the fecal *Bacteroides* spp. QPCR due to inhibition of the sample. Finally, during the wet weather and base flow sampling events, there were six instances where quantification of the fecal *Bacteroides* spp. marker was not possible due to inhibition of the QPCR. It is typically more common to observe QPCR inhibition during wet weather events, due to the presence of high molecular weight (e.g., humic and fulvic acids and refractory organic matter) that is washed into riverine systems from stormwater runoff (e.g., Watson and Blackwell, 2000). Humic and fulvic acids are from decomposition, they are refractory, high molecular weight compounds that effectively "blanket" the enzymes required for QPCR. Humic and fulvic acids would come from degradation of upstream agricultural material and decay of any of the plant vegetation that is in or overhanging the river. High turbidity can also act as a physical block to QPCR. No enteroviruses were present in main stem river and tributary samples during the wet season. One sample collected at River Mouth 1 during Wet Event 1 was positive for enteroviruses during wet season.

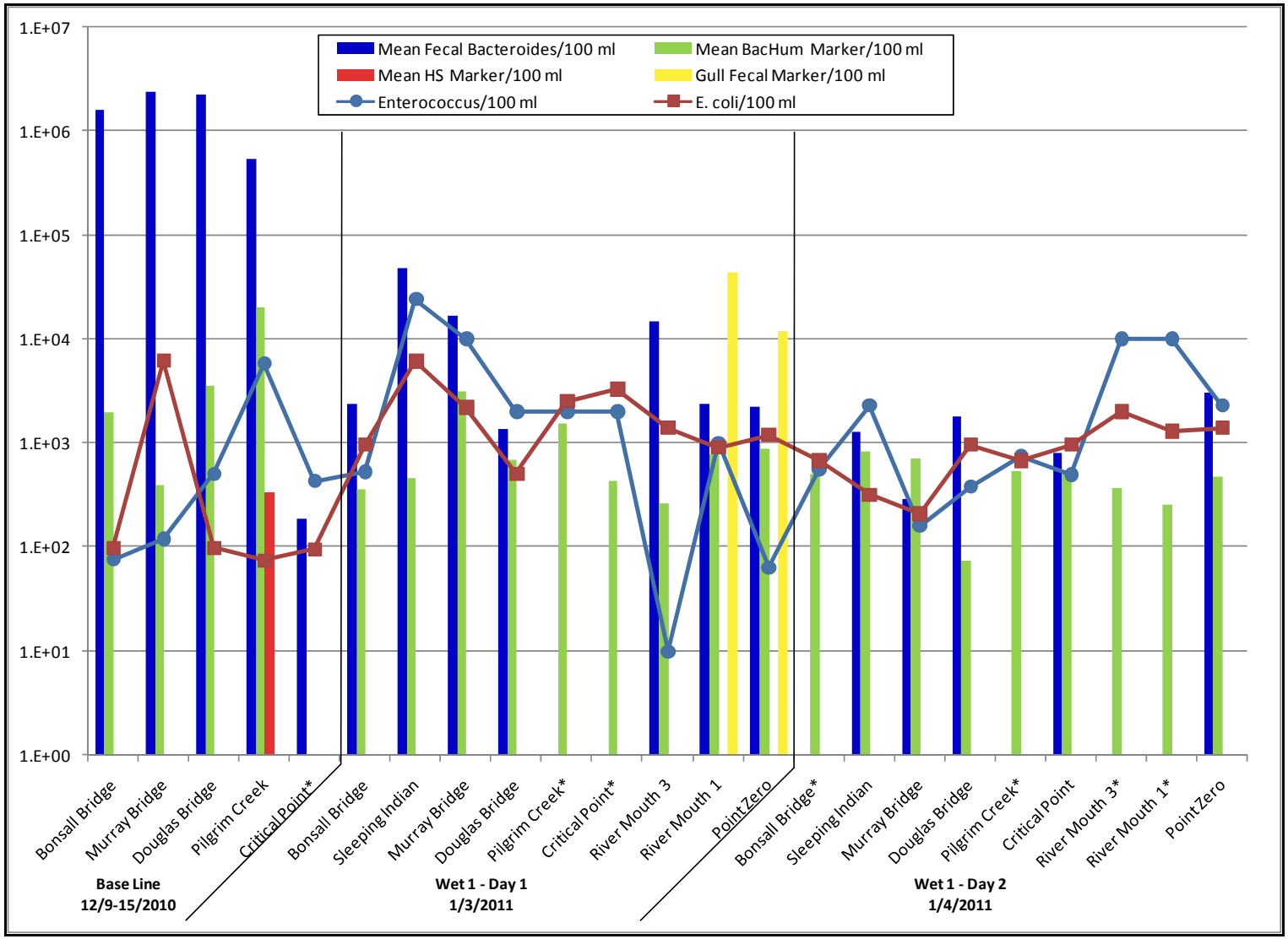
Although progress has been made on characterizing the delivery patterns of microbial contaminants during wet and dry weather, there are limitations to the data presented. First, and foremost, characterization of storms over a range of storm size and duration is necessary. The observations of multiple microbial source tracking molecular markers for human-related bacterial sources being positive during the August time frame (concomitant to the existence of a sand berm in the river mouth) indicates the potential for residence time, flushing resuspension, and river mouth transport, especially in relation to reservoir populations of FIB, to be important components for study.

Figure 3-28: Dry Season FIB and QPCR Results Summary



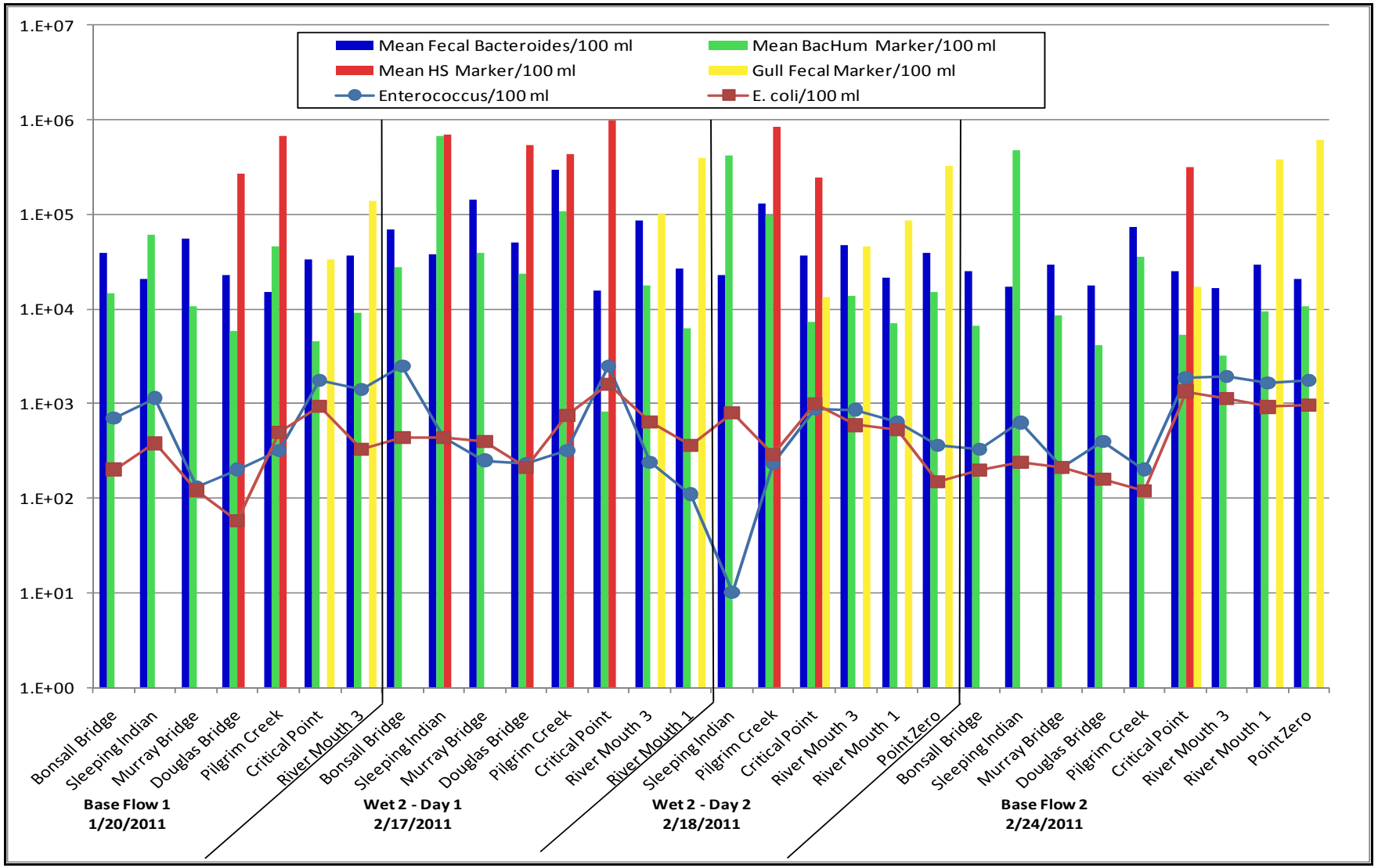
* Asterisked sampling events yielded samples that exhibited inhibition of the QPCR analyses for molecular markers. Because inhibition was present, markers that were not detected should not be interpreted as zero values.

Figure 3-29: Wet Season Baseline/Wet Event 1 FIB and QPCR Results Summary



* Asterisked sampling events yielded samples that exhibited inhibition of the QPCR analyses for molecular markers. Because inhibition was present, markers that were not detected should not be interpreted as zero values.

Figure 3-30: Wet Season Base Flow 1/Wet Event 2/Base Flow 2 FIB and QPCR Results Summary



Seasonal Comparison

Fecal *Bacteroides* spp. concentrations were high across both dry and wet weather sampling events during this study. It is not directly possible to compare the resulting concentration of fecal *Bacteroides* spp. per 100 ml for dry versus wet weather because of methodological differences in sample processing. The wet weather samples contained a range of inhibitory compounds that can interfere with QPCR, resulting in a reduction in amplification. QPCR can be prone to inhibition in the presence of specific inhibitory compounds such as humic and fulvic acids (Watson and Blackwell, 2000). Unfortunately, these compounds are often present in the highest concentrations during periods of strong stormwater runoff as observed during this study. Briefly stated, dry weather samples that were analyzed for fecal *Bacteroides* spp. were processed using a simple bead beating approach (simple mechanical lysis of the sample, but no further DNA purification step). Wet weather samples were subjected to a full DNA purification step with the use of a commercial DNA extraction kit. This difference in the sample processing conducted between dry and wet weather samples makes it difficult to analyze shifts in fecal *Bacteroides* spp. concentrations over the entire study period. Commercial DNA extraction kits purify the DNA, but they also cause concomitant loss of the total DNA signal, making the recovery much lower (about one tenth) that of bead beating.

3.2.4 Examining the Relationship between Bacteria and Flow

The MST Program was designed to examine the distribution of specific FIB and fecal bacteria source markers over a wide geographic area. Given this, it is important to remember that repeat sampling at any one given location was limited (i.e., sampling at the tributary of Pilgrim Creek occurred only 10 times, and of those, only eight samples were analyzed for both enterovirus and MST molecular markers). The data were generally found to be not normal so the non-parametric Spearman rank correlation test was used in place of the Pearson product moment correlation test. Results from statistical analyses using Spearman rank correlation reveal significant differences in the relationships between flow and FIB (*E. coli* and *Enterococcus* sp. concentrations) and molecular marker (fecal *Bacteroides* spp. and BacHum marker) concentrations (Tables 3-10 and 3-11).

Table 3-10: Spearman's Rank Correlation – Dry Season Data

Variable	Flow			
	n	ρ	Critical Value	Significant at α=0.10
E. coli	25	0.086	0.337	No
Enterococcus sp.	31	-0.001	0.301	No
Fecal *Bacteroides* spp.	10	0.280	0.564	No
BacHum Marker	10	-0.354	0.564	No

There was no significant relationship established between either the FIB or the molecular markers and flow during the dry season (90-percent confidence interval), as presented in Table 3-10. There was a positive significant correlation between *E. coli* and flow during wet weather at a 90 percent confidence interval as presented in Table 3-11. Overall, the relationship of the FIB to flow was positive although *Enterococcus* sp. was insignificant and weak. On the contrary, it is interesting to note that the relationship to flow with the molecular markers was significant and negative during wet season, whereas concentration of molecular markers decreased with an increase in flow (Table 3-11).

Table 3-11: Spearman's Rank Correlation – Wet Season Data

Variable	Flow			
	n	ρ	Critical Value	Significant at α=0.10
E. coli	39	0.309	0.267	Yes
Enterococcus sp.	39	0.195	0.267	No
Fecal *Bacteroides* spp.	36	-0.462	0.279	Yes
BacHum Marker	36	-0.396	0.279	Yes

The fecal *Bacteroides* spp. had the strongest relationship to flow during the wet season, with a p value of -0.462. This finding is likely dominated by the amount of sampling conducted in specific areas where flow was measured (i.e., not the river mouth), and the generally low sample size for correlative analysis (n=31). Even given the caveats for the observed statistical relationship, the strong negative relationship between a marker of human-related bacterial sources and flow may be indicative of the importance of human-related bacterial sources stemming from low flow areas or lower dilution (i.e., small tributaries). Dilution may be playing a role, or the bacterial signal may be stronger, therefore, it is a high concentration to start with and discharge plays a relatively lesser role. Additional data is needed to further characterize the relationship between concentration and flow. Specifically, the higher the number of samples collected, or *n*, the more the statistical power of the relationship would increase dramatically.

3.2.5 Bacterial Loading Patterns

Loading estimates for dry and wet weather indicate an important departure from the concentration results. Loading of FIB and molecular markers during wet season was two to four orders of magnitude greater than dry season loading, as shown in Tables 3-8 and 3-9. This dramatic difference in loading patterns persisted regardless of whether sampling was conducted during a storm event, or after the storm event had passed (base flow), indicating the importance of considering the loading data carefully for appropriate BMP selection in given locations.

There were also some differences in the loading patterns of *E. coli*, *Enterococcus* sp., and fecal *Bacteroides* spp. between storms, and the two storm events sampled were of very different total rainfall amounts. However, with only two storm events sampled, and without integrated flow measurements over the course of storms, it is difficult to know if this trend is real. Previously, rainfall amounts have been shown to be significantly correlated with conventional FIB loads (Stumpf et al. 2010). In the SLR River, it remains to be seen if this pattern would also hold true. Several studies have found significant correlations between conventional FIB concentrations and stormwater flow (e.g., Davis et al. 1977, Stumpf et al. 2010).

Table 3-12: Dry Season Loading Estimates

Event	Date	Site Description	*E. coli* Daily Load (MPN/day)	*Enterococcus* sp. Daily Load (MPN/day)	Fecal *Bacteroides* spp. Daily Load (CE/day)	BacHum Marker Mean Daily Load (CE/day)
Dry 1	6/18/2008	Bonsall Bridge	2.57E+11	1.94E+10	NA	NA
Dry 1	6/18/2008	Sleeping Indian	1.19E+09	7.66E+08	NA	NA
Dry 1	6/18/2008	Murray Bridge	1.18E+10	1.34E+10	NA	NA
Dry 1	6/18/2008	Douglas Bridge	2.26E+11	5.74E+10	NA	NA
Dry 1	6/18/2008	Pilgrim Creek	DRY	DRY	DRY	DRY
Dry 1	6/18/2008	Critical Point	1.15E+10	9.47E+09	NA	NA
Dry 1	6/19/2008	Bonsall Bridge	4.05E+09	2.16E+10	NA	NA
Dry 1	6/19/2008	Sleeping Indian	1.94E+09	7.55E+08	NA	NA
Dry 1	6/19/2008	Murray Bridge	7.77E+09	3.83E+10	NA	NA
Dry 1	6/19/2008	Douglas Bridge	1.95E+11	1.12E+11	NA	NA
Dry 1	6/19/2008	Pilgrim Creek	3.33E+08	6.34E+07	NA	NA
Dry 1	6/19/2008	Critical Point	6.57E+09	1.75E+10	NA	NA
Dry 2	7/23/2008	Bonsall Bridge	2.30E+10	1.99E+09	NA	NA
Dry 2	7/23/2008	Sleeping Indian	3.18E+08	2.33E+08	NA	NA
Dry 2	7/23/2008	Murray Bridge	2.32E+08	4.63E+08	NA	NA
Dry 2	7/23/2008	Douglas Bridge	1.59E+08	2.56E+07	NA	NA
Dry 2	7/23/2008	Critical Point	9.83E+09	3.67E+08	NA	NA
Dry 2	7/24/2008	Bonsall Bridge	2.24E+10	1.60E+09	NA	NA
Dry 2	7/24/2008	Sleeping Indian	2.84E+07	1.19E+08	NA	NA
Dry 2	7/24/2008	Murray Bridge	2.24E+08	1.16E+09	NA	NA
Dry 2	7/24/2008	Douglas Bridge	1.26E+07	6.30E+06	NA	NA
Dry 2	7/24/2008	Critical Point	2.81E+09	6.86E+08	NA	NA
Dry 3	5/18/2010	Bonsall Bridge	NA	2.53E+10	2.50E+15	9.76E+12
Dry 3	5/18/2010	Sleeping Indian	NA	5.92E+08	1.33E+09	2.79E+10
Dry 3	5/18/2010	Murray Bridge	NA	1.25E+11	1.72E+16	5.49E+12
Dry 3	5/18/2010	Douglas Bridge	NA	3.78E+10	9.00E+15	3.93E+13
Dry 3	5/18/2010	Pilgrim Creek	NA	9.12E+09	2.08E+13	2.84E+11
Dry 3	5/18/2010	Critical Point	NA	9.79E+09	5.57E+15	1.61E+13
Dry 3	5/19/2010	Bonsall Bridge	7.09E+10	3.46E+10	4.57E+15	8.98E+11
Dry 3	5/19/2010	Critical Point	2.54E+10	2.59E+10	9.39E+15	2.65E+08
Dry 3	5/20/2010	Bonsall Bridge	7.09E+10	7.02E+10	5.87E+15	7.57E+12
Dry 3	5/20/2010	Critical Point	3.18E+10	1.09E+10	7.46E+15	1.56E+13

Table 3-13: Wet Season Loading Estimates

Event	Date	Site Description	*E. coli* Daily Load (MPN/day)	*Enterococcus* sp. Daily Load (MPN/day)	Fecal *Bacteroides* spp. Daily Load (cell equivalents/day)	BacHum Marker Mean Daily Load (DNA copies/day)
Baseline	12/9/2010	Bonsall Bridge	3.84E+09	2.98E+09	6.28E+13	1.41E+09
Baseline	12/9/2010	Sleeping Indian	DRY	DRY	DRY	DRY
Baseline	12/9/2010	Murray Bridge	1.12E+12	2.19E+10	4.35E+14	1.64E+09
Baseline	12/9/2010	Douglas Bridge	1.54E+10	4.30E+10	3.25E+14	7.16E+09
Baseline	12/9/2010	Pilgrim Creek	2.34E+09	1.81E+11	1.68E+13	1.18E+10
Baseline	12/15/2010	Critical Point	6.23E+13	2.79E+14	1.23E+14	6.49E+11
Wet 1	1/3/2011	Bonsall Bridge	5.08E+12	2.80E+12	1.28E+13	1.93E+12
Wet 1	1/3/2011	Sleeping Indian	NA	NA	NA	NA
Wet 1	1/3/2011	Murray Bridge	1.31E+13	5.93E+13	9.91E+13	1.86E+13
Wet 1	1/3/2011	Douglas Bridge	3.35E+12	1.32E+13	8.91E+12	4.51E+12
Wet 1	1/3/2011	Pilgrim Creek	2.49E+12	2.00E+12	9.97E+08	1.56E+12
Wet 1	1/3/2011	Critical Point	2.23E+13	1.36E+13	6.76E+09	2.97E+12
Wet 1	1/4/2011	Bonsall Bridge	3.30E+12	2.71E+12	4.85E+09	2.46E+12
Wet 1	1/4/2011	Sleeping Indian	NA	NA	NA	NA
Wet 1	1/4/2011	Murray Bridge	1.20E+12	9.18E+11	1.65E+12	4.06E+12
Wet 1	1/4/2011	Douglas Bridge	6.36E+12	2.52E+12	1.19E+13	4.97E+11
Wet 1	1/4/2011	Pilgrim Creek	8.76E+11	9.66E+11	0.00E+00	6.95E+11
Wet 1	1/4/2011	Critical Point	7.59E+12	3.96E+12	6.42E+12	4.14E+12
Base Flow 1	1/20/2011	Bonsall Bridge	3.63E+11	1.27E+12	7.10E+13	2.65E+13
Base Flow 1	1/20/2011	Sleeping Indian	2.15E+07	6.46E+07	1.17E+09	3.46E+09
Base Flow 1	1/20/2011	Murray Bridge	2.53E+11	2.76E+11	1.16E+14	2.24E+13
Base Flow 1	1/20/2011	Douglas Bridge	1.38E+11	4.75E+11	5.41E+13	1.40E+13
Base Flow 1	1/20/2011	Pilgrim Creek	4.43E+10	2.84E+10	1.34E+12	4.09E+12
Base Flow 1	1/20/2011	Critical Point	2.11E+12	3.91E+12	7.51E+13	1.01E+13
Wet 2	2/17/2011	Bonsall Bridge	6.39E+11	3.63E+12	9.98E+13	4.00E+13
Wet 2	2/17/2011	Sleeping Indian	1.63E+08	1.63E+08	1.39E+10	2.50E+11
Wet 2	2/17/2011	Murray Bridge	6.36E+11	3.97E+11	2.25E+14	6.22E+13
Wet 2	2/17/2011	Douglas Bridge	3.62E+11	3.97E+11	8.56E+13	4.01E+13
Wet 2	2/17/2011	Pilgrim Creek	4.22E+10	1.82E+10	1.70E+13	6.09E+12
Wet 2	2/17/2011	Critical Point	2.91E+12	4.55E+12	2.79E+13	1.51E+12
Wet 2	2/18/2011	Bonsall Bridge	1.40E+11	4.43E+10	NA	NA
Wet 2	2/18/2011	Sleeping Indian	3.27E+09	4.14E+07	9.46E+10	1.75E+12
Wet 2	2/18/2011	Murray Bridge	1.78E+11	1.02E+11	0.00E+00	0.00E+00
Wet 2	2/18/2011	Douglas Bridge	9.43E+10	7.43E+10	NA	NA
Wet 2	2/18/2011	Pilgrim Creek	1.78E+10	1.41E+10	7.89E+12	5.99E+12
Wet 2	2/18/2011	Critical Point	2.05E+12	1.81E+12	7.45E+13	1.52E+13

Table 3-13 Wet Season Loading Estimates (continued)

Event	Date	Site Description	*E. coli* Daily Load (MPN/day)	*Enterococcus* sp. Daily Load (MPN/day)	Fecal *Bacteroides* spp. Daily Load (cell equivalents/day)	BacHum Marker Mean Daily Load (DNA copies/day)
Base Flow 2	2/24/2011	Bonsall Bridge	3.76E+11	6.23E+11	4.70E+13	1.24E+13
Base Flow 2	2/24/2011	Sleeping Indian	8.20E+07	2.15E+08	5.95E+09	1.65E+11
Base Flow 2	2/24/2011	Murray Bridge	4.58E+11	4.58E+11	6.28E+13	1.88E+13
Base Flow 2	2/24/2011	Douglas Bridge	3.93E+11	9.82E+11	4.37E+13	1.01E+13
Base Flow 2	2/24/2011	Pilgrim Creek	8.19E+09	1.36E+10	5.02E+12	2.42E+12
Base Flow 2	2/24/2011	Critical Point	3.72E+12	5.16E+12	6.85E+13	1.47E+13

In this study, samples were collected twice over the extent of the hydrograph, and two storm events were monitored. The percentages of the total loads that occurred during the first 24 hours of the storm events are presented in Tables 3-14 and 3-15. The bacterial load from the first 24 hours of the event was compared to the total load of the event. This simple examination demonstrates that there is a trend toward delivery of microbial contaminants in the first half of the storm event. It may indicate if there is a first flush effect, aiding in the selection and design of appropriate BMPs.

To conduct this assessment with increased resolution, typically as many as 5 to 10 samples are collected over the course of a single storm event to determine FIB concentrations (and other markers), while fully characterizing flow and hydrograph characteristics (Stumpf et al. 2010). For example, this will allow an assessment of first flush conditions at multiple increments over the hydrograph, such as the first 30 minutes, 60 minutes, and 90 minutes.

Table 3-14: Wet Event 1 First Flush Analysis

Site Type	Site	Percent of Total Load[(a)]			
		E. coli	*Enterococcus* sp.	Fecal *Bacteroides* spp.	BacHum Marker Mean
Main Stem	Bonsall Bridge	61%	51%	100%	44%
Tributary	Sleeping Indian	NA	NA	NA	NA
Main Stem	Murray Bridge	92%	98%	98%	82%
Main Stem	Douglas Bridge	35%	84%	43%	90%
Tributary	Pilgrim Creek	74%	67%	100%	69%
Main Stem	Critical point	75%	77%	0%	42%

(a) Percent of total load that occurred during the first 24 hours of the storm event.
NA = Not Analyzed

Table 3-15: Wet Event 2 First Flush Analysis

Site Type	Site	Percent of Total Load[(a)]			
		E. coli	***Enterococcus* sp.**	**Fecal *Bacteroides* spp.**	**BacHum Marker Mean**
Main Stem	Bonsall	82%	99%	NA	NA
Tributary	Sleeping Indian	5%	80%	13%	13%
Main Stem	Murray Bridge	78%	80%	NA	NA
Main Stem	Douglas Bridge	79%	84%	NA	NA
Tributary	Pilgrim Creek	70%	56%	68%	50%
Main Stem	Critical Point	59%	72%	27%	9%

(a) Percent of total load that occurred during the first 24 hours of the storm event.
NA = Not Analyzed

3.2.6 Community Analysis Using TRFLP

The bacterial community analysis was conducted on paired water and sediment samples using terminal restriction fragment length polymorphism (TRFLP) to characterize the relationship between the bacterial communities in the water column and sediment of the river mouth. For the community analyses, it is important to note that the dataset is limited by the number of samples were analyzed with limited spatial (only river mouth) and temporal (only two dry sampling events) coverage for this component. Some interesting patterns are indicated, and more work (with upstream samples or wet samples) may be helpful in the future. The following results and discussions are related only to the samples included in this component.

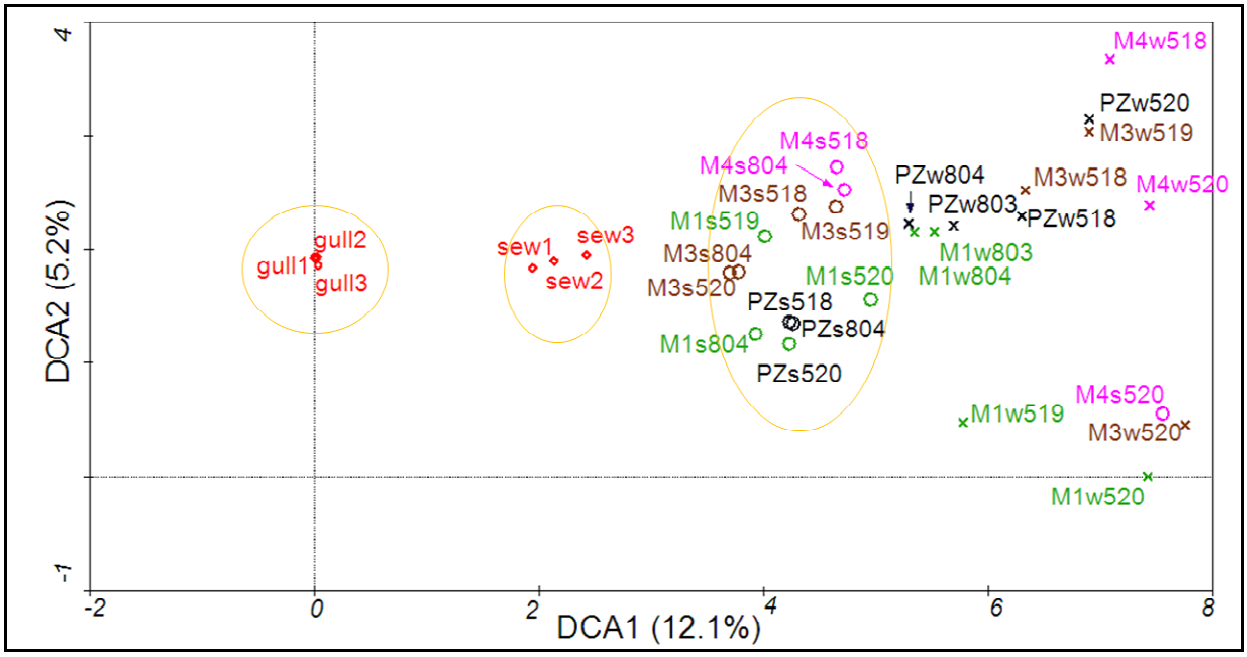
To conduct an assessment of the likelihood for known fecal sources to contribute to the community analyses patterns that were observed, known fecal material from seagulls and from sewage treatment plants was analyzed separately, but, included in the bacterial community analysis. The sources of the material were composited gull fecal matter from Orange County beaches, and sewage influent combined from three major sewage treatment plants in Orange County, CA. When considering the known fecal sources, it appears that gull feces were not a major fecal source to the river mouth water samples (Figure 3-31). Along the first detrended correspondence axis (DCA), gull samples grouped tightly together, but >5 SD units away from the water samples, indicating little similarity between microbial community composition in gull and water samples. An example of raw TRFLP data is included (Figure 3-32).

Sewage influent generally does not appear to be a major source to the water samples either (Figure 3-31). Along the first DCA, the three sewage samples are grouped together, and are far away from the May water samples (>3SD), indicating no major overlap between microbial communities between sewage and

water samples. On the contrary, the sewage samples are more closely related to the sediment samples (2 SD).

A comparison of three bacterial community raw DNA profiles as shown in Figure 3-32 does indicate some level of similarity between sewage and sediment samples, potentially indicating the importance of re-suspension in the river mouth area, especially during times when the berm is closed (i.e., the hydrological connection between the river mouth and Point Zero is limited). The results of this analysis are limited because neither local (City of Oceanside) sewage influent nor local bird populations were used as known fecal sources. The inclusion of these types of known samples would be prudent in any future bacterial community analysis approach.

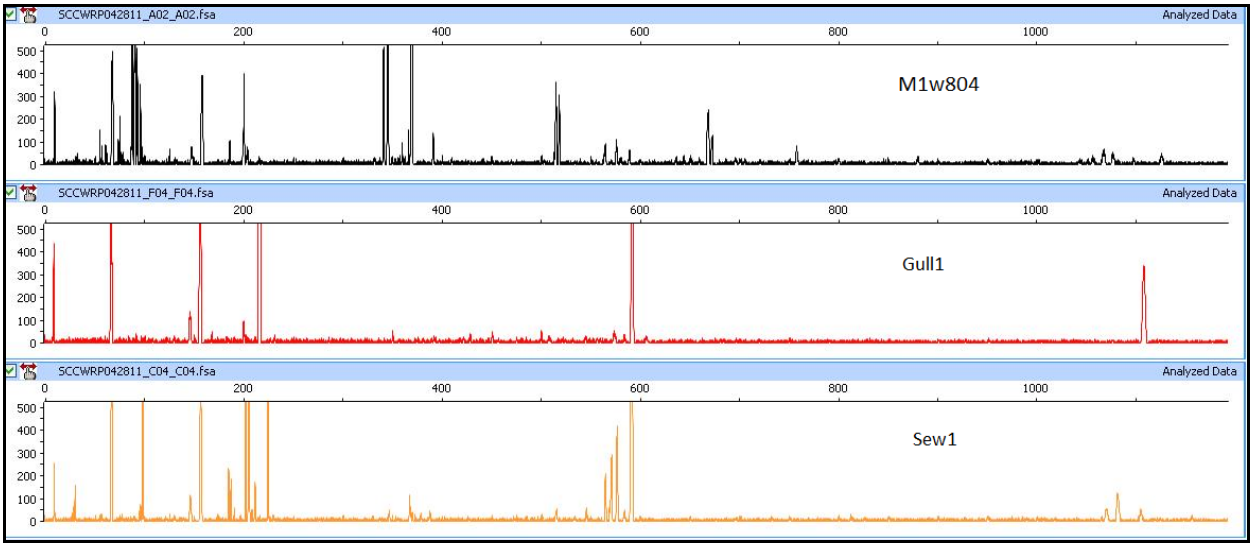
Figure 3-31: DCA Plot with All Samples



Gull 1, 2, 3 are known composite gull fecal material from Orange County beaches.
Sew 1, 2, 3 are known fecal material from three sewage treatment plants in Orange County.
Project Samples were assigned identification numbers according to the following naming convention: Site Name Abbreviation – Sample Type Code – Date

Site Name Abbreviations	Sample Type Code	Date
Point Zero (PZ) River Mouth 1 (M1) River Mouth 2 (M2) River Mouth 3 (M3) River Mouth 4 (M4)	There are two sample type codes: 's' for sediment 'w' for water	May, 18, 19, 20, 2010 (518, 519, 520) August 3 and 4, 2010 (803, 804)

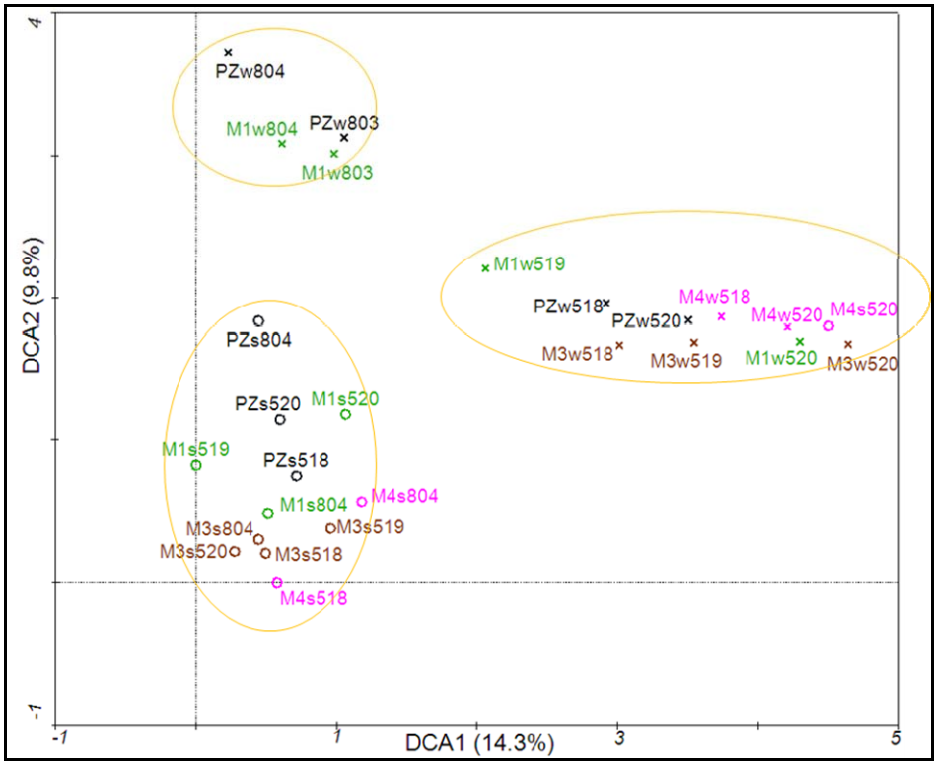
Figure 3-32: Raw TRFLP Data



Gull 1is one known composite gull fecal material from Orange County beaches.
Sew 1 is one known fecal material from three sewage treatment plants in Orange County.
M1w804 is one water sample collected at River Mouth 1 on August 4, 2010.

Overall, sediment does not appear to contribute significantly to bacteria in the water column during the May 2010 dry weather sampling event (Dry Event 3) as seen in Figure 3-33. The sediment bacterial community appears to be fairly stable/similar over these two sampling events, as indicated by their grouping together, with the exception of M4s520. The August 2010 water samples are grouped together and appear to be more similar to sediment (along the first DCA) than the May 2010 water samples, indicating that the May 2010 samples may have been more influenced from upstream (which was not included for bacterial community analysis) than from sediment in the river mouth. The four August 2010 water samples also have slightly higher *Enterococcus* sp. and *E. coli* concentrations, while the FIB for the May 2010 samples were mostly around detection limits.

Figure 3-33: DCA Plot for SLR Samples Only



Project Samples were assigned identification numbers according to the following naming convention: Site Name Abbreviation – Sample Type Code – Date

Site Name Abbreviations	Sample Type Code	Date
Point Zero (PZ) River Mouth 1 (M1) River Mouth 2 (M2) River Mouth 3 (M3) River Mouth 4 (M4)	There are two sample type codes: 's' for sediment 'w' for water	May, 18, 19, and 20, 2010 (518, 519, 520) August 3 and 4, 2010 (803, 804)

3.2.7 Summary of Overall Findings

Several observations can be made when examining the microbial contaminants investigated for this project holistically (FIB and molecular markers for microbial source tracking, and bacterial community analysis). First, during dry weather, there were days, particularly during the August sampling event, for which all three markers of human-related bacterial sources were positive and quantifiable and the *Enterococcus* sp. concentrations were all below the single sample standard for recreational waters. There was only one instance where enteroviruses were positive for the entire study, and this was in the river mouth (concentration >10,000 enterovirus PFU/100 ml). There were also high concentrations of gull bacteria at the river mouth and at Point Zero, indicating the potential for the presence of pathogens stemming from bird feces. Even though pathogens could be present, the lack of hydrological connection because of the sand berm likely reduces the exposure of the ocean swimming public to potential

pathogens during "berm closed" periods. The bacterial community analysis indicated some linkage between sediment and water samples during the August dry weather period, as evidenced by the proximity of the samples horizontally along the "DC1" axis (Figure 3-33). In addition, the examination of TRFLP patterns from selected composited gull fecal and sewage influent source samples, indicates that the gull samples grouped a distance away from the ambient environmental samples (Figure 3-31), with the sewage samples grouping close to (within 2 standard deviation), of the August sediment samples. While this data provides an interesting look at the river mouth from a community perspective, further work would need to be done on a higher number of samples, collected over a range of conditions, and analyzed in conjunction with fecal source samples from local entities (the fecal source samples used here were collected in Orange County, California).

During wet weather, there was a substantial increase in FIB concentrations from dry weather. It is interesting to note that FIB concentrations had a positive, although generally weak correlation with flow. On the contrary, fecal *Bacteroides* spp. and BacHum marker, two fecal markers quantified via QPCR, had significant and negative relationships to flow. These relationships may be important, even though it is based on a small total number of sampling events for this study. Loading estimates for dry and wet weather indicate an important departure from the concentration results. Loading of FIB and molecular markers during wet season were two to four orders of magnitude higher than dry season loading. During base flow events, FIB concentrations and loading were similar to FIB levels and loadings during storm events. Base flow conditions proved to be critical periods within the river system as it relates to bacterial sources. Additional data is needed to further characterize the relationship between FIB concentrations and flow and to verify the wet season patterns indicated in this study.

3.3 QUALITY ASSURANCE/QUALITY CONTROL (QA/QC) RESULTS

Samples were collected according to the Lower San Luis Rey River Source Identification Project QAPP and have been compared to Project data quality objectives (DQOs) for field and laboratory Quality Assurance/Quality Control (QA/QC) samples. Laboratory blanks achieved 100 percent completeness and acceptance. Positive control and negative control results were accepted for 100 percent of samples however, samples were analyzed for 88 percent and 59 percent of the 17 batches analyzed, respectively. During dry season events, positive controls were run each consecutive day of sampling whereas, negative controls were run on the first day of each event per the request of the Lead Professional Investigator. Positive and negative controls were not run with two analytical batches associated with Wet Event 1 due to a communication error with the laboratory. The original microbiological laboratory closed down in December 2010 and new laboratory was contracted prior to Wet Event 1 in January 2011 and positive and

negative controls were not clearly requested by the Project Coordinator. Laboratory QA/QC samples are presented in Table 3-16.

Table 3-16: Summary of Laboratory QA/QC Samples

Type	Batch Count	Analysis Frequency DQO	Analysis Frequency Completeness	Acceptance DQO	Percent Acceptance Achieved
Lab Blank	17	One per batch	100%	<10% MDL	100% Pass
Negative Control	10	One per batch	59%	Lot must pass	100% Pass
Positive Control	15	One per batch	88%	Lot must pass	100% Pass

Field blank and field duplicate samples were collected at a frequency of 7 and 9 percent, respectively, which exceeded the completeness DQO of 5 percent of all program samples. All field blanks collected met the acceptance DQO; however, 37 percent of field duplicates collected had a relative percent difference (RPD) of less than 25 percent. A RPD of less than 25 percent is the recommended criteria as set forth by the SWAMP 2008 QAPP for pathogens. There is however, discussion within the environmental monitoring community that a RPD of less than or equal to 50 percent may be acceptable for field duplicates due to the heterogeneous nature of bacteria populations in surface water ("National Beach Guidance and Required Performance Criteria for Grants" June 2002, page H-14, USEPA). If this secondary RPD criterion was used, 74 percent of field duplicates would meet acceptance standards. Field QA/QC samples are presented in Table 3-17.

Table 3-17: Field Data Quality Objectives

Type	Samples Collected	Collection Frequency DQO	Actual Collection Frequency	Acceptance DQO	Percent Acceptance Achieved
Field Blank	16	5% of all program samples	7% of all program samples	<RL	100%
Field Duplicate	20	5% of all program samples	9% of all program samples	RPD <25% RPD <50%	37% 74%

4.0 CONCLUSIONS AND RECOMMENDATIONS

4.1 SUMMARY OF CONCLUSIONS

The Lower SLR River Bacterial Source Identification Project provided a broad characterization of bacterial concentrations throughout the SLR River and river mouth during wet and dry seasons. Both anthropogenic and non-anthropogenic sources were identified as contributing bacterial sources. Concentrations and loading patterns for FIB and human-specific markers were notably higher during the wet season in the river and tributaries. A summary of conclusions is provided below for the river mouth, main stem, and tributary locations:

River Mouth:

- FIB concentrations were generally below WQOs in the river mouth during the summer months when human exposure and health risks are highest at the Pacific Ocean.
- Although FIB concentrations were below WQOs, multiple MST molecular markers for human-related bacterial sources were present in August when the river mouth was closed to ocean inputs and SLR River flow was significantly reduced. Human sources of contamination have been identified subsequently in this area, including an active sewer pipe. Other factors could be important to the retention of fecal contamination in this area, including low residence time and hydrologic conditions in the river mouth during low flow.
- The bacterial community analysis showed a lack of relationship between the sediment and water samples in May when the river mouth is open to tidal exchange. Conversely, there was a strong relationship between the sediment and water samples in August when the river mouth is closed. The sediment bacterial community is stable during the dry season and was not analyzed during the wet season. It should be noted that the bacterial community analysis was only conducted on river mouth samples and no analysis was conducted to evaluate the relationship between the SLR River's main stem and tributaries and the river mouth monitoring locations.
- No enteroviruses were recorded during the dry season.
- A strong signal of gull feces was found in the river mouth during the wet and dry seasons.
- During the dry season, average FIB concentrations at river mouth sites were generally less than the range of concentrations for developed watersheds, based on the SCCWRP Natural Loadings Study (Stein and Yoon, 2007). River Mouth 2 and 4 had average *Enterococcus* sp. concentrations and average *E. coli* concentrations typically found within the natural watershed range.
- FIB concentrations significantly increased in the wet season resulting in a higher frequency of WQO exceedances.
- There was evidence of potential human-related bacterial sources (a minimum of two out of three markers) at River Mouth 1 and River Mouth 3 throughout the wet season, while at Point Zero there was evidence of potential human-related bacterial sources (a minimum of two out of three markers) at a lower frequency.
- One sample collected at River Mouth 1 during Wet Event 1 was positive for enteroviruses.

- During the wet season, the average concentrations at all monitored sites were less than the 25th percentile concentration for developed watersheds, based on a comparison with the SCCWRP Natural Loadings Study.
- Of the 74 samples from the DEH AB411 program, 12 results for *Enterococcus* sp, and 4 results for fecal coliform were above the respective WQOs, whereas no total coliform results exceeded the WQO.

SLR River Main Stem:

- The MST Program study data at the SLR main stem reinforced trends in elevated FIB concentrations seen in the data collected by the Joint Monitoring Program.
- In May of 2010, there were potential (two of three markers) human-related bacterial sources present at Bonsall Bridge, Douglas Bridge, and the Critical Point.
- During the dry season, average FIB concentrations at main stem sites were generally less than the range of concentrations for developed watersheds, based on the SCCWRP Natural Loadings Study. During the dry season, average *Enterococcus* sp. concentrations at the Critical Point, and average *E. coli* concentrations at Murray Bridge, were found to be within the natural watershed range.
- No enteroviruses were detected in samples at the main stem monitoring locations during dry or wet seasons.
- During the wet season, the Douglas Bridge and Critical Point locations exhibited strong positive signals for all three MST molecular markers of human fecal *Bacteroides* spp.
- FIB concentrations and loadings did not change significantly in the river during base flow conditions.
- A trend toward delivery of bacterial loadings in the first half of the storm event was demonstrated by a simple first flush analysis. This indicates a possible first flush effect and may aid in the future selection and design of appropriate BMPs.
- During the wet season the average concentrations at all monitored sites were less than the 25th percentile concentration for developed watersheds. Bonsall Bridge, Douglas Bridge, and Point Zero also had average *Enterococcus* sp. concentrations that fell within the natural watershed range.

Tributaries:

- The MST Program study data at the SLR tributaries reinforced trends in elevated FIB concentrations seen in the data collected by the Joint Monitoring Program.
- In May of 2010, there were potential human-related bacterial sources present at Sleeping Indian and Pilgrim Creek.
- During the dry season, average *Enterococcus* sp. concentrations at tributary sites were generally less than the range of concentrations for developed watersheds based on the SCCWRP Natural Loadings Study. However, Sleeping Indian and Pilgrim Creek had average dry season *E. coli* concentrations greater than the 75th percentile concentration for developed watersheds.
- No enteroviruses were detected in samples at the tributaries during dry or wet seasons.

- During the wet season, Sleeping Indian and Pilgrim Creek exhibited strong positive signals for all three MST molecular markers of human fecal *Bacteroides* spp.
- FIB concentrations and loadings did not change significantly in the tributaries after storms passed during base flow conditions.
- The assessment of flow relative to concentrations of FIB and human-specific markers indicates the importance of bacterial sources stemming from low flow areas or low dilution (i.e., tributaries).
- A trend toward delivery of bacterial loadings in the first half of the storm event was demonstrated by a simple first flush analysis. This indicates a possible first flush effect and may aid in the future selection and design of appropriate BMPs.
- Both tributaries (Sleeping Indian and Pilgrim Creek) had average dry season *E. coli* concentrations greater than the 75th percentile concentration for developed watersheds. However, the average *Enterococcus* sp. concentrations at tributary sites were less than the 25th percentile concentration for developed watersheds.

To identify the locations with the highest levels of bacterial contamination the following factors were taken into consideration: average percent exceedance of FIB WQOs, frequency and presence of multiple fecal *Bacteroides* spp. human-specific markers, presence of gull feces, and presence of enteroviruses (Table 4-1). Individual factors were assigned equal weight and a site was considered high risk based on the frequency of medium and high levels of concern. Sleeping Indian, Douglas Bridge, Pilgrim Creek, Critical Point, and River Mouth 3 are considered high risk locations for probable human-related sources. River Mouth 1 needs to be considered for future study since it had moderate to high levels of human-related sources based on the results of the *Bacteroides* assays and a positive result for enteroviruses during a storm event.

Table 4-1: Bacterial Hot Spot Assessment Matrix

Monitoring Locations		Dry Season June 18, 19 & July 23, 24, 2008 May 18, 19, 20 & August 3,4, 2010							Storm Events January 3, 4, 2011 February 17, 18, 2011							Baseline and Base Flow Events December 9, 15, 2010 January 20 & February 24, 2011						
		Average % Exceedance of FIB WQOs	Number of samples analyzed by molecular, gull, and viral	Fecal *Bacteroides* spp. Assay: 1 out of 3 human-specific markers	Fecal *Bacteroides* spp. Assay: 2 out of 3 human-specific markers	Fecal *Bacteroides* spp. Assay: 3 out of 3 human-specific markers	High concentrations of Gull bacteria	Presence of Enteroviruses	Average % Exceedance of FIB WQOs	Number of samples analyzed by molecular, gull, and viral	Fecal *Bacteroides* spp. Assay: 1 out of 3 human-specific markers	Fecal *Bacteroides* spp. Assay: 2 out of 3 human-specific markers	Fecal *Bacteroides* spp. Assay: 3 out of 3 human-specific markers	High concentrations of Gull bacteria	Presence of Enteroviruses	Average % Exceedance of FIB Rec-1 WQOs	Number of samples analyzed by molecular, gull, and viral	Fecal *Bacteroides* spp. Assay: 1 out of 3 human-specific markers	Fecal *Bacteroides* spp. Assay: 2 out of 3 human-specific markers	Fecal *Bacteroides* spp. Assay: 3 out of 3 human-specific markers	High concentrations of Gull bacteria	Presence of Enteroviruses
Upstream	Bonsall Bridge	30	3	1	2	--	NA	--	60	3	1	2	--	NA	--	50	3	--	3	--	NA	--
	*Sleeping Indian	65	1	--	1	--	NA	--	75	4	--	3	1	NA	--	50	2	--	2	--	NA	--
	Murray Bridge	20	1	1	--	--	NA	--	50	3	--	3	--	NA	--	67	3	--	3	--	NA	--
	Douglas Bridge	45	1	--	1	--	NA	--	63	3	--	2	1	NA	--	50	3	--	2	1	NA	--
	*Pilgrim Creek	17	1	--	1	--	NA	--	88	4	2	--	2	NA	--	67	3	--	1	2	NA	--
	Critical Point	7	3	1	2	--	NA	--	100	4	1	1	2	NA	--	83	2	--	1	1	NA	--
	River Mouth 3	0	2	--	--	2	2	--	88	4	1	3	-	2	--	50	2	--	2	--	1	--
	River Mouth 1	0	3	1	2	--	3	--	88	4	1	3	-	3	1	33	1	--	1	--	1	--
Downstream	Point Zero	0	2	--	2	--	2	--	50	3	-	3	-	2	--	33	1	--	1	--	1	--

Level of % Exceedance:

Low	0- 25 %
Medium	25-50%
High	>50%

Level of Molecular Indicators:

Low	1 result of 1 or 2 markers
Medium	2 results of 1 or 2 markers
High	3 results of 2 markers or > 1 result of 3 markers

Notes:

NA – Analysis Not Requested

(--) symbol – Not present

*Denotes a Tributary monitoring location

4.2 RECOMMENDATIONS

The SLR River MST Program was designed as the first phase in source identification to assess the magnitude and extent of elevated FIB and to characterize sources on a broad scale. This study focused on assessing the potential of anthropogenic (human-specific) and non-anthropogenic (gull) sources within the SLR Watershed. The results indicate that both types of sources are present. To focus on the anthropogenic sources, follow-up investigations targeting human sources on a smaller scale are required. While broad management measures are identified, the results from the follow-up source investigations will allow a more refined look at these measures and will these provide further information to help select the most feasible and effective management strategies necessary to reduce bacterial concentrations.

4.2.1 Follow-up Source Investigations

In order to address potential management options, further monitoring is required to increase the data resolution and quantity at a given site. This will provide a stronger basis for making management decisions. The following recommendations may be considered when designing follow-up source investigations within the Sleeping Indian and Pilgrim Creek sub-drainage areas.

- To identify possible sources of overflow or leaks within the respective tributary drainage areas, conduct a desktop GIS analysis of City sewer infrastructure and septic (onsite) wastewater systems. Field surveys may be prompted to identify leaks or illicit connections such as dye testing or closed circuit television (CCTV).
- To identify potentially failing septic systems, design a comprehensive monitoring and inspection program. Based on available resources, monitoring septic systems may utilize viral tracing techniques in conjunction with dye testing to track fate and transport of the contaminants. Viral tracing has proven to be more effective at determining the condition of septic systems than standard dye testing alone, which can rapidly, dilute in the system (Feldner, 2007). Viral tracing is implemented by seeding a viral indicator such as MS2 coliphage (single stranded RNA virus that infects *E. coli*) into septic systems, then tracking it using both QRTPCR and plaque assay methods. Monitoring includes collection of water samples from monitoring wells near leach lines, nearby ditches, and receiving surface waters to analyze for FIB and viral tracer(s) (Conn, 2011).
- Higher resolution data is required for an evaluation of loading and possible implementation of structural treatment control BMPs. This may be accomplished through continuous flow and higher resolution FIB sampling at the outlets during the wet season to characterize conditions over the course of the hydrograph and continuous flow and higher resolution FIB sampling at the outlet over a 24 hour period during dry weather (including base flow conditions and dry season, when flow is present).
- Follow up source identification in the drainage area during base flow. Collect samples upstream isolating major inputs to the tributary. Conduct targeted visual surveys adjacent to major inputs to document potential bacterial sources.

The following recommendations may be considered when designing follow-up source investigations at the main stem locations.

- To identify possible sources of overflow or leaks along the main stem river with emphasis on infrastructure that crosses the river, conduct a desktop GIS analysis of City sewer infrastructure and septic systems. Field surveys may be prompted to identify leaks or illicit connections such as dye testing or closed circuit television (CCTV) surveys.
- An Exfiltration Study may be conducted on high priority sections of pipe as an additional method to identify leaks, cracks, or displaced pipe on a sewer main. First, the City needs to prioritize force mains that have the highest potential to impact the river. Based on available resources, an exfiltration assessment may include testing the flow rate before and after the section of interest to calculate the change in flow rate as a result of exfiltration, in conjunction with CCTV surveys, and/or other methods to identify leaks.
- To identify areas of homeless encampments, conduct homeless population surveys. Homeless encampments were observed under bridges and tree canopy at main stem river locations during sampling.
- To understand bacterial levels between main stem locations such as Murray Bridge and Douglas Bridge, conduct a survey to identify any major inputs and conduct sampling at multiple locations between the two locations and at major inputs.

In addition to impacts from tributaries, other sources such as gulls, groundwater, and sediment may be contributing to the overall bacterial sources at the river mouth. Further studies would enable specific sources of human-related bacterial sources in the river mouth to be isolated. The following recommendations may be considered when designing follow-up source investigations in the river mouth.

- To identify possible sources of overflow or leaks within drainage areas adjacent to the river mouth, conduct desktop GIS analysis of City sewer infrastructure located west of the Critical Point.
- Characterize the flow patterns within the river mouth system to understand the general fate and transport of fecal bacteria sources.
- To further characterize the relationship between the sediment and water bacterial communities in the Lower SLR River and river mouth, conduct bacterial community analysis of paired water and sediment samples. Collect samples at monitoring locations in the river and river mouth during multiple events throughout the dry season when the river mouth is open and closed. Include gull and/or sewage samples from the City of Oceanside to improve the comparison of environmental samples with potential local sources.
- Monitor during the wet season after storm flows to better understand the role of re-suspension and flushing on increased FIB concentrations.
- Implement a groundwater study to gain a better understanding of groundwater dynamics and its relationship to surface water in SLR River during base flow conditions.

4.2.2 Assess Relationship between River Mouth and Ocean

To contribute to future achievement of Bacterial TMDL, it will be important to assess the interaction between the river mouth and the Pacific Ocean. The following monitoring recommendations will help further define the association between the river mouth and AB411 monitoring location.

- To characterize the dispersal of the bacterial and viral material from the SLR River outlet along the shoreline and determine the public health risk. Monitor additional sample locations along the beach shoreline between the river mouth and the AB411 compliance point on multiple consecutive days. Sampling would occur at a regularly spaced grid such as Point Zero, shoreline locations at 5 feet south, 25 feet south, 50 feet south and AB411 site (75 feet south), as well as a control site located approximately, 25 feet north.
- Focus sampling during the wet season and base flow conditions when the highest frequency of exceedances was recorded at the AB411 site.
- As higher resolution data are collected over the duration of storms, site-specific differences in loading and waste load delivery can be better assessed. This data can be used to guide BMP selection. Conduct pollutograph monitoring at a river mouth location to characterize bacteria over the course of a storm event and higher frequency sampling over a 24 hour period during base flow conditions.
- Employ an approach to allow for accurate loading calculations and assessment of bacterial loads and flux over the course of the monitoring period. Utilize acoustic Doppler current profilers (ADCPs) to collect continuous flow measurements during sampling events.

4.2.3 Management Measures

With additional data, focused management measures may be selected to reduce bacterial concentrations. Potential management strategies can be divided into three tiers, as detailed below. The three tiers were identified to consider the associated feasibility, data availability, difficulty, and time frame for implementation of management measures.

<u>Tier 1 Source Controls</u>

Given the elevated FIB concentrations and human-specific markers present during dry season and base flow events, source controls are considered the most feasible management measures for rapid implementation to address the relatively low flow volumes and help begin reducing bacterial concentrations. In addition to short-term management measures, long-term capital improvement projects have been suggested to address difference sources. Examples of source controls:

- Focused educational and outreach within these drainage areas based on activities and known land use types.
- Street sweeping and catch basin cleaning.

- Additional source controls may be identified based on the follow-up investigations, such as sewer/infrastructure repairs or elimination of an illicit connection.
- To identify potentially failing septic (onsite) wastewater systems viral tracking, dye testing, inspections and/or other methods may be utilized. Once identified, an inspection and maintenance schedule may be developed and revised to prioritize the septic systems in the SLR watershed, based on threats to groundwater and receiving water quality. Based on available resources and feasibility, a capital improvement plan for conversion of septic systems to sanitary sewers may be developed in appropriate areas.
- To address the human-related bacterial sources present in the river mouth during the dry season when it is closed to ocean inputs and SLR River flow is significantly reduced, evaluate the feasibility of hydraulic modifications to the river mouth and/or SLR River outlet to reduce residence time and increase flushing during the dry season. A feasibility study should be implemented after additional monitoring has been conducted to better understand the hydrology of the river mouth and retention of the FIB in the river mouth. Based on available resources, the feasibility study would identify benefits and constraints for current and alternative hydraulic configurations of the river mouth.

Tier 2 Decentralized Treatment Controls

Tier 2 decentralized treatment controls are designed to mitigate pollutants in runoff on a small, local scale. Main inputs identified during follow-up source investigations contributing elevated FIB concentrations to the tributaries and/or river mouth may be treated. Examples of decentralized treatment controls are low impact development related controls, such as bioswales and bioretention basins for small drainages and targeted small scale treatment systems.

Tier 3 Centralized Treatment Controls

If Tiers 1 and 2 have been implemented and TMDL end points have not been reached, centralized, regional, large-scale treatment BMPs may need to be considered. Treating high flow volumes associated with rainfall events can be costly, however, implementing treatment controls during base flow conditions may be a potentially more cost-effective method to initially target high risk locations identified during this study and any follow-up source investigations. However, if additional monitoring substantiates a first-flush effect then a feasibility study with conceptual level designs for treatment of the initial bacterial load would be beneficial. The tributaries may be the areas to focus initial management efforts for reducing microbial inputs into the SLR River and river mouth. BMP considerations at these locations could be instituted prior to these tributaries entering the SLR River, allowing for manageable treatment of volumes or flows. These include treatment train BMPs such as detention and/or infiltration basins, biofiltration, and/or UV treatment.

5.0 PROJECT PERFORMANCE

5.1 SUCCESSES AND FAILURES

Project completeness and success was determined using the measurement tools outlined in Table 1 of the PAEP. The MST Program, Joint Monitoring Program, and a visual observations program were implemented between 2008 and 2011 in accordance with the QAPP and MP. All samples collected by the MST Program and Joint Monitoring Program were analyzed for fecal indicator bacteria analyses. A multi-tiered analytical approach using QPCR methodologies was utilized to identify potential sources of fecal bacteria sources at monitoring locations with elevated FIB results. This project was successful in attaining its goals as described below:

- Goal 1: Assess where and what sources and activities have contributed to bacterial impairment of SLR River mouth. Anthropogenic and non-anthropogenic sources were identified as contributing bacterial sources. A chart of bacterial hot spots is provided in Section 4.0.
- Goal 2: Analyze potential bacterial source elimination or reduction practices that are targeted at identified sources. A discussion of recommended follow-up source investigation studies and management measures is provided in Section 4.0.
- Goal 3: Contribute to future achievement of bacterial TMDL objectives by effectively targeting sources.

Based on the MST data and City Joint Monitoring data, exceedances of the single-sample maximum Rec-1 WQOs appear to be occurring during wet and dry weather and have the potential to contribute to exceedances at the beach. A recommended monitoring approach to characterize the relationship between the river mouth and TMDL compliance point is outlined in Section 4.0.

The Lower SLR River Source Identification Project was a broad scale effort to assess the magnitude and extent of bacterial levels along the lower SLR River within the City of Oceanside. The Project utilized an adaptable approach that can easily be modified for similar microbial source tracking studies.

5.2 LESSONS LEARNED

A summation of lessons learned during the implementation of visual observations, sampling, and molecular source tracking is provided below.

5.2.1 Visual Observations

- The original format of visual observations was more qualitative and after the initial event, the Visual Observation Sheet was restructured to provide more quantitative information with increased detail to facilitate a comparison of overall sources between zones and events. Visual observations should include as much quantifiable data as possible.
- Visual observations were conducted mostly by Project team members, the City, and City of Vista personnel due the difficulty of scheduling volunteer activities during work days and with limited notice during the wet season. Schedules of private citizens should be taken into account when planning a volunteer program.

5.2.2 Sampling Approach

- It is important to note that the dataset is limited by the number of samples analyzed and flow measurements (maximum 10 samples per site) and by limited events monitored (only two – 3 dry sampling events per site, 2 storm events and 2 days of dry weather during wet season). Increased resolution would provide a more robust data set to evaluate site-specific seasonal and temporal loading patterns.
- For storm events, consider designing the monitoring program to account for short response times in tributary watersheds and longer response time in the main stem river. If possible, utilize equipment with remote sensing capabilities to trigger sample collection based on site-specific conditions.
- If the appropriate safety conditions exist and budget is available, sampling timeframe could be expanded to a 24 hour period. This would allow field crews to collect samples based the site-specific hydrograph.

5.2.3 Molecular Source Tracking

- In 2008, six dry season samples from main stem river and tributary locations were selected for further molecular analysis, but were not analyzed as a result of the stop work order in December 2008. Therefore, there were limited dry season samples collected at main stem and tributary locations. It is recommended that samples are processed within 30 days.
- Any future testing for human pathogenic enterovirus could incorporate filtrations at two volumes, to keep inhibition of the Q-RTPCR reaction controllable, while increasing the sensitivity of the assay.
- The molecular source tracking methodology was updated in 2010. Additional genetic tests are available to characterize the non-human sources and newer indicators may become available prior to the start of other programs.

5.3 FUTURE PROMOTION AND NEXT STEPS

In addition to the Clean Water Program Newsletter and Oceanside Magazine articles published during the implementation of the Project, the City of Oceanside and the Project team have been accepted to present the results of this project at the 2011 California Stormwater Quality Association (CASQA) conference.

This will provide an opportunity to disseminate the findings of the study, describe the microbial source tracking tools and approach, and share the next steps in the City's progress toward addressing the Bacteria I TMDL.

The results of this project will be used immediately by the SLR TMDL Responsible Parties in the development of the Comprehensive Load Reduction Plan (CLRP) addressing bacteria. The Project data will be used to focus the initial phase of CLRP implementation by assisting in the prioritization of source and sub-watershed activities.

With preliminary results indicating human sources, the City of Oceanside took a proactive approach and immediately began to implement a key recommendation from this project; the desktop GIS analysis of the City's sewage infrastructure. The Clean Water Program began work with the City's Sewer Division to map and identify the maintenance history of the City's targeted sewage infrastructure. The tasks have been divided into four phases:

1. First, a map was created to identify the maintenance history and location of older sections of pipe near the river mouth and sections crossing the River. This task was completed in August 2011.
2. Second, Clean Water Program and Sewer Division staff compiled information from GIS sewage infrastructure maps, the initial maintenance history search, and staff knowledge to identify the potential human bacteria sources for each water quality monitoring site sampled through the Project. This task was also completed in August 2011 and a summary is provided below.
3. In the third phase, the City will conduct a more resource intensive process of identifying the maintenance history of sewage infrastructure that runs parallel to the River throughout its jurisdictional boundaries. These sections of pipe will then be prioritized for either CCTV surveys and/or cleaning. The research and prioritization task is scheduled to be completed within a year.
4. The final phase will be to complete the CCTV surveys and/or cleaning of the prioritized lines as resources are available in order of priority. It is anticipated that this task will be completed within five years.

Below is a summary of the initial human source identification and site-specific recommendations for further study. Sewage infrastructure within the City's jurisdiction not only includes City sewer lines and force mains, but also sewage infrastructure from Camp Pendleton military base, Fallbrook Public Utilities District (FPUD), and Rainbow Municipal Water District (RMWD). Septic systems within the City are regulated and permitted by the County of San Diego Department of Environmental Health.

River Mouth:

- As of July 2011, a probable human source in the river mouth may have been identified. A private sewer force main, for a condominium structure built in 2009, (not maintained by the City) on the south side of the river mouth, just west of Interstate 5 was found to be leaking. In June 2011, a sewage overflow was identified by the condominium complex crews. All surface spills were contained on the property. Ground water contamination and seepage near the river may have occurred, but there is no way of confirming this due to repairs being made as of June 2011. All required reporting information was filed by the property owner and repairs began immediately. While information is still being collected, preliminary assessments indicate the leak may have been present during the time of the 2010 and 2011 dry and wet weather events. The possibility of confirmation is low.
- A 10" City force main is attached to the railway trestles and crosses the river mouth east of the river mouth sampling locations. There is no access point for a CCTV survey, but external visual observations are completed periodically.
- A 16" Camp Pendleton force main is attached to the Interstate 5 Bridge further east of the river mouth locations. Per the maintenance agreement between the City and Camp Pendleton, the City conducts external visual observations of the force main periodically.
- Results from the first phase of the sewer system survey identified approximately 4,000 feet of aging sewage infrastructure on the north side of the River east of Interstate 5. This section of 10" clay pipe was constructed in 1953. A CCTV survey was completed in 2004 and the section was last cleaned in 2009, indicating that the pipe is being properly maintained and that no major problems have been identified. However, erosion from the cliffs above and the River to its south have made the easement road inaccessible and, thus, visual observations for a portion of this line is not possible at this time. A rehabilitation of this line and/or rehabilitation of the easement road have been identified as priority projects as resources are made available.

Critical Point:

- The area directly surrounding the Critical Point has little sewage infrastructure. A sewer line owned and maintained by Fallbrook Public Utilities District (FPUD) is south of the River and parallels Highway 76. The flows are composed of tertiary treated wastewater and are used by Caltrans for irrigation purposes. It is unlikely that this treated wastewater is a potential human source as it is disinfected prior to distribution.
- There is a commercial area and a monastery to the north. Because there are no sewer connections from these areas, it is likely that these land uses utilize portable restroom facilities or septic systems. More research is recommended to identify if these uses are a probable source.
- Approximately one mile upstream of the Critical Point is the next force main crossing the River. A 24" iron pipe constructed in 1958 carries flows from the neighborhood north of the River. As with other force mains CCTV and other internal visual inspections are not feasible at this time due to the amount and velocity of flow. However, external visual inspections are completed periodically.

Pilgrim Creek Tributary:

- An additional map was created by the Sewer Division specifically for the Pilgrim Creek drainage area. The area contains two wastewater treatment plants; the City's San Luis Rey Wastewater

Treatment Plant and a treatment plant in Camp Pendleton. The City's plant includes approximately 20 force main and gravity lines crossing or near the Creek. Additional sources include the FPUD sewer line crossing the Creek near the Camp Pendleton boundary and any potential discharges from the drainage area upstream of the City's boundary within the Camp Pendleton military base, including the treatment plant. In addition, a City golf course using reclaimed water for irrigation is also within the drainage area.

- Because of the complex system, further monitoring, potentially including a source tracking study utilizing human genetic markers, is recommended as resources become available for this drainage area to pinpoint the sources of bacteria during dry and wet weather.

Douglas Bridge:

- FPUD's 16" iron sewer outfall line crosses the River and is attached to the Douglas Bridge. FPUD is responsible for maintenance of this line; however, occasional visual inspections are conducted by the Sewer Division. Any required maintenance is then referred to FPUD.

College Bridge:

- A 24" clay pipe constructed in 1994 is attached to the College Bridge. This section of sewage infrastructure was last inspected in 2009. External visual inspections are completed periodically.
- Within a mile upstream of College Bridge, the majority of the City sewage infrastructure ends. Land uses upstream of this point change to agricultural and rural residential. Wastewater in this area is treated by septic systems, which are permitted and regulated by the County of San Diego Department of Environmental Health.
- At the eastern terminus of the City's maintained sewage infrastructure on the north side of the River, RMWD maintains a line that discharges their sewage into the City's infrastructure. This line runs parallel to the River under North River Road. Rainbow is responsible for maintenance and inspection of this portion of line.

Sleeping Indian Tributary:

- There are no City sewer lines within the Sleeping Indian drainage area.
- The Rainbow Municipal Water District infrastructure does transect the downstream portion of the Sleeping Indian drainage area.
- Wastewater in this area is treated by septic systems, which are permitted and regulated by the County of San Diego Department of Environmental Health.

Bonsall Bridge:

- This area is outside of the City's jurisdictional boundary and therefore a sanitary sewer assessment was not completed in this area.

6.0 REFERENCES

Ahmed, W., Goonetilleke, A., Powell, D., & Gardner, T. (2009). Evaluation of multiple sewage-associated *Bacteroides* PCR markers for sewage pollution tracking. *Water Research*, 43(19): 4872-4877.

Bernhard, A. E. & Field, K. G. (2000). A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides-Prevotella* genes encoding 16S rRNA. *Applied and Environmental Microbiology*, 66(10): 4571-4574.

Bertrand-Krajewski, J., Chebbo, G., & Saget, A. (1998). Distribution of pollutant mass vs. volume in stormwater discharges and the first flush phenomenon. *Water Research*, 32 (8), 2341e2356.

Cao, Y., Cherr, G. N., Cordova-Kreylos, A. L., Fan, T. W.-M., Green, P. G., Higashi, R. M., LaMontagne, M. G., Scow, K. M., Vines, C. A., Yuan, J., & Holden, P. A. (2006). Relationships between sediment microbial communities and pollutants in two California salt marshes. *Microbial Ecology*, Volume 52, 619–633.

City of Oceanside, County of San Diego, and City of Vista, Watershed Urban Runoff Management Program (WURMP). January 2010. *San Luis Rey WURMP Annual Report for Fiscal Year 2008 - 2009, Attachment B: SLR Watershed Water Quality Monitoring Program.*

City of Oceanside, County of San Diego, and City of Vista, Watershed Urban Runoff Management Program (WURMP). March 2008. *Watershed Urban Runoff Management Program.*

Conn, K. E., Habteselassie, M. Y., Blackwood, A. D., and R. T. Noble. (2011). Microbial water quality before and after the repair of a failing onsite wastewater treatment system adjacent to coastal waters. Journal of Applied Microbiology. July 2011.

Converse, R. R., Blackwood A.D., Kirs, M., Griffith, J. F., & Noble, R. T. (2009). Rapid QPCR-based assay for fecal *Bacteroides* spp. as a tool for assessing fecal contamination in recreational waters. *Water Research*, 43, 4828-4837.

Davis, E., Casserly D. M., & Moore, J. D. (1977). Bacterial relationships in stormwaters. *Water Research Bulletin*, 13, 895-905.

Feldner, R., Power, C., Scanlon, B., Frischer, M., Richardson, J. (2007). Bacterial Source Tracking in yhe Vernon River Watershed. Proceedings of the 2007 Georgia Water Resources Conference, held March 27–29, 2007, at the University of Georgia.

Fuhrman J. A., Liang, X., & Noble, R. T. (2005). Rapid detection of enteroviruses in small volumes of natural waters by real-time quantitative reverse transcriptase PCR. *Applied and Environmental Microbiology*, 71(8): 4523-4530.

Gregory, J. B., Litaker, W. A., and Noble, R. T. (2006). A rapid one-step quantitative reverse transcriptase PCR assay for enterovirus with a competitive internal positive control. *Applied and Environmental Microbiology*. 72 (6): 3960-3967.

Gupta, K., & Saul, A. J. (1996). Specific relationships for the first flush load in combined sewer flows. *Water Research*, 20 (5), 1244e1252.

Heaney, C. D., Sams, E., Dufour, A., Brenner, K., Haugland, R., Wymer, L., Wing, S., Marshall, S., Serre, M., Seed, R., Beach, M., Love, D., Noble, R. T., & Wade, T. J. (2011). Fecal indicators in sand, sand contact, and risk of enteric illness among beach-goers. Submitted. *Journal of Epidemiology*.

Kildare, B. J., Leutenegger, C. M., McSwain, B. S., Bambic, D. G., Rajal, V. B., & Wuertz, S. (2007). 16S rRNA-based assays for quantitative detection of universal, human-, cow- and dog-specific fecal *Bacteroidales*: a Bayesian approach. *Water Research*, 41(16):3701–3715.

Lee, J. H., Bang, K. W., Ketchum, L. H., Choe, J. S., & Yu, M. J. (2002). First flush analysis of urban storm runoff. *Science of the Total Environment*, 293 (1e3), 163e175.

Lu, J., Santo Domingo, J. W., Lamendella, R., Edge, T., & Hill, S. (2008). Phylogenetic diversity and molecular detection of bacteria in gull feces. *Applied and Environmental Microbiology*, 74: 3969-3976.

Noble, R. T., & Fuhrman, J. A. (2001). Enteroviruses detected in the coastal waters of Santa Monica Bay, CA: Low correlation to bacterial indicators. *Hydrobiologia*, 460:175-184.

Parker, J. K., McIntyre, D., & Noble, R. T. (2010). Characterizing fecal contamination in stormwater runoff in coastal North Carolina, USA. *Water Research*, 44, 4186-4194.

Rantz, S. E., et al. (1982). Measurement and Computation of Streamflow: Volumes 1 and 2. U.S. Geological Survey Water-Supply Paper 2175. United States Government Printing Office, Washington D.C.

SDRWQCB (San Diego Regional Water Quality Control Board). (2008a). Resolution No. R9-2008-0028. Amendment to the Water quality Control Plan for the San Diego Basin (9) to Incorporate Implementation Provisions for Indicator Bacteria Water Quality Objectives to Account for Loading from Natural Uncontrollable Sources within the Context of a Total Maximum Daily Load. May 14, 2008.

Seurinck, S., Defoirdt, T., Verstraete, W., & Siciliano, S. D. (2005). Detection and quantification of the human-specific HF183 *Bacteroides* 16S rRNA genetic marker with real-time PCR for assessment of human faecal pollution in freshwater. *Environmental Microbiology*, 7(2), 249–259.

Stein, E., & Yoon, V. K. (2007). Assessment of water quality concentrations and loads from natural landscapes. Technical Report 500. Southern California Coastal Research Project (SCCWRP), Costa Mesa, California.

Stoeckel, D. M., & Harwood, V. J. (2007). Performance, design and analysis in microbial source tracking studies. *Applied and Environmental Microbiology*, 73(8): 2405-15.

Stumpf, C. H., Piehler, M. F., Thompson, S., & Noble, R. T. (2010). Loading of fecal indicator bacteria in North Carolina tidal creek headwaters: Hydrographic patterns and terrestrial runoff relationships. *Water Research*, 44, 4704-4715.

USGS Website. USGS 11042000 San Luis Rey River at Oceanside Ca. Viewed April 1, 2011. http://waterdata.usgs.gov/ca/nwis/uv/?site_no=11042000&PARAmeter_cd=00065,00060.

Wade, T. J., Sams, E., Brenner, K. P., Haugland, R., Chern, E., Beach, M., Wymer, L., Rankin, C. C., Love, D., Li, Q., Noble, R. T., & Dufour, A. P. (2010). Rapidly measured indicators of recreational water quality and swimming-associated illness at marine beaches. Submitted. *Journal of Epidemiology*.

Watson, R. J., & Blackwell, B. (2000). Purification and characterization of a common soil component which inhibits the polymerase chain reaction. *Canadian Journal of Microbiology*, 46:633-642.

For more information, please contact:

Mo Lahsaie, Ph.D., REHS
Clean Water Program Coordinator
Water Utilities Department
300 North Coast Highway
Oceanside, CA 92054
Tel: (760) 435-5803
Fax: (760) 435-5814
E-MAIL: mlahsaie@ci.oceanside.ca.us

www.oceansidecleanwaterprogram.org